Postharvest moisture loss in Japanese plums

By

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: April 2019

DEDICATION

to Elke Kritzinger

my sister

because you carry my heart with you

you carry it in your heart

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SUMMARY

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Plums exported from South Africa reach overseas markets after a long sea freight period. Yet consumers still expect fruit to be in perfect condition upon arrival at the supermarket. While care is taken to limit moisture loss throughout the handling chain, fruit still show the negative effects thereof. Reduced fruit quality due to moisture loss may lead to rejection of export consignments at overseas markets, causing major financial losses for South African producers.

The aim of this study was to investigate the role of the fruit cuticle in determining moisture loss and susceptibility to shrivel development in Japanese plum cultivars.

Peel permeability differed between farms, seasons, cultivars, orchards and developmental stage. In general, the water vapour permeance of the peel was higher in cultivars that are susceptible to moisture loss and shrivelling. However, this was not true in all cases and measuring pre-harvest water vapour permeance of the peel to predict shrivel susceptibility was only successful in some cultivars.

Lenticel numbers differed between seasons and cultivars and clearly contribute to moisture loss, but this contribution differs between cultivars. As the number of open lenticels could not explain all the variation in peel permeability between cultivars, cuticle composition must play an important role in determining peel permeability.

Cuticular composition differed significantly between cultivars and seasons. The compound 2,4-bis (dimethyl benzyl) phenol was present in high concentration in both cultivars. We propose that the combination of a rigid cuticle, due to high phenol content, fewer tri-hydroxy acids, and high primary alcohol content, and its smaller intercellular spaces, reduces ‘Songold’ cuticle deformation due to excessive postharvest moisture loss. Since the hypodermal cells of ‘Songold’ are closer together, their dehydration and collapse might not lead to significant shrinkage compared to the other cultivars. The cuticle is rigid, which means that it is less likely to collapse when the supporting cells underneath it shrink and collapse due to moisture loss.

Packaging solutions to reduce moisture loss need to be optimized for individual cultivars since they vary so much in terms of susceptibility to moisture loss and shrivel. Using Low-Density Poly-Ethylene packaging with 92 or 72 micro-perforations might be a viable option to reduce moisture loss, while still preventing excessive in-package humidity, decay and chilling injury. In seasons when high rates of moisture loss are experienced, the use of these bags might reduce the number of consignments rejected at overseas markets.

This study showed the complex interplay of different cuticle characteristics in response to or as a result of, moisture loss. It would be interesting to investigate how environmental signals lead to a certain cuticular response – which genes are involved, how these genes activated and so forth. Elucidating some of the mechanisms involved in the functioning and response of this complex biopolymer might enable manipulation of the cuticle to improve fruit quality and extend shelf life or to select and breed cultivars that are not prone to cuticular defects.

OPSOMMING

Na-oes vogverlies in Japanese pruime

Pruime wat vanaf Suid-Afrika na oorsese markte uitgevoer word, spandeer lang periodes in opberging.Verbruikers verwag egter dat vrugte in perfekte kondisie by supermarkte aanland. Alhoewel vogverlies so veel as moontlik beperk word in die koueketting, affekteer die negatiewe effek van vogverlies steeds vrugkwaliteit. Verlaagde kwaliteit van vrugte as gevolg van vogverlies lei tot afkeur van vrugte by aankoms in oorsese markte. Dit lei tot geweldige finansiële verliese vir Suid-Afrikaanse steenvrug-produsente.

Die doel van hierdie studie was om die rol van die kutikula in vogerverlies en verrimpeling van Japanese pruim kultivars te ondersoek.

Skildeurlaatbaarheid het beduidend verskil tussen seisoene en kultivars. Die skildeurlaatbaarheid van kultivars wat sensitief is ten opsigte van vogverlies en verrimpeling was beduidend hoër as die deurlaatbaarheid van nie-sensitiewe kultivars. Hierdie bevindinge was egter nie van toepassing op al die kultivars nie. Die bepaling van vooroes skildeurlaatbaarheid om na-oes verripeling te voorspel was dus net suksesvol in sekere kultivars.

Die aantal lentiselle het beduidend verskil tussen seisoene en kultivars en het duidelik ‘n bydrae gelwer tot vogverlies. Hierdie bydrae het egter verskil tussen kultivars. Aangesien die hoeveelheid oop lentiselle nie al die variasie in skildeurlaatbaarheid tussen kultivars kon verklaar nie, speel die samestelling van die kutikula duidelik ‘n rol in die bepaling van skildeurlaatbaarheid.

Die samestelling van die kutikula het ook beduidend verskil tussen kultivars en seisoene. ‘n Komponent, naamlik 2,4-bis(dimetiel benzyl) fenol, het in hoë konsentrasies voorgekom in albei kultivars. ‘n Kombinasie van ‘n rigiede kutikula, as gevolg van hoë fenol konsentrasies, tri-hidroksie sure, en primêre alkohole, saam met die kleiner intersellulêre ruimtes van ‘Songold’, verminder die kanse van misvorming as gevolg van oormatige vogverlies in hierdie kultivar. Aangesien die hipodermale selle van ‘Songold’ nader aan mekaar is, sal hul dehidrasie en ineenstorting moontlik nie lei tot soveel misvorming en verrimpeling as die ander kultivars nie. Die kutikula is

meer rigied, wat beteken dat dit minder geneig sal wees om ineen te stort wanneer die ondersteunende selle onder die kutikula ineenstort as gevolg van vogverlies.

Plastiek verpakking om vogverlies te verminder moet volgens individuele kultivars aangepas word, aangesien daar so baie variasie is tussen kultivars in terme van gevoeligheid tot vogverlies en verrimpeling. Die gebruik van Lae Digtheid Poli-Etileen sakke met 72 of 92 mikro-perforasies het vogvervlies verminder sonder om vrugkwaliteit negatief te beïnvloed. In seisoene wannneer vogverlies en verrimpeling hoog is, sal gebruik van hierdie sakke die aantal versendings wat by oorsese markte afgekeur word, kan verminder.

Hierdie studie het bewys dat daar ‘n komplekse interaksie tussen die verskillende eienskappe van die kutikula is in reaksie op, of as gevolg van, vogverlies. Dit sal interessant wees om te die omgewings-seine wat tot verandering van die kutikula lei te ondersoek en te bepaal watter gene betrokke is, hoe hulle geaktiveer word, ensovoorts. Kennis van die meganismes betrokke by die funksionering en reaksies van hierdie komplekse polimeer kan die manipulasie van die kutikula om vrugkwaliteit en raklewe te verleng of kultivars te teel wat nie geneig is tot kutikula defekte nie.

NOTE

This thesis is a compilation of chapters, starting with a literature review, followed by five research papers. Each paper is prepared as a scientific paper for submission to *Scientia Horticulturae*. Repetition or duplication between papers might therefore be necessary.

GENERAL INTRODUCTION AND OBJECTIVES

Plums produced in South Africa are mostly exported by sea, which requires a sea freight period of nearly three weeks (Theron, 2015). Early season plum cultivars are stored for approx. 35 days, while some late season cultivars can be stored for approx. 56 days to allow the stock to reach the overseas markets. Even though South African plums need to be stored for such extended periods, consumers still have very high expectations with regards to quality when buying the fruit. However, some cultivars are susceptible to development of a shrivelled appearance at the pedicel end of the fruit during cold storage, which has a negative impact on the appearance of the fruit.

After harvest, fruit continue to lose water, but since the water cannot be replaced naturally, the fruit must rely on its internal water content available at harvest (Mahajan et al., 2008; Sastry, 1985; Wilson et al., 1995). Continued moisture loss from fresh produce leads to shrinkage, shrivelling, textural changes and mass loss. Some products can lose between 5 and 10 % of their fresh mass before they start to wilt and becomes unusable. Excessive mass loss and shrivel of exported plums can render the product completely worthless, leading to significant financial losses for South African stone fruit producers. Postharvest moisture loss from the fruit must therefore be limited as much as possible.

Transpiration rate is influenced by temperature, relative humidity (RH), fruit surface area, respiration rate and air movement over the fruit (Holcroft, 2015; Mahajan et al., 2008). The RH of the ambient atmosphere has a considerable effect on the moisture loss of fresh products during storage. Transpiration rate increases with increasing temperature and decreasing RH. Yet, high RH can lead to the accumulation of a thin layer of moisture on the fruit surface, which can increase susceptibility to decay. As fruit still respire after harvest, it releases water vapour during the respiration process and, if allowed to accumulate in the packaging, it can further enhance microbial growth.

To maintain optimal fruit quality, the current recommended handling protocol for South African plums is the removal of field heat directly after harvest, using forced air cooling to reduce the pulp temperature to 15°C within 3 h (HORTGRO, 2015). Fruit must be packed on the day of harvest and then force air cooled to a pulp temperature of -0.5°C within 24 to 36 h. Since many of the factors that influence postharvest moisture loss

(cultivar, season, environment, preharvest factors etc.) are difficult, if not impossible, to control completely, it is of the utmost importance to minimize moisture loss during the entire handling chain from orchard to consumer. Still, moisture loss and shrivelling of stone fruit is a significant postharvest problem in the South African stone fruit industry.

The fruit cuticle is the primary barrier to moisture loss (Riederer and Schreiber, 2001; Yeats and Rose, 2013). Yet, to our knowledge, no research has been done to investigate the structural and compositional qualities of Japanese plum cuticles and how these properties affect postharvest moisture loss and shrivel development.

In Paper 1 we determined the peel water vapour permeabilities of a range of plum cultivars to establish if permeability controls shrivel incidence.

In Paper 2 we investigated whether structural differences exist between the epicuticular waxes of a cultivar that is susceptible to shrivel versus a cultivar that is not susceptible to shrivel, using scanning electron microscopy (SEM). The contribution of lenticels to postharvest moisture loss and shrivel was also explored.

Paper 3 identified the chemical composition of the cuticular waxes and cutin, as well as their relative amounts in a shrivel susceptible and a non-susceptible cultivar.

To compare cuticle and epidermal microstructure between two cultivars, scanning electron microscopy and light microscopy were employed in Paper 4.

As a possible prevention of postharvest moisture loss, novel perforated bag technology was applied in Paper 5.

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LITERATURE REVIEW

The fruit cuticle as a barrier to moisture loss

1. Introduction

The plant cuticle is as a continuous extracellular layer that covers the above-ground organs of plants (Belding et al., 1998; Koch and Ensikat, 2008). It is a polymeric, lipid membrane that is synthesized by the epidermal cells and located on top of the epidermal cell walls (Belge et al., 2014a; Holloway, 1982; Schreiber and Schönherr, 2009). The main function of the cuticle is to act as a barrier to extreme transpirational moisture loss, while still allowing gas exchange and transpiration to be controlled by the stomata (Riederer and Schreiber, 2001; Yeats and Rose, 2013).

Excessive transpirational moisture loss can cause water deficits, malfunctioning of many cellular processes during growth and development, and loss of turgor pressure within the cells (Taiz and Zeiger, 2010). Turgor pressure is essential, since it is involved in physiological processes, such as cell enlargement and stomatal opening, and it contributes to the mechanical strength and stability of non-lignified tissues.

Postharvest mass loss can lead to shrivelling of the fruit surface and changes in wax structure (Maguire et al., 1999a; Veraverbeke et al., 2001a, 2001b). Shrinkage of fresh products due to moisture loss leads to quality deterioration and a loss in saleable mass (Sastry, 1985b). In ‘Conference’ pears, small amounts of moisture loss can lead to shrivelling at the stem end of the fruit, which reduces the commercial value of the fruit (Nguyen et al., 2006). During dehydration of a fruit (at shelf-life of 20˚C), the fruit shrinks, the water from the cells is released into the intercellular spaces, and cell volume decreases. Transpirational moisture loss also determines the water balance of fruit, which, in turn, determines several quality parameters at harvest, including fruit size and sugar content (Lescourret et al., 2001). Moisture loss from fruit continues even after harvesting of the fruit (Holcroft, 2015; Mahajan et al., 2008). Since the moisture lost from the fruit cannot be replaced anymore, the fruit continue to lose moisture, leading to mass loss, textural changes, wilting and shrivelling (Holcroft, 2015; Mahajan et al., 2008; Nguyen et al., 2006). Fruit quality is further affected by a reduction in firmness, glossiness and shelf-life (Kissinger et al., 2005).

In addition to acting as a barrier to moisture loss, the cuticle has several secondary functions that are associated with its status as the outermost layer of above-ground organs. It forms a physical barrier against pests and pathogens (Knoche and Peschel, 2007). In many species, epicuticular crystals prevent dust and other debris from blocking sunlight, effectively turning the cuticle into a self-cleaning surface. The cuticle also screens excessive UV light and it plays a significant role in development by physically establishing organ boundaries (Belding et al., 1998; Riederer, 2006; Yeats and Rose, 2013).

As the distinct functions of the cuticle have already been discussed by numerous authors (Dominguez et al., 2011; Lee and Priestley, 1924; Martin, 1964; Martin and Juniper, 1970a; Riederer, 2006; Yeats and Rose, 2013), the aim of this review is to focus on the properties of the cuticle that render it an effective barrier to moisture loss. Since research on fruit cuticles is limited, research on leaf cuticles is often included in this review. Since the cuticle is the fruits' most important defence against moisture loss, knowledge about its composition and structure, and, more importantly, the influence of the cuticle on postharvest fruit moisture loss, is vital to understand the mode of action of postharvest fruit moisture loss. This knowledge could enable producers to improve management throughout the cold chain to prevent moisture loss and maintain fruit quality.

2. Cuticle structure

The cuticle is composed of three separate groups of lipid substances: insoluble, polymeric cutins and cutans, which form the scaffolding of the membrane, and soluble lipids, referred to as waxes (Holloway, 1982; Pollard et al., 2008; Yeats and Rose, 2013). Epicuticular waxes form the outermost layer of the cuticle. Waxes are also embedded within the cutin matrix, forming intracuticular waxes (Holloway, 1982; Schreiber and Schönherr, 2009). Beneath the layer of epicuticular wax is a layer referred to as the cuticle proper (CP) (Fig. 1). This layer is composed of the cutin matrix and contains no cellulose or cell wall materials (Commenil et al., 1997; Holloway, 1982). Cutin forms a rigid meshwork of inter-esterified hydroxy fatty acids (Walton and Kolattukudy, 1972) and therefore provides most of the mechanical strength to the cuticle (Petracek and Bukovac, 1995). The physical and chemical properties of the

cuticle are determined by its cutin and wax components, which is the primary area of interaction between the plant and its environment.

Beneath the cuticle proper lies one or more cuticular layers (CL). Under these layers is a layer of pectin that is continuous with the anticlinal walls of the epidermal cells (Martin and Juniper, 1970b). The number, thickness and distinction between the various cuticle layers varies among species as well as stage of development (Holloway, 1982; Pollard et al., 2008; Yeats and Rose, 2013). Together, the three different layers are referred to as the cuticular membrane (CM), of which the cuticle proper forms most of the CM when it is fully developed (Martin and Juniper, 1970b).

3. Cuticle composition and biosynthesis

3.1 Waxes

The outermost layer of the cuticle is formed by the epicuticular waxes, which consist of two main classes of substances: linear long-chain aliphatic compounds and cyclic terpenoids (Schreiber and Schönherr, 2009; Yeats and Rose, 2013). The aliphatic compounds include long-chain alcohols, aldehydes, fatty acids, ketones (El-Otmani et al., 1989; Schreiber and Schönherr, 2009; Yeats and Rose, 2013). Crystalline domains form when the long aliphatic chains assemble in lattices, while amorphous zones form in between these domains, consisting of chain ends, functional groups, short-chain aliphatics and non-aliphatic compounds (Reynhardt and Riederer, 1994; Riederer and Schneider, 1990). The size and spatial arrangement of the crystalline and amorphous domains determine the mobility of permeating water and solute molecules within the cuticle.

Epicuticular waxes give the surface of an organ distinct properties (Yeats and Rose, 2013). Wax blooms, formed by densely packed microcrystalline areas have two main functions (Schreiber and Schönherr, 2009). Light reflection reduces heat damage to leaves or fruit and decreases the wettability of the fruit surface, which prevents leaching of solutes from the apoplast during rain. The glossy appearance of some leaves and fruit types (e.g. tomatoes), are formed by wax films, while wax crystals account for the dull, glaucous (greyish coloured, powdery bloom) appearance found in broccoli leaves and Arabidopsis stems. The visible waxy bloom is caused by the reflection and scattering of light on the surface of the wax crystal deposits (Martin and

Juniper, 1970a). A bloom, however, does not necessarily indicate excessive waxiness – some surfaces without a bloom may still contain a large amount of wax, like plum fruit that are not waxier than apples or pears, but show a more definite bloom. The wax structures on plums just scatter light more effectively than the platelets of wax that occur on apples or pears.

Since epicuticular waxes are easily extracted and examined by light and electron microscopy, their properties, structure and chemistry have been well studied (Schreiber and Schönherr, 2009). The chemical composition of the wax may differ between areas on a single plant and the fine structure may vary correspondingly (Baker, 1982). Therefore, the epicuticular waxes are classified into different groups according to their fine structure (Jeffree, 2006). The most common morphologies are amorphous films, grains or granules, plates (simple or crenate, polygonal or rounded or spiky, prostate or erect), filaments, rods, tubes with a hollow centre, elongated flattened ribbons and plates. The link between wax morphology and the underlying chemical and genetic basis is not yet completely understood.

Biosynthesis of the long-chain aliphatic compounds occurs in the plastids of epidermal cells and starts with the de novo synthesis of C_{16} and C_{18} fatty acids (Schreiber and Schönherr, 2009; Yeats and Rose, 2013). These fatty acid compounds are then converted to CoA thioesters by a long-chain acyl-coenzyme A synthase (LACS) isozyme and are ultimately transferred to the ER (Yeats and Rose, 2013), where the C_{16} acyl-CoA serves as a substrate for the fatty acid elongase (FAE) complex. Through successive addition of two carbons per cycle (derived from malonyl-CoA), the ultimate products of this complex are very-long-chain fatty acids (VLCFAs). Therefore, elongated fatty acids predominantly have even–numbered carbon chains (Schreiber and Schönherr, 2009). Oxidation leads to the formation of aldehydes and primary alcohols, also with even numbered chain-length. Alkane synthesis involves a decarboxylation step, and thus they are characterised by odd-numbered chain lengths. Secondary alcohols are synthesised from alkanes and are therefore also odd-numbered.

The contributions of epicuticular and intracuticular waxes on permeability of the cuticle is not known (Schreiber and Schönherr, 2009). To determine this, epicuticular waxes would have to be removed quantitatively without disturbing intracuticular waxes. As

solvents very rapidly penetrate the cuticle, separating the epicuticular and intracuticular waxes is very difficult. Therefore, most studies of plant cuticles involve a combined analysis of both the epicuticular and intracuticular waxes.

3.2 Cutin

It is very difficult to isolate cutin in a pure state from plant material because it cannot be extracted with any solvent (Holloway, 1982). However, since the polymers are located mainly in the cuticular membrane which can usually be isolated through enzymatic or chemical treatments that disrupt the pectinaceous components of the cell walls, the cutin monomers can easily be extracted. Incubation with pectinase enzymes, buffered at pH 3.4-4, are generally used for cuticle isolation. The isolated cuticular membrane is released through the disruption of the lateral walls of the epidermal cells and not by dissolution of the 'pectin layer' present at the junction between cuticular membrane and cell wall. The key step in the isolation of cutin from the cuticular membrane, is the removal of all soluble components (waxes) by extraction with organic solvents (e.g. chloroform). Waxes embedded within the cutin matrix are difficult to remove and requires lengthy extraction with chloroform-methanol solutions. As all plant cutins are insoluble polyesters, they can be depolymerized by any of the common reagents used to cleave ester bonds, but cutins are depolymerized most rapidly using alcoholic solutions of alkali.

Like the wax precursors, the cutin precursors are synthesized in the plastids of epidermal cells (Yeats and Rose, 2013). Cutin monomers are usually C_{16} and C_{18} ω-hydroxy fatty acids that can contain one or two additional mid-chain hydroxyl groups or an epoxy group (Holloway, 1982; Pollard et al., 2008). The first step of cutin monomer biosynthesis is the *de novo* synthesis of fatty acids. The following three steps of biosynthesis occur in the endoplasmic reticulum (ER) and consists of ω-hydroxylation, mid-chain hydroxylation and the synthesis of an acyl-CoA intermediate. Self-polymerization of these monomers produces a linear polyester chain (Pollard et al., 2008). The mid-chain hydroxyls can also attach to ω-hydroxy fatty acid monomers through esterification, leading to a branched structure. However, it is still unknown whether cutin exists as multiple discrete polymer molecules that may be anchored to the cell wall or whether it is a highly cross-linked continuum. It is also still unclear how branching or cross linking of cutin affects cuticle functions (Yeats and Rose, 2013).

In the cuticle of some species, once all the wax and cutin components have been removed, some residual material remains; this is referred to as cutan (Heredia, 2003; Jeffree, 1996; Pollard et al., 2008). This depolymerisation-resistant residue represents cutin monomers held together by non-ester bonds (Pollard et al., 2008). Being an amorphous solid, cutan is highly resistant to further degradation without loss of chemical information and the monomers are thought to be held together by ether-and C-C bonds. Although the cuticles of some species appear to completely lack cutans, in other species, cutin and cutan can occur in any ratio, differing in their relative abundance at different stages of cuticle development (Tegelaar et al., 1996).

3.3 Precursor assembly

After synthesis of the wax and cutin precursors is completed, they are exported from the ER, across the plasma membrane, through the cell wall, and onto the cuticular membrane. To reach the surface, the precursors must pass through the plasmalemma and the outer wall of the epidermal cells (Martin and Juniper, 1970c). Movement through the plasmalemma occurs via diffusion, and the solubility of these precursors in the lipids of the plasmalemma facilitate the movement of large molecules. Most of these transport processes are poorly understood, although trafficking of both wax and cutin precursors across the plasma membrane has been shown to depend on ATP-binding cassette (ABC) transporters (Yeats and Rose, 2013). The last step of cutin synthesis is the incorporation of the hydroxy-acyl monomers into the polymer to form the cutin framework. Again, the mechanism of this step has not been completely uncovered.

4. Fruit cuticle development

There are significant species- and cultivar- related differences in cuticle composition and susceptibility to moisture loss, as observed in sweet cherries, apples, tomatoes and peaches (Belge et al., 2014a, 2014b; Lara et al., 2014; Leide et al., 2007; Martin and Juniper, 1970c). Relative to leaves, the cuticles of fruit are thicker and there is more cuticle per surface area (Martin and Juniper, 1970b; Parsons et al., 2013). These differences between fruit and leaves are thought to be related to differences in susceptibility to moisture loss between the organs (Parsons et al., 2013). Young fruit have well-developed waxy cuticles and more wax and cutin are rapidly laid down as the fruit increase in size (Martin and Juniper, 1970c). Cutin, however, does not play a

significant role as a barrier to moisture loss, but rather acts as a framework into which the intracuticular waxes are deposited (Isaacson et al., 2009). A stronger or larger cutin matrix may therefore allow for the deposition of more waxes.

The cuticle develops and thickens as the fruit develops and enlarges (Commenil et al., 1997; El-Otmani et al., 1989; Martin and Juniper, 1970c). The primary waxes that cover the ovaries after anthesis flatten and spread onto the surface as fruit growth continues (Commenil et al., 1997). The epicuticular wax layer initially consists of mostly small, individual, upright wax platelets, called secondary waxes. At maturity, only remnants of the primary wax structures are still visible. The wax platelets are rougher and densely distributed at harvest. Most fruit with a prominent waxy bloom such as figs, grapes and some varieties of prune, do not develop the bloom until the fruit have reached, or are approaching maturity (Martin and Juniper, 1970c). The epicuticular wax of mature ‘D’Agen’ plums, taken from regions of the skin that showed visible bloom, consists of an outer crystalline layer with an underlying amorphous layer (Storey and Price, 1999a). These epicuticular waxes have a closely packed, granular structure, overlying a more amorphous layer. However, there are major differences in the crystalline form of the epicuticular wax on the bloom and non-bloom side of the fruit. Crystalline wax granules on the non-bloom side are finer and less dense compared to the bloom-side of the fruit. The formation of the bloom over the fruit surface is probably influenced by the microclimate around the fruit, such as the effect of incident radiation on skin temperature. This agrees with observations in apples, where the cuticle is much thicker on the blush side of the fruit (Konarska, 2013).

Even after harvest, during cold storage, cuticle properties can still change. The cuticle yield of both sweet cherries and peaches increases during cold storage, providing evidence that cuticular thickening continues after harvest (Belge et al., 2014a, 2014b). In sweet cherries, wax alkanes tend to decrease during cold storage, while the triterpene content remains stable (Belge et al., 2014a). Consequently, the ratio of triterpenes to alkanes increases, with ursolic and oleic acids representing the two main components. Reduced wax alkanes and enhanced triterpenoids lead to an increase in amorphous waxes, which in turn, impaired the water barrier properties of the cuticle (Isaacson et al., 2009). In fact, the ratio of alkanes to triterpenoids plus sterols correlates inversely with dehydration rates in pepper fruit

In apples, both the structure and composition of the cuticular wax change during cold storage (Veraverbeke et al., 2001a). The wax layer limits post-harvest moisture loss by changes in its composition and distribution or by covering cracks and stomata. (Parsons et al., 2012). In some cultivars, there is a continuous accumulation of waxy or greasy materials on the surface of the cuticle even after harvest, probably to further reduce moisture loss. However, continued accumulation of these materials can give the fruit a greasy and sticky appearance (Veraverbeke et al., 2001b). Therefore, changes in wax properties can have major economic consequences, in a positive way due to reduction of excessive moisture and mass loss, but eventually having a negative effect on fruit appearance, as a greasy layer forms over the fruit surface. These changes are mostly related to hydrolysis of the ester fraction (Parsons et al., 2012). Hydrolysis of esters leads to increased free fatty acids, especially C_{16} and C_{18} fatty acids. During cold storage, many metabolic processes in fruit are enhanced or redirected in response to low temperatures and it is suggested that cuticle formation is one such process (Belge et al., 2014a). Developmentally regulated changes in the n-alkane constituents of cuticular waxes during fruit ripening is one of the major determinants of the permeability of the fruit cuticle (Leide et al., 2007; Martin and Juniper, 1970c). Nevertheless, more wax does not necessarily result in reduced permeability of the fruit peel, because cuticular wax composition and structure, rather than quantity, predominantly affect the barrier properties of fruit cuticles (Belge et al., 2014b; Leide et al., 2007). Thus, the assumption that fruit with thicker cuticles or a visible waxy bloom will be less prone to moisture loss, is incorrect.

5. Factors that influence cuticles

5.1 Factors influencing moisture loss

Moisture loss through the fruit peel and cuticle is governed by Fick's first law of diffusion (Nobel, 1999). This law states that the rate of moisture loss depends on a combination of three factors. First, the contribution of the surface area of the fruit peel to moisture loss. Moisture loss from a fruit is significantly influenced by its size, since size influences the total surface area and volume of the fruit (Wills et al., 1989). More moisture loss occurs from products with a high surface area to volume ratio (e.g. lettuce), compared to produce with a lower surface to volume ratio (e.g. plums). Similarly, immature or small fruit have a larger surface to volume ratio than large fruit

or fruit that reached the end of their growth stage. Larger fruit also lose less moisture on a per unit mass basis than smaller and/or immature fruit.

The second factor is the driving force behind moisture loss. Moisture loss is primarily controlled by the difference in the water vapour pressure between the intercellular air spaces inside the fruit and of the air in the environment surrounding the fruit (Thompson, 1992). The partial pressure of water vapour of the air inside the intercellular air spaces of the fruit is assumed to be very close to saturation, with a RH of more than 99 % (Ben-Yehoshua, 1987). Compared to the intercellular air spaces of the fruit, the water vapour of the surrounding air is usually lower than saturation, depending on temperature and the moisture content of the air (Wills et al., 1989). This generates a vapour pressure difference (VPD) between the fruit and its environment, which drives moisture loss from the fruit (Lara et al., 2014; Maguire et al., 2001; Wills et al., 1989). The third factor that affects the rate of fruit moisture loss, is the water vapour permeability of the fruit peel /cuticle (Nobel, 1999). The permeability of the fruit peel is influenced by the composition and structure of the cuticle (Riederer and Schneider, 1990). Cutin provides a framework into and onto which waxes are deposited to form a structure than can reduce the rate of evaporation from plant cells approx. 25-fold. (Isaacson et al., 2009).

However, the cuticular barrier is not infallible. To prevent moisture loss, the cuticular membrane must remain intact (Knoche and Peschel, 2007). This can be quite difficult, especially with the cuticular membranes of fruit, which are characterized by almost continuous surface expansion until maturity. Cuticle deposition often cannot keep pace with fruit surface expansion and the rates of cutin and wax deposition also varies. This results in thinning of the cutin matrix on the enlarging fruit surface. In contrast, wax deposition continues at a constant rate until harvest. In European plums, the deposition of wax and cutin occurs simultaneously, up to approx. 71 days after full bloom, but thereafter the deposition of cutin stops almost completely.

As the fruit surface continues to expand, the cutin matrix becomes thinner, resulting in considerable strain on the cuticle. This can lead to the formation of micro-cracks, disrupting the water-barrier characteristic of the cuticle and creating avenues for moisture loss from the fruit. Micro-cracking occurs predominantly in the pedicel region, with higher structural strain on the cuticle in this region than on the cheek of the fruit.

In addition, postharvest shrivelling is primarily observed in the pedicel region on European plums. Thus, a relationship between micro-crack incidence and an increase in moisture loss, leading to shrivelling at the pedicel end of European plums, was suggested by Knoche and Peschel (2007).

Other openings in the fruit surface also act as avenues of moisture loss, e.g. wounds inflicted during handling or transport, abrasions on the tree, stomata and lenticels (Mitchell and Kader, 1989; Wills et al., 2007). Similarly, damage to the fruit surface caused by pests and diseases will increase the likelihood of moisture loss (Wills et al., 2007). Although some wound healing can occur with damage to the peel during the growth and development of the fruit, the capacity for wound healing decreases as the plant organs mature and damage inflicted during or after harvest generally remain unprotected and act as avenues for moisture loss.

5.2 Barrier properties of the cuticle

A common misconception is that a thick cuticle is associated with lower water permeability and thus increased tolerance to water stress (Yeats and Rose, 2013). However, the water permeability of cuticles from a range of species show that there is no correlation with either the thickness of the cuticle or the amount of wax (Riederer and Schreiber, 2001). Similarly, the amount of cutin is not necessarily an indication of the cuticular water permeability (Isaacson et al., 2009; Yeats and Rose, 2013). In contrast with cutin, extensive removal of wax from tomato fruit indicated that waxes contribute about 95 % of the cuticle-mediated resistance to water diffusion (Burghardt and Riederer, 2006; Leide et al., 2007). Within waxes, specific compound classes seem to be associated with the water barrier properties of the cuticle. The more nonpolar components, such as alkanes, tend to be associated with increased barrier properties, while non-aliphatic wax compounds, such as triterpenoids, are less effective water barriers (Buschhaus and Jetter, 2012; Leide et al., 2007; Parsons et al., 2013). These observations have been confirmed in a range of cuticle types, from Arabidopsis, tomatoes, peppers etc. This is consistent with a supposition that cuticular waxes form either crystalline or amorphous domains within the cuticle, with aliphatic compounds forming crystalline ‘rafts’ that are water-resistant, forcing water to diffuse by an indirect route through the amorphous domains that are formed by more polar and cyclic waxes (Reynhardt and Riederer, 1994; Riederer and Schreiber, 1995;

Rogiers et al., 2004). The mobility of molecules within a polymer depends on the rate of molecular motion of the polymer chain (Kerstiens, 2006). Molecular motion leads to the random formation of openings in the polymer network, which allows diffusing molecules to move into those openings (Kerstiens, 2006, 1996a). Amorphous zones form more such openings to allow diffusion.

In pepper cultivars, a poor correlation was found between total fruit wax amount and moisture loss (Parsons et al., 2013). However, there is a significant positive correlation between the proportion of total free fatty acids and the rate of moisture loss, thus, higher moisture loss rates occur when high amounts of free fatty acids are present. Significant negative correlations were found between *n*-alkane amounts and water loss, as well as the ratio of total alkanes to combined non-aliphatic compound amounts, which confirms that the chemical composition of wax is a likely determinant of fruit moisture loss.

6. The effect of environment on cuticles

Peel permeability and cuticle composition vary significantly between species, cultivars and individual fruit (Lara et al., 2014; Whitelock et al., 1994). In apples and Japanese plums, peel permeability also varies between fruit maturities, orchards and growing areas (Maguire et al., 2000; Theron, 2015). Since cuticle composition is primarily determined genetically and environmentally, significant changes in permeability on this level will involve selective breeding and controlled growing conditions.

The quantity of wax, its chemical composition and surface morphology are controlled by several biological and physical factors like temperature, light, humidity, age and the position of the fruit in the tree canopy (Konarska and Agata, 2013). Small variations in environmental conditions can cause changes in epicuticular wax morphology, while larger variations (e.g. 20˚C increase in temperature) are required for changes in the chemical composition of the wax (Latimer and Severson, 1997).

6.1 Temperature

The most pronounced differences in wax ultrastructure occur because of temperature changes (Baker, 1974). Increasing temperatures lead to a greater tendency for the wax to develop over, rather than project from the surface of the cuticle. Wax on the leaves of brussels sprouts grown at 15°C developed as hollow tubes that projected

from the leaf surface. When the growing temperature increased to 21°C, wax structure changed to a composite arrangement of tubes and dendrites. These tubes are orientated at 90° angles to the surface, while the dendrites lie parallel to the surface. The structure of wax on leaves grown at 35°C appeared as a meshwork of very large dendrites.

Temperature also influences the chain length composition of the hydrocarbons and free fatty acids, but it has little influence on the composition of aldehydes and free and esterified primary alcohols. Still, Baker (1974) found that the waxes of brussels sprout plants grown at 15°C showed higher alkane and lower aldehyde contents than the wax from brussels sprouts grown at 35°C (at the same RH and radiant energy rate). The lower alkane content in Arabidopsis leaf cuticles and tomato fruit cuticles exposed to higher temperatures, reduces the barrier properties of the cuticle (Buschhaus and Jetter, 2012; Leide et al., 2007), thus explaining why cuticular water vapour permeability increases as temperature increase (Schönherr et al., 1979). Furthermore, low alkane content in the cuticular waxes leads to the formation of more amorphous wax domains, as seen in grape berries (Reynhardt and Riederer, 1994; Riederer and Schreiber, 1995; Rogiers et al., 2004), possibly explaining the morphological observations by Baker (1974).

A phase transition occurs in the cuticular membrane between 30˚C to 39 ˚C (Riederer and Schreiber, 2001; Schreiber, 2001). Above this phase transition, a drastic increase in cuticular permeability occurs - likely due to changes in the structure of the cuticular waxes (Schreiber, 2002). On a clear day in the orchard, the fruit surface temperature of apples is often up to 10°C above ambient temperature (Glenn et al., 2002; Schrader et al., 2001). Thus, fruit surface temperatures can easily increase to more than 30˚C to 39 ˚C, causing structural changes in the cuticle. In the temperature range between 66˚C to 74 ˚C, the epicuticular waxes reach a visible melting point (Bain and Mcbean, 1968; Schreiber and Riederer, 1996). In addition, the cutin matrix increases in volume (Schreiber and Schönherr, 1990). This is thought to cause defects in the wax barrier, which might contribute to increased cuticular permeability with increasing temperatures. These high temperatures can change cuticle permeability irreversibly, probably due to rearrangement of the lipids when they melt and then re-solidify (Schönherr et al., 1979). However, such elevated temperatures and consequent

irreversible changes in cuticle permeability are not likely to occur under normal growing conditions.

6.2 Light exposure and radiation

Differences in wax ultrastructure are observed between citrus fruit exposed to sunlight versus fruit deeper in the canopy (El-Otmani et al., 1989). The density of fine crystalline wax platelets on the fruit surface is highest in shaded parts of the fruit. In other positions, the wax platelets tend to be smoother and less fringed. This is because exposure leads to a faster transition from crystalline-to-amorphous waxes.

The structure of the epicuticular wax of grape berries also changes with fruit age (Rogiers et al., 2004). The waxes are normally crystalline, but rather soft, and can be altered or removed by the impact of rain, abrasion by wind-blown particles, or contact and rubbing against other berries or leaves. Sun-exposed berries have larger areas of amorphous wax compared to shaded berries and wax is also more amorphous in areas of contact between berries. The more amorphous wax on sun-exposed berries is likely due to higher temperatures experienced by these berries that can often be up to 15°C higher than the ambient temperature. As fruit continue to grow and develop, the structure of the wax crystals and plates changes, and the more amorphous wax areas can be directly related to the degree of exposure to sunlight and/or temperature (El-Otmani et al., 1989).

As with high temperatures, excessive UV-B exposure can lead to the formation of significantly more cuticle and wax (Rosenquist and Morrison, 1989; Steinmuller and Tevini, 1985). In apples, increased cuticle thickness on the sun-exposed side of the fruit occurs to reflect or absorb excessive light radiation (Solovchenko and Merzlyak, 2003). This increased reflection of light due to enhanced cuticle thickness can reduce tissue temperatures, thus reducing vapour pressure deficit between the tissue and the air, which reduces transpirational water loss (Shepherd and Griffiths, 2006). Thus, both temperature and light exposure / radiation, affect plant cuticles. In support of this, barley leaves grown in the dark, have about 2.5 times less epicuticular wax compared to light-grown leaves (Giese, 1975). If the temperature of the dark-grown barley is increased, almost 40 % more wax is present. If dark-grown plants are moved to the light the rate of wax synthesis is stimulated, indicating that the amount of wax on the cuticle controls the synthesis and extrusion of wax lipids through a regulated feed-

back system. Light regulates the biosynthesis and extrusion of wax in several ways and determines the quantity of wax per unit surface area. Light also affects the chain length distributions composing each wax class. In wax classes that arise from decarboxylation and reductive pathways, longer chain lengths are observed in light-grown plants.

6.3 Relative humidity

Lower relative humidity stimulates the production of wax and a higher density of wax crystals in *Brassica*, nasturtium leaves, and *Eucalyptus* leaves (Baker, 1974; Koch et al., 2006). Maximum wax deposition occurs under conditions of high radiant energy and low humidity (Baker, 1974).

Some plant species do not show significant changes in wax composition in response to changes in RH (e.g. *Eucalyptus*), while others (e.g. *Brassicas)* show substantial changes when grown under conditions of high relative humidity (Koch et al., 2006). Ketones and primary alcohols tend to increase, while secondary alcohols and aldehydes are reduced. The changes in chemical composition of the waxes due to RH can be explained in view of the wax biosynthetic pathway. According to this model, ketones are synthesized through the decarboxylation of alkanes to secondary alcohols, followed by oxidation/hydroxylation of the secondary alcohols. Thus, the decrease in secondary alcohols observed at high RH could be due to the synthesis of ketones. In turn, the lower content of aldehydes could be explained by a reduction of aldehydes to primary alcohols. Furthermore, at very high relative humidity (98 %) water and wax diffusion through the cuticle is reduced, since the driving force for cuticular transpiration will be close to zero, and therefore the plant will be incapable of accumulating more epicuticular wax. At low RH, the driving forces for transpiration are high and this may lead to a higher accumulation of cuticular waxes, which will then reduce cuticular transpiration as an adaptation to environmental stress.

6.4 Water stress

Water relations have a marked effect on cuticle formation (Skoss, 1955). A heavier wax layer is a common response to water stress (Bain and Mcbean, 1967; Bondada et al., 1996). Tree tobacco plants undergoing water stress develop twice as much cuticle as those that are not stressed (Skoss, 1955), while in cotton and Arabidopsis plants, the total wax concentration increases (Bondada et al., 1996; Kosma et al.,

2009). Both qualitative and quantitative differences in wax composition are observed between well-watered and water-stressed plants, with stressed plants showing an increase in long-chain alkanes. Besides waxes, water deficit also causes an increase of 65 % in Arabidopsis leaf cutin monomers (Kosma et al., 2009). However, unlike with wax induction, nearly all the cutin monomers increase. Drought acclimation might involve synthesis of a larger cutin framework to support more concentrated intracuticular packing of crystalline wax regions. Increased cross-linking of the cutin polymer can prevent hydrogen bonding of water molecules to unlinked, oxygenated cutin functional groups, and in this way slow the diffusion of water.

6.5 Wind

Exposure to chronic wind leads to the formation of thicker leaf cuticles and more variability in leaf water permeability (McArthur et al., 2010). Wind exposure can damage leaf cuticles, making them more prone to moisture loss. However, the thickening of the cuticle is not a response to reduce moisture loss, but rather to reduce mechanical damage, which can indirectly lead to high moisture loss. Wind damage can also lead to a loss of turgor in some leaf epidermal cells and chronic exposure to wind can lead to changes in the form of the epicuticular waxes e.g. smoothing of the epicuticular wax (Wilson, 1984).

Wind stress or abrasion to leaf surfaces reduces the amount of C_{29} components in the waxes (Latimer and Severson, 1997). This is either because the biosynthetic pathways are inhibited, or due to physical removal of the waxes. The hydrocarbon content of wind-damaged leaves is approx. 36% than unstressed leaves. However, cuticles can recover to a certain extent after exposure to wind and moisture stress.

7. Conclusion

Due to the diverse functions of the plant cuticle, it is highly variable in terms of chemical composition and structure. However, this review focussed only on the role of the cuticle as a barrier against moisture loss, specifically under postharvest conditions.

Specific wax compound classes are associated with the water barrier traits of the cuticle in a range of commodities, including tomatoes, apples, cherries, peppers, and peaches. Aliphatic components, such as alkanes, are associated with increased barrier properties as these components form crystalline lattices that block water

movement. Non-aliphatic compounds tend to form more amorphous wax zones, which are more permeable to water vapour. However, there is a lack of information about the cuticles of Japanese plums.

Japanese plums exported from South Africa are especially prone to quality deterioration due to the extended shipping times required to reach overseas markets. During these storage periods, the fruit lose moisture, leading to mass loss, shrivel, and a loss in firmness and glossiness. As these traits are unacceptable to consumers and because fruit are sold by mass, significant financial losses are incurred when plum consignments are rejected.

It has been shown that cuticle properties change during cold storage, possibly to protect the fruit from more moisture loss. According to our knowledge, no studies have been done to investigate the contribution of the cuticle to moisture loss susceptibility between different plum cultivars, or how the cuticle changes during cold storage.

To manage postharvest fruit quality, a critical investigation into cuticle development is required. Determining the differences in cuticle composition and morphology between plum cultivars susceptible to moisture loss, versus those that are less susceptible might enable breeders to develop cultivars with more effective water-barriers. Furthermore, knowledge of how the fruit cuticle changes during development and cold storage, may aid producers in determining optimal handling protocols to maintain high fruit quality.

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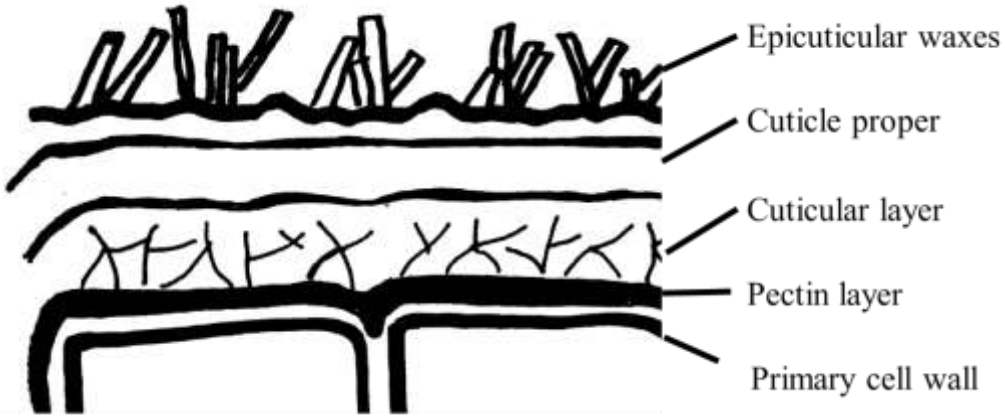

Fig. 1. A hypothetical depiction of cuticle structure adapted from Cohen et al., 2017.

PAPER 1

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Peel water vapour permeance of Japanese plums as indicator of susceptibility to postharvest shriveling

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ABSTRACT

Moisture loss and postharvest shrivelling of some Japanese plum cultivars result in significant financial losses in the South African stone fruit industry. Even though fruit are stored at optimal temperatures and packaging solutions are implemented to reduce shrivel, the incidence of shrivel is still unacceptably high in susceptible cultivars. Fruit peel water vapour permeance (P_{H2O}) can be calculated to determine the proneness of a cultivar to moisture loss. Knowledge of the status of the P_{H2O} prior to harvest and the variation between cultivars, orchards and seasons could indicate whether newly developed cultivars are prone to postharvest shrivel. This could assist in determining the optimum handling protocols for susceptible cultivars to reduce potential moisture loss. The P_{H2O} of various cultivars were determined during 2015/16 and 2016/17. In addition, to establish whether a relationship exists between postharvest fruit moisture loss and shrivel, weight loss and shrivel incidence was recorded on individual fruit of the cultivars Sapphire, Laetitia and African Delight™ during 2016/17. P_{H2O} varied between seasons, cultivars and orchards. In 'African Rose', 'Ruby Sun' 'Ruby Star' and 'Sapphire', high P_{H2O} corresponded with known shrivel susceptibility. 'Songold', 'Fortune' and 'Angeleno' are not prone to shrivel and these cultivars had a low P_{H2O}. However, 'Laetitia' and 'African Delight™' had low P_{H2O}, even though both cultivars are prone to shrivel. Pre-harvest moisture loss and P_{H2O} could therefore not be used to predict shrivel susceptibility successfully for evaluated cultivars.

Peel water vapour permeance of Japanese plums as indicator of susceptibility to postharvest shrivelling

Abstract

Moisture loss and postharvest shrivelling of some Japanese plum cultivars result in significant financial losses in the South African stone fruit industry. Even though fruit are stored at optimal temperatures and packaging solutions are implemented to reduce shrivel, the incidence of shrivel is still unacceptably high in susceptible cultivars. Fruit peel water vapour permeance (P_{H2O}) can be calculated to determine the proneness of a cultivar to moisture loss. Knowledge of the status of the P_{H2O} prior to harvest and the variation between cultivars, orchards and seasons could indicate whether newly developed cultivars are prone to postharvest shrivel. This could assist in determining the optimum handling protocols for susceptible cultivars to reduce potential moisture loss. The P_{H2O} of various cultivars were determined during 2015/16 and 2016/17. In addition, to establish whether a relationship exists between postharvest fruit moisture loss and shrivel, mass loss and shrivel incidence was recorded on individual fruit of the cultivars Sapphire, Laetitia and African Delight™ during 2016/17. P_{H2O} varied between seasons, cultivars and orchards. In 'African Rose', 'Ruby Sun' 'Ruby Star' and 'Sapphire', high P_{H2O} corresponded with known shrivel susceptibility. Songold, Fortune and Angeleno are not prone to shrivel and these cultivars had a low P_{H2O}. However, Laetitia and African Delight™ had low P_{H2O}, even though both cultivars are prone to shrivel. Pre-harvest moisture loss and P_{H2O} could therefore not be used to predict shrivel susceptibility successfully for evaluated cultivars.

Key words: maturity; moisture loss; *Prunus salicina* Lindl.; shrivel; mass loss

1. Introduction

Accumulated postharvest moisture loss leads to the manifestation of shrivelling in various fresh commodities (Crisosto et al., 1995; Crouch, 1998; Maguire et al., 2000; Mitchell and Crisosto, 1995; Mitchell et al., 1963; Edmond et al., 2007). However, some fruit or even cultivars of the same fruit type, are more prone to moisture loss and/or shrivelling than others. The driving force of postharvest moisture loss is determined by the vapour pressure deficit (VPD) between the fruit and the environment (Mitchell and Crisosto, 1995). VPD is controlled by the temperature and relative humidity (RH) of the air surrounding the fruit, the temperature of the fruit and velocity of the air moving over the fruit. Under high VPD conditions, water evaporating from fruit cells saturate the intercellular spaces within the fruit, creating a RH of nearly 100%. Water vapour then diffuses along the concentration gradient from this area of high vapour pressure to the surrounding atmosphere which is at a lower vapour pressure. The fruit peel and waxy cuticle act as the main protection against excessive moisture loss (Dietz et al., 1985; Konarska, 2013; Wills et al., 1989). This is especially important after harvest, when the fruit do not receive additional moisture from the tree via the peduncle to replace moisture lost to the atmosphere.

Loss in mass after harvest is mostly due to the loss of water vapour through evaporation and, to a lesser extent, the loss of carbon in the respiration process (Lara et al., 2014; Pieniazek, 1944). Thus, mass loss can be used as a measure of fruit moisture loss (Shibairo et al., 1997). Furthermore, the sensitivity of fruit to moisture loss is influenced by both external and internal factors. External factors include temperature, RH and air movement over the product (Pieniazek, 1944; Shibairo et al., 1997). Internal factors include the surface area to fresh mass ratio of the fruit, (Díaz-Pérez et al., 2007; Konarska, 2013), cuticle composition and the presence of open stomata, lenticels, cracks or wounds (Sastry, 1985a). Maturity also influences moisture loss, with immature and over mature fruit losing moisture at a faster rate than mature fruit. Together, the internal factors influencing moisture loss determine the water vapour permeance (P_{H2O}) of the peel, which is a measure of how easily water vapour can move out of the fruit (Maguire et al., 1999a). Although packaging solutions exist to reduce shrivel in Japanese plums, shrivel incidence is still unacceptably high for

most cultivars. This is most likely due to accumulated moisture loss, starting as soon as the fruit are harvested.

The diffusion of water vapour across the cuticle requires water to dissolve in the lipophilic medium of the cuticle at the cell wall/cuticle interface, diffusion in the solid matrix, followed by desorption from the outer surface of the cuticular membrane (Kerstiens, 1996b). Variation in the composition and molecular structure of the cuticle and cuticular waxes was therefore responsible for the variation in the P_{H2O} between cultivars and orchards (Maguire et al., 1999b). Structural and compositional changes occur in the fruit cuticle during development, which affects the permeance of the cuticle (Karbulková et al., 2008). In addition, environmental factors can also influence the P_{H2O} of fruit, with temperature being the main contributor (Riederer and Schreiber, 2001). The water permeability of the cuticular membrane can change rapidly when the lipids undergo a phase transition due to increasing temperatures (Schönherr et al., 1979). Above specific species-related transition temperatures, these changes can be irreversible, thus permanently changing the permeability of the cuticle. As the cuticular waxes contribute about 95% of the cuticle-mediated resistance to water diffusion in tomato fruit (Burghardt and Riederer, 2006; Leide et al., 2007), it appears that changes in lipid composition of the cuticle may have a significant impact on the P_{H2O}.

Fanta et al. (2014) developed a model to compute deformation of cells in tissues that are experiencing water loss. Loss of turgor of an individual cell also affects neighboring cells (Fanta et al., 2014). When cells lose turgor, the cell wall relaxes, and all neighboring cells are also deformed. This may be the main reason why fruit shrivel after water loss (Nguyen et al., 2006; Veraverbeke et al., 2003), because the cuticle is relatively inelastic, and it maintains its surface area, even during water loss (Fanta et al., 2014). Thus, when the underlying cells shrink and deform during moisture loss, the fruit peel gets a shriveled appearance. This might explain why some cultivars are more prone to shrivel than others. A cultivar with high P_{H2O} might have stronger underlying cells or smaller intercellular spaces, which are more resistant to deformation during moisture loss, making it less prone to shrivel.

Limited information currently exists on the effect of pre-harvest factors on fruit permeability. Knowledge of fruit peel permeability and the influence of season and

cultivar on permeability will contribute towards optimizing handling protocols for cultivars that are susceptible to shrivel.

2. Materials and methods

2.1 Trial layout and site selection

2.1.1 Trial 1: 2015/16

The trial compared peel permeability of five Japanese plum cultivars, namely: African Rose (shrivel prone), Ruby Sun (shrivel prone), Fortune (not shrivel prone), Angeleno (not shrivel prone), Ruby Star (shrivel prone) from pre-optimum to optimum maturity. The optimum harvest date for each cultivar is presented in Table 1.

Fruit were sampled from commercial farms in the Franschhoek, Paarl and Simondium regions of the Western Cape. Five orchards were used for each cultivar and five uniform trees per orchard were randomly chosen for sampling. The same trees were used on each sampling date to avoid added variation due to tree differences. For ‘African Rose’ the farms La Terra de Luc (33°53'51.2"S 19°06'32.8"E), Keerweder (33°55'40.5"S 19°07'53.2"E), Môrelig (33°46'13.2"S 18°55'30.4"E), Bergvliet (33°49'54.2"S 18°57'51.6"E) and Babylonstoren (33°49'17.6"S 18°55'47.9"E) were used, and for ‘Ruby Sun’ La Terra de Luc, two orchards at Môrelig, Vredenburg (33°45'28.1"S 18°56'49.7"E) and Bergvliet were used. For ‘Fortune’, Keerweder, Bourgogne (33°55′33.26″S 19°07′03.02″E), La Bri (33°55'25.7"S 19°07'06.7"E), Cabrierre (33°54′58.42″S 19°07′08.17″E) and Bergvliet were used while for ‘Angeleno’ La Terra de Luc, Bergsig (33°56'15.9"S 19°07'04.2"E), Bourgogne, La Bri and Cabrierre were used. In the case of ‘Ruby Star’ the farms were La Terra de Luc, Bourgogne, La Bri, Cabrierre and Bergvliet. On each sampling date, five visually unblemished fruit, of equivalent size and maturity (fruit peel ground colour), was harvested per tree. Fruit sampling occurred on a weekly basis, starting at different weeks before the anticipated optimum harvest (cultivar dependent) and continuing until after the optimal harvest date, depending on fruit ripening. After harvesting, fruit were carefully placed into plastic bags and transported to the laboratory at the Department of Horticultural Science, Stellenbosch University. The fruit reached the laboratory within two to three hours after harvest. During harvesting and transport,

care was taken to handle fruit as little as possible and to prevent disturbance of the waxy bloom.

2.1.2 Trial 2 (2015/16 and 2016/17)

The trial was carried out on 'Laetitia' and 'Songold' plums, sampled from the Welgevallen Research Farm, Stellenbosch, South-Africa (33°56′50.68″S 18°52′14.98″E) over two consecutive seasons (2015/16 and 2016/17). In addition, in 2016/2017, 'Sapphire' and 'African Delight™' (cv. ARC PR00-29), were sampled from Morgenzon Farm in Helshoogte, Stellenbosch, South-Africa (33°55'29.33" S 18°55'48.446" E). Fruit sampling was done weekly, from approx. three weeks before the anticipated optimum harvest date until the optimum harvest date. The optimum harvest date for each cultivar is presented in Table 1.

On each sampling date, 100 visually unblemished fruit of the same size and ground colour were picked from randomly selected trees for each cultivar. Fruit were transported in plastic bags to the laboratory at the Department of Horticultural Science, Stellenbosch University.

2.1.3 Trial 3 (2016/17)

To establish whether a relationship exists between fruit moisture loss and shrivel in 'Sapphire', 'Laetitia', and 'African Delight™, 180 fruit at optimum maturity per cultivar were collected from a commercial pack-house in Franschhoek, South-Africa (33°54'24.4"S 19°06'47.0"E). The fruit were randomly divided into three groups of 60 fruit per cultivar and packaged in cartons according to commercial standards. The fruit were packed into count 30 pulp trays. Two layers were packed per carton and were covered with a perforated, high density polyethylene (HDPE) shrivel sheet (± 48 perforations with 5m diameter) to reduce moisture loss and a white corrugated sheet was placed on top to protect the fruit (PPECB, 2014). Twenty randomly selected fruit per carton were labelled and weighed (XB 320M, Precisa Instruments Ltd., Switzerland). Fruit were cold-stored in regular atmosphere at RH of ± 95% according to standard practice as follows: 'Sapphire' 10 days at -0.5 °C, 7 days at 7.5 °C and then 25 days at -0.5 °C; 'Laetitia' 10 days at -0.5°C, 7 days at 7.5°C and then 32 days at -0.5°C. 'African Delight™' was stored at a commercially used single-temperature regime of 56 days at -0.5°C. After cold storage, fruit were weighed again and

individually scored (visually) for shrivel incidence (Fig.1). This was repeated until more than 10% of the fruit showed shrivelling symptoms. Fruit were stored at -0.5 ˚C continuously to force shrivel development.

2.2 Measurements

In the laboratory, each fruit was numbered, and the diameter recorded diagonally across the fruit suture with a digital calliper (Mitutyo, Japan). The shape of the fruit was assumed to be spherical. Afterwards each fruit was weighed using a balance accurate to 0.001 g (XB 320M, Precisa Instruments Ltd., Switzerland).

Fruit were then placed in pulp fruit trays and allowed to reach an internal temperature of 20 °C (approx. 5 hours) in a temperature conditioned room. Fruit were subsequently placed in a plastic container and subjected to an airflow of ≈ 0.5 m s^{-1} at 20 °C and average RH of 60% for a 16-hour period. Atmospheric RH and temperature were recorded using a HygrochronTM iButton (CST electronics, Sandton) and pulp temperature was recorded using a Thermocron® iButton. The HygrochronTM iButton was placed on the underside of the lid of the container to record the RH and air temperature, while the Thermocron® iButton was inserted into one fruit which was not part of the experiment, to record pulp temperature at 5-minute intervals during the 16-hour period. Finally, the individual fruit were weighed again after the 16-hour period and the difference in mass was used to calculate the rate of moisture loss, assuming that respiration did not have a significant effect on mass loss due to the relatively short duration over which the test was performed.

Determination of the water vapour permeance of the fruit peel (P_{H2O}) of each fruit

The P_{H2O} of the fruit peel was calculated using Fick's first law of gas diffusion according to equation 1.

$$P_{H2O} = r'H_2O / (\Delta pH_2O * A) \quad \text{(Eq. 1)}$$

Where:

$r'H_20$ = rate of moisture loss (mol s^{-1})

ΔpH_20 = difference in the water vapour pressure inside and outside the fruit (Pa)

A = area of the fruit surface (m^2)

2.3 Statistical analysis

A completely randomised design with five orchards and five trees per orchard was used in Trial 1. Data of all sampling dates were analysed using a cross-nested model to determine the contribution of each factor (harvest date, cultivar, orchard, tree and fruit) to the water vapour permeance of the fruit.

Water vapour permeance data of Trial 2 were analysed with a one-way analysis of variance (ANOVA), including cultivar and weeks before harvest as factors. ANOVA-generated P-values and the significant differences between means were determined using Fisher's least significant differences (LSD) test with a 95% confidence interval. Trial 3 is presented as frequency plots with the number of shrivelled and non-shrivelled fruit and the percentage of mass loss per fruit as factors.

3. Results

3.1 Trial 1

P_{H20} values for the different cultivars varied considerably. Generally, P_{H20} of 'African Rose' (shrivel prone) increased from three weeks before optimum harvest maturity, through optimum harvest maturity until one week later. There was a decrease in P_{H20} either during the 2nd or 3rd week after optimum maturity (Fig. 2A).

In the case of 'Ruby Sun' (shrivel prone), the P_{H20} was relatively stable from six to two weeks before optimum maturity (Fig. 2B). From around one week prior to optimum maturity, P_{H20} increased until two weeks after optimum maturity in three orchards. In another orchard, it decreased from optimum maturity, while in another it started to decrease one week after optimum maturity.

In 'Fortune' (not prone to shrivel) P_{H20} trends differed from the other four cultivars (Fig. 2C). In this case, the P_{H20} increased from four to three weeks before optimum maturity, but then stabilised for three weeks. One week prior to optimum maturity, orchard 5 displayed a sharp increase in P_{H20} until optimum maturity, while the other four orchards displayed a slight decrease in P_{H20} until optimum maturity. During the one week after

optimum maturity, all orchards showed a decrease in P_{H20} followed by a slight increase again, except for orchard 5.

For ‘Angeleno’ (not prone to shrivel) the P_{H20} was very variable and changed on a weekly basis (Fig 2D). The general trend showed little change in P_{H20} prior to optimum maturity until one week before optimum maturity, when there was an increase in P_{H20}. After optimum maturity, the changes in P_{H20} were again very variable. In ‘Ruby Star’ (shrivel prone) the P_{H20} steadily increased at a slow rate prior to optimum maturity (Fig. 2E). In two orchards the P_{H20} increased at a faster rate one week before optimum maturity, while in the other three orchards, this only occurred after optimum maturity. In the three orchards where the increase in P_{H20} occurred slightly earlier, P_{H20} decreased during the second week after optimum maturity, while the other two orchards showed a further increase in P_{H20}.

In general, the P_{H20} of ‘Angeleno’ and ‘Fortune’ (not prone to shrivel), remained lower than in the three shrivel prone cultivars over the sampling periods (Fig. 3). ‘African Rose’, ‘Ruby Sun’ and ‘Ruby Star’ all had relatively high P_{H20} and are known to be shrivel prone.

3.2 Trial 2

The trends in P_{H20} for ‘Songold’ and ‘Laetitia’ differed between the two cultivars but were similar over the two seasons (Fig. 4A and 4B). In 2015/16, P_{H20} of ‘Laetitia’ increased between three to two weeks prior to optimum maturity, then remained stable and decreased again from one week prior to optimum maturity until harvest (Fig. 4A). In 2016/17, there was a slight decrease in P_{H20} between three and two weeks prior to optimum maturity (Fig. 4B), after which P_{H20} remained stable and then decreased up to the harvest date, which corresponds with the trend of the first season. However, the actual P_{H20} values were slightly higher for ‘Laetitia’ during 2016/17. In 2015/16, P_{H20} of ‘Songold’ remained stable between three and two weeks prior to optimum maturity and then increased significantly until the harvest date (Fig. 4A). The same trend was seen in 2016/17 (Fig. 4B). ‘African Delight’, only included in 2016/17, showed an increase in P_{H20} between three to two weeks prior to optimum maturity and then a decrease until harvest. The P_{H20} of ‘Sapphire’ (also only included in 2016/17)

decreased between three to two weeks prior to optimum maturity, then increased up to harvest.

With reference to peel permeability, ‘Sapphire’ had the highest permeability, followed by ‘African Delight’. ‘Songold’ and ‘Laetitia’ had the lowest peel permeability and only differed from each other at optimum maturity, when peel permeability of ‘Songold’ was higher.

3.3 Trial 3

After the standard cold-storage period, 6.7% of the 60 ‘Sapphire’ fruit were shrivelled (data not shown). The fruit were cold-stored for another week, after which shrivel incidence was 11.7% and after an additional week of storage, it increased to 15%. A frequency plot of the number of shrivelled and non-shrivelled fruit and the percentage of mass loss per fruit, indicated no relationship moisture loss and shrivel (Fig.5A).

‘Laetitia’ fruit did not show any signs of shrivelling after cold-storage (data not shown). After an additional week of cold storage, 5% of the fruit were shrivelled and after another week, 10% of the fruit were shrivelled. The fruit were not stored for longer due to over maturity. Like ‘Sapphire’, no relationship between moisture loss and shrivel incidence could be established (Fig.5B).

‘African Delight™’ fruit had a 23% incidence of shrivel after cold-storage (data not shown) and therefore no further cold storage was applied. The frequency plot for ‘African Delight™’ differed from the other cultivars (Fig. 5C). Fruit with moisture loss between 0 - 1% generally did not shrivel, while more shrivel was noticed in fruit with slightly more moisture loss, but there were also fruit with more moisture loss that did not show signs of shrivel. In all cultivars, fruit continued to lose moisture during the cold storage period.

4. Discussion

Mass loss occurs during storage, regardless of the type of fruit or vegetable that is stored (Nunes and Edmond, 2007). However, the rate of moisture loss depends on the type of crop and is primarily related to the physiological and morphological characteristics of the product.

Values for P_{H20} varied between cultivars and trends in P_{H2O} over time also varied between orchards. This agrees with the observations in apples where a five-fold difference between the lowest and highest measured P_{H2O} was recorded (Maguire et al., 2000). Differences between farms, orchards, individual trees, as well as fruit on the same tree were also reported to affect fruit peel permeability in apple (Maguire et al., 2000) and Japanese plum (Theron, 2015). In the Japanese plum cultivars, African Delight™, Laetitia, and Songold, fruit to fruit variance contributed more than 45% to the total variation in $P_{H2O,}$ harvest date contributed more than 20% to the total variation, and orchard differences accounted for more than 15% of the total variation in P_{H2O} (Theron, 2015).

Since apple cultivars with higher P_{H2O}, particularly in later harvests, are thought to be more prone to shrivel during storage (Maguire et al., 2000), it was expected that plums would behave similarly. Greater initial P_{H2O} values of apples and larger increases during the growing season are thought to explain the differences in shrivel incidence between seasons. In general, the P_{H20} of the cultivars Angeleno, Fortune and Songold, not prone to shrivel (Fig. 3 and Fig. 4), remained lower than the other cultivars. The cultivars prone to shrivel had the highest P_{H20} (Fig. 3 and Fig. 4). However, 'Laetitia' (shrivel prone) performed like 'Angeleno' (not shrivel prone) and 'African Delight™' (shrivel prone) performed similar to 'Fortune' (not shrivel prone) and had lower P_{H20} than the other shrivel susceptible cultivars.

The lack of a strong relationship between moisture loss and shrivel (Fig.5) indicates that moisture loss during storage might not be the only factor causing shrivel. Measuring pre-harvest P_{H2O} to predict whether a cultivar is shrivel-prone seems to only be successful in some cultivars (Sapphire, African Rose, Ruby Star and Ruby Sun) (Fig. 2). These cultivars had a higher P_{H2O} and were prone to postharvest moisture loss and shrivel, while cultivars with low P_{H2O} (Angeleno, Fortune, and Songold) were not prone to shrivel. However, Laetitia and African Delight™ did not comply with this criterion. Both cultivars are susceptible to moisture loss and shrivel but had P_{H2O} values similar to the cultivars that are not prone to shrivel. It is possible that the epidermal cells of these cultivars became deformed more easily during moisture loss than the other cultivars (Fanta et al., 2014). P_{H2O} values will therefore give an indication

of whether a cultivar is prone to moisture loss, but not necessarily whether it is prone to shrivel.

5. Conclusion

The aim of this study was to establish whether pre-harvest determination of peel vapour permeance in Japanese plums could indicate and therefore be applied to predict whether a cultivar would be susceptible to postharvest shrivelling or not. Peel vapour permeance was highly variable between cultivars, over time and between different sites. This explained the variation in shrivel incidence between individual fruit often noticed in boxes. In agreement with findings in apples, some of the plum cultivars with high peel permeability were also more prone to shrivel.

However, this was not true for all the cultivars that were evaluated, indicating that these results cannot be generalised across all plum cultivars. ‘Laetitia’ and African Delight™’ had low peel permeability even though they are known to be shrivel prone. Differences in permeability are thus more likely due to differences in cuticle composition, since the cuticle is the main barrier to moisture loss. A direct link between postharvest moisture loss and shrivel incidence could not be established in the scope of this project.

As moisture loss is cumulative and fruit start losing moisture in the orchard as soon as it is harvested, peel permeability and moisture loss may not be the only factors contributing towards shrivel. The underlying cells might also play a role as moisture loss from the cells may lead to deformations of the epidermal cells. This may result in the shrivelled appearance of the fruit peel, since the cuticle is relatively inelastic and will not shrink along with the underlying cells. This may be cultivar dependant. However, this was also not quantified in the present study and is recommended for future research.

Thus, pre-harvest peel water vapour permeance is not recommended as a method to determine or predict shrivel susceptibility in Japanese plums.

6. Acknowledgements

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Table 1

Optimum harvest dates of the cultivars in 2015/16 and 2016/17.

Cultivar	Harvest 2015/16	Harvest 2016/17
African Rose	19 Nov 2015	
Ruby Sun	17 Dec 2015	
Fortune	24 Dec – 31 Dec 2015	
Angeleno	25 Feb – 3 Mar 2016	
Ruby Star	11 Feb – 18 Feb 2016	
Sapphire	-	13 Dec 2016
Laetitia	18 Jan 2016	16 Jan 2017
Songold	8 Feb 2016	31 Jan 2017
African Delight™	-	13 Feb 2017

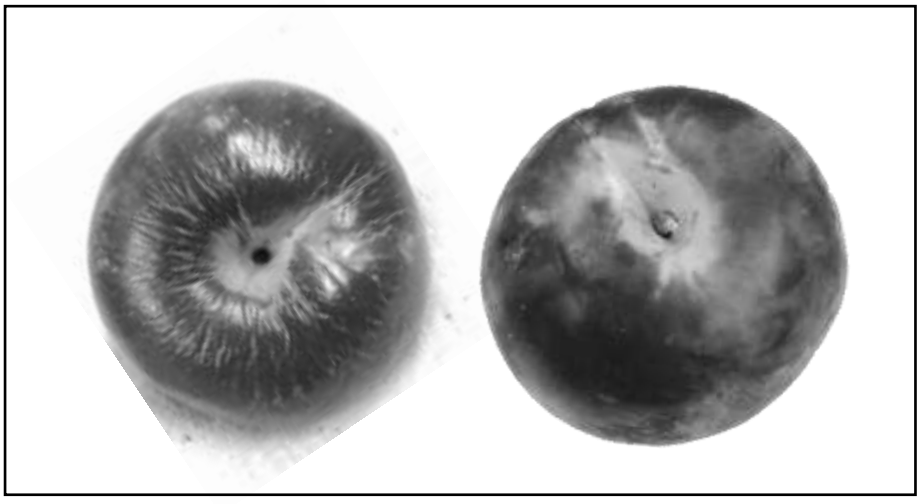


Fig. 1. Shrivelled versus non-shrivelled 'African Delight™' plum

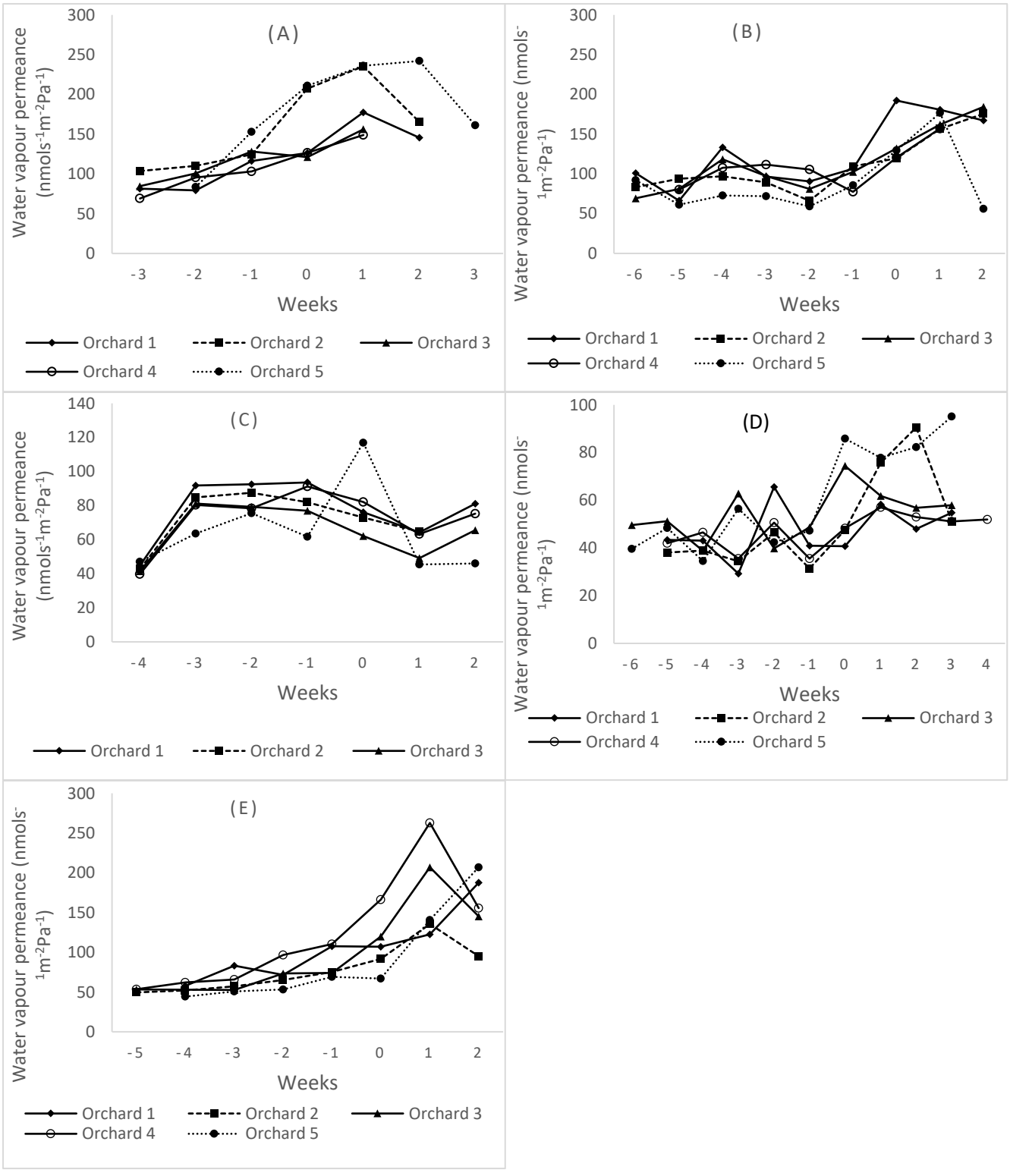


Fig. 2. Water vapour permeance of fruit from five different orchards harvested in different weeks relative to the commercial (optimum) harvest maturity (0 weeks) of the cultivars (A) African Rose, (B) Ruby Sun, (C) Fortune, (D) Angeleno and (E) Ruby Star in 2015/16. Negative week-values indicate weeks before optimum maturity, while positive values indicate weeks after optimum maturity.

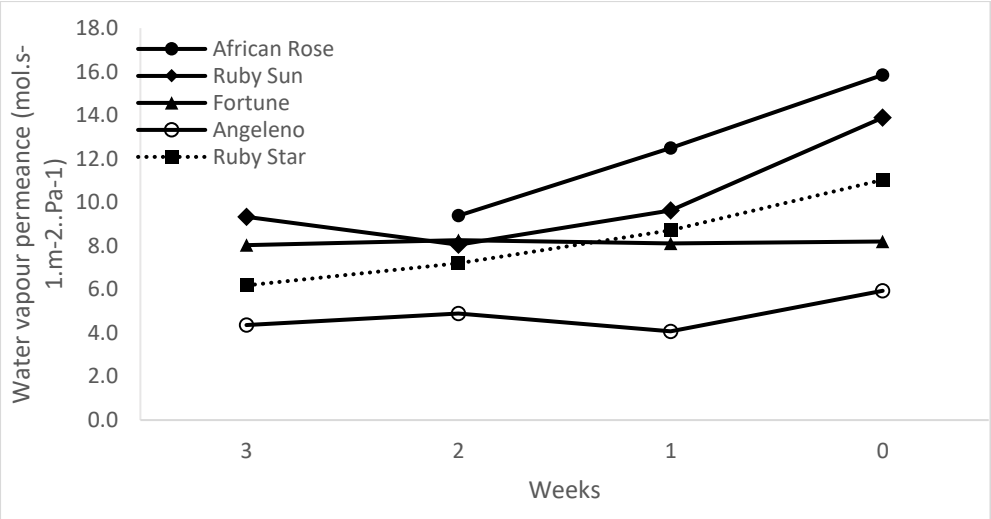
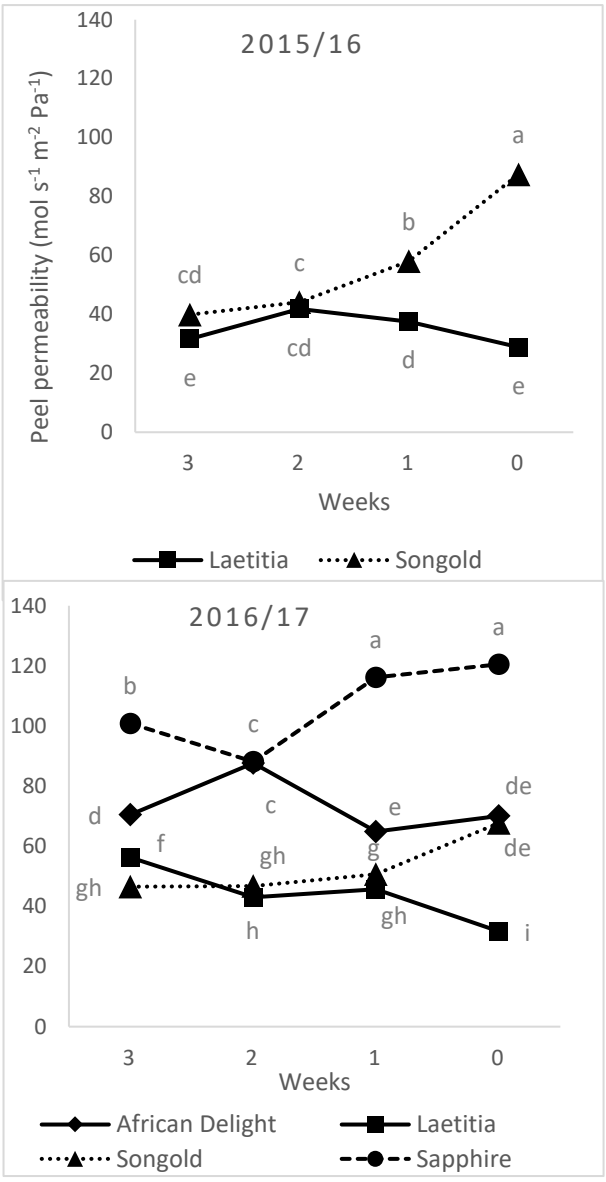


Fig. 3. Changes in water vapour permeance of ‘African Rose’, ‘Ruby Sun’, ‘Fortune’, ‘Angeleno’ and ‘Ruby Star’ fruit harvested from three weeks before harvest, to commercial (optimum) harvest maturity (0 weeks) in 2015/16. ‘African Rose’ could only be harvested from two weeks before commercial harvest maturity.



(A)

Parameter	p-value
Weeks before harvest (WBH)	< 0.0005
Cultivar	< 0.0001
WBH x Cultivar	< 0.0001

(B)

Parameter	p-value
Weeks before harvest (WBH)	< 0.0005
Cultivar	< 0.0001
WBH x Cultivar	< 0.0001

Fig. 4. Interaction between weeks before harvest and cultivar on mean peel permeability from 3 weeks before harvest to optimal harvest date (0 weeks before harvest), for 2015/16 (A) and 2016/17 (B) separately. In 2015/16 only 'Laetitia' and 'Songold' were investigated, while 'African Delight™' and 'Sapphire' were added in 2016/17. Seasons were not compared statistically, but rather cultivars and dates within a season.

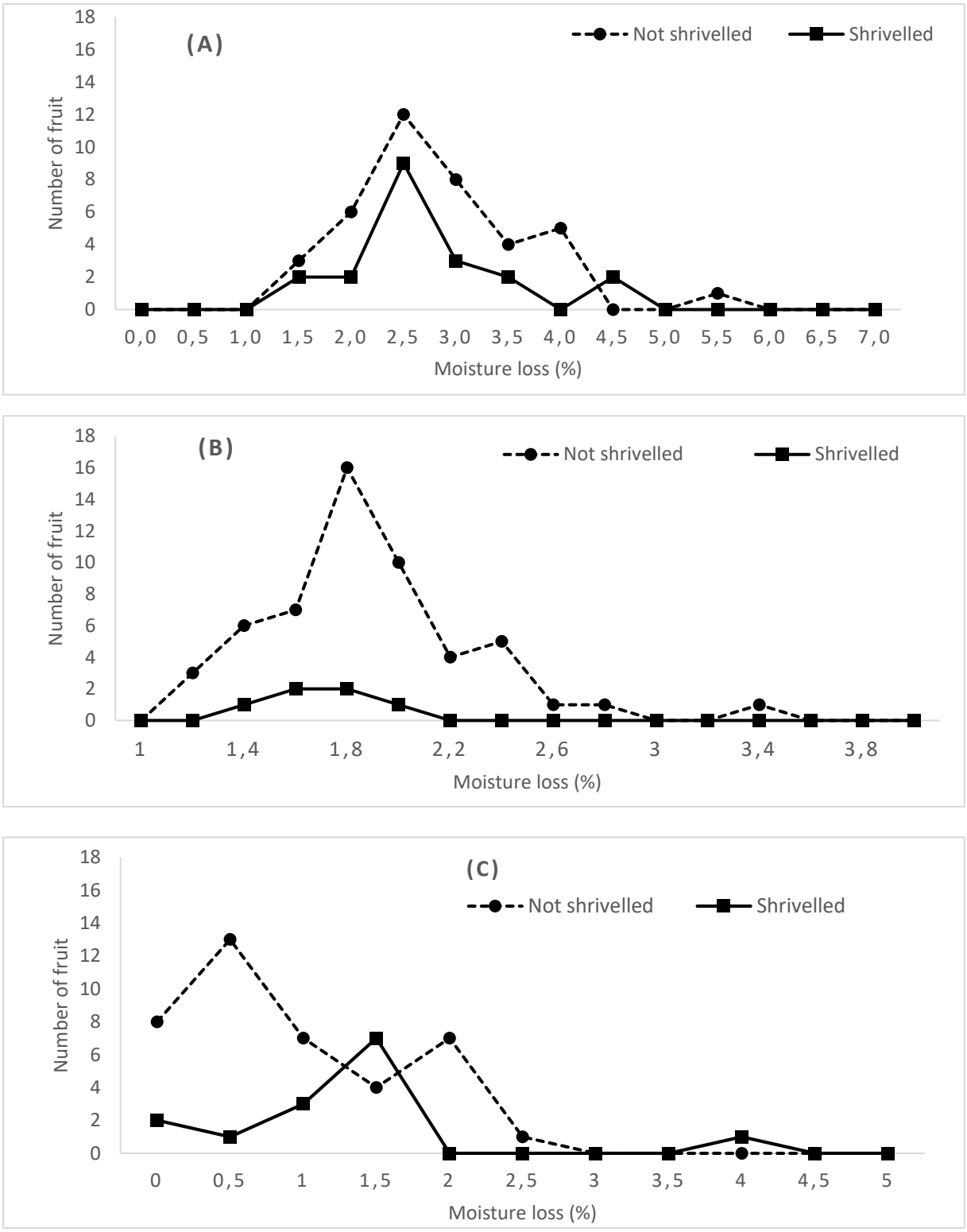


Fig. 5. Frequency plot of percentage moisture loss versus number of fruit shrivelled / not shrivelled for (A) ‘Sapphire’, (B) ‘Laetitia’ and (C) ‘African Delight™ in 2016/2017.

PAPER 2

Quantification of lenticels in Japanese plum cultivars and their effect on peel water vapour permeance

Abstract

Fruit peel water vapour permeance is an indicator of the propensity of fruit to lose moisture. Permeability is affected by the cuticle, as well as stomata, lenticels and cracks in the cuticle. Stomata plays a significant role in controlling the transpiration of leaves. Since lenticels on fruit often form from non-functional stomata, this study determined whether they contribute to peel permeability in Japanese plums and if this varies between cultivars. In 2015/16 as well as 2016/17, 'Laetitia' and 'Songold' were investigated and in 2016/17, 'African Delight™' and 'Sapphire' were added to the study. Fruit were sampled from three weeks before commercial harvest until the commercial harvest date. On each sampling date, the peel permeability of individual fruit was determined and the number of open lenticels, quantified. Lenticels and peel permeability both differed significantly between cultivars and seasons. Significant correlations between permeability and the number of open lenticels were found only in 'Songold' and 'Sapphire'. The percentage of open lenticels are therefore not the determining factor of peel permeability / tendency to lose moisture in plums. Cuticle composition seems to play a more important role in determining fruit moisture loss.

Keywords:

fruit cuticle; moisture loss; *Prunus salicina* Lindl.*;* transpiration; mass loss

1. Introduction

Fruit mass loss is mostly due to the loss of water vapour through transpiration and to a lesser extent, the loss of carbon in the respiration process (Pieniazek, 1944). Therefore, peel water vapour permeance (P'_{H20}) is a good indicator of the propensity of a product to lose moisture, as it quantifies the ease with which water vapour can escape from the fruit (Maguire et al., 2000).

The rate of transpiration and thus, moisture loss, is influenced by both external and internal factors. External factors include temperature, relative humidity (RH) and air movement over the fruit surface (Pieniazek, 1944), while internal factors include cuticle composition and openings in the fruit peel such as wounds, cracks, stomata and lenticels (Maguire et al., 2000; Veraverbeke et al., 2003b).

The cuticle is a lipid membrane, consisting of cutin and waxes that covers the epidermal cells (Belge et al., 2014a; Holloway, 1982; Schreiber and Schönherr, 2009). Its main function is to act as a barrier to transpirational moisture loss, while still allowing gas exchange and transpiration controlled by the stomata (Riederer and Schreiber, 2001; Yeats and Rose, 2013). The waxes associated with the cuticle are the main determinants of the hydrophobic nature of the cuticle (Díaz-Pérez et al., 2007) and the rate of moisture loss through transpiration can be reduced up to 25 times (Wills et al., 2007). This becomes especially important after harvest, when fruit do not receive additional moisture from the tree to replace the moisture lost to the atmosphere (Díaz-Pérez et al., 2007). So, the state of the fruit peel at harvest can have a significant effect on its postharvest quality (Léchaudel et al., 2013).

In leaves, the cuticle blocks moisture loss to such an extent that transpiration occurs mostly through the stomata (Veraverbeke et al., 2003b; Yeats and Rose, 2013). However, in fruit than contain stomata, the guard cells generally become non-functional as the fruit mature (Tamjinda et al., 1992). In sweet cherries, the stomata are non- functional by Stage III of fruit growth and are fixed in a partially open position (Peschel et al., 2003). Lenticels often develop from these non-functional stomata (Dietz et al., 1989; Everett et al., 2008; Veraverbeke et al., 2003b). They are sunken openings that are larger than the stomata and, because they do not have functional guard cells, they form open pores in the fruit peel. However, lenticels can be packed

with suberised cells or become covered with epicuticular wax, which effectively blocks / closes it (Turketti et al., 2012). The cuticle layer is usually thinner over lenticels and the small holes / openings formed by the lenticels, cause discontinuities in the cuticle (Tamjinda et al., 1992). Therefore, these small holes in the cuticle can act as avenues for water vapour to escape the fruit and are actively involved in transpiration and respiration (Dietz et al., 1989; Léchaudel et al., 2013; Veraverbeke et al., 2003b).

Very few studies have investigated the effect of fruit lenticels on moisture loss and, according to our knowledge, this is the first study on Japanese plum lenticels. Significant difference exist in the degree of lenticular transpiration between apple cultivars (Pieniazek, 1944). Yet, the number and appearance of lenticels are not the only factors that determine transpiration. Apple cultivars with very few, inconspicuous lenticels can have a similar transpiration rate to a cultivar that is characterised by numerous (1500 per fruit) and large lenticels. In nectarines and mangoes, a direct link has been found between transpiration rate of fruit and the number of lenticels (Dietz et al., 1989; Wu et al., 2003).

Theron (2015) reported that the plum cultivars African Delight™, Laetitia and Sapphire have open lenticels that possibly contribute to postharvest moisture loss. These cultivars, especially African Delight™, are very prone to postharvest moisture loss and shrivelling. Therefore, the aim of this study was to determine whether the number of lenticels differs between cultivars and whether moisture loss, via the lenticels, contribute towards water vapour permeance of the fruit peel.

2. Materials and methods

2.1 Trial layout and site selection

The study was carried out on Japanese plum cultivars, Laetitia and Songold, sampled from Welgevallen Experimental farm, Stellenbosch, South-Africa (33°56′50.68″S 18°52′14.98″E) over two consecutive seasons (2015/16 and 2016/17). In 2016/2017, ‘Sapphire’ and ‘African Delight™ (cv. ARC PR00-29)’, sampled from Morgenzon farm in Helshoogte, Stellenbosch, South-Africa (33°55’29.33” S 18°55’48.446” E), were added. Fruit sampling occurred weekly, from approx. 3 weeks before the anticipated commercial harvest date until the commercial harvest date. On each sampling date,

100 blemish free fruit of similar size and background colour were picked from randomly selected trees for each cultivar. Fruit were transported in plastic bags to the laboratory at the Department of Horticultural Science, Stellenbosch University.

2.2 Determining peel water vapour permeability

After arrival at the laboratory, the individual fruit were numbered, and their diameter was recorded with a digital calliper (Mitutoyo, Japan) to calculate the surface area of the fruit, assuming the fruit shape to be that of a sphere. According to the method used by Theron (2015), each fruit was then weighed using a balance accurate to 0.001 g (XB 320M, Precisa Instruments Ltd., Switzerland). The fruit were placed in an air-conditioned room for approx. 3 h to reduce the pulp temperature to 20°C before the water vapour permeance (P'_{H2O}; mol s^{-1} m^{-2} Pa^{-1}) was determined for each fruit, over a 16 h period. P'_{H2O} was calculated in a constant environment in a plastic container (20°C, 60 % relative humidity and an air velocity of ~ 0.5 m s^{-1}) using the steady state solution of Fick's first law of diffusion (Taiz and Zeiger, 2010):

$$P'_{H20} = r'_{H20} / \Delta p_{H2O} A \qquad \text{(Eq. 1)}$$

Where:

r'H20 = rate of moisture loss (mol. s^{-1})

ΔpH20 = difference in the water vapour pressure inside and outside the fruit (Pa)

A = area of the fruit surface (m^2)

r'_{H2O} (the rate of water loss; mol. s^{-1}) was calculated by weighing each fruit before and after the 16 h treatment, without adjusting rates of mass loss for the contribution of respiration. Δp_{H2O} (the difference in partial pressure of water vapour between the environment and inside the fruit; Pa) was calculated by means of psychrometric relationships from average relative humidity and temperature data. Mean pulp and air temperature, and relative humidity over the 16 h period, was logged every 15 min with Thermocron® (inserted in a fruit that was not part of the trial) and Hygrochron™ (placed in the air surrounding the fruit) iButtons (CST electronics, Sandton).

After the peel permeability of each fruit was determined, 15 (2015/2016 season) and 20 fruit per cultivar (2016/2017 season), per sampling date, were randomly selected to be examined by means of microscopy. The fruit were fist submerged in an aqueous solution of 0.25% methylene blue (Sigma-Aldrich) and kept at room temperature for 1-2 h. The stain only penetrates open lenticels and stains the underlying tissues (Harker and Ferguson, 1988) creating blue halos around them. Afterwards, fruit were rinsed with water, patted dry, and sections of peel were cut along the equator of the fruit - where most of the lenticels are located (Theron, 2015). Fruit were examined under a stereo-microscope (Leica KL 200 LED, Switzerland) at a 1.25x enlargement. Images of the sections (three per fruit) were captured using Leica Application Suite Software (Version 3.70, Leica Microsystems Limited, Switzerland). The total number of lenticels, as well as the number of open lenticels, were counted and expressed as the percentage of open lenticels per fruit area. A peel section with open lenticels stained a dark blue colour, while closed lenticels remain unstained, is shown in Figure 1. Sections were inspected to make sure that the stain had penetrated through the peel and into the underlying cells (Fig. 2).

2.3 Statistical analysis

The number of lenticels were expressed as means with standard errors. Pearson Product-Moment Correlation Coefficient analyses were performed for each cultivar and sampling date using Statsoft Statistica, version 13 (Statsoft, Inc., 2011) to determine whether there was a relationship between peel permeability and the number of open lenticels per fruit surface area.

3. Results

In 2015/2016, lenticels for ‘Laetitia’ could only be quantified from 2 weeks before harvest (WBH) (Table 1). Fruit surface area decreased slightly from two weeks before harvest until the commercial harvest date, but this was likely due to sampling error. There were no significant differences in the percentage of open lenticels between sampling dates. The total number of lenticels per fruit surface area did not differ significantly between 1 WBH and the commercial harvest date, but was significantly higher at 2 WBH. The number of open lenticels per fruit surface area did not differ significantly between sampling dates. Peel water vapour permeability decreased

significantly between 1 WBH and 2 WBH, then remained stable from 1 WBH until the optimum harvest date. Poor, non-significant correlations were found between peel permeability and the number of open lenticels per fruit. The size of the lenticels did not differ significantly between sampling dates.

For ‘Songold’, lenticels were quantified from 3 WBH until the harvest date (Table 2). Fruit surface area was significantly lower at 3 WBH compared to the following sampling dates. The percentage of open lenticels was significantly lower at 3- and 2 WBH compared to 1 WBH, after which it remained stable until harvest. ‘Songold’ had a higher percentage of open lenticels compared to ‘Laetitia’. The total number of lenticels did not differ significantly between sampling dates, but was almost four times higher than that of ‘Laetitia’. The number of open lenticels per fruit surface area in ‘Songold’ was significantly higher at 1 WBH and on the commercial harvest date, compared to 2 WBH and 3 WBH. On each sampling date, the number of open lenticels was nearly 14 times higher than in ‘Laetitia’. In ‘Songold’, peel permeability did not differ significantly between 3- and 2 WBH. At 1 WBH and on the optimum harvest date, permeability did not differ significantly, but was significantly higher compared to 2- and 3 WBH. Permeability was higher than that of ‘Laetitia’ at 1 WBH and on the commercial harvest date. The lenticel area of ‘Songold’ did not change significantly during the sampling period, but was larger than that of ‘Laetitia’. Poor, non-significant correlations were found between the number of open lenticels and peel permeability at 2- and 3 WBH. However, at 1 WBH and on the commercial harvest date, there were strong positive correlations between peel permeability and open lenticel numbers. As the number of open lenticels increased, the peel permeability also increased. At harvest, 90 % of the variability in peel permeance could be explained by the number of open lenticels.

In 2016/2017, the surface area of ‘Laetitia’ fruit did not differ significantly between sampling dates, except at 1 WBH, when it was significantly higher, though this might have simply been due to natural variation between fruit (Table 3). The percentage of open lenticels was the highest at 3 WBH and increased significantly between successive sampling dates. There were no significant differences in the total number of lenticels per fruit surface area in 2016/17, though it was higher than in 2015/16. The number of open lenticels per fruit surface area decreased significantly from 3 WBH

until the commercial harvest date. In 2016/17, peel permeability in ‘Laetitia’ decreased significantly between 3 WBH and 2 WBH, then increased significantly between 2 WBH and 1 WBH, followed by a significant decrease. Lenticel area was significantly larger at 3 WBH compared to the other sampling dates and the lowest at 2 WBH. On the commercial harvest date, lenticel area could not be determined as the number of open lenticels was too low.

In 2016/17, the fruit surface area in ‘Songold’ did not differ significantly between 2 WBH and the commercial harvest date, but was significantly smaller at 3 WBH (Table 4). The percentage of open lenticels was significantly higher at 2 WBH than at 3 WBH, decreased significantly between 1- and 2 WBH and then, increased significantly between 1 WBH and the commercial harvest date. Between 2 WBH and the commercial harvest date, ‘Songold’ had more open lenticels than ‘Laetitia’. In 2016/17, ‘Songold’ had more lenticels per fruit surface are compared to ‘Laetitia’. At 1 WBH, the total number of lenticels in ‘Songold’ was significantly higher compared to the other sampling dates, nearly three times higher than on the preceding sampling dates. Open lenticel numbers increased dramatically between 3 WBH and the commercial harvest date and was higher than ‘Laetitia’ from 2 WBH.

Peel permeability in ‘Songold’ did not change significantly between 1 - and 3 WBH, but was significantly higher on the commercial harvest date compared to the start of the sampling period. Permeability was lower than in 2015/16 at 1 WBH and on the commercial harvest date. In 2016/17, the permeability of ‘Songold’ was higher than that of ‘Laetitia’. Lenticel area on the commercial harvest data of ‘Songold’ was significantly higher than on the preceding sampling dates and lowest at 3 WBH. Significant correlations between peel permeability and number of open lenticels were found at 2 - and 3 WBH, yet these correlations were not strong enough to explain the variation completely.

In 2016/17, there were significant differences in the fruit surface area of ‘African Delight™’ fruit between sampling dates (Table 5). At 3 WBH and on the commercial harvest date, fruit surface area was significantly higher than at 1- and 2 WBH. The percentage of open lenticels did not differ significantly between sampling dates, except at 3 WBH, when it was significantly higher. ‘African Delight™’ had more open lenticels

compared to ‘Laetitia’ on all sampling dates, but compared to ‘Songold’ was only higher at 1 - and 3 WBH. The total number of lenticels and the number of open lenticels showed a dramatic increase at 2 WBH. On all other sampling dates, the total number of lenticels did not differ significantly, while the number of open lenticels was significantly lower at 1 WBH and on the optimum harvest date compare to 3 WBH. ‘African Delight™’ had more lenticels than ‘Laetitia’, but less than ‘Songold’. Peel permeability of ‘African Delight™’ increased significantly between 3 WBH and 2 WBH and was significantly lower at 1 WBH and on the optimum harvest date compared to 2 WBH. Permeability was higher than that of ‘Laetitia’ and ‘Songold’. The lenticels were similar in size, or slightly larger than those of ‘Songold’. At 3 WBH and 1 WBH, peel permeability had a significantly positive correlation with the number of open lenticels and explained more than 50 % of the variation. Poor, non-significant correlations were present at 2 WBH and the optimum harvest date.

‘Sapphire’, the second cultivar added in 2016/17, showed some variation in fruit size, but this was likely due to natural variation between the fruit (Table 6). The percentage of open lenticels increased significantly over the sampling period, but was quite similar to that ‘African Delight™’. The total number of open lenticels did not differ significantly between sampling dates, except at 2 WBH, when it was significantly lower. Sapphire had more lenticels than ‘African Delight™’, similar to ‘Songold’. The number of open lenticels increased slightly over the sampling period. Between 3 WBH and 2 WBH there were no significant differences in the number of open lenticels. It increased, but not significantly, between 1 WBH and the optimum harvest date. Peel permeability did not differ significantly at 2- or 3 WBH, but was significantly higher at 1 WBH and on the commercial harvest date. Lenticel area did not change significantly between 3- and 1 WBH but was significantly higher on the commercial harvest date. Lenticel area was similar to those of ‘African Delight™’ lenticels. Significant positive correlations were observed between peel permeability and the number of open lenticels at from 2 WBH until the optimum harvest date. The number of open lenticels accounted for 49 to 65 % of the variance in peel permeability.

4. Discussion

Peel permeability determines the tendency of a fruit to lose moisture (Maguire et al., 2000) and cuticle composition, stomata, lenticels, cracks and wounds all contribute to

the permeability of the fruit peel and therefore, to the amount of moisture loss (Maguire et al., 2000; Veraverbeke et al., 2003b). Fruit moisture loss becomes especially important after harvest, since the moisture lost, cannot be replenished by the plant anymore (Díaz-Pérez et al., 2007). As moisture loss has a significantly negative impact on postharvest fruit quality, a better understanding of the factors that contribute to peel permeability may enable growers and exporters to mitigate their negative effects.

The water vapour permeance of a peel changes during fruit development and ripening (Díaz-Pérez et al., 2007; Theron, 2015). Furthermore, differences between farms, orchards, individual trees, fruit on the same tree and fruit developmental stage all affect apple and plum peel permeability (Maguire et al., 2000; Theron, 2015).

Our results clearly indicate significant differences in the number of open lenticels between seasons and cultivars - agreeing with Theron (2015), who postulated that this difference in the number of open lenticels contributed to moisture loss. This may explain why significant positive correlations between peel permeability and the number of open lenticels were seen in ‘Songold’, ‘African Delight™’, and ‘Sapphire’ on specific sampling dates. The lack of a significant correlation between number of open lenticels and peel permeability in ‘Laetitia’ may indicate that in this cultivar, the contribution of the lenticels to moisture loss plays a less important role than the cuticle. Since lenticels do not explain all the variation in peel permeability, cuticle composition needs to be investigated further, as it was reported to play an important role in peel permeability (Karbulková et al., 2008).

‘Songold’ had more open lenticels and higher peel permeability compared to ‘Laetitia’ in both seasons, which was unexpected, as ‘Laetitia’ is considered more prone to postharvest moisture loss and shrivelling. This disagrees with findings by Theron (2015) who found no visible cracks or open lenticels in ‘Songold’ when using fluorescent microscopy. The high permeability and higher percentage of open lenticels seen in ‘African Delight™’ and ‘Sapphire’ correspond with their known susceptibility to moisture loss and subsequent shrivel development.

Our results indicate that the number of lenticels is genetically predetermined to a certain extent, since ‘Laetitia’ and ‘Songold’ develop and mature only a few weeks apart and yet show substantial differences in lenticel numbers. This agrees with

variation reported for sweet cherry cultivars (Lane et al., 2000; Peschel et al., 2003). However, since fruit peel permeability differed between seasons, environmental conditions clearly also play a significant role in peel permeability and lenticel development. Turketti et al. (2012) reported that the number of lenticels in apples were affected by climatic factors. Higher maximum temperatures during the growing season lead to the development of more lenticels on fruit. Thus, our observations of variation in lenticel numbers between seasons for Japanese plums confirmed previous findings in apple, although climate parameters were not specifically quantified in the present study.

5. Conclusion

This study set out to determine whether, and to what extent, open lenticels on the fruit surface contribute to moisture loss of Japanese plums. Since ‘Laetitia’ is more prone to post-harvest moisture loss and shrivelling compared to ‘Songold’, we hypothesized that ‘Laetitia’ would have a larger number of open lenticels per fruit area, thus exacerbating the loss of moisture from the fruit.

In both seasons, ‘Songold’ had significantly more open lenticels and higher peel permeability than ‘Laetitia’, which contradicts the assumption that ‘Laetitia’ is more prone to moisture loss if it is related to peel permeability. The postharvest shrivelling of ‘Laetitia’ must therefore be influenced by more factors than just moisture loss. From 1 WBH ‘Songold’ showed significant positive correlations between open lenticels and peel permeability, yet these parameters did not correlate significantly in ‘Laetitia’ and ‘African Delight™’. The contribution of cuticle composition and/or structure must therefore play a more important role in determining peel permeability in these cultivars and need to be investigated further.

Seasonal differences have a significant effect on peel permeability and lenticel quantities, but number of lenticels also seems to be genetically determined in Japanese plums. This study shows that open lenticels do contribute to moisture loss in Japanese plums, but the extent to which it contributes differs between seasons and cultivars. Based on this anatomical study, the proneness of Japanese plum cultivars to shrivelling and moisture loss cannot be related directly to the number of open lenticels per fruit as primary indicator of this disorder.

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Table 1

Summary of 'Laetitia' lenticel data in 2015/16, from 2 weeks before harvest (WBH) until the commercial harvest date. Values indicate mean with standard error. R^2 -values indicate the Pearson Product-Moment Correlation Coefficient analyses between peel permeability and the number of open lenticels, while the p-value shows the significance of the correlation.

WBH	Fruit surface area (cm^2)	Open lenticels (%)	Total lenticels per fruit area	Open lenticels per fruit area	Permeability ($nmols^{-1}m^{-2}Pa^{-1}$)	R^2	p-value	Lenticel area (cm^2)
2	64.18 ± 1.39	12.9 ± 2.9	647.6 ± 47.9	79.2 ± 16.0	53.44 ± 2.11	-0.44	0.0960	-
1	62.0 ± 1.39	14.6 ± 5.4	495.1± 45.8	63.4 ± 18.8	27.83 ± 2.68	0.20	0.9530	0.0050 ± 0.0021
0	59.59 ± 1.7	13.1 ± 3.2	541.9 ± 43.9	70.3 ± 20.3	33.42 ± 2.40	0.36	0.1870	0.0070 ± 0.0014

Table 2

Summary of lenticel data for 'Songold' lenticel data in 2015/16, from 3 weeks before harvest (WBH) until the commercial harvest date. Values indicate mean with standard error. R^2 -values indicate the Pearson Product-Moment Correlation Coefficient analyses between peel permeability and the number of open lenticels, while the p-value shows the significance of the correlation.

WBH	Fruit surface area (cm^2)	Open lenticels (%)	Total lenticels per fruit area	Open lenticels per fruit area	Permeability ($nmols^{-1}m^{-2}Pa^{-1}$)	R^2	p-value	Lenticel area (cm^2)
3	69.62 ± 7.62	29.9 ± 4.9	1817.4 ± 155.1	375.6 ± 65.4	53.20 ± 15.17	-0.30	0.3370	0.0102 ± 0.0017
2	86.64 ± 1.86	21.5 ± 2.3	1761.5 ±142.3	365.3 ± 44.4	49.78 ± 8.15	0.36	0.2010	0.0099 ± 0.0028
1	82.87 ± 3.10	44.7 ± 8.34	1766.6 ± 145.3	843.0 ± 203.5	80.48 ± 22.62	0.84	<0.0001	0.0154 ± 0.0033
0	87.35 ± 1.91	50.3 ± 6.4	1692.1 ± 139.6	923.4 ± 176.0	95.64 ± 14.09	0.90	<0.0001	0.0107 ± 0.0022

Table 3

Summary of lenticel data for ‘Laetitia’ in 2016/2017, from 3 weeks before harvest (WBH) until the commercial harvest date. Values indicate mean with standard error. R^2 -values indicate the Pearson Product-Moment Correlation Coefficient analyses between peel permeability and the number of open lenticels, while the p-value shows the significance of the correlation.

WBH	Fruit surface area (cm^2)	Open lenticels (%)	Total lenticels per fruit area	Open lenticels per fruit area	Permeability ($nmols^{-1}m^{-2}Pa^{-1}$)	R^2	p-value	Lenticel area (cm^2)
3	53.80 ± 1.45	31.0 ± 3.7	721.6 ± 35.8	221.6 ± 28.5	57.39 ± 1.57	-0.16	0.5070	0.0726 ± 0.165
2	56.51 ± 1.36	9.4 ± 3.3	756.9 ± 44.1	65.4 ± 22.5	42.72 ±1.71	0.32	0.1670	0.0034 ± 0.0011
1	64.56 ± 2.08	4.6 ± 1.3	748.8 ± 42.4	32.2 ± 9.7	47.40 ±1.64	0.06	0.800	0.0071 ± 0.0019
0	54.78 ± 3.33	0.2 ± 0.1	709.2 ± 62.1	1.2 ± 0.9	30.03 ±1.67	-0.12	0.6330	-

Table 4

Summary of lenticel data for 'Songold' in 2016/2017, from 3 weeks before harvest (WBH) until the commercial harvest date. Values indicate mean with standard error. R^2 -values indicate the Pearson Product-Moment Correlation Coefficient analyses between peel permeability and the number of open lenticels, while the p-value shows the significance of the correlation.

WBH	Surface area (cm^2)	Open lenticels (%)	Total lenticels per fruit area	Open lenticels per fruit area	Permeability ($nmols^{-1}m^{-2}Pa^{-1}$)	R^2	p-value	Lenticel area (cm^2)
3	72.32 ± 1.02	6.0 ± 1.2	2140.5 ± 78.4	120.3 ± 24.1	47.74 ± 1.68	0.51	0.022	0.0029 ± 0.0005
2	87.17 ± 1.69	38.0 ± 3.7	1770.7 ± 91.7	653.3 ± 61.6	49.80 ± 2.98	0.49	0.027	0.0075 ± 0.0005
1	87.99 ± 2.02	19.2 ± 2.2	5893.5 ± 252.9	1117.4 ± 121.9	51.39 ± 3.04	-0.25	0.297	0.0065 ± 0.0027
0	83.85 ± 5.05	57.4 ± 3.2	1943.7 ± 138.8	1107.5 ± 97.8	59.42 ± 3.92	0.30	0.231	0.0200 ± 0.0026

Table 5

Summary of lenticel data for 'African Delight' in 2016/2017, from 3 weeks before harvest (WBH) until the commercial harvest date. Values indicate mean with standard error. R^2-values indicate the Pearson Product-Moment Correlation Coefficient analyses between peel permeability and the number of open lenticels, while the p-value shows the significance of the correlation.

WBH	Surface area (cm^2)	Open lenticels (%)	Total lenticels per fruit area	Open lenticels per fruit area	Permeability ($nmols^{-1}m^{-2}Pa^{-1}$)	R^2	p-value	Lenticel area (cm^2)
3	104.63 ± 1.75	53.5 ± 4.05	1041.7 ± 69.9	542.1 ± 56.7	73.44 ± 4.18	0.52	0.0190	0.01518 ± 0.0026
2	89.53 ± 5.02	30.6 ± 3.7	3671.4 ± 238.7	1045.8 ± 177.2	87.65 ± 5.87	0.06	0.8130	0.0077 ± 0.0015
1	93.16 ± 1.59	31.5 ± 2.9	1039.3 ± 63.9	325.9 ± 32.9	66.17 ± 1.97	0.65	0.00201	0.0122 ± 0.0014
0	101.80 ± 2.67	31.0 ± 2.6	931.2 ± 66.6	289.2 ± 29.3	71.07 ± 5.08	0.06	0.8180	0.0233 ± 0.0040

Table 6

Summary of lenticel data for 'Sapphire' in 2016/2017, from 3 weeks before harvest (WBH) until the commercial harvest date. Values indicate mean with standard error. R^2 -values indicate the Pearson Product-Moment Correlation Coefficient analyses between peel permeability and the number of open lenticels, while the p-value shows the significance of the correlation.

WBH	Surface area (cm^2)	Open lenticels (%)	Total lenticels per fruit area	Open lenticels per fruit area	Permeability ($nmols^{-1}m^{-2}Pa^{-1}$)	R^2	p-value	Lenticel area (cm^2)
3	60.11 ± 0.98	24.4 ± 3.2	1859.0 ± 106.3	447.6 ± 62.6	99.42 ± 2.48	-0.38	0.0970	0.0123 ± 0.0032
2	76.20 ± 2.01	34.5 ± 2.2	1410.6 ± 106.9	504.2 ± 56.7	86.93 ± 3.83	0.65	0.0030	0.0117 ± 0.0019
1	91.46 ± 1.77	31.2 ± 3.7	1862.2 ± 94.5	586.5 ± 80.2	134.5 ± 7.66	0.53	0.0240	0.0187 ± 0.0033
0	75.52 ±4.51	47.0 ±6.4	1653.4 ±115.0	723.0 ±83.3	140.72 ±13.15	0.49	0.035	0.0287 ± 0.0035

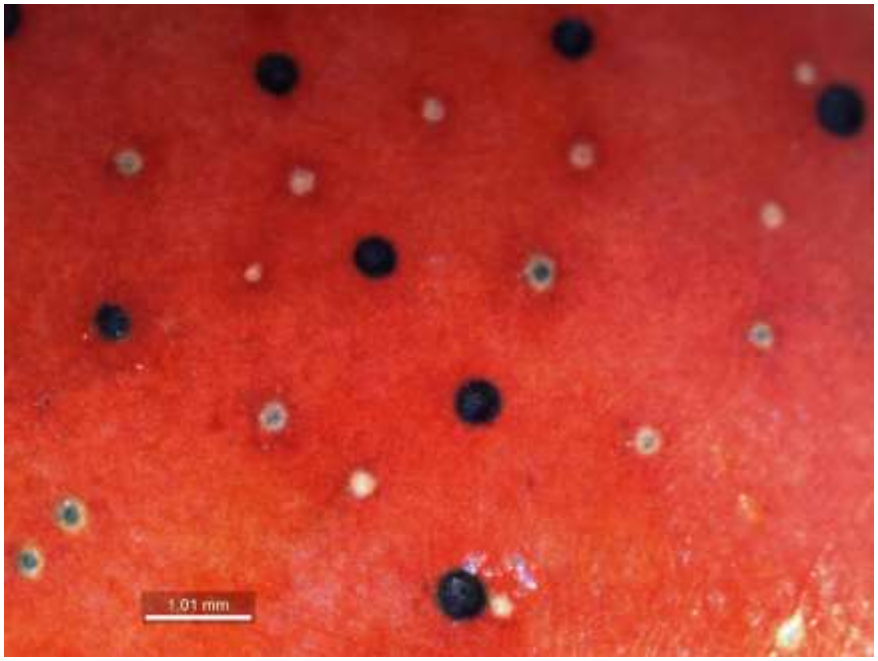

Fig. 1. ‘Laetitia’ peel section micrograph showing open lenticels forming dark blue halos after being stained with Toluidine Blue.

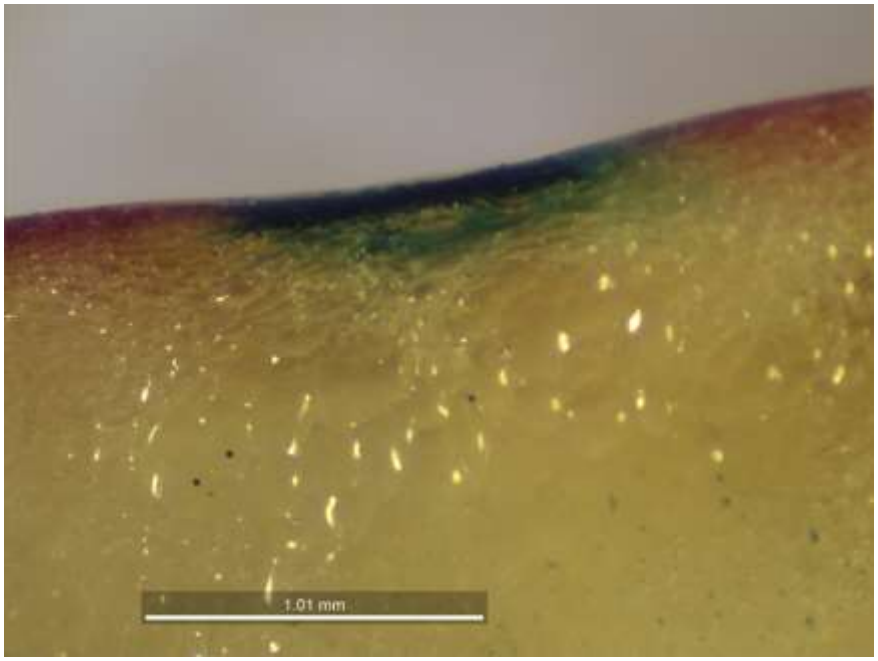

Fig. 2. Penetration of Toluidine Blue stain through the open lenticel into the hypodermal tissue.

PAPER 3

Fruit cuticle composition in two Japanese plum cultivars and its connection to postharvest shrivel development

Abstract

The fruit cuticle serves as the most important barrier against moisture loss and protection against mechanical damage and insects. The composition of the cuticle, which includes waxes and cutin, greatly influences the susceptibility to moisture loss in other fruit. The aim of this study was to determine whether cuticle composition is related to postharvest moisture loss. Sixty fruit were sampled from 'Songold' and 'Laetitia' orchards, from three weeks before the commercial harvest date, after cold storage and a simulated shelf-life period, during two consecutive seasons. There was a marked difference in shrivel incidence between cultivars and seasons. Wax and cutin monomers were extracted separately and compounds identified using mass spectroscopy. High relative concentrations of the primary alcohols in the waxes was reported to reduce the permeability of the cuticle and prevent shrivel. However, principal component analyses could not separate the cultivars or seasons along these parameters. The cutin matrix may also contribute towards shrivel development. The higher concentration of tri-hydroxy acids measured in 'Laetitia' caused increased hydration and flexibility of the cutin matrix. The decrease in total wax content during cold storage in 2017/18 may have increased the flexibility of the cuticle even more. As fruit lose moisture during cold storage, the epidermal and hypodermal cells become dehydrated and collapse. The more flexible cuticles of 'Laetitia' deform with the epidermal cells, causing a shrivelled appearance, while the more rigid cuticles of 'Songold' can resist this deformation and maintain its original shape.

Key words: cuticle; cutin; moisture loss; postharvest; *Prunus salacina* (Lindl.); shrivel; wax

1. Introduction

The cuticle forms a heterogeneous, continuous protective layer, covering all aerial surfaces of terrestrial plants, including fruit (Belding et al., 1998; Gazzola et al., 2004). It is the first and most important barrier against moisture loss, while also protecting fruit from insects, pathogens and excessive UV radiation. Cuticles are composed of two main components, namely cutin and wax. Cutin consists of long-chain C_{16} and C_{18} ω-hydroxy and epoxy-hydroxylated fatty acids linked via ester bonds and forms the structural matrix or scaffolding of the cuticle (Heredia, 2003; Kolattukudy, 2001; Lara et al., 2015). Intracuticular waxes are embedded within this matrix and epicuticular waxes cover the surface of the matrix. The waxes consist of different classes of very long-chain aliphatics, including alkanes, alcohols, fatty acids, aldehydes and esters, mixed with triterpenoids and other cyclic compounds (Lara et al., 2015; Matas et al., 2004). Cutin and waxes usually predominate the cuticle composition, but another constituent of some cuticles, a non-hydrolysable polymer called cutan, can also occur (Gupta et al., 2006).

The protective function of fruit cuticles is not only important on the tree, but also during long-term storage, especially in limiting moisture- and mass loss (Lara et al., 2014; Veraverbeke et al., 2001a). During cold storage, fruit continue to lose moisture across the epidermis due to the vapour pressure deficit between the epidermal cells and the surrounding atmosphere (Jenks et al., 1994; Veraverbeke et al., 2003a). Both cuticle structure and composition change during cold storage (Konarska and Agata, 2013; Veraverbeke et al., 2001a) and the cuticular wax layer can minimise the loss of moisture through changes in its composition and distribution or by covering cracks and open stomata. The continued and excessive moisture loss that often occurs in fresh products during storage, can lead to the development of a shrivelled appearance (Nguyen et al., 2006; Veraverbeke et al., 2001a).

Cutin and wax monomers are all synthesised in the plastids of the epidermal cells and share the same pool of precursors (Lara et al., 2015). However, the composition and structure of cuticular membranes are highly variable between species (Martin and Rose, 2014). In fruit, significant differences also exist between cultivars of the same species, organs on the same plant and even different parts of an individual fruit (Konarska and Agata, 2013; Martin and Rose, 2013; Sala, 2000; Veraverbeke et al.,

2001a). This explains why the cuticular changes that occur during storage and shelf-life are also quite variable.

In Japanese plums exported from South Africa, moisture loss during the extended periods of shipping required to reach overseas markets, has a very detrimental effect on fruit quality. Consignments are often rejected due to excessive mass loss (since the fruit are sold per mass) and the development of a shrivelled appearance at the stem end of the fruit. However, moisture loss also has other negative effects, including a loss in firmness and glossiness, colour changes, limpness, reduced shelf-life and reduced nutritional value (Finger et al., 1995; Holcroft, 2015; Sastry, 1985b; Smith et al., 2006; Wilson et al., 1995). Since the cuticle is the primary barrier to moisture loss and is known to affect the quality of other fresh products (Lara et al., 2014; Nguyen et al., 2006; Veraverbeke et al., 2003b), information about its composition and how it changes during storage, could provide insight into managing moisture loss and partly explain why some Japanese plum cultivars are more prone shrivelling than others.

2. Materials and methods

2.1 Site description

During 2016/17 fruit were sampled from ‘Laetitia’ and ‘Songold’ on ‘Marianna’ rootstock on the Welgevallen Experimental farm (33°56′50.68″S 18°52′14.98″E), Stellenbosch University, South Africa. In 2017/18, fruit were sampled from The Firs, Devonvalley, Stellenbosch (33°54'55.6"S 18°49'11.4"E) from ‘Laetitia’ and ‘Songold’ on ‘FG967’ rootstock.

2.2 Sampling

Fruit were sampled from three weeks before the anticipated harvest date, until the commercial harvest date. On each sampling date, 60 fruit of similar size and background colour were selected at random, per cultivar. Fruit were handled carefully and placed in polyethylene bags (10 per bag) to prevent excessive rubbing of fruit against one another and transported to the laboratory at the Department of Horticultural Science, Stellenbosch University.

On arrival at the laboratory, the diameter of each fruit was recorded with a digital calliper (Mitutyo, Japan) and fruit surface area was determined with the assumption

that fruit were spherical. On the commercial harvest date, 250 fruit per cultivar were sampled. Sixty fruit were randomly selected for 'at harvest' cuticle extraction. The remaining fruit were packed by inserting a perforated, high density polyethylene (HDPE) shrivel sheet into a carton (38.5 x 30 x 10.5 cm open-top) and then inserting two layers of fruit, packed in count-30 pulp trays. The shrivel sheets were neatly folded over the fruit after packing and a corrugated paper sheet was placed on top to prevent the sheets from moving or opening during cooling. Three cartons per cultivar were packed and cold-stored according to a commercially used intermittent warming regime (PPECB, 2017). 'Laetitia' was stored for 10 days at -0.5°C, followed by 7 days at 7.5°C and a further 32 days at -0.5°C. This was followed by a simulated shelf-life of 7 days at 10°C. 'Songold' was stored for 10 days at -0.5°C, followed by 9 days at 7.5°C and a further 23 days at -0.5°C, also followed by a simulated shelf-life of 7 days at 10°C. The plastic shrivel sheet was removed before the fruit were placed under shelf–life conditions to prevent condensation and fruit decay. After the cold storage period, 60 fruit, selected at random from the three cartons, were used for analysis. Shrivel (%) was quantified on the remaining 120 fruit after cold storage and again after the simulated shelf-life period. Shrivel was counted when shrivelled skin extended over the shoulder of the fruit.

2.3 Cuticle isolation

The 60 fruit of each cultivar were randomly divided into four replicates of 15 fruit and two peel disks per fruit were carefully excised using a cork borer (13 mm i.d). The 30 disks per replicate were pooled for enzymatic isolation adapted from methods used in peaches and sweet cherries (Belge et al., 2014a;b). These methods were also adapted for wax extraction, cutin extraction, and GC-MS.

The enzyme solution was prepared by adding 1.6 $U.ml^{-1}$ cellulase from *Trichoderma reesei* (C2730, SIGMA) and 100 $U.ml^{-1}$ pectinase from *Aspergillus aculeatus* (E6287, SIGMA) to 50mM citrate buffer (pH 4) and including 1 mM NaN_3 (0. 065$g.L^{-1}$) to the solution to prevent microbial growth. Peel disks were incubated in this solution (100ml per 30 disks) for approx. five days at 37°C (in a water bath). After incubation, the flasks were lightly shaken to remove any exocarp material still attached to the cuticular membranes (CM) and then washed with a citrate buffer (pH 4.0, 50 mM) at 37°C until no material was left in suspension. Disks were then thoroughly rinsed in distilled water

to remove any residual materials and dried overnight at 40°C. After drying, samples were weighed on a microbalance and stored in specimen jars until further use.

2.4 Wax extraction protocol

To extract the intra- and epicuticular waxes from the dried CM, samples were shaken in chloroform (1 mg sample mL^{-1}) for 24h at room temperature, followed by incubation in an ultrasonic bath for 15 min. The process was repeated two more times. After extraction, and filtration the extracts were concentrated at 45°C using a rotary evaporator. The waxes were transferred to pre-weighed vials and dried completely, then weighed on a microbalance. The free hydroxyl and carboxyl groups in the wax samples were converted into their trimethylsilyl (TMSi) ethers and esters respectively, by derivatising with N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine for 60 min at 80°C. After derivatization, eicosane (C20) and dotriacontane (C32) were added as internal standards and vortexed to ensure sufficient mixing.

2.5 Cutin extraction

The dewaxed cuticular membranes (that remained after filtration) were hydrolysed to extract the cutin, by adding 3ml of 1 M HCL in 100% MeOH (2.94 ml HCl in 30 ml MeOH) to 10 mg of CM. At this stage, the samples turned a light pink colour. Samples were esterified in the same solvent for 2h at 80°C and cooled to room temperature. After cooling, the non-hydrolysable part (cutan) was removed from the solution. The cutan was air-dried and weighed. After removing the cutan, 2ml of saturated NaCl (SIGMA, 0.375 $g.ml^{-1}$) was added to the methanolysate, followed by 2 ml of hexane to extract the cutin monomers and the samples were shaken for 10 min. The solution separated into a light pink fraction at the bottom, with a transparent fraction on top. After shaking, the transparent fraction was carefully removed using a pipette and placed into pre-weighed vials. An additional 2 ml of Hexane was added to the remaining fraction and the process was repeated. This was done three times. The pooled transparent fraction was then concentrated using a rotary evaporator and weighed. The insoluble part of the cuticular membrane that remained after cutin extraction, cutan, was not analysed further.

The free hydroxyl and carboxyl groups in the wax samples were converted into their trimethylsilyl (TMSi) ethers and esters respectively, by derivatising with N, O-

bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine for 60 min at 80°C. After derivatization, eicosane (C20) and dotriacontane (C32) were added as internal standards and vortexed to ensure sufficient mixing.

2.6 GC-MS

The mixtures were injected (1 µl) in split-less mode into a gas chromatography-mass spectrometry (GC-MS) system to identify was composition. GC equipment (6890N, Agilent technologies network) coupled to an Agilent technologies inert XLE/CI Mass Selective Detector (MSD) (5975, Agilent technologies Inc., Palo Alto, CA). The GC-MS system was coupled with a CTC Analytics PAL autosampler. Separation of the components was performed on a ZB-Semi-volatile (30 m x 0.25 mm, 0.25 µm) Zebron 7HG-G027-11-GGA capillary column. The oven was set at 100°C (2 min), and initially raised at 15°C min^{-1} to 180°C, then raised at 5°C min^{-1} to 250°C and kept constant for 3min, then raised again at 20°C min^{-1} to 320°C and kept constant for 12 min. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. Compounds were identified by matching their electron ionization mass spectra (EI-MS) (70 eV, *m/z* 40-650) with those from the NIST 08 MS library.

2.7 Statistical analysis

Due to logistics, only three replicate samples per treatment was used for analyses and therefore results were reported as means with standard error bars. A second season was added for evaluation to add robustness to the interpretation. Principal component analyses were performed in Statsoft Statistica for wax and cutin compounds separately to reduce the number of variables and identify which variables are important in describing the variation. Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks.

3. Results

3.1 Cuticle

In 2016/17, the mass of the cuticular membrane per fruit surface area did not differ significantly between the cultivars at either 2- or 3 WBH (Fig. 1A). One week before harvest, ‘Songold’ had a significantly higher amount of cuticular membrane than ‘Laetitia’, but on their respective harvest dates, there were no significant differences. In ‘Laetitia’, the cuticular membrane mass was significantly higher after the cold

storage period, than on the commercial harvest date. In ‘Songold’, the cuticular membrane mass also increased significantly during cold storage, though not as much as ‘Laetitia’. During the simulated shelf-life period of 7 days at 10˚C, no significant changes occurred in either cultivar. Thus, after cold storage and shelf-life, the cuticular membrane of ‘Laetitia’ was significantly thicker than that of ‘Songold’.

In 2017/18, ‘Songold’ had a significantly higher cuticular membrane mass per fruit surface area than ‘Laetitia’ at 2- and 3 WBH (Fig. 1B). At 1 WBH, there were no significant differences between the cultivars. From the commercial harvest date until the end of shelf-life, the cuticular membrane mass remained stable in both cultivars, with ‘Laetitia’ significantly higher than ‘Songold’.

In 2016/2017 the total amount of extracted wax increased significantly in ‘Laetitia’ during cold storage (Fig 2A). In 2017/18 the total amount of extracted wax in ‘Laetitia’ was significantly lower compared to ‘Songold’ (Fig. 2B). On their respective harvest dates, there were no significant differences in total amount of extracted wax between ‘Laetitia’ and ‘Songold’. Thus, the wax content of ‘Laetitia’ increased significantly from 1 WBH until the commercial harvest date, while it remained stable in ‘Songold’. After cold storage ‘Laetitia’ had significantly more wax than ‘Songold’, but the total amount of extracted wax had decreased significantly, in both cultivars, during storage. At the end of the shelf-life period in 2017/18 the amount of cuticular wax was significantly higher in ‘Laetitia’ than ‘Songold’. The total amount of wax in ‘Laetitia’ had increased during shelf-life, while that of ‘Songold’ decreased.

In 2016/17 ‘Songold’ had a significantly higher amount of cutin than ‘Laetitia’ at 2- and 3 WBH (Fig. 3A). From 1 WBH until their respective commercial harvest dates, the cutin mass did not differ significantly between the cultivars. In ‘Laetitia’, higher amount of cutin increased significantly during cold storage, then remained stable during shelf-life. The same trend was seen in ‘Songold’, though not significantly. The amount of cutin was significantly higher in ‘Laetitia’ than ‘Songold’ after cold storage and shelf-life. In 2017/18 there was a high degree of variation in cutin mass between replicates (Fig. 3B). There were no significant differences in cutin mass between the cultivars from 1- to 3 WBH. From their commercial harvest dates until the end of shelf-life, ‘Laetitia’ had a significantly more cutin than ‘Songold’ and cutin mass remained stable in both cultivars.

3.2 Cuticular waxes

The different compounds identified in cuticular wax and cutin samples are summarised in Table 1. In 2016/17 the relative percentage of lauric acid in the wax samples did not differ significantly between the cultivars from 3 WBH until the commercial harvest date (Fig. 4A). After cold storage and shelf-life, 'Songold' had a significantly lower amount of lauric acid than 'Laetitia', though the relative percentage remained stable within each cultivar during this time. In 2017/18 there were no significant differences in lauric acid content between the two cultivars, except at 2 WBH, when it was significantly higher in 'Songold' (Fig. 4B)

The relative percentage of myristic acid (Fig. 5A and B) and pentadecanoic acid (Fig. 6A and B) followed the same trend during both seasons. Like lauric acid, the relative percentage of myristic acid and pentadecanoic acid did not differ significantly between the cultivars from 3 WBH until the commercial harvest date. After cold storage and shelf-life, the myristic- and pentadecanoic acid content was significantly higher in 'Songold' compared to 'Laetitia'. A different trend was seen in 2017/18 (Fig. 5B and 6B). From 1- to 3 WBH 'Songold' waxes had a significantly higher myristic acid and pentadecanoic acid content than 'Laetitia'. On the commercial harvest date, the relative percentage of both myristic- and pentadecanoic acid did not differ significantly between the cultivars. In 'Songold' the myristic- and pentadecanoic acid content increased significantly during cold storage, becoming significantly higher than 'Laetitia', then decreased again during shelf-life.

The heptadecanoic acid content was significantly higher in 'Laetitia' at 3 WBH in 2016/17 but there were no significant differences between the cultivars at 1- or 2 WBH (Fig. 7A). On their respective commercial harvest dates, 'Laetitia' waxes had a significantly higher heptadecanoic acid content than 'Songold', but it decreased significantly during cold storage, while it increased in 'Songold'. After cold storage and the simulated shelf-life period, the heptadecanoic acid content remained stable in each cultivar, with 'Songold' having a significantly higher content than 'Laetitia'. In 2017/18 the heptadecanoic acid content followed the same trend as the pentadecanoic acid content from 3 WBH until the commercial harvest date (Fig. 7B). Between 1- and 3 WBH it was significantly higher in 'Songold' compared to 'Laetitia', while it did not differ significantly on the commercial harvest date but had increased significantly in 'Laetitia'.

There were no significant differences between the cultivars after cold storage, but ‘Laetitia’ increased significantly during shelf-life and was significantly higher than ‘Songold’ by the end of shelf-life.

The relative abundance of oleic acid in the cuticular waxes was significantly higher in ‘Laetitia’ on all sampling dates in 2016/17, except after cold storage, when no oleic acid was detected in ‘Laetitia’ (Fig. 8A). Oleic acid content decreased significantly in ‘Laetitia’ during cold storage, while it remained the same in ‘Songold’. In 2017/18 there were no significant differences in oleic acid content between the cultivars on any of the sampling dates (Fig. 8B). Between 3 WBH and the commercial harvest date, there was almost no oleic acid present in either of the cultivars.

In 2016/17, the relative abundance of 2,4-bis (dimethyl benzyl) phenol did not differ significantly between the cultivars at 2- or 3 WBH (Fig. 9A). For the rest of the sampling period, ‘Songold’ had a significantly higher abundance of this phenol compared to ‘Laetitia’. ‘Songold’ showed a decline in the 2,4-bis (dimethyl benzyl) phenol content between 1 WBH and the end of shelf-life, while ‘Laetitia’ did not follow a clear pattern. In 2017/18 a different trend was observed (Fig. 9B). From 1- until 3 WBH, ‘Songold’ had a significantly higher phenol content than ‘Laetitia’. From the commercial harvest date until the end of shelf-life, ‘Laetitia’ had a significantly higher phenol content compared to ‘Songold’. ‘Songold’ showed a decline in 2,4-bis (dimethyl benzyl) phenol between harvest and the end of shelf-life, as in 2016/17, while ‘Laetitia’ also followed the same trend as the previous season, a slight decrease during cold storage, followed by an increase during shelf-life.

In 2016/17 there was almost no tetratriacontane in the wax samples of either cultivar (Fig. 10A). Yet, on the commercial harvest date, ‘Songold’ had a significantly higher abundance of tetratriacontane compared to ‘Laetitia’, and compared to the previous sampling dates, and it remained stable until the end of shelf-life. In ‘Laetitia’, almost no tetratriacontane was detected at harvest or after cold storage, though it was significantly higher after shelf-life. In 2017/18 the tetratriacontane content in ‘Laetitia’ was much higher than the previous season (Fig. 10B). There were no significant differences between cultivars at either 2- or 3 WBH. From 1 WBH until the end of shelf-life, the tetratriacontane content in ‘Songold’ was significantly higher than that of ‘Laetitia’.

The other alkane, tetratetracontane, showed a different pattern compared to tetratriacontane (Fig. 11). In 2016/17, 'Laetitia' had a significantly higher tetratetracontane content compared to 'Songold' at 2- and 3 WBH (Fig 11A). At 1 WBH, there were no significant differences between cultivars. For the rest of the sampling period, no tetratetracontane was detected in 'Songold'. In 'Laetitia', the relative abundance of tetratetracontane decreased significantly, to zero, during cold storage and increased again during shelf-life. In 2017/18 no tetratetracotane was detected in either cultivar (Fig. 11B).

In 2016/17, the tetracosanol content was very low in both cultivars between 1- and 3 WBH and there were no significant differences between cultivars (Fig. 12A). From the commercial harvest date until the end of shelf-life, the relative abundance of tetracosanol was significantly higher in 'Songold' compared to 'Laetitia'. In 2017/18, no tetracosanol was detected in 'Laetitia' between 1- and 3 WBH and it was therefore significantly higher in 'Songold' during this time (Fig. 12B). For the remainder of the sampling period, there were no significant differences between the cultivars and the tetracosanol content remained relatively stable.

Like tetracosanol, the hexacosanol content was low in both cultivars between 1- and 3 WBH in 2016/17 (Fig. 13A). On the commercial harvest date, the relative abundance of hexacosanol was significantly higher in 'Songold'. Both cultivars showed a significant increase in hexacosanol during cold storage, then remained stable during shelf-life. During this time, there were no significant differences between the cultivars. In 2017/18, 'Laetitia' had a significantly higher hexacosanol content than to 'Songold' at 3 WBH, 1 WBH and on the commercial harvest date (Fig. 13B). 'Songold' had a significantly higher hexacosanol content than 'Laetitia' at 2 WBH and after the cold storage period.

The secondary alcohol, nona-10-cosanol (Fig. 14), was quite high throughout the sampling period. In 2016/17, the relative abundance remained stable in 'Laetitia' between 3 WBH and the commercial harvest date (Fig. 14A). Between 2- and 3 WBH, there were no significant differences between cultivars. At 1 WBH and on the commercial harvest date, 'Laetitia' had a significantly higher nona-10-cosanol content compared to 'Songold'. During cold storage, there was a significant decrease in 'Laetitia', while the nona-10-cosanol content in 'Songold' remained stable until the end

of shelf-life. In ‘Laetitia’ however, there was a significant increase in nona-10-cosanol during shelf-life. In 2017/18 ‘Songold’ had a significantly higher nona-10-cosanol content compared to ‘Laetitia’ from 3 WBH until the commercial harvest date (Fig. 14B). During cold storage and shelf-life, the alcohol remained stable in ‘Laetitia’, while it decreased in ‘Songold’.

The relative abundance of octacosanol was quite low in both seasons (Fig. 15). It increased slightly in ‘Songold’ between 1- and 3 WBH, then remained stable until the commercial harvest data when it did not differ significantly from ‘Laetitia’. After cold storage, no octacosanol was detected in ‘Laetitia’ but it increased significantly during shelf-life. In ‘Songold’ it remained stable during cold storage and shelf-life. In 2017/18 octacosanol was only detected in ‘Songold’ after cold storage and shelf-life.

The triacontanol content in the wax samples of both cultivars was low in 2016/17 and completely absent in 2017/18 (Fig. 16). No significant differences were seen in triacontanol content at 2- or 3 WBH, while it was significantly higher in ‘Songold’ at 1 WBH. For the rest of the sampling period, no triacontanol was detected in ‘Songold’ while it increased significantly in ‘Laetitia’ between harvest and the end of shelf-life.

In 2016/17 the oleanolic acid content remained very low in ‘Laetitia’, only increasing significantly during shelf-life (Fig. 17A). In ‘Songold’ the oleanolic acid content was significantly higher than ‘Laetitia’ between 1 WBH and the end of shelf-life. In 2017/18 the oleanolic acid content was higher than in 2016/17 in both cultivars (Fig. 17B). Between 3 WBH and the commercial harvest date, ‘Laetitia’ had a significantly higher oleanolic acid content than ‘Songold’. After cold storage, there were no significant differences in oleanolic acid content between treatments, while after shelf-life, ‘Songold’ had a significantly higher content.

Almost no ursolic acid was detected in ‘Laetitia’ samples between 3 WBH and the commercial harvest date in 2016/17 (Fig. 18A). In ‘Songold’, ursolic acid was only detected from 1 WBH and it was significantly higher than that of ‘Laetitia’ for the remainder of the sampling period. In 2017/18, the ursolic acid content in ‘Laetitia’ was significantly higher than ‘Songold’ between 1- and 3 WBH (Fig. 18B). On the commercial harvest date, the ursolic acid content in ‘Laetitia’ waxes was significantly lower than on the preceding dates, though it did not differ significantly from ‘Songold’.

After cold storage and shelf-life, ‘Songold’ had a significantly higher ursolic acid content than ‘Laetitia’.

In 2016/17 the maslinic acid content in ‘Laetitia’ was barely detectable between 3 WBH and the commercial harvest date but increased to 1 % during the simulated shelf-life period (Fig. 19A). From 1 WBH until the end of shelf-life, ‘Songold’ had a significantly higher maslinic acid content than ‘Laetitia’. In 2017/18 ‘Laetitia’ had very low levels of maslinic acid throughout the sampling period (Fig. 19B). ‘Songold’ wax samples had a similarly low maslinic acid content, except after cold storage and shelf-life, when it was significantly higher than ‘Laetitia’ and the preceding sampling dates.

Like the other triterpenoids, very low levels of corosolic acid were detected in ‘Laetitia’ and ‘Songold’ between 3 WBH and the respective commercial harvest dates of the cultivars in 2016/17 (Fig. 20A). It increased significantly in ‘Songold’ during cold storage. After shelf-life, there were no significant differences between the cultivars. In 2017/18, the corosolic acid content in ‘Songold’ was very low between 3 WBH and the commercial harvest date, while it was significantly higher in ‘Laetitia’ between 1- and 3 WBH (Fig. 20B). On the commercial harvest date, both cultivars had a very low corosolic acid content and it remained low in ‘Laetitia’ until the end of shelf-life, while it increased significantly in ‘Songold’.

Table 2 shows the total relative concentration of components within each wax class in 2016/17. In this season, the shrivel incidence was very low in both cultivars and did not differ significantly. The total free fatty acid concentration was significantly higher in ‘Laetitia’ compared to ‘Songold’ on all sampling dates. The primary alcohols (1˚) in both cultivars increased from 1 WBH until the end of cold storage but was significantly higher in ‘Songold’. Secondary alcohols (2˚) formed the largest component of the cuticular waxes in both cultivars. This grouped contained only nonacosan-10-ol. In ‘Laetitia’, the nonacosan-10-ol concentration decreased significantly during cold storage, while it remained stable in ‘Songold’. At harvest, ‘Laetitia’ had a significantly higher concentration of nonacosan-10-ol than ‘Songold’, but it was significantly lower than ‘Songold’ after cold storage. From 1 WBH until the end of shelf-life, the alkane and triterpenoid content of ‘Songold’ was significantly higher than ‘Laetitia’. ‘Songold’ also contained a significantly higher concentration of phenols than ‘Laetitia’ between 1 WBH and the end of cold-storage but it decreased significantly during shelf-life.

Table 3 summarises the total relative concentration of components within each wax class in 2017/18. ‘Laetitia’ had significantly more shrivel than ‘Songold’, but both cultivars were above the acceptable cut-off value of 10 % required for export. During this season, the fatty acid concentration was lower in both cultivars compared to 2016/17. ‘Laetitia’ had a significantly lower concentration of fatty acids than ‘Songold’ between 3 WBH and the commercial harvest date, did not differ significantly after cold storage, and had significantly more free fatty acids than ‘Songold’ after the simulated shelf-life period. The relative concentrations of both primary- and secondary alcohols were lower in both cultivars during 2017/18. In ‘Laetitia’ and ‘Songold’ the primary alcohol concentration increased significantly between 1 WBH and the end of cold storage but was still higher in ‘Songold’. In ‘Laetitia’, the secondary alcohols decreased significantly between 1 WBH and the end of cold storage, while it did not change significantly during cold storage of ‘Songold’. Total alkane concentration was higher in 2017/18 in both cultivars, with ‘Laetitia’ still significantly lower than ‘Songold’. Triterpenoid concentration was also higher in 2017/18 compared to 2016/17. ‘Laetitia’ had a significantly higher triterpenoid content than ‘Songold’ on all sampling dates, which is in contrast with the previous season, when ‘Songold’ had the higher triterpenoid content. Lastly, phenol content was also higher in 2017/18 compare to 2016/17. On all sampling dates except the commercial harvest date, ‘Laetitia’ had a significantly lower relative phenol concentration compared to ‘Songold.

3.3 Cutin components:

Due to a laboratory error, the ‘Laetitia’ cutin samples at 2 WBH were lost.

In 2016/17 there was a high relative abundance of 9,10-dihydroxy octadecanedioic acid in the cutin samples of both ‘Laetitia’ and ‘Songold’, but a lot of variance was seen between the replicates (Fig. 21A). It was significantly higher in ‘Songold’ at 1- and 3 WBH, while no significant differences were observed on the commercial harvest date. A significant increase occurred in ‘Songold’ during the cold storage period and then it remained stable until the end of shelf-life. However, in ‘Laetitia’ there was only a slight increase during cold storage and it ended up not differing significantly from ‘Songold’ after shelf-life. In 2017/18 the abundance of 9,10-dihydroxy octadecanedioic acid was almost undetectable in ‘Laetitia’ between 1- and 3 WBH. From 3 WBH until the commercial harvest date, the content was significantly higher in ‘Songold’ (Fig. 21B).

After cold storage there were no significant differences between the two cultivars, while the abundance of 9,10-dihydroxxy octadecanedioic acid was higher in ‘Laetitia’ after the simulated shelf-life period.

In 2016/17, one of the unidentified compounds (“Unknown 1”), showed a decrease from 3 WBH until the commercial harvest date in both ‘Laetitia’ and ‘Songold’ (Fig. 22A). At 2- and 3 WBH, there were no significant differences in the relative abundance of this compound between the cultivars. One week before harvest and after cold storage, ‘Songold’ had a significantly higher abundance of “Unknown 1” compared to ‘Laetitia’, but ‘Laetitia’ was significantly higher on the commercial harvest date. In 2017/18 the pattern of relative abundance over the sampling period looked quite different (Fig. 22B). Here ‘Laetitia’ had a significantly higher abundance of the component on the commercial harvest date, ‘Songold’ was significantly higher at 3 WBH, and there were no significant differences on the other sampling dates. However, at 2 WBH, this compound was not detected in ‘Laetitia’ samples, thus ‘Songold’ had a significantly higher abundance here.

In 2016/17, the relative abundance of the second unidentified compound, “Unknown 2”, was significantly higher in ‘Laetitia’ at 3 WBH and on the commercial harvest dates of the two cultivars (Fig. 23A). On the other sampling dates there were no significant differences between the cultivars, yet after the shelf-life period ‘Laetitia’ had a significantly higher abundance of “Unknown 2”. In 2017/18 ‘Laetitia had a significantly higher abundance of Unknown 2 at 3 WBH, 1 WBH and on the commercial harvest date, while there were no significant differences between the cultivars on the other sampling dates (Fig. 23B).

In 2016/17, the 9,10-epoxy octadecanedioic acid content in the cutin samples did not differ significantly between the two cultivars at 3 WBH, 1 WBH or on the commercial harvest date (Fig. 24A). At 2 WBH, after cold storage and after the simulated shelf-life, ‘Songold’ had a significantly higher relative abundance of 9,10-epoxy octadecanedioic acid compared to ‘Laetitia’. In 2017/18 ‘Songold’ had a significantly higher abundance of this compound again, except on the commercial harvest date, when it was very high in ‘Laetitia’ as well as after shelf-life (Fig. 24B)

During 2016/17, ‘Laetitia had a significantly higher relative abundance of 9,10,18-trihydroxy octadecanoic acid compared to ‘Songold’, except at 1 WBH, when no

significant differences were seen (Fig. 25A). In the second season, there were no significant differences at 3 WBH and the contents in ‘Laetitia’, was significantly higher at 1 WBH and on the commercial harvest date (Fig. 25B). During cold storage, the relative abundance of 9,10,18- trihydroxy octadecanoic acid decreased significantly in ‘Laetitia’ and increased significantly in ‘Songold’. During the simulated shelf-life period, ‘Laetitia’ showed a slight increase while ‘Songold’ showed a significant decrease, resulting in ‘Laetitia’ having a significantly higher abundance of 9,10,18- trihydroxy octadecanoic acid after shelf-life.

The same phenol that was identified in the wax samples, 2,4-bis (dimethyl benzyl) phenol, was also present in the cutin samples, and it constituted a large portion of the total cutin (Fig. 26). There were no significant differences in phenol content between ‘Laetitia’ and ‘Songold’ between 1- and 3 WBH in 2016/17 (Fig. 26A). On their respective harvest dates, ‘Songold’ had a significantly higher phenol content than ‘Laetitia’, but after cold storage and shelf-life, ‘Laetitia’ had a significantly higher relative abundance of this compound. In 2017/18 ‘Laetitia’ had a significantly higher phenol content than ‘Songold’ at 1- and 3 WBH (Fig. 26. B). On the commercial harvest date of ‘Laetitia’, almost no phenol was detected, but it increased significantly during cold storage and was significantly higher than the phenol content in ‘Songold’. During the simulated shelf-life, the phenol content in ‘Laetitia’ decreased significantly, while it increased significantly in ‘Songold’, with the phenol content in ‘Songold’ being significantly higher than that of ‘Laetitia’ after shelf-life.

3.4 Principal component analysis (PCA)

The PCA bi-plot of the first two principal components for cuticular waxes is shown in Fig. 27. PC1 and PC2 accounted for 35 % and 21 % respectively.

At harvest in 2016/17, ‘Laetitia’ and ‘Songold’ were separated along PC2, but cultivars did not differ in 2017/18, or after cold storage in either season. In both seasons, ‘Laetitia’ separated along PC1 at harvest, but did not differ after cold storage. ‘Songold’ also separated along PC1 between the seasons at harvest and did not differ after cold storage. In 2016/17, ‘Laetitia’ separated along PC1 at harvest and after storage, but not in 2017/18. ‘Songold’ showed no separation for either season or sampling time.

Oleanolic acid and ursolic acid were correlated positively, but these components did not contribute to the variation, since they were along the horizontal axis. There was a positive correlation between maslinic acid and tetracosanol, and between octacosanol and corosolic acid. Separated along PC2 from the aforementioned components were tetratetracontane, nonacosan-10-ol, oleic acid and triacontanol, all showing positive correlations.

In 2016/17 ‘Laetitia’ had similar response patterns at harvest and after cold storage. These groups (‘Laetitia’ at harvest and after cold storage) had more oleic acid, triacontanol, nonacosan-10-ol and tetratetracontane than the other groups (‘Laetitia 2017/18 at harvest and after storage, and ‘Songold’ at harvest and after storage in 2016/17 and 2017/18). The other groups had similar response patterns and had higher levels of octacosanol, corosolic acid, maslinic acid and tetracosanol.

The PCA bi-plot of the first two components for cutin explained 55 % and 23 % respectively (Fig. 28). There were no differences in cutin composition between cultivars, seasons, or sampling dates, except for ‘Laetitia’ at harvest, in 2017/18. The PCA did not indicate a separation between parameters. Except for outliers from ‘Laetitia’ at harvest in 2016/17 and 2017/18, all other parameters clustered together. “Unknown 1” and 9,10 epoxy octadecanedioic acid were correlated positively with one another, while “Unknown 2” and 9,10,18 trihydroxy octadecanoic acid were correlated positively.

4. Discussion

The aim of this study was to determine whether differences exist in the chemical composition of two Japanese plum cultivars, ‘Laetitia’ and ‘Songold’, and whether this can explain the susceptibility of these cultivars to moisture loss and/or shrivelling during storage. These cultivars were grown under the same climatic conditions and harvested approximately two weeks apart, yet there were significant differences in peel permeability and propensity for postharvest shrivel development. Since the most significant changes in fruit quality occur between the commercial harvest date and the end of the storage period, the focus of this discussion was on these dates.

The mass of the cuticular membrane increased significantly during cold storage in ‘Laetitia’ 2016/17 (Fig. 1) and can be explained by the significant increase in both the

wax and cutin components of the membrane. Changes were less pronounced in ‘Songold’, but increases were still observed in these parameters in 2016/17. This confirms that cuticle thickening, and active cuticle synthesis occurred during cold storage, similar to what was found in the peach cultivars ‘Jesca’ and ‘October Sun’ (Belge et al., 2014b). Yet, in 2017/18, in both cultivars, the mass / thickness of the cuticular membrane remained unchanged during the storage period (Fig. 1), while total wax content per fruit decreased significantly (Fig. 2 and Fig. 3).

The total amount of wax and cutin isolated per fruit surface area, from both cultivars at harvest, was significantly higher in 2017/18 than in 2016/17 and may explain the lack of further increases. Although not quantified in this study, it may have been due to depletion of wax and cutin precursors or biochemical inhibition of their synthesis (Curry, 2009).

The quantity of wax, its chemical composition and surface morphology are controlled by several biological and physical factors (Baker, 1974). Environmental factors, developmental stage and the position of a fruit on the tree can influence the structure and composition of the cuticle. Therefore, seasonal differences in cuticle composition and amount are to be expected as confirmed by Veraverbeke et al. (2001) for apple, but only certain compounds e.g. some alkanes, were reported to be affected.

All fatty acids identified in ‘Laetitia’ and ‘Songold’ cuticular waxes are present in peaches as well, however the peach cultivars Jesca and October Sun contained higher amounts of hexadecanoic acid (palmitic acid) (Belge et al., 2014b). In both ‘Laetitia’ and ‘Songold’, as with peaches, the total amount of fatty acids increased during shelf-life. However, PCA only identified oleic acid as an important variable in describing the variation between seasons and cultivars. Only oleanolic acid and ursolic acid were identified in the triterpenoid fraction of peach cuticles, while in this study, corosolic and maslinic acid were also identified. The most important triterpenoid identified in apple and sweet cherry cultivars was ursolic acid and the main primary alcohol, was hexacosanol (Belding et al., 1998; Belge et al., 2014a). Our results for plums confirmed these reports. The PCA identified all the triterpenoids, oleanolic -, ursolic -, maslinic - and corosolic acids as important variables in separation between seasons and cultivars in this study.

Only two alkanes were identified in this study on plums, whereas a range of alkanes between C_{12} and C_{31} is present in peach fruit cuticular waxes at harvest (Belge et al., 2014b). In addition, very low relative concentrations (0.006 %) were present in peaches confirming reports by Barthlott et al., (1998). It is possible that such low concentrations could not be determined accurately in our study and were therefore not identified.

In ‘Laetitia’ and ‘Songold’, the main wax components identified were alcohols, specifically the secondary alcohol, nonacosan-10-ol (Fig. 14). This alcohol was not identified in peaches (Belge et al., 2014), while its presence in apple cultivars varies to such an extent that it could be used to differentiate between cultivars (Belding et al., 1998). The nonacosan-10-ol content in apples can be the most important factor in determining differences in surface characteristics among cultivars (Veraverbeke et al., 2001a). Furthermore, the relative concentration of nonacosan-10-ol was higher in apple cultivars not susceptible to moisture loss, compared to susceptible cultivars which did not contain any nonacosan-10-ol. In 2017/18, when ‘Songold’ had a significantly lower incidence of shrivel than ‘Laetitia’, the nonacosan-10-ol concentration was significantly higher at harvest. Yet, in 2016/17, when no significant differences were observed in shrivel incidence, the nonacosan-10-ol content was significantly lower in ‘Songold’ compared to ‘Laetitia’. Thus, nonacosan-10-ol content on the commercial harvest date is probably not the only factor contributing to shrivel development in Japanese plums.

Although phytosterols are also often found in cuticular waxes (Belge et al., 2014a, 2014b; Parsons et al., 2013), none were detected in ‘Laetitia’ and ‘Songold’. Interestingly, a high relative concentration of 2,4-bis (dimethyl benzyl) phenol was identified in the wax and cutin samples of ‘Laetitia’ and ‘Songold’ on all sampling dates. However, phenolics are usually only present in very low concentrations (Domínguez et al., 2011). According to our knowledge, this is the first identification of this compound in plum fruit. This phenol also occurs in a variety of other products, including Brazilian cherries, Natsugumi fruit, the stems of castor plants and leaf extracts of bitter melon (Lee et al., 2007; Malaman et al., 2011; Panlilio et al., 2012; Salem et al., 2017), where it is commonly associated as a compound with antioxidant capabilities. In the cutin matrix, phenols provide increased rigidity as it restricts the mobility of the polyester

chains (España et al., 2014; Lopez-Casado et al., 2007) and increases the resistance to deformation (Lopez-Casado et al., 2007). The increase in the phenol content of 'Laetitia' during storage possibly contributes towards increasing the rigidity of the cuticle to prevent its deformation, while 'Songold' cuticles may already be adequately rigid at harvest. This increase in rigidity of 'Laetitia' may thus be too late to sufficiently resist deformation of the peel during harvest, resulting in shrivelling and/or moisture loss.

Specific compound classes are associated with water-resistant properties of the cuticle. Alkanes in particular, are associated with lower cuticular permeability, while non-aliphatic wax compounds, such as triterpenoids, are a less effective water barrier (Buschhaus and Jetter, 2012; Leide et al., 2007; Vogg et al., 2004). The ratio between n-alkanes and triterpenoids is thought to explain the predisposition of a product towards moisture loss (Belge et al., 2014a, 2014b; Isaacson et al., 2009; Parsons et al., 2013). In 2016/17, the cuticular permeability of 'Laetitia' was significantly lower than 'Songold' at harvest (Chapter 1), while the alkane: triterpenoid ratio was significantly higher 1.54 vs 0.71 (data not shown). This agrees with findings in 'Jesca' and 'October Sun' peaches, where the cultivar with the higher ratio of alkanes: triterpenoids was less prone to moisture loss after five days at 20˚C (Belge et al., 2014b). However, shrivel incidence did not differ significantly between the cultivars in 2016/17. So, while the presence of more alkanes in the cuticular wax could explain the lower peel permeability, it could not explain shrivel incidence.

In 2017/18, the alkane: triterpenoid ratio did not differ significantly between cultivars at harvest (1.27 vs 1.35, data not shown), while shrivel incidence was significantly higher in 'Laetitia'. Unfortunately, permeability data were not available for 2017/18. Since the relationship between moisture loss and shrivelling is not directly correlated (Chapter 1), it is possible that these results can explain the difference in moisture loss between 'Laetitia' and 'Songold' but not necessarily the difference in shrivel incidence.

In a range of pepper cultivars, moisture loss was shown to have a significant negative relationship with primary alcohols and total alkanes (Parsons et al., 2013). This approach offers an improved explanation for shrivelling incidence in Japanese plums. In 2016/17, the relative concentration of primary alcohols increased significantly during cold storage in both cultivars and shrivel incidence was low. In 2017/18, when shrivel

incidence was high in both cultivars, the relative concentration of primary alcohols did not change significantly but was low in both cultivars. Thus, primary alcohol content at harvest may indicate potential shrivel incidence in Japanese plums. However, this requires further investigation and validation.

Despite the differences between cultivars, seasons and sampling dates (Fig. 27), a relationship between the different wax components and shrivel incidence (Table 2 and Table 3) could not be determined. In 2016/17, when shrivel incidence (%) was low in both cultivars, cultivars were separated along PC 2 at harvest and after cold storage, showing that their wax composition differed significantly. Yet, in 2017/18, when shrivel incidence was higher in both cultivars and 'Laetitia' had a significantly higher shrivel incidence (%) than 'Songold', wax composition did not differ significantly between cultivars at harvest or after cold storage.

In 2017/18, there was a higher incidence of shrivel compared to 2016/17 and shrivel incidence was significantly higher in 'Laetitia' compared to 'Songold'. Since the wax composition alone did not provide a mode of action for proneness to shrivel, the cutin matrix clearly plays an important role. 'Laetitia' had a significantly higher tri-hydroxy acid content compared to 'Songold' at harvest in both seasons, making it more flexible (Marga et al., 2001). In 'Laetitia', the cuticle seems to be flexible at harvest and then remains this way during cold storage or become more rigid. Yet, in 'Songold', the cuticle is probably rigid at harvest and only becomes more flexible during cold storage. The flexibility of 'Laetitia' may make deformation easier, therefore the fruit might develop a shrivelled appearance if the hypodermal cells shrink due to cumulative moisture loss during storage. The rigid cuticles of 'Songold' on the commercial harvest date may reduce their deformation and consequent shrivelling, even though cuticle flexibility increases during cold storage.

In 2017/18 the amount of wax isolated from the cuticular membranes decreased during cold storage in both cultivars and shrivel incidence was significantly higher in this season (Table 2). Thus, when the amount of wax decreased during cold storage (low RH, low temperatures and continued moisture loss from the fruit surface) in both cultivars, their cuticles became more flexible. This may have allowed an increase in shrivelling (Nguyen et al., 2006; Sastry, 1985) via water vapour lost from the epidermis which leads to the dehydration and shrinkage of the cells just below the fruit surface

(Díaz-Pérez et al., 2007; Nguyen et al., 2006. These anatomical changes should be monitored and quantified in future to validate this hypothesis.

5. Conclusion

This study showed significant differences in cuticle composition between Japanese plum cultivars. It supports the interaction between genetic and environmental factors in determination of cuticular properties reported for other crops. It was not possible to identify a single compound or group of compounds that clearly relates to shrivel development. The causes of shrivel may thus be a combination of factors and not only due to cuticle composition. As the composition of the cutin matrix and the cuticular waxes determine the permeability, flexibility and strength of the cuticle, continued evaporative moisture loss during cold storage leads to dehydration and collapse of the epidermal and hypodermal cells that may result in shrivel development if the cuticle is flexible enough to deform with the dehydrated cells. Further investigation of cuticle strength and flexibility, combined with anatomical studies of the epidermal and hypodermal cells, may provide more detailed information about the mechanisms of this phenomenon.

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Table 1

Components identified in the cuticular waxes and cutin matrix of ‘Laetitia’ and ’Songold’ plums in 2016/17 and 2017/18.

Wax components	Chemical formula
Fatty acids:	
Lauric acid	$C_{12}H_{24}O_2$
Myristic acid	$C_{14}H_{28}O_2$
Pentadecanoic acid	$C_{15}H_{30}O_2$
Heptadecanoic acid	$C_{17}H_{34}O_2$
Oleic acid	$C_{18}H_{34}O_2$
Phenols:	
2,4-bis (dimethyl benzyl) phenol	$C_{24}H_{26}O$
Alkanes:	
Tetratriacontane	$C_{34}H70$
Tetratetracontane	$C_{44}H90$
Alcohols:	
Tetracosanol	$C_{25}H_{50}O$
Hexacosanol	$C_{25}H_{54}O$
Nona-10-cosanol (secondary alcohol)	$C_{29}H_{60}O$
Octacosanol	$C_{28}H_{58}O$
Triacontanol	$C_{30}H_{62}O$
Triterpenoids:	
Oleanolic acid	$C_{30}H_{48}O_3$
Ursolic acid	$C_{30}H_{48}O_3$

Maslinic acid	$C_{30}H_{48}O_3$
Corosolic acid	$C_{30}H_{48}O_3$
Cutin components	
9,10-dihidroxy octadecanedioic acid	
Unknown 12	
Unknown 22	
9,10-epoxy octadecanedioic acid	
9,10,18-trihydroxyoctadecanoic acid	
2,4-bis (dimethyl benzyl) phenol	

Table 2

Summary of the total relative concentration of each chemical class in the cuticular wax of ‘Laetitia’ and ‘Songold’ respectively in 2016/17. Dates are indicated as weeks before harvest (WBH). H – optimum harvest date; C – after cold storage of six weeks for ‘Laetitia’ and 7 weeks for ‘Songold’; S – after a shelf-life period of 7 days at 10˚C. Significant differences between cultivars are indicated in lower case letters for the different classes separately. Only cultivars were compared and not changes over sampling dates. Shrivel in ‘Laetitia’ and ‘Songold’ after cold storage was 4 ±1 % and 2 ± 1 % respectively.

Table 3

	Fatty acids		Primary alcohols		Secondary alcohols		Alkanes		Triterpenoids		Phenols	
	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold
3 WBH	5.90 ± 0.14	2.43 ± 0.13	5.38 ± 0.43	4.13 ± 0.47	77.92 ± 2.90	85.02 ± 1.73	4.86 ±0 .77	3.10 ± 0.78	1.64 ± 0.96	2.27 ± 0.85	4.30 ± 1.26	3.06 ± 1.00
2 WBH	2.57 ± 0.14	1.98 ± 0.05	4.43 ± 0.93	4.23 ± 0.34	81.04 ± 2.95	82.59 ± 2 .51	5.02 ± 0.82	3.38 ± 0.51	2.28 ± 1.13	3.12 ± 0.09	4.66 ± 1.47	4.70 ± 0.75
1 WBH	2.39 ± 0.23	1.54 ± 0.12	2.27 ± 0.36	7.19 ± 1.16	87.34 ± 2.05	23.30 ± 7.78	2.47± 0.42	4.37 ± 0.73	1.45 ± 0.56	18.41 ± 4.98	4.08 ± 0.77	18.66 ± 9.10
H	2.62 ± 0.32	1.14 ± 0.18	4.26 ± 0.42	7.56 ± 0.33	81.99 ± 1.86	48.72 ± 2.37	3.32 ± 0.57	12.61 ± 0.20	2.15 ± 2383	17.66 ± 2.71	5.67 ± 1.01	12.19 ± 3.88
C	0.57 ± 0.04	0.94 ± 0.08	5.81 ± 0.57	10.09 ± 0.49	34.00 ± 5.37	47.69 ± 0.53	0.00	12.26 ± 0.28	9.98 ± 0.56	18.23 ± 1.20	3.08 ± 0.31	10.80 ± 1.77
S	1.44 ± 0.03	0.99 ± 0.06	18.4 ± 1.16	9.23 ± 0.22	53.24 ± 2.70	49.33 ± 0.25	4.30 ± 0.25	12.33 ± 0.13	10.73 ± 0.59	18.65 ± 1.10	11.9 ± 0.37	9.48 ± 1.14

Summary of the total relative concentration of each chemical class in the cuticular wax of ‘Laetitia’ and ‘Songold’ respectively in 2017/18. Dates are indicated as weeks before harvest (WBH). H – optimum harvest date; C – after cold storage of six weeks for ‘Laetitia’ and 7 weeks for ‘Songold’; S – after a shelf-life period of 7 days at 10˚C. Only cultivars were compared and not changes over sampling dates. Shrivel in ‘Laetitia’ and ‘Songold’ after cold storage was 16 ±1 % and 12 ± 1 % respectively.

	Fatty acids		Primary alcohols		Secondary alcohols		Alkanes		Triterpenoids		Phenols	
	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold	Laetitia	Songold
3 WBH	0.17 ± 0.02	0.41 ± 0.02	2.82 ± 0.08	2.48 ± 0.15	39.24 ± 0.49	55.52 ± 1.96	10.10 ± 0.20	11.04 ± 0.39	44.10 ± 0.49	12.79 ± 1.70	3.57 ± 0.43	17.76 ± 2.80
2 WBH	0.18 ± 0.001	1.02 ± 0.08	2.73 ± 0.05	6.78 ± 0.18	39.06 ± 0.95	49.01 ± 1.14	10.53 ± 0.27	11.06 ± 0.23	42.70 ± 0.73	15.03 ± 1.08	4.81 ± 0.61	17.10 ± 1.30
1 WBH	0.22 ± 0.009	0.75 ± 0.07	4.22 ± 0.71	3.20 ± 0.37	39.36 ± 0.98	57.27 ± 2.45	11.04 ± 0.35	13.77 ± 0.41	45.17 ± 0.66	14.19 ± 1.53	0.00	10.81 ± 1.94
H	0.68 ± 0.06	0.67 ± 0.05	3.40 ± 0.18	2.60 ± 0.18	55.91 ± 1.37	62.35 ± 1.06	11.12 ± 0.48	13.03 ± 0.1	11.94 ± 1.43	9.63 ± 0.38	16.95 ± 1.72	11.72 ± 0.18
C	0.88 ± 0.11	1.00 ± 0.06	3.03 ± 0.42	5.09 ± 0.24	56.98 ± 1.95	52.32 ± 2.05	11.01 ± 0.0.28	12.62 ± 0.32	14.05 ± 1.46	18.62 ± 1.26	14.05 ± 1.84	10.37 ± 0.75
S	1.44 ± 0.07	1.20 ± 0.05	3.41 ± 0.44	5.48 ± 0.33	51.90 ± 1.06	44.29 ± 1.73	10.06 ± 0.21	14.51 ± 0.07	12.95 ± 1.46	29.70 ± 1.11	20.25 ± 1.87	4.82 ± 0.67

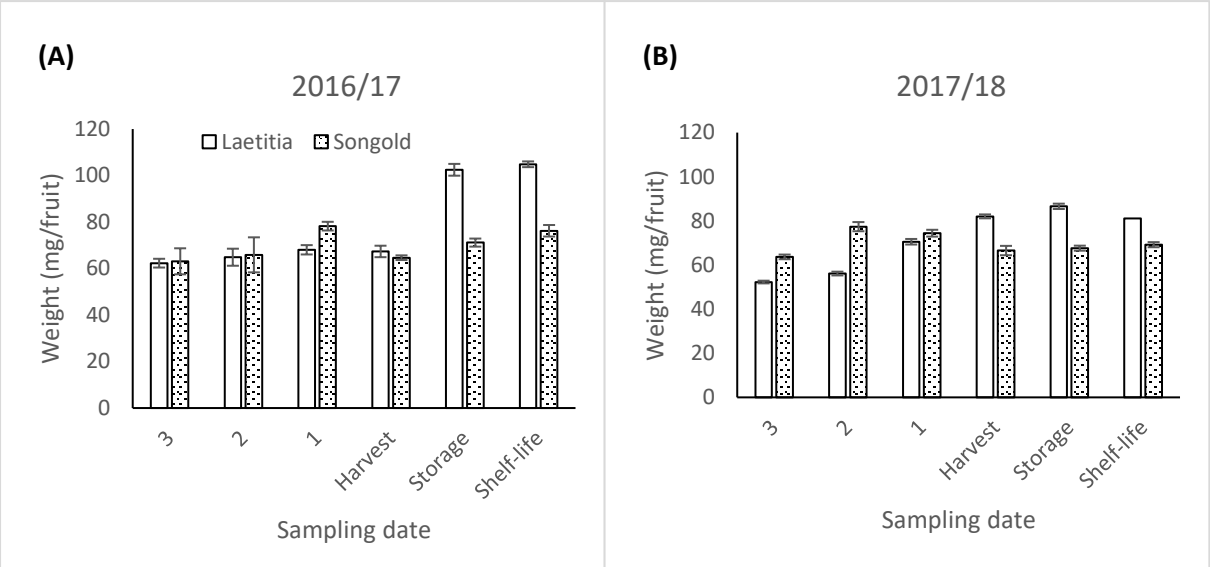

Fig. 1. Cuticular membrane (mg. fruit^{-1}) isolated from 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Bars represent means with standard error.

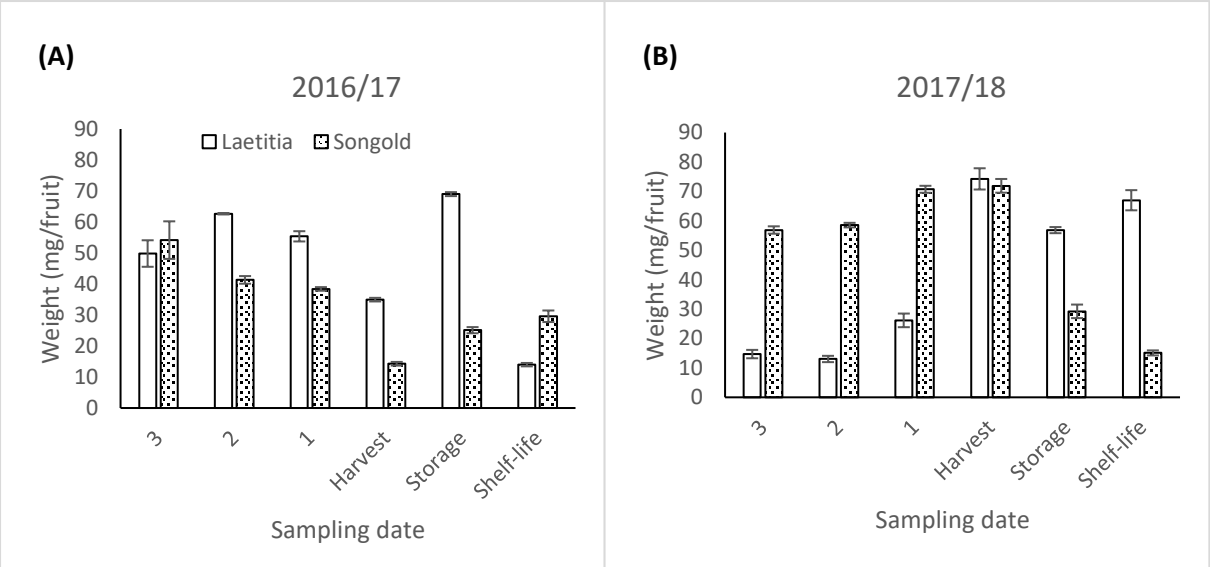

Fig. 2. Wax (mg. fruit^{-1}) isolated from 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Bars represent means with standard error.

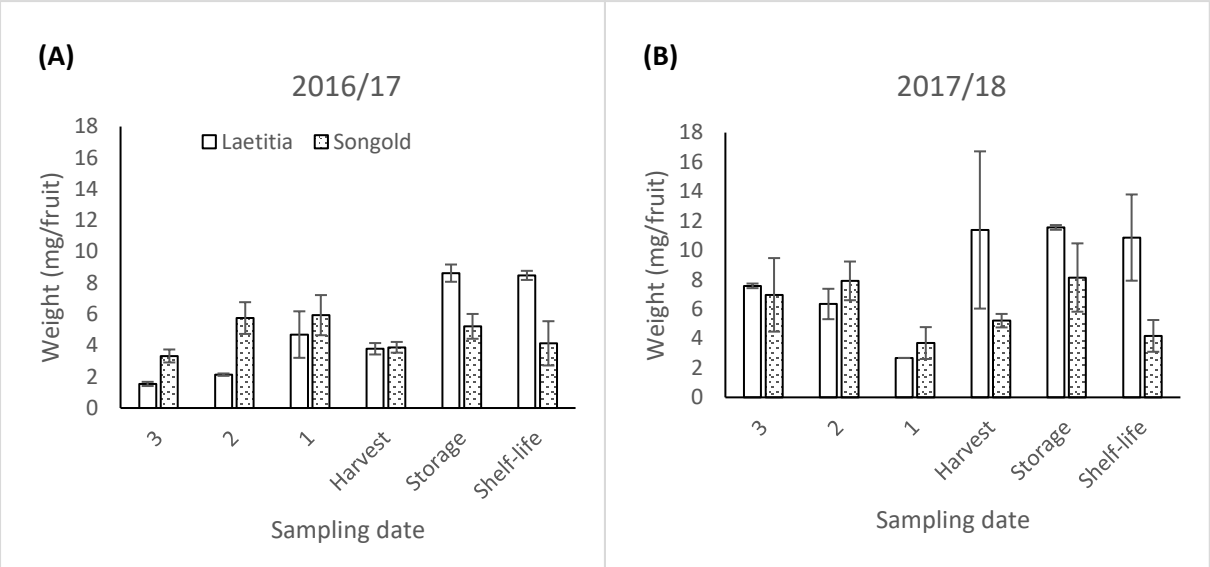

Fig. 3. Cutin (mg. fruit^{-1}) isolated from 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Bars represent means with standard error.

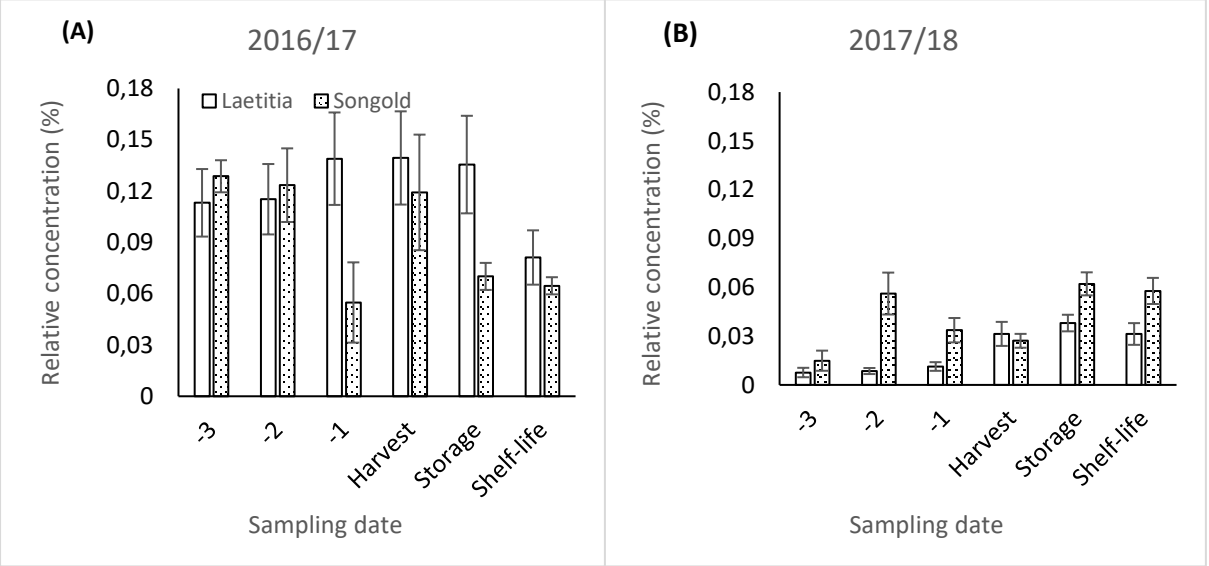

Fig. 4. Relative concentration of lauric acid identified in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

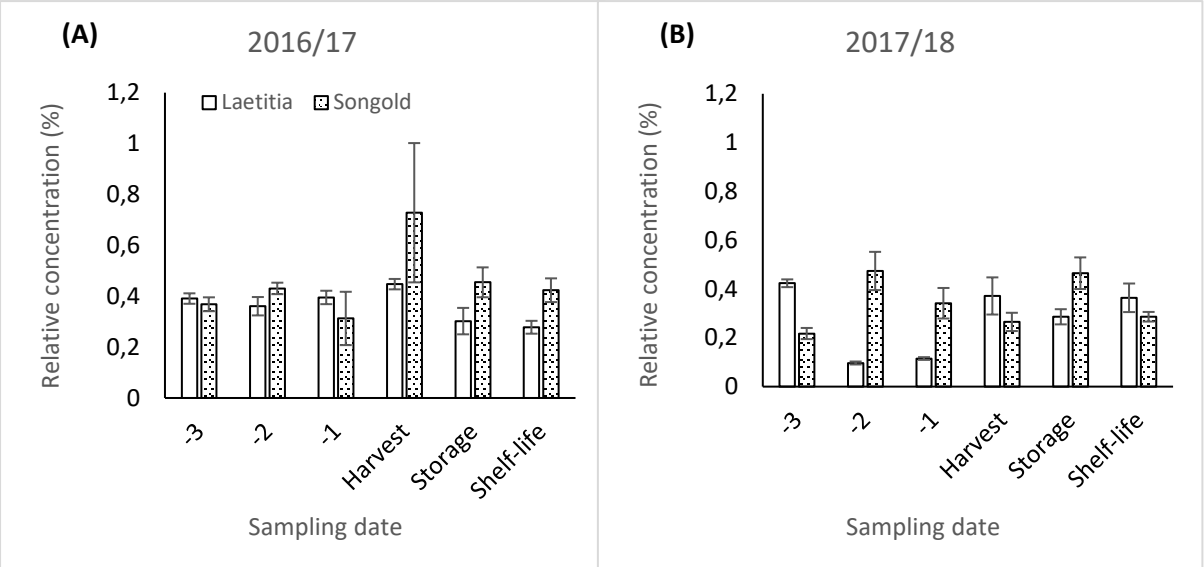

Fig. 5. Relative concentration of myristic acid identified in 'Laetitia' and 'Songold' plums 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

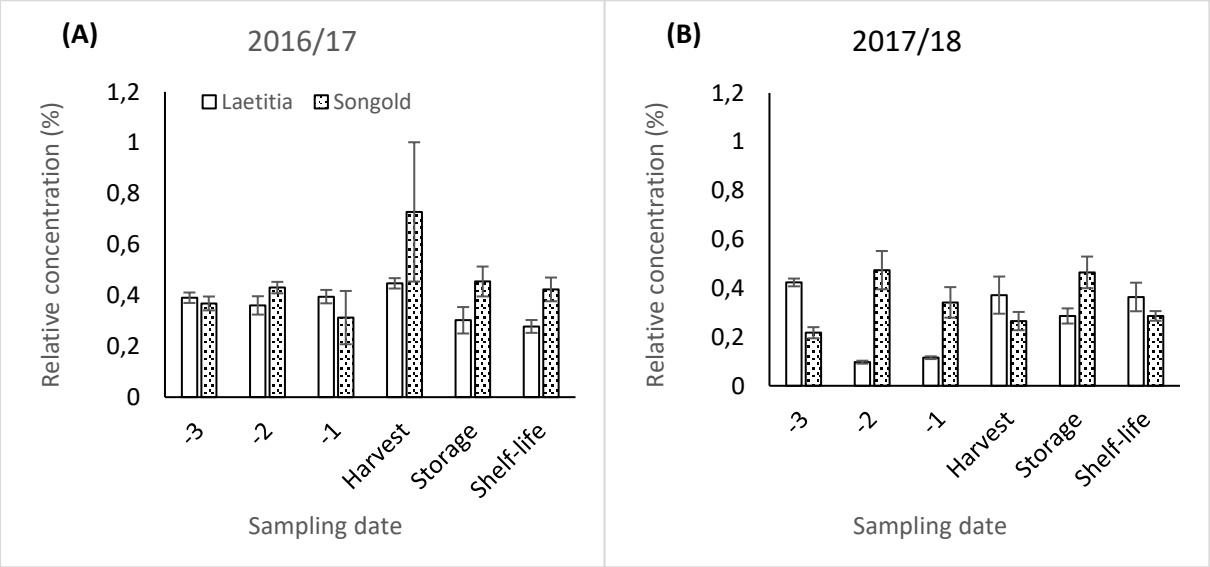

Fig. 6. Relative concentration of pentadecanoic acid identified in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was Calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

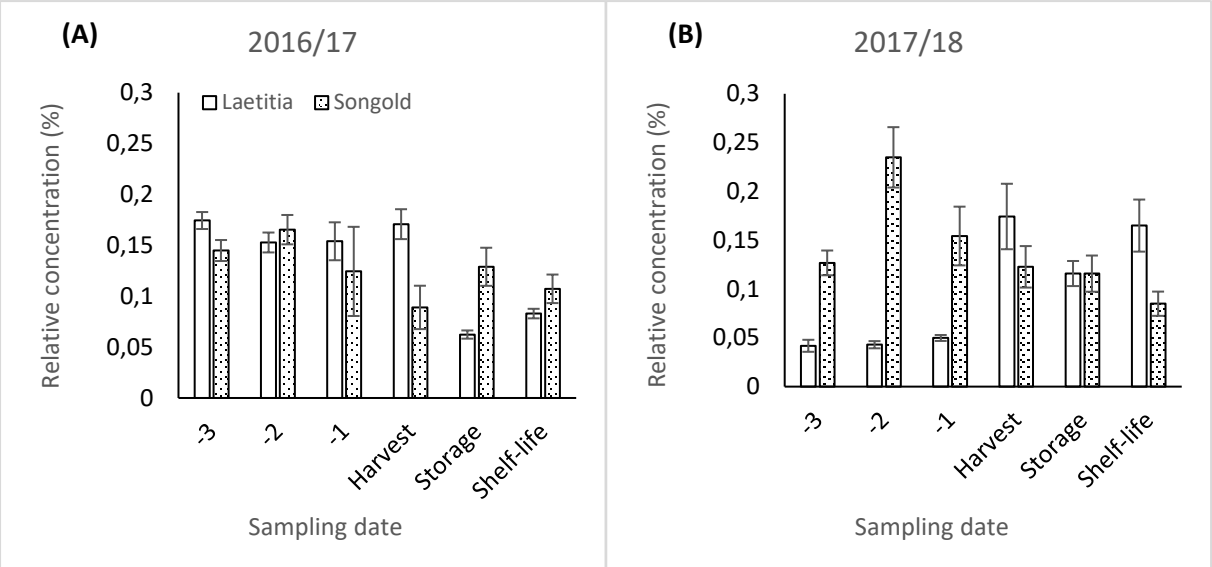

Fig. 7. Relative concentration of heptadecanoic acid in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

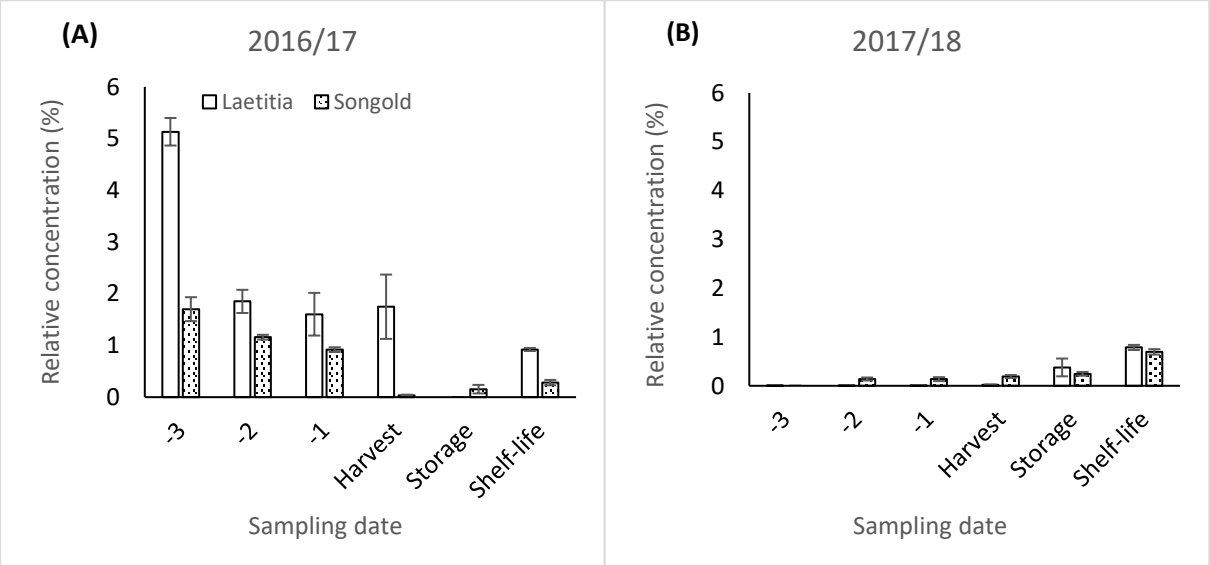

Fig. 8. Relative concentration of oleic acid in the cuticular waxes of 'Laetitia' and 'Songold' plums 2016/17 (A) and 2017/18 (B). Concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

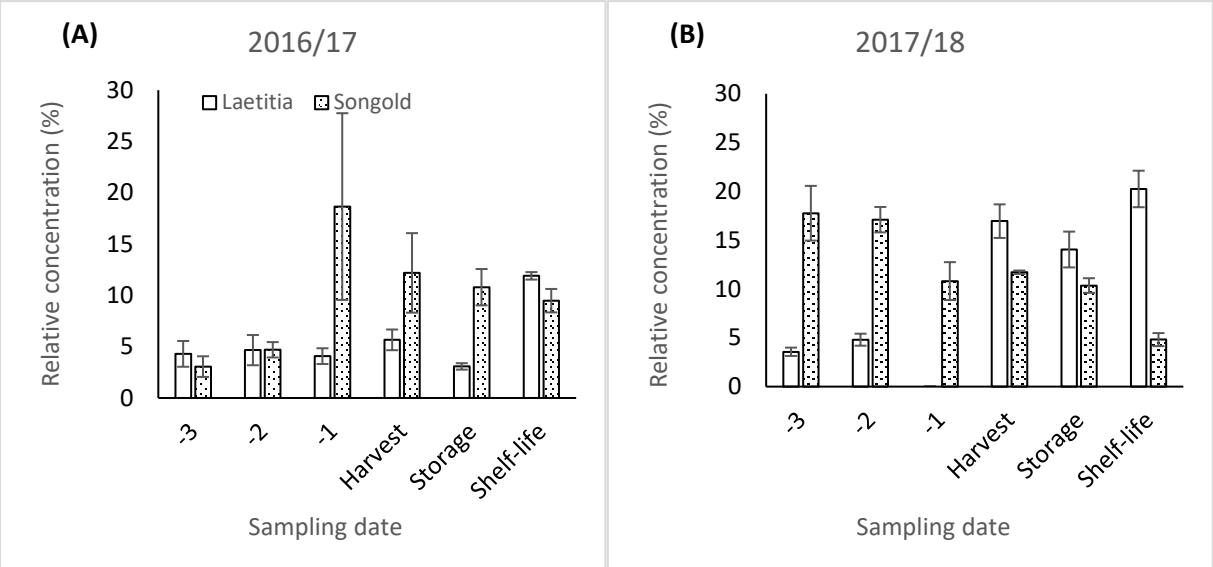

Fig. 9. Relative concentration of 2,4-bis (dimethyl benzyl) phenol in the cuticular waxes of 'Laetitia' and 'Songold' 2016/17 (A) and 2017/18 (B). Concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks Bars represent means with standard error.

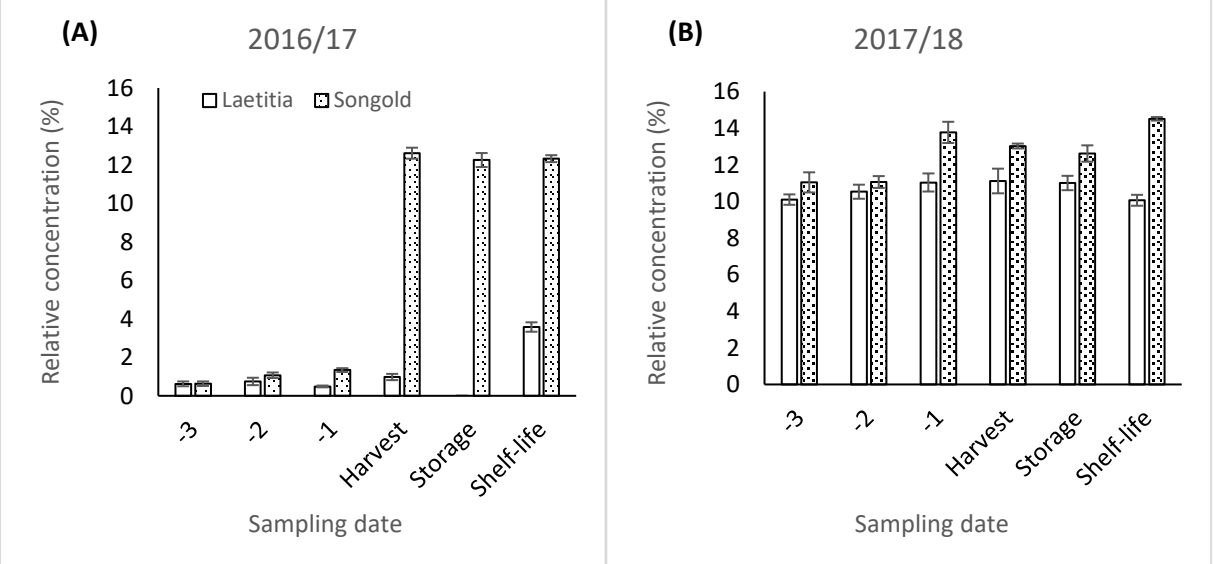

Fig. 10. Relative concentration of tetratriacontane in the waxes of 'Laetitia' and 'Songold' in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

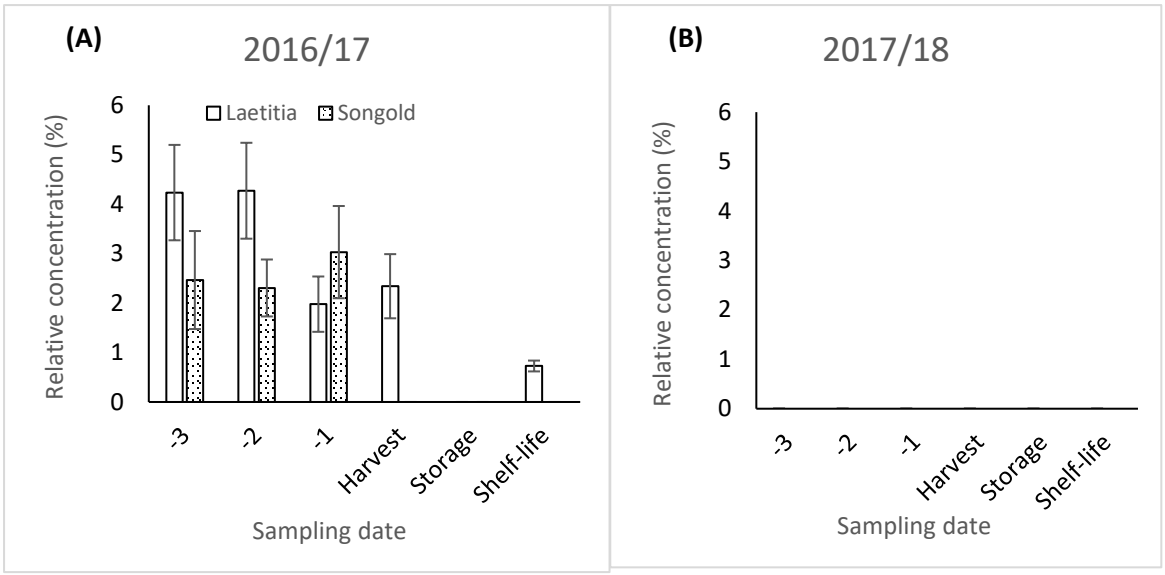

Fig. 11. Relative concentration of tetratetracontane in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

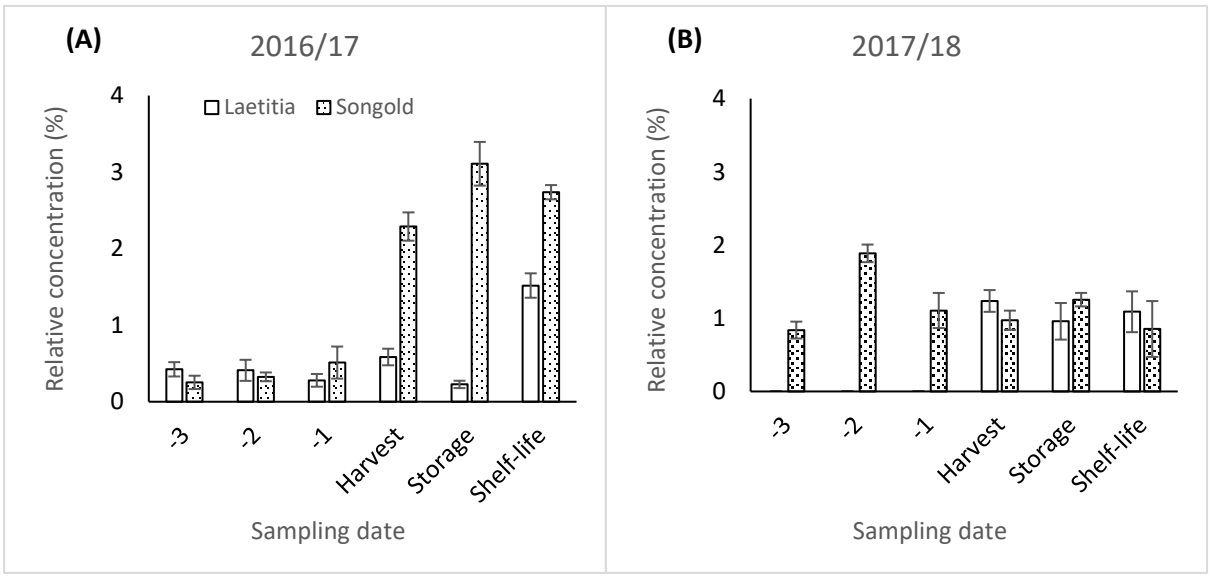

Fig. 12. Relative concentration of tetracosanol in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

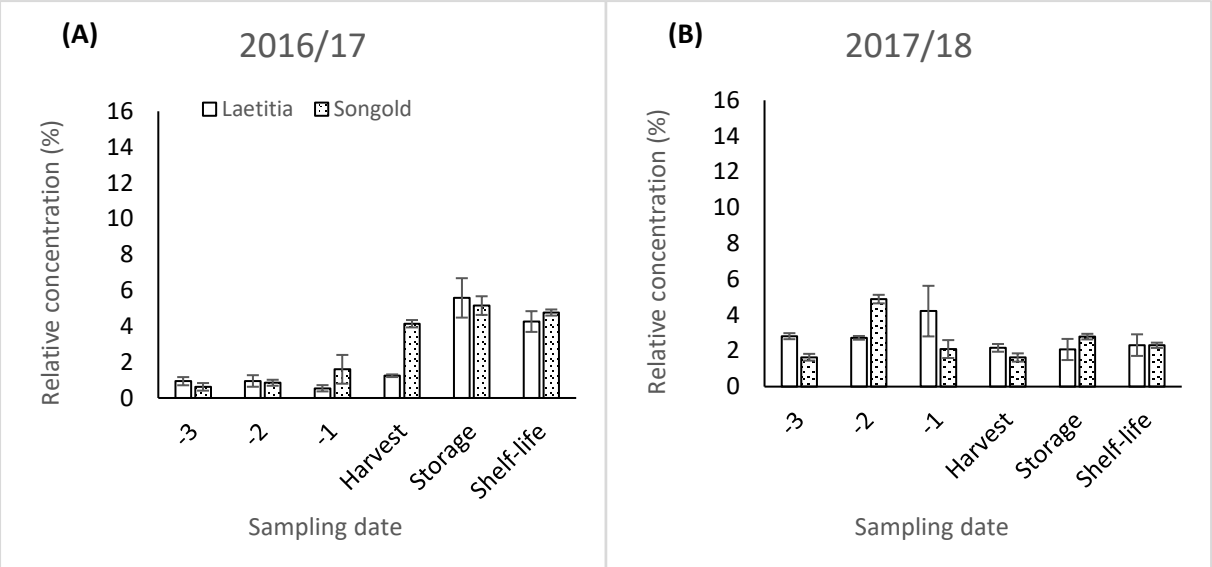

Fig. 13. Relative concentration of hexacosanol in the cuticular waxes of 'Laetitia' and 'Songold' in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

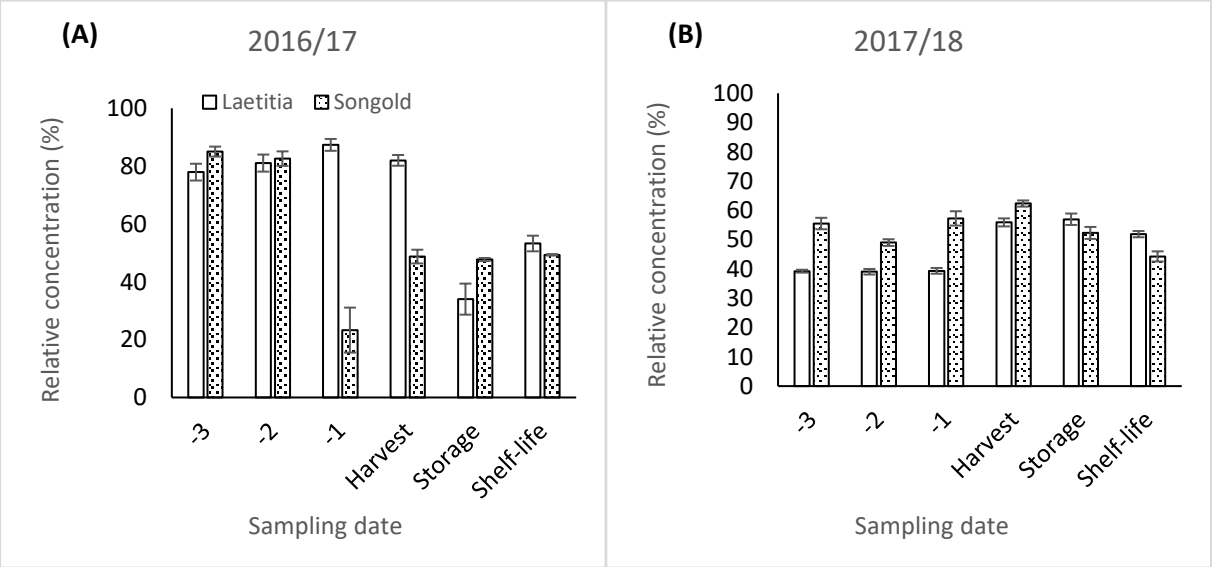

Fig. 14. Relative concentration of nonacosan-10-ol in the cuticular waxes of 'Laetitia' and 'Songold' in 2016/17 (A) and 2017/18 (B). Concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

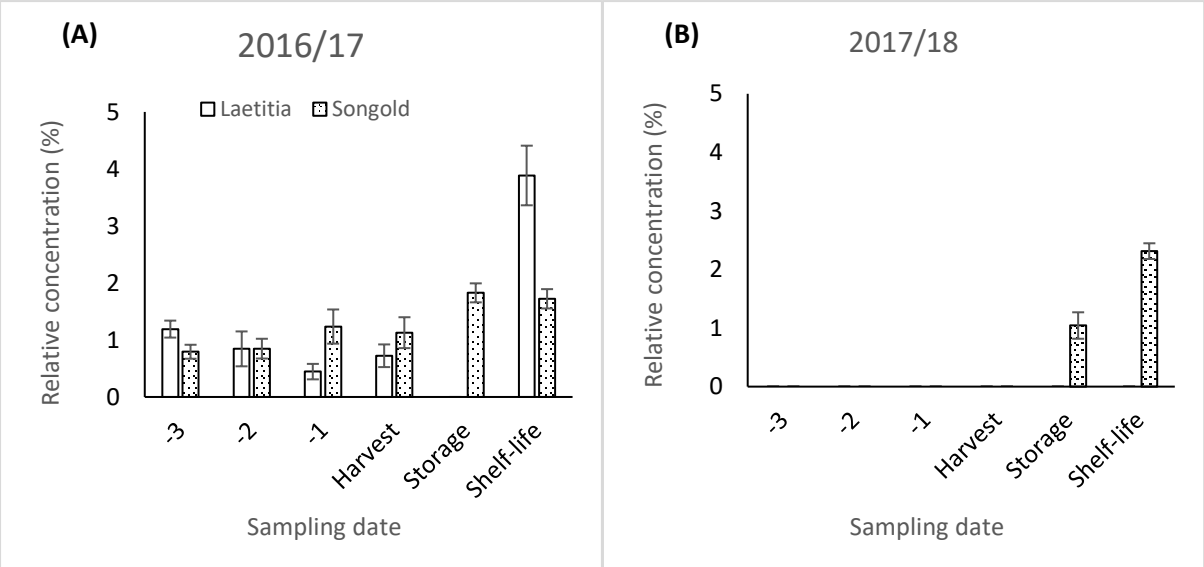

Fig. 15. Relative concentration of octacosanol in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

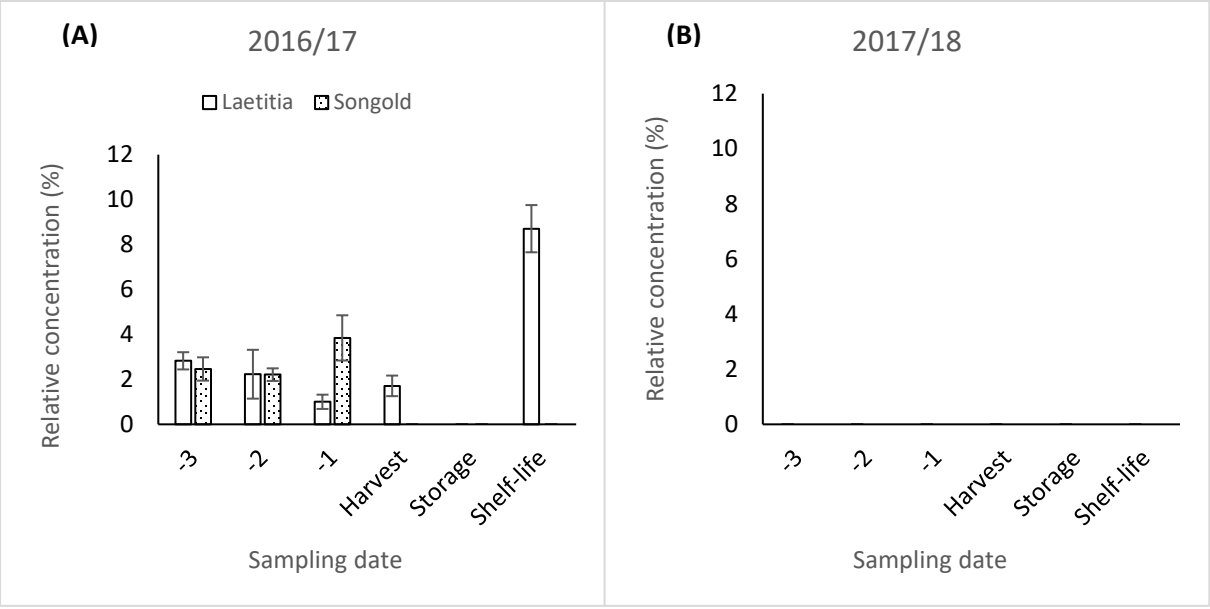

Fig. 16. Relative concentration of triacontanol in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

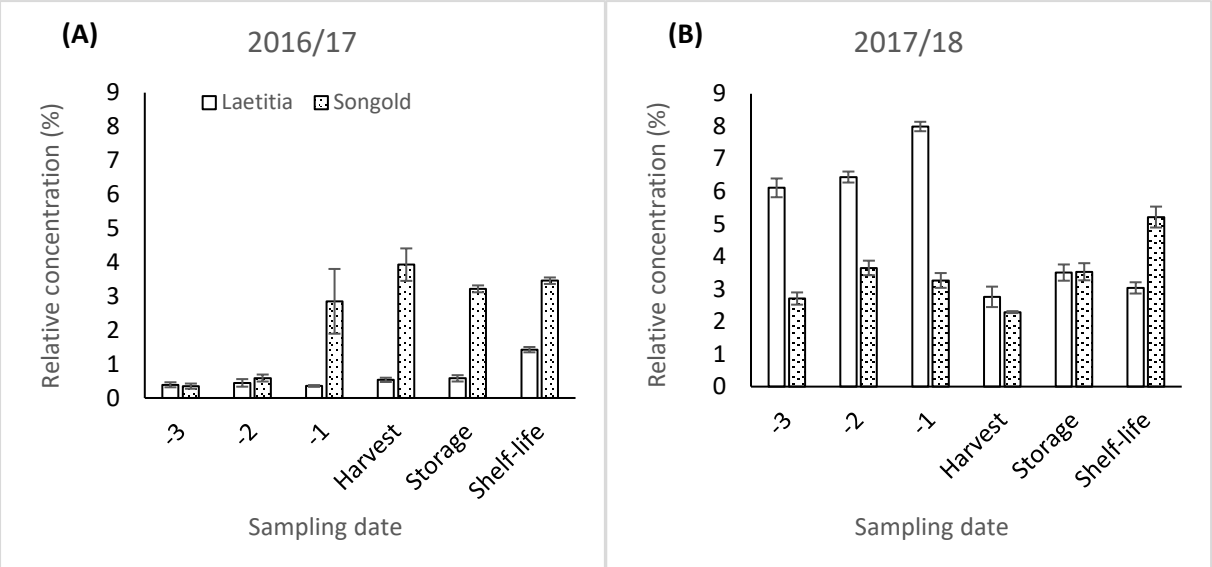

Fig. 17. Relative concentration of oleanolic acid in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

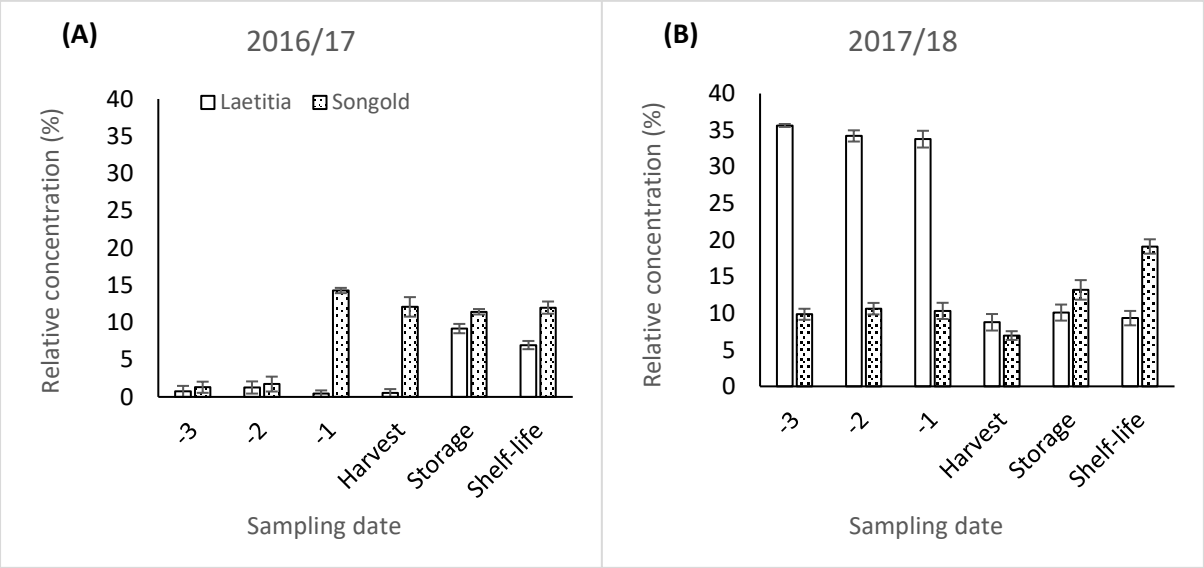

Fig. 18. Relative concentration of ursolic acid in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

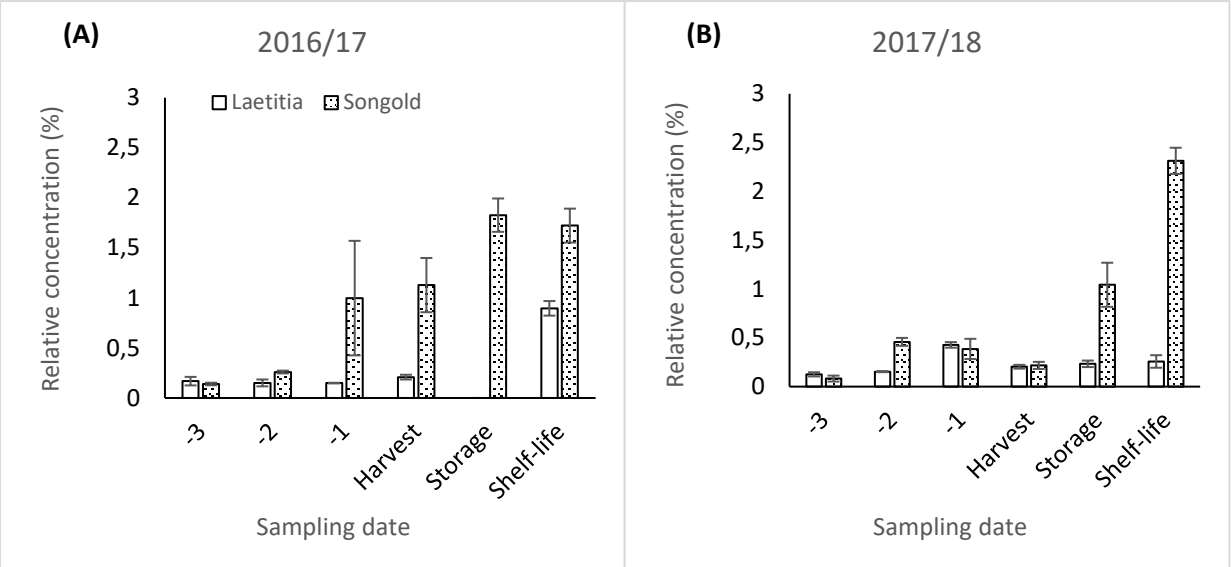

Fig. 19. Relative concentration of maslinic acid in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

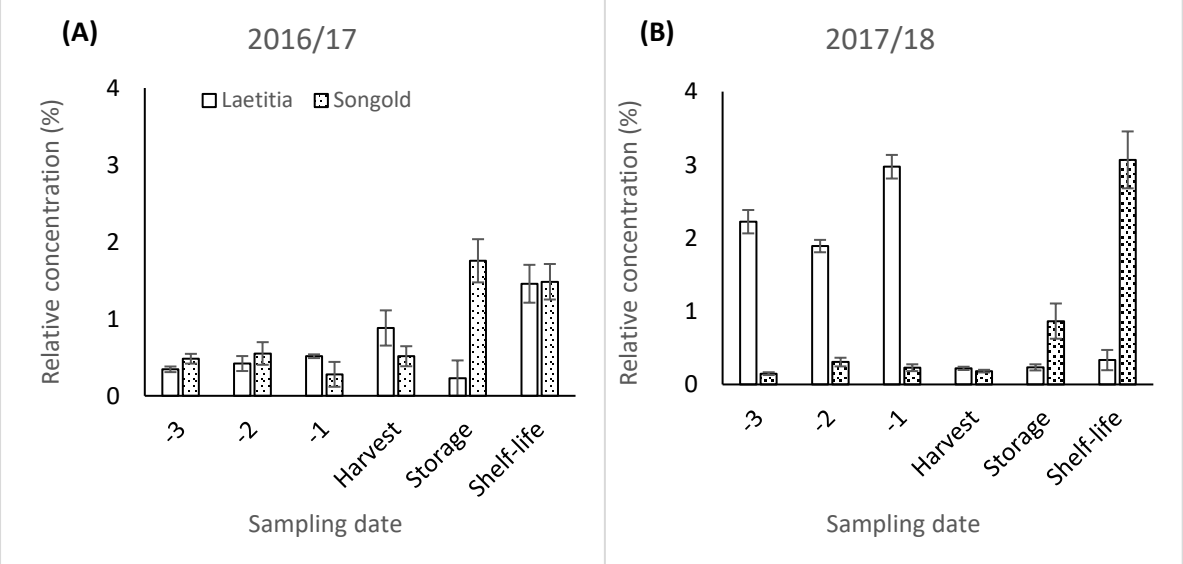

Fig. 20. Relative concentration of corosolic acid in the cuticular waxes of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks.. Bars represent means with standard error.

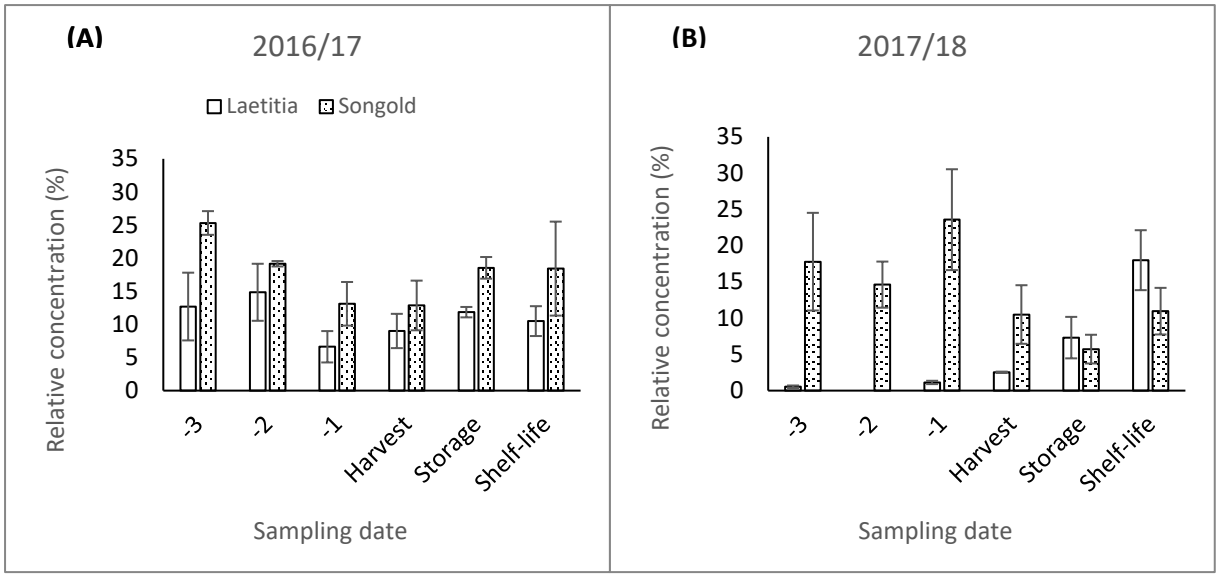

Fig. 21. Relative concentration of 9,10-dihydroxy octadecanedioic acid in the cutin of 'Laetitia' and 'Songold' in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

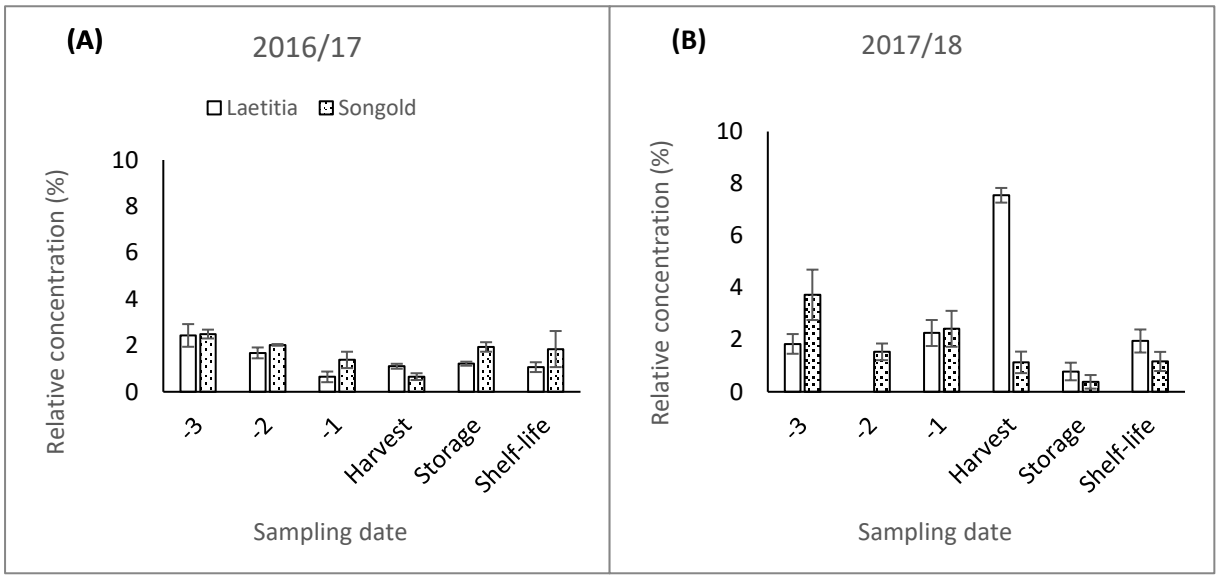

Fig. 22. Relative concentration of "Unknown 1" identified in the cutin of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

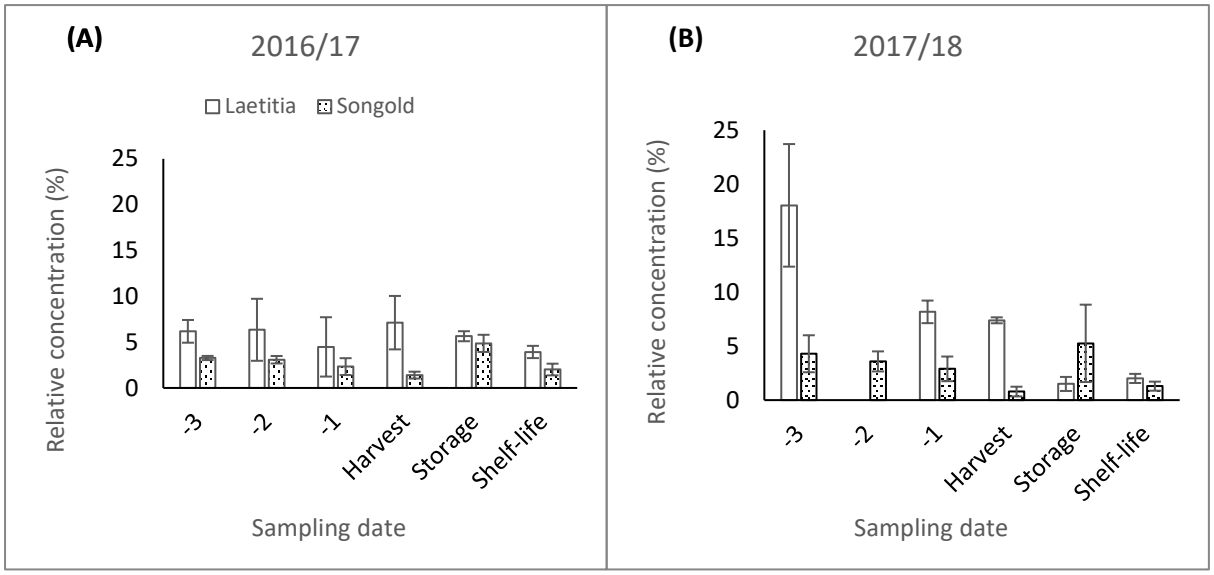

Fig. 23. Relative concentration of “Unknown 2” acid identified in the cutin of ‘Laetitia’ and ‘Songold’ plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

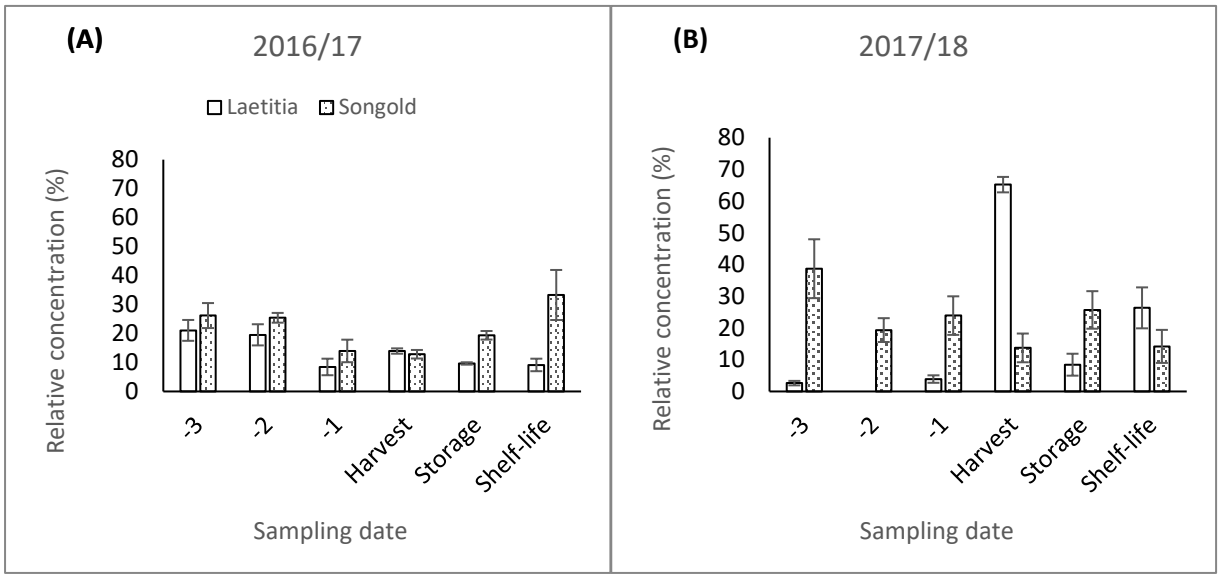

Fig. 24. Relative concentration of 9,10 epoxy octadecanedioic acid in the cutin of ‘Laetitia’ and ‘Songold’ in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

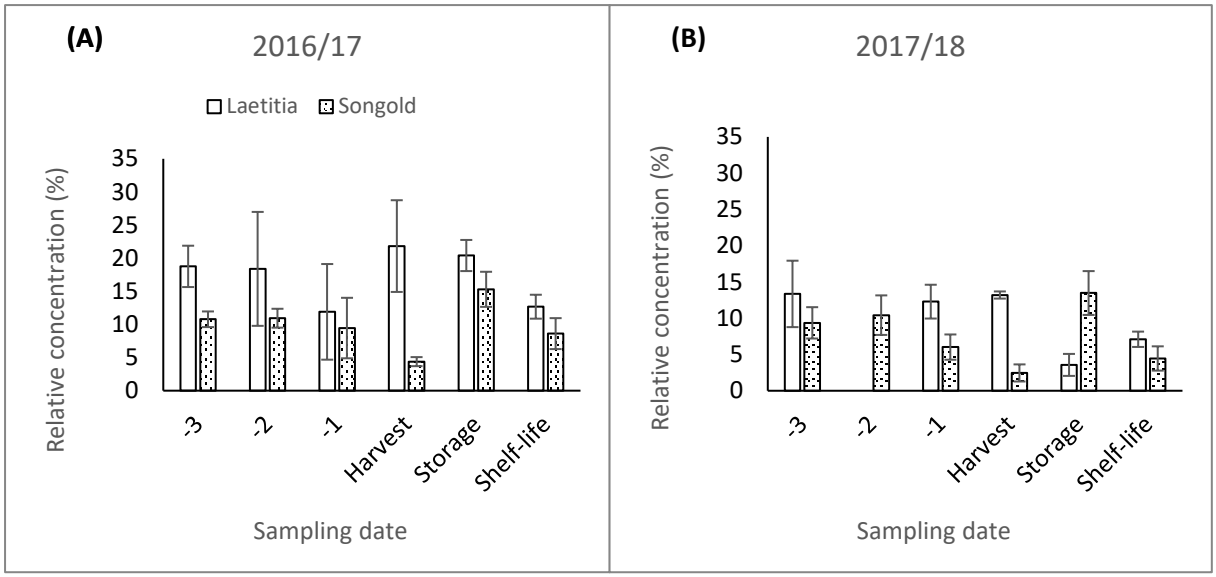

Fig. 25. Relative concentration of 9,10,18 trihydroxy octadecanoic acid in the cutin of 'Laetitia' and 'Songold' in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

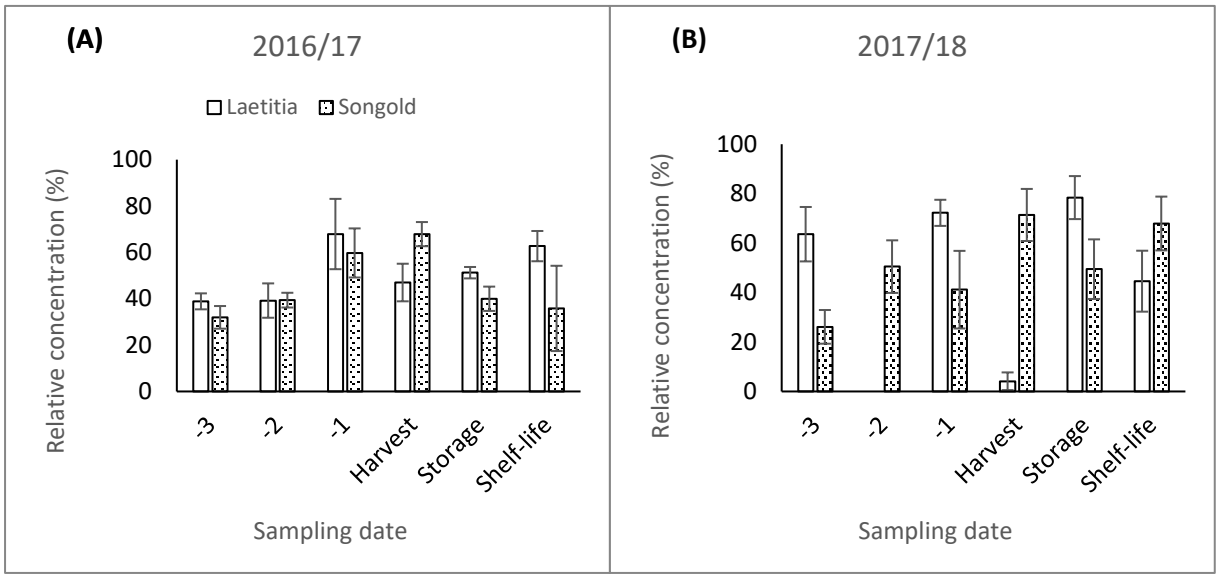

Fig. 26. Relative concentration of 2,4 bis (dimethyl benzyl) phenol identified in the cutin of 'Laetitia' and 'Songold' plums in 2016/17 (A) and 2017/18 (B). Relative concentration was calculated using the ratio of integrated area of each peak and total area of all integrated peaks. Bars represent means with standard error.

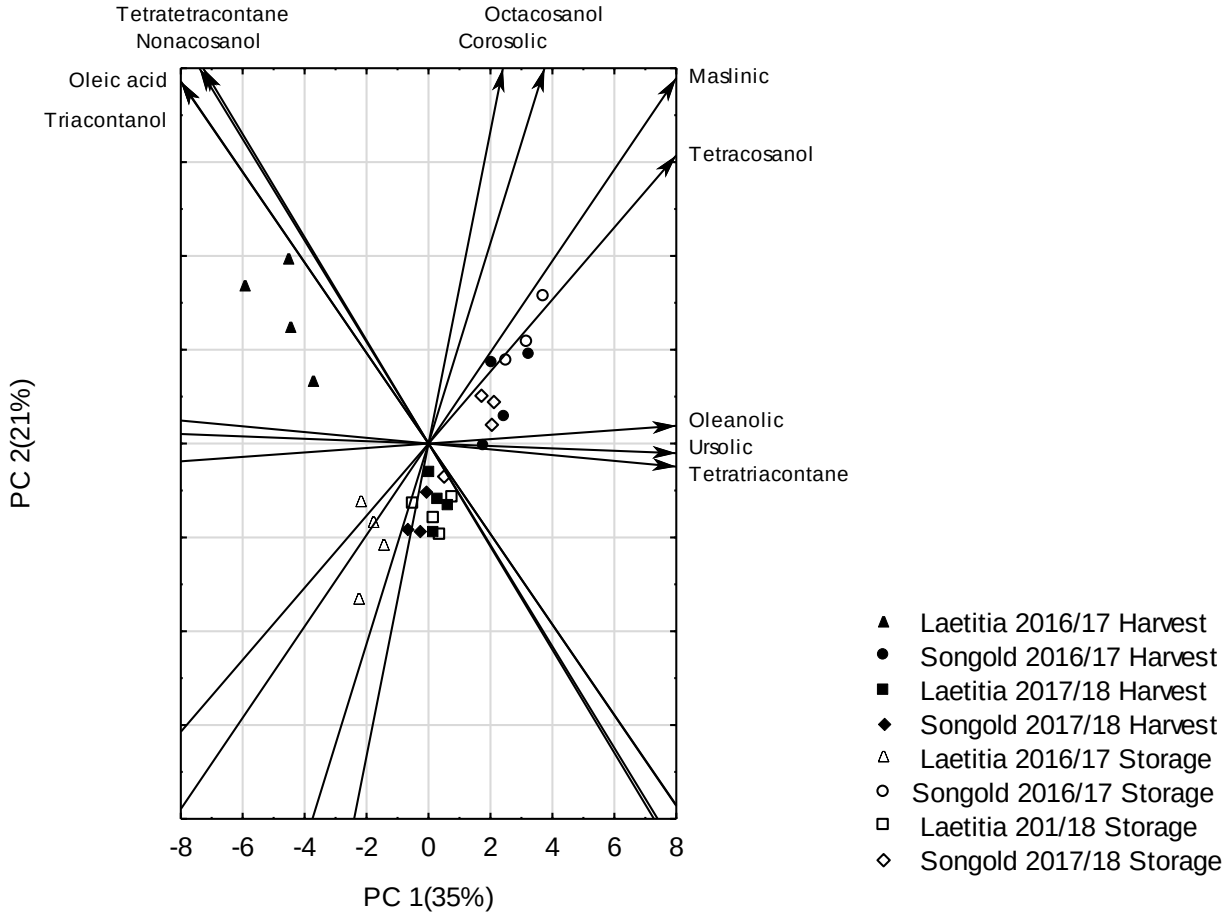

Fig. 27. Principal component (PC) bi-plot of the wax constituents of ‘Laetitia’ and ‘Songold’ on their respective commercial harvest dates and after cold storage of seven and six weeks at -0.5˚C for the respective cultivars, in 2016/17 and 2017/18. The PCA explains 56% of the variation.

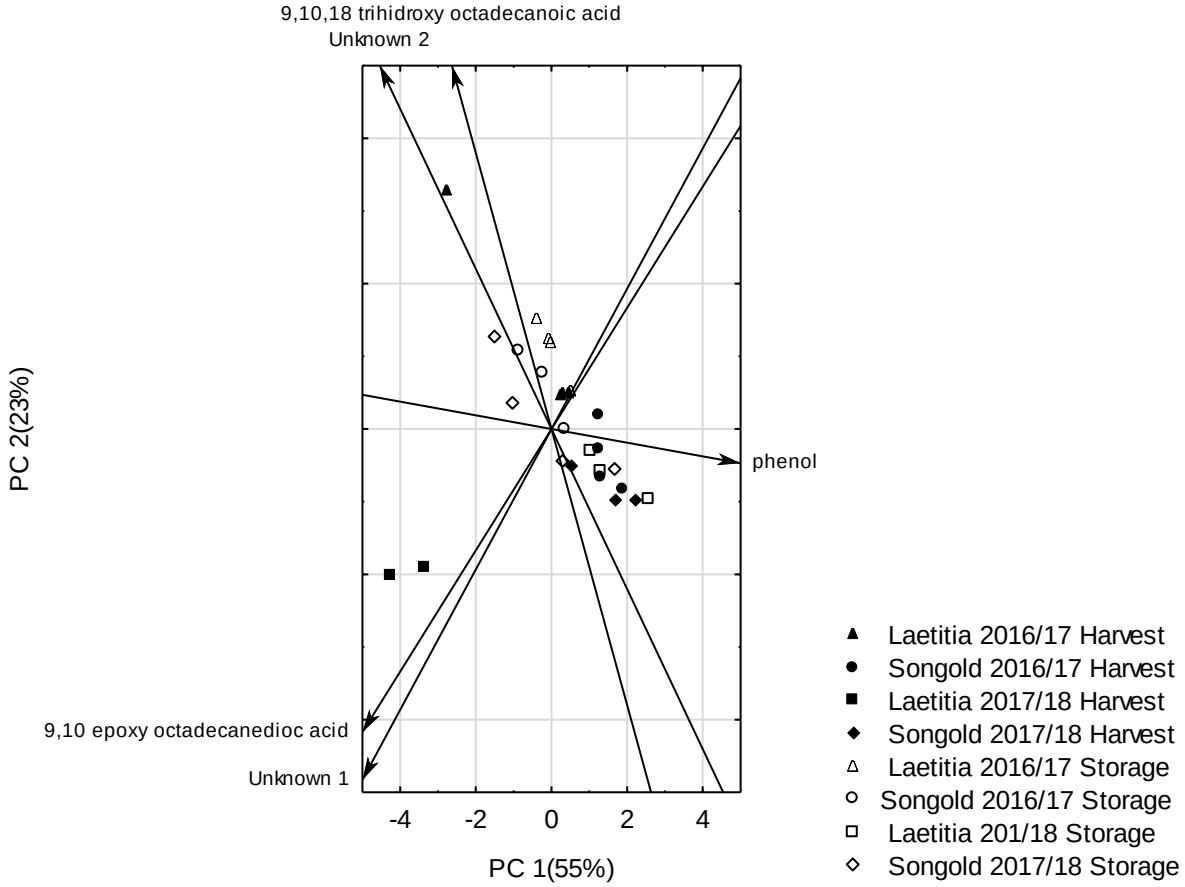

Fig. 28. Principal component (PC) bi-plot of the cutin constituents of ‘Laetitia’ and ‘Songold’ on their respective commercial harvest dates and after cold storage of seven and six weeks at -0.5˚C for the respective cultivars, in 2016/17 and 2017/18. The PCA explains 78% of the variation.

PAPER 4

Microstructure of the cuticle, epidermis, and hypodermis of Japanese plum cultivars

Abstract

The microstructure of the cuticle is strongly related to its chemical composition and differs significantly between species and cultivars. The cuticle is the fruits' most important defence against abiotic and biotic stress, yet its role in determining postharvest fruit quality in Japanese plums, is unclear. In 2016/17 fruit sampling for scanning electron microscopy occurred every fortnight in 2016/17, starting at approx. 60 days after full bloom (DAFB) until commercial harvest and the end of cold storage for 'Songold' and 'Laetitia'. On each sampling date, three sections from three fruit per cultivar were used for microscopy. In 2017/18 light microscopy was performed on 'Laetitia', 'Songold' and 'African Delight™' to visualise and measure cuticle thickness and the size of the epidermal and hypodermal cells on the commercial harvest date. The cuticular microstructure of 'Laetitia' and 'Songold' changed dramatically during fruit growth and development, as well as the cold storage period. The epicuticular waxes consist of densely packed microgranules interspersed with non-entire platelets. A significant portion of the cuticular waxes consisted of hollow, nonacosano-10-tubules. In both cultivars, but more so in 'Laetitia', the cuticular waxes seem to flatten and become smoother during cold storage. While no differences were seen in the number or size of epidermal cells between 'Laetitia', 'Songold' or 'African Delight™', 'Songold' had significantly more hypodermal cells than the other cultivars, indicating that the cells are more closely packed together, which might explain why this cultivar is less prone to showing signs of shrivelling.

Keywords: intercellular spaces; light microscopy; moisture loss; SEM; shrivel

1. Introduction

The cuticle forms the first barrier to protect fruit from abiotic and biotic stress (Domínguez et al., 2011; Gouret et al., 1993; Segado et al., 2016). Its main functions include the reduction of moisture loss, protection from insects and pathogens and to provide mechanical support to the fruit surface (Lara et al., 2014).The microstructure of the cuticle is strongly related to its chemical composition (Koch and Ensikat, 2008) and it differs significantly between species, cultivars and even different sides of the same fruit (Lara et al., 2014; Rosenquist and Morrison, 1989). Furthermore, the composition and structure of the cuticular waxes are controlled by environmental factors including temperature, exposure to sunlight and relative humidity (Baker, 1974).

A function of the fruit cuticle that has been somewhat overlooked is its role in determining and maintaining postharvest fruit quality (Lara et al., 2014; Segado et al., 2016). Since temperature and relative humidity have significant effects on cuticle composition and structure on the tree, they also play very important roles in maintaining postharvest fruit quality during cold storage (Lara et al., 2014). The mechanical properties of the fruit peel can determine its susceptibility to cracking, pest and pathogen penetration and physical damage (Konarska, 2015), all affecting the appearance, consumer preference and postharvest life of the fruit. Changes in cuticle composition are also responsible for the unacceptable sticky, waxy appearance that some apple cultivars develop during cold storage (Veraverbeke et al., 2001a).

Since plums exported from South Africa spend extended periods of time in transit to reach overseas markets, they often reach their destinations with a visibly shrivelled appearance and significant mass loss (due to moisture loss). However, this disorder is more prevalent in certain cultivars. These cultivar differences in shrivel and moisture loss may be related to differences in cuticle structure or composition. The composition and structure of apple fruit cuticles change significantly during cold storage and shelf-life, as well as between cultivars (Veraverbeke et al., 2001a). Extended cold storage of apples leads to thickening of the wax layer and changes in its ultrastructure (Konarska, 2013). In addition, microcracks often develop in the cuticle during storage and can act as avenues of moisture loss.

The aim of this study was to examine the cuticular microstructure of three plum cultivars with varying susceptibility to shrivelling, from shortly before harvest, until after cold storage: African Delight™ which is the most susceptible cultivar to postharvest shrivel development and hairline cracks also form at the pedicel end of the fruit (Theron, 2015); 'Laetitia' which is very susceptible without forming hairline cracks and Songold', which is the least prone to shrivel. We propose that differences in cuticle and peel structure partly explain the cultivar differences to shrivelling susceptibility.

2. Materials and methods

In 2016/17, fruit samples were obtained from Welgevallen Experimental farm (33°56′50.68″S 18°52′14.98″E) and in 2017/18, from the Firs, Devonvalley, Stellenbosch (33°54'55.6"S 18°49'11.4"E). Fruit sampled in 2016/17 were used to perform Scanning Electron Microscopy (SEM) of the cuticle surface. In 2017/18, light microscopy was used to visualise the cuticle, hypodermal, and epidermal cells.

2.1 Sampling in 2016/17

Fruit sampling occurred every fortnight, starting at approx. 60 days after full bloom (DAFB) until commercial harvest. On each sampling date, three fruit of similar size and background colour were selected per cultivar for 'Laetitia' and 'Songold'. To minimize disturbance of the fruit surface, fruit were only handled at their pedicel and stylar ends. Fruit were placed in pulp trays and transported to the laboratory at the Department of Horticultural Science, Stellenbosch University for further analyses.

On the commercial harvest date (121 DAFB for 'Laetitia and 130 DAFB for 'Songold), 180 visually unblemished, export-quality, size A fruit (50 – 55 mm diameter) were harvested and packed in two count-30 pulp trays, in each of three cartons (38.5 x 30 x 10 cm) and covered with a perforated, high density polyethylene (HDPE) shrivel sheet to reduce moisture loss. A white corrugated sheet was placed on top to protect the fruit (PPECB, 2017). 'Laetitia' and 'Songold' were stored according to commercial intermittent warming regimes (PPECB, 2017). Following cold storage, sampling of three fruit of similar size and background colour per cultivar, was repeated.

2.2 Scanning Electron Microscopy (SEM)

Two 5 x 10 mm peel sections were cut from the bloom side on the cheeks of each of the three fruit per cultivar (‘Laetitia’ and ‘Songold), using a sharp razor blade. The sections were then plunge frozen in liquid propane (Afrox, South Africa) according to methods adapted from Dobro et al., (2010). According to this method, water and/or organic molecules in samples are cooled rapidly, allowing the molecular arrangement and sample ultrastructure to be preserved before ice crystals can form. While liquid nitrogen is commonly used for this purpose, its thermal conductivity is not low enough to remove heat from samples before ice crystals are produced. Therefore, propane gas was used because its thermal conductivity is 300-400 times higher than that of liquid nitrogen. Liquid nitrogen (Afrox, South Africa) was however used as the primary coolant to first liquefy the propane gas and keep it cool during the sample- freezing procedure. For this trial, liquid nitrogen was poured into a Styrofoam cooler box fitted with a copper plate in the base, onto which a copper pipe is attached. As soon as the liquid nitrogen stopped bubbling and the pipe was cooled, propane gas was slowly released into the copper pipe to condense the gas until the liquid propane filled approx. half of the pipe. Using tweezers, the thin peel sections were held in the liquid propane for approx. 30 sec until completely frozen. Samples were then placed in 5 ml tubes and covered with Parafilm. Small perforations were made in the Parafilm before the samples were placed in a container filled with liquid nitrogen. The peel sections were then vacuum dried at -80°C and 2 mBar for 96h. The dried samples were stored in a sealed container silica beads until further use. To prepare samples for scanning electron microscopy (SEM) analyses, a small square was cut from each peel section and mounted onto an aluminium stub with double-sided, adhesive, energy conductive, carbon tape. Stubs were then sputter-coated with gold for 6 min. to render the material conductive. SEM analysis was performed at the Central Analytic Facility at Stellenbosch University. In-lens surface imaging (using a secondary electron type detector) was performed using a Zeiss MERLIN FEG® Scanning Electron Microscope with a GEMINI II® column. Beam conditions during the image analysis were 5 kV, with a working distance of 4 mm and Inlens-probe current of approx. 250 pA. The samples were examined at 2 µm magnification (500 x).

2.3 Sampling in 2017/18:

In 2017/18, ‘African Delight’ was included for microscopic analysis along with ‘Laetitia’ and ‘Songold’. Fruit were sampled from three weeks before the anticipated optimum harvest date (WBH), until commercial harvest. On the selected dates, 10 fruit of similar size and background colour were sampled at random, per cultivar. At the Department of Horticultural Science, Stellenbosch University, three fruit of similar size were chosen for light microscopy and preserved in formaldehyde until further use. Three longitudinal sections (approx. 3 mm x 2 mm) from pedicel end of each fruit were cut by hand using a sharp razor blade and stained with 0.01% Toluidine Blue (staining pectin in the cell walls) and Sudan III (a lipid-specific stain that stains cutin bright orange) (Isaacson et al., 2009; Schreiber and Schönherr, 2009). Cuticle thickness, cell size, cell shape and intercellular spaces were determined in a 0.5 μm^{-2} area.

2.4 Statistical analyses

One-Way analysis of variance (ANOVA) was performed at the 95% confidence limit, to determine the differences between cultivars for each of the parameters measured on the light microscopy images, namely, epidermal cell length (µm), epidermal cell height (µm), number of epidermal cells, number of hypodermal cells, and cuticle thickness (µm).

3. Results

3.1 Scanning Electron Microscopy

At 60 DAFB, the epicuticular wax morphology of ‘Laetitia’ presented as small, wax ‘droplets’, covering an amorphous crust (Fig.1). The density of the ‘droplets’ was variable over the surface of the sample and interspersed with non-entire platelets (Fig.2). At 60 DAFB (8 WBH), ‘Songold’ wax ‘droplets’ were also observed, but they were elongated, appearing more like tubules in some cases (Fig. 3). The crystalloid structures were more densely packed than in ‘Laetitia’ and more fused together to form dense clusters. In contrast to ‘Laetitia’, platelets were not as prevalent in ‘Songold’. At 75 DAFB (5 WBH), the crystalloid structures in ‘Laetitia’ resembled tubules and many had fused together to form dense clusters (Fig.4). More non-entire platelets were present at this time than at 60 DAFB. A similar trend was observed in ‘Songold’ at 6 WBH, however, the platelets appeared slightly more upright than those of ‘Laetitia’ (Fig. 5). ‘Laetitia’ had fewer tubule clusters at 92 DAFB (4 WBH) and more of the

amorphous crust was visible than on the preceding dates (Fig. 6). Some of the tubules appeared melted or flattened slightly, while non-entire plates were still present. At 92 DAFB (5 WBH) ‘Songold’ still had densely packed tubules, interspersed with non-entire platelets, now at various angles to the surface (Fig. 7). At 109 DAFB (2 WBH), the hollow tubules were clearly visible in ‘Laetitia’ (Fig. 8). The tubules were connected in a dense network, forming an intricate, multi-dimensional layer.

At one week before the commercial harvest date of ‘Laetitia’ (115 DAFB) and 2 WBH for ‘Songold’, the ultrastructure of the cuticle appeared similar to that of the previous week in some areas, while in other areas, the wax layer had a melted appearance and formed a thick crust rather than a tubular network (Fig. 9, Fig. 10). This variation could have been caused by sampling error and/or damage to the cuticle during sampling preparation.

At 121 DAFB, one week before the commercial harvest date, ‘Songold’ still had densely packed granules and tubules, interspersed with non-entire platelets (Fig. 11). At 121 DAFB in ‘Laetitia’ (Fig. 12) and 130 DAFB in ‘Songold’ (Fig. 13), the tubule clusters were slightly less dense in some areas. As a result, the amorphous crust could be seen below but the platelets were still clearly visible. After cold storage of ‘Laetitia’ the tubules were significantly flattened in some areas, almost as if melted into the crust (Fig 14). Yet some platelets were still visible, and the tubule clusters were also still present in smaller areas. After cold storage, the cuticular ultrastructure of ‘Songold’ looked slightly less ‘melted’ than ‘Laetitia’ (Fig. 15). Even though tubule density was lower than at harvest, it did not appear as flat as in ‘Laetitia’. In some areas, the amorphous crust showed extensive cracks (Fig. 16).

3.2 Light Microscopy

On the commercial harvest dates of ‘Laetitia’, ‘Songold’ and ‘African Delight™’, all had one row of epidermal cells and approx. three to four layers of hypodermal cells. The cuticle stained as an orange layer on top of the epidermal cells. ‘Laetitia’ had small epidermal cells with elongated hypodermal cells (Fig. 17) and the cuticle immediately above the epidermal cells could be clearly identified after staining (Fig. 18). In ‘Songold’, the hypodermal cells appeared flatter than those of ‘Laetitia’ (Fig. 19), while the cuticle covered more of the epidermal cells, extending in between the cells as well

(Fig. 20). In ‘African Delight™’, the hypodermal cells were not as neatly arranged in rows as with the two other cultivars (Fig. 21), but the cuticle was similar to that of ‘Laetitia’ (Fig. 22).

The average epidermal cell length and height did not differ significantly between cultivars (Table 1). `Laetitia’ had more epidermal cells compared to the other cultivars, but not significantly so. The number of hypodermal cells differed significantly between cultivars. ‘Songold’ had significantly more hypodermal cells than the other cultivars. Cuticle thickness did not differ significantly between cultivars.

4. Discussion

4.1 Scanning Electron Microscopy

This study quantified the cuticular microstructure of plum cultivars with varying susceptibilities towards shrivelling during fruit development, from pre- to post harvest.

Fruit cutin and wax composition changes dramatically during growth and development as more precursors are synthesised and the cuticle structure changes to keep up with the rapid expansion of the fruit surface (Lara et al., 2014; Martin and Rose, 2014). In European plums (*Prunus domestica* L.), the first indication of wax development is seen in 8 mm sized fruit – before this, the fruit surface appears smooth (Bain and Mcbean, 1968). As with grape berries, the structure of the epicuticular waxes of both ‘Laetitia’ and ‘Songold’ changed with fruit age / maturity (Rogiers et al., 2004). In the orchard, the area of the peel that is exposed to sunlight ages earlier, which leads to a faster transition from crystalline to amorphous waxes (El-Otmani et al., 1989). The sun-exposed areas tend to have high fruit surface temperatures, as fruit surface temperature can often be up to 15°C higher than the ambient temperature (Rogiers et al., 2004). Although fruit surface temperatures were not quantified in the current study.

The densely packed ‘droplets’ or microgranules observed during the early stages of development in both ‘Laetitia’ and ‘Songold’ are considered to be crystalloid structures (Barthlott et al., 1998; Jeffree et al., 1975) and agree with findings in European plums (*Prunus domestica* L.) (Konarska, 2015; Storey and Price, 1999b). In ‘Bluefire’ European plums, large, vertical platelets interspersed the microgranules in a disorganised fashion (Konarska, 2015). In contrast, the platelets in ‘Laetitia’ and ‘Songold’ are non-entire, as they do not have distinct edges.

Two types of tubules, differing in chemical composition, are present in plant cuticles namely nonacosan-10-ol and β-diketone tubules (Barthlott et al., 1998; Koch and Ensikat, 2008). Nonacosan-10-ol tubules are arranged in clusters and branch at right-angles from the surface, while β-diketone tubules branch in more acute angles. The tubules in 'Laetitia' and 'Songold' resembled nonacosan-10-ol tubules. This was also confirmed in Chapter 3 where one of the major components of the cuticular waxes was indeed identified as nonacosan-10-ol.

As the chemical constituents of the waxes determine their micromorphology, cuticular waxes can form either crystalline or amorphous domains (Lara et al., 2014; Riederer and Schreiber, 1995; Rogiers et al., 2004). Aliphatic compounds form crystalline 'rafts' that are water-resistant. This forces water molecules and other polar metabolites to diffuse through the amorphous domains that are formed by more polar and cyclic waxes. Crystalline zones in the cuticle block the movement of water because they have very limited molecular motion within the polymers, so there are no gaps through which the diffusing molecules can move (Riederer and Schreiber, 1995).

The 'flattening' of the wax layer, after cold storage in 'Laetitia' and to a lesser extent, 'Songold', seem to be a common postharvest occurrence. Although Lara et al., (2014) reported that the wax layer becomes smoother in order to cover cracks that are present in the cuticle during storage and possibly protect the fruit from moisture loss, this was not investigated during this study.

4.2 Light microscopy

The peels of apple fruit and European plums consist of a single- or double-layered epidermis, a cuticle and a multi-layered hypodermis (Konarska, 2015, 2013). In all three Japanese plums cultivars, Laetitia, Songold, and African Delight™, the peel consisted of a single layer of epidermal cells, with three to four layers of hypodermal cells below. Although the apple cuticle can extend into the anticlinal walls of the epidermal cells to form cuticular pegs, this was only found in 'Songold' and not as clearly as in apples. This agrees with observations in European plums (Konarska, 2015). Apples therefore have stronger peels that are reinforced by the cuticle to a greater extent than that of plums.

The fact that cuticle thickness did not differ significantly between the cultivars does not indicate that they have similar peel permeabilities and rates of moisture loss. Cuticle thickness does not determine cuticular transpiration rate or permeability as the composition and structure of the cuticle plays a more important role (Riederer and Schreiber, 2001). The significantly higher number of hypodermal cells in 'Songold' indicate that its intercellular spaces are much smaller compared to the other cultivars, though it is uncertain why. As moisture loss is driven by the vapour pressure deficit between the intercellular spaces in fruit and the surrounding atmosphere (Lara et al., 2014; Maguire et al., 2001; Wills et al., 1989), the smaller intercellular spaces of 'Songold' may slow down moisture loss and dehydration of the hypodermal cells, explaining why 'Songold' is less prone to moisture loss than the other cultivars.

5. Conclusion

The cuticular microstructure of 'Laetitia' and 'Songold' changed dramatically during fruit growth and development, as well as the cold storage period. Such changes are expected, as more cuticular membrane is synthesised during fruit growth to enable cuticle expansion to maintain the rapid increase in the fruit surface area. Both environmental and genetic factors play a significant role in determining cuticle structure. In 'Laetitia' and 'Songold', the epicuticular waxes consist of densely packed microgranules interspersed with non-entire platelets. In 'Songold' the microgranules are more densely packed than in 'Laetitia', and the platelets are more upright. This is likely due to differences in wax composition between the cultivars. A significant portion of the cuticular waxes consisted of hollow, nonacosano-10-ol-tubules. In both cultivars, but more so in 'Laetitia', the cuticular waxes seem to flatten and become smoother during cold storage, which is likely a response to fill and cover cracks to prevent moisture loss. While no differences were seen in the number or size of epidermal cells between 'Laetitia', 'Songold' or 'African Delight™', 'Songold' had significantly more hypodermal cells than the other cultivars, indicating that the cells are more closely packed together. According to our knowledge, this is the first time that the microstructure of the fruit cuticle and epidermis has been described in Japanese plums. These results contribute to our understanding of why 'Songold' is less susceptible to shrivel development and the interplay between the fruit cuticle and epidermal/ hypodermal cells in determining some aspects of fruit quality. Future

studies can build on these findings to determine how the fruit cuticle, or storage conditions, can be manipulated to ensure optimal fruit quality and postharvest life.

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Table 1

Cuticle and epidermal cell measurements of ‘Laetitia’, ‘Songold’, and ‘African Delight™’ in a 0.5 µm^2 area in 2017/18. Significant differences are indicated in lower case letters.

Cultivar	Epidermal cell length (µm)	Epidermal cell height (µm)	Number of epidermal cells	Number of hypodermal cells	Cuticle thickness (µm)
Laetitia	0.129 ns	0.069 ns	7.22 ns	7.44 b	0.078 ns
Songold	0.132	0.059	6.22	10.00 a	0.093
African Delight™	0.108	0.061	6.56	8.00 b	0.0800
Pr > F	0.2211	0.3774	0.3520	**0.0064**	0.3080

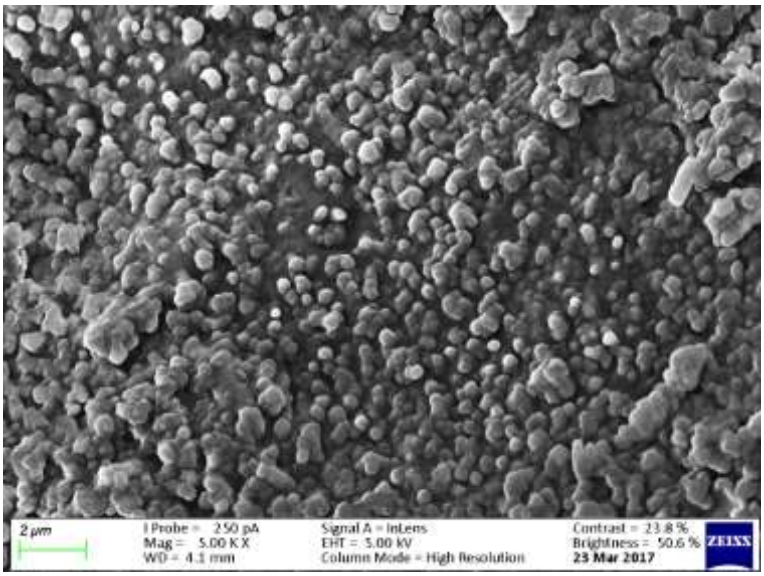

Fig.1. At 7 weeks before harvest (60 days after full bloom) the epicuticular wax morphology of ‘Laetitia’ presented as small wax ‘droplets’ or microgranules covering an amorphous crust below.

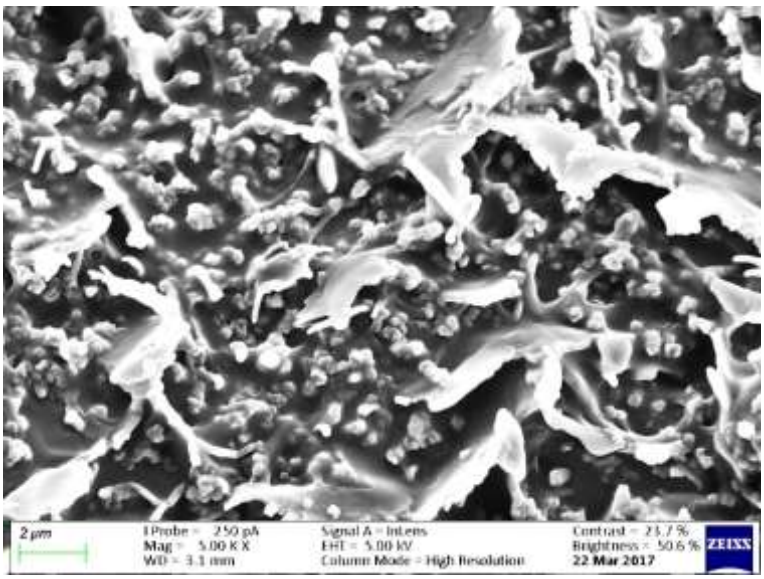

Fig. 2. At 7 weeks before harvest (60 days after full bloom ‘Laetitia’ also had areas where the microgranules were less dense and interspersed with non-entire platelets.

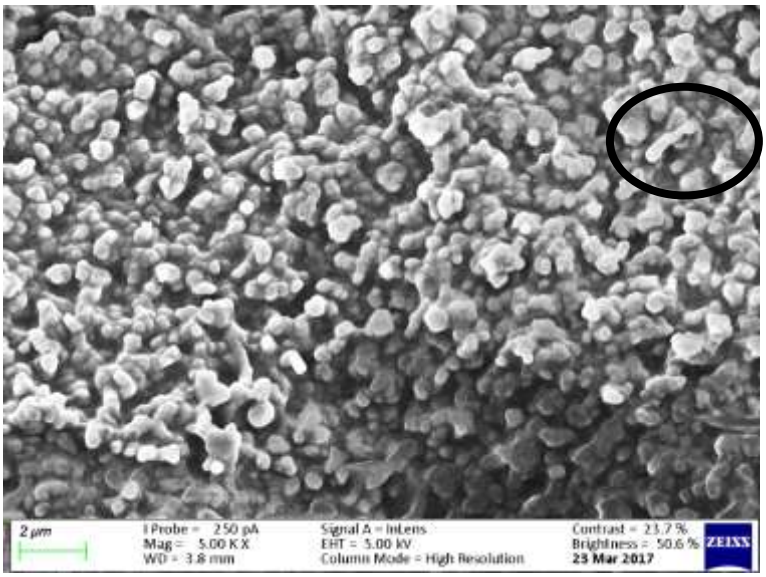

Fig. 3. At 8 weeks before harvest (60 days after full bloom) ‘Songold’ wax microgranules were elongated, appearing more like tubules in some cases (as seen in circle). The crystalloid structures were densely packed and fused together, forming dense clusters.

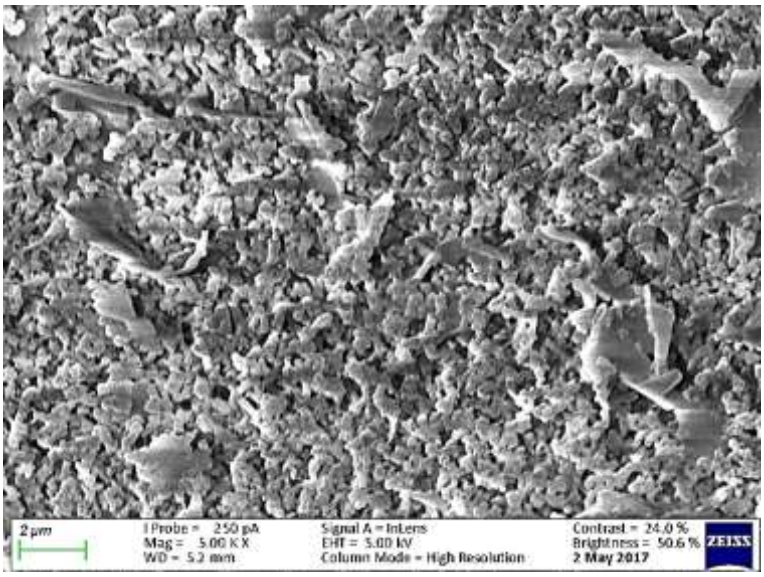

Fig. 4. At 5 weeks before harvest (75 days after full bloom) the crystalloid structures in ‘Laetitia’ presented as tubules and many had fused together to form dense clusters.

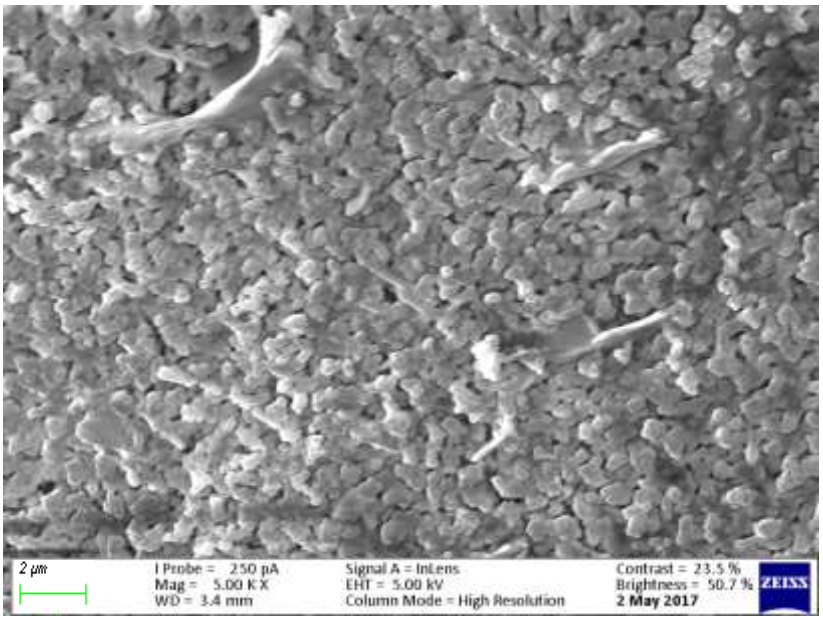

Fig. 5. At 6 weeks before harvest, ‘Songold’ microgranules started to present as tubules, but the platelets appeared slightly more upright than those of ‘Laetitia’.

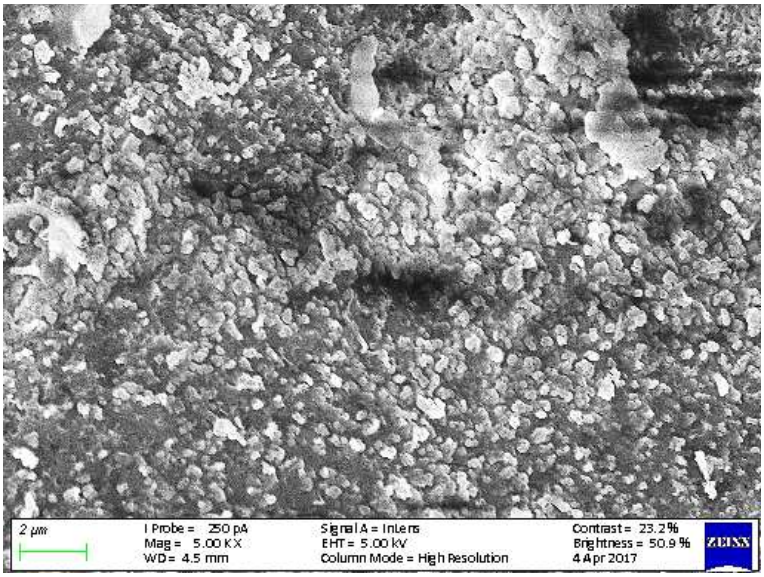

Fig. 6. ‘Laetitia’ had fewer tubule clusters at 4 weeks before harvest (92 days after full bloom) and more of the amorphous crust was visible than on the preceding dates. Some of tubules seem to have melted or flattened slightly.

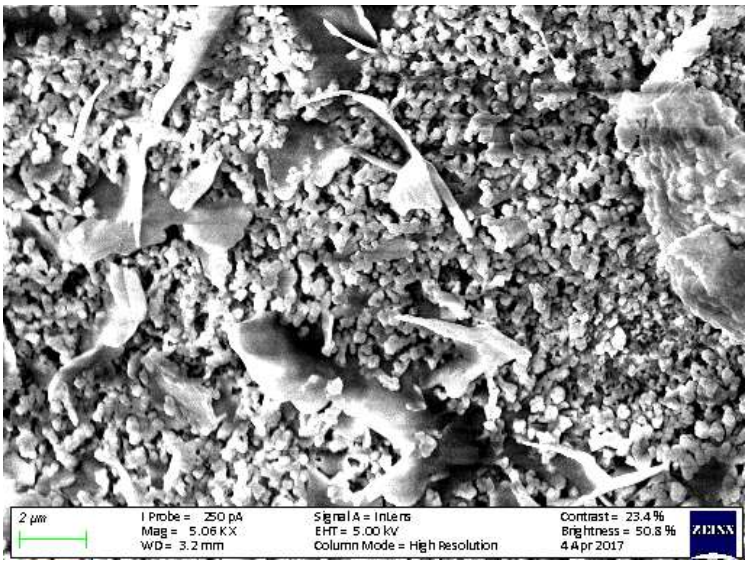

Fig. 7. At 5 weeks before harvest (92 days after full bloom) ‘Songold’ still had densely packed tubules, interspersed with non-entire platelets, now at various angles to the surface.

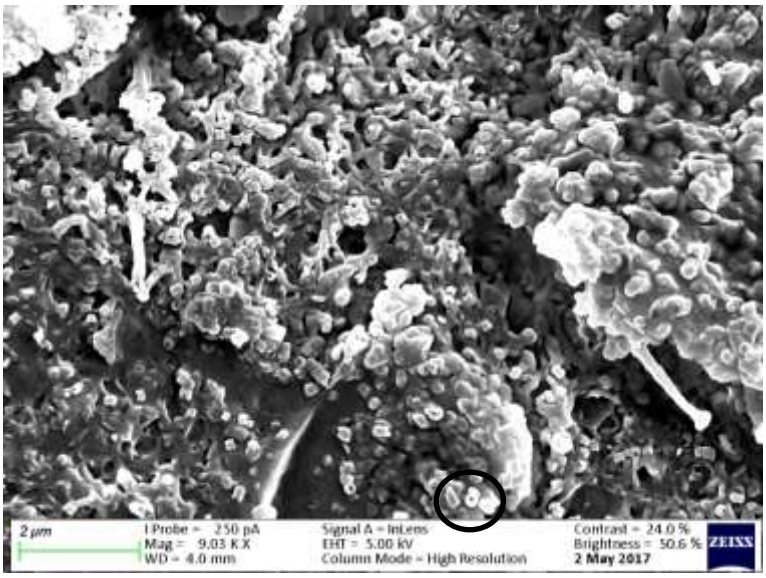

Fig. 8. At 2 weeks before harvest (109 days after full bloom), the hollow tubules were clearly visible in ‘Laetitia’ at higher magnification (9.03 K X) (as seen in circle). The tubules were connected in a dense network, forming an intricate, multi-dimensional layer.

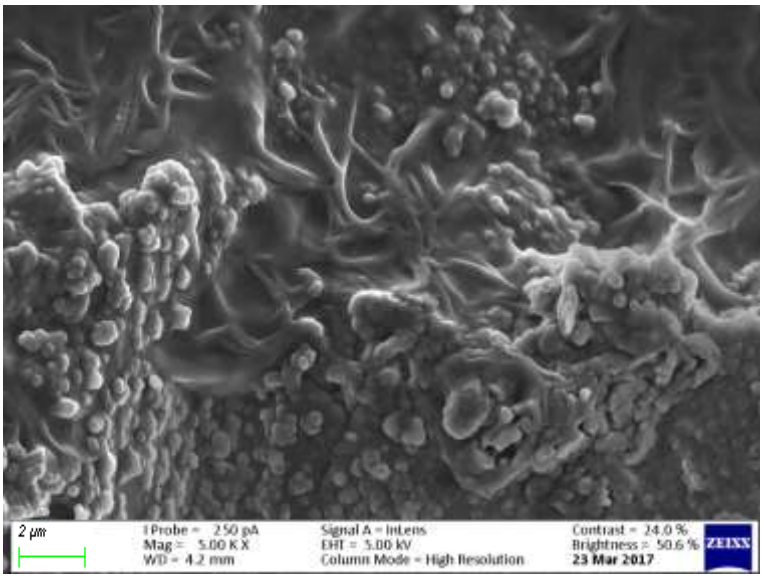

Fig. 9. At one week before the commercial harvest date of ‘Laetitia’ (115 days after full bloom) the ultrastructure of the cuticle did not differ from the previous week, while in others, the wax layer had a melted appearance and formed a thick crust rather than a tubular network

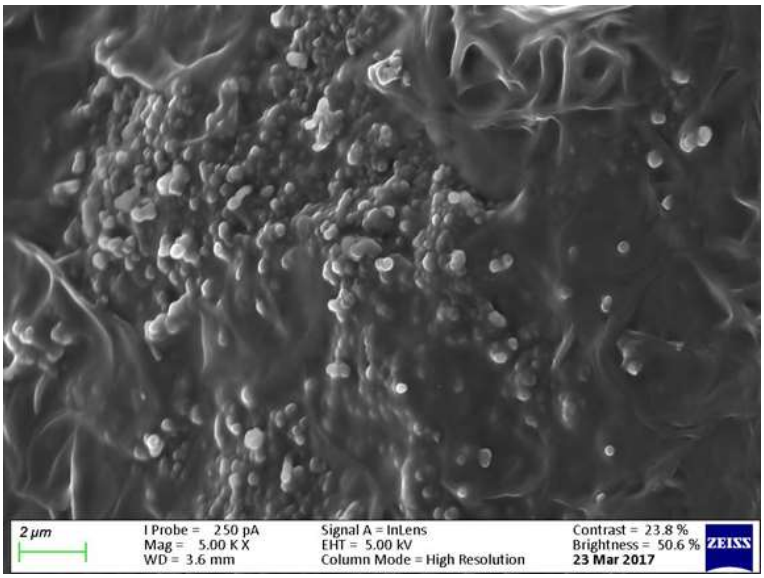

Fig. 10. At 2 weeks before harvest, the ultrastructure of ‘Songold’ cuticles did not differ from the previous week, while in others, the wax layer had a melted appearance and formed a thick crust rather than a tubular network

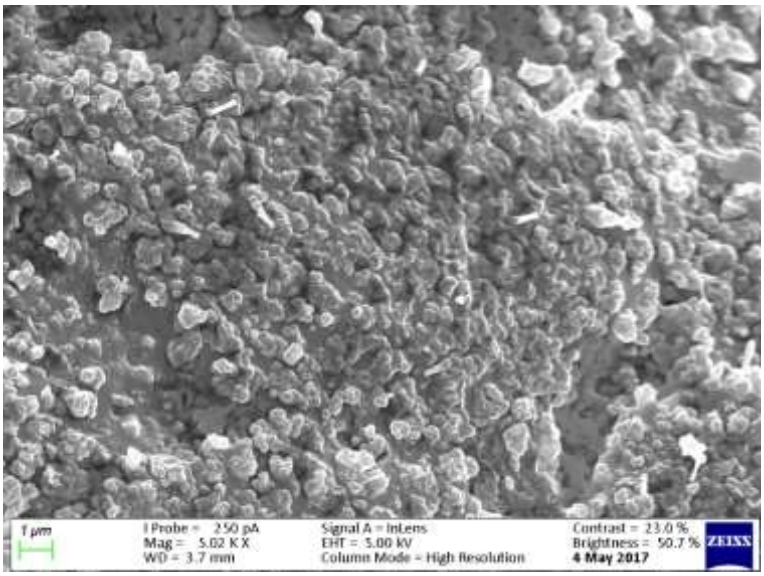

Fig. 11. At 121 days after full bloom, one week before the commercial date, ‘Songold’ had densely packed granules and tubules, with non-entire platelets.

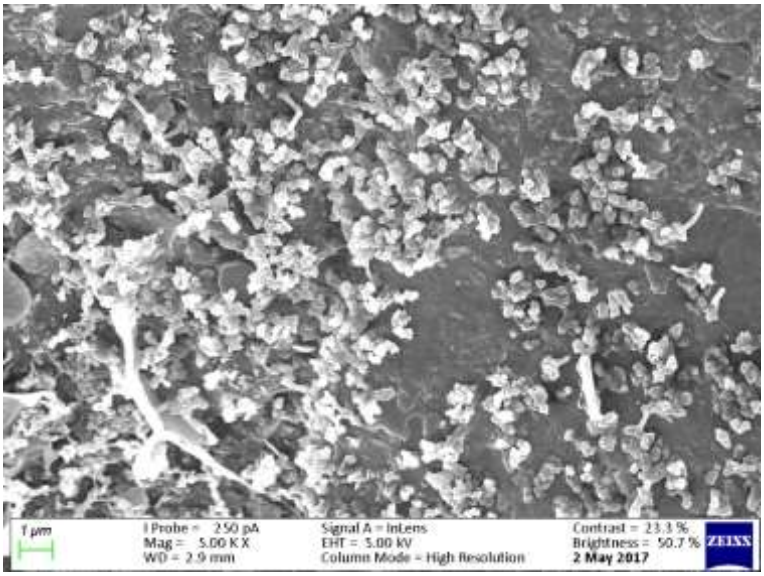

Fig.12 At 121 days after full bloom, the commercial harvest date of ‘Laetitia’, the tubule clusters were slightly less dense in some areas, showing the amorphous crust below.

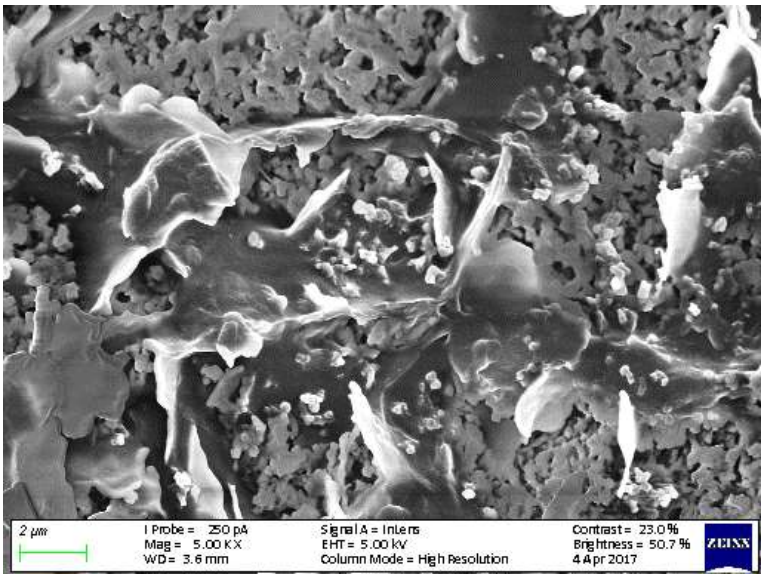

Fig.13. At 130 days after full bloom, the commercial harvest dates of ‘Songold’, the tubule clusters were less dense in some areas, showing the amorphous crust below but the platelets were still clearly visible.

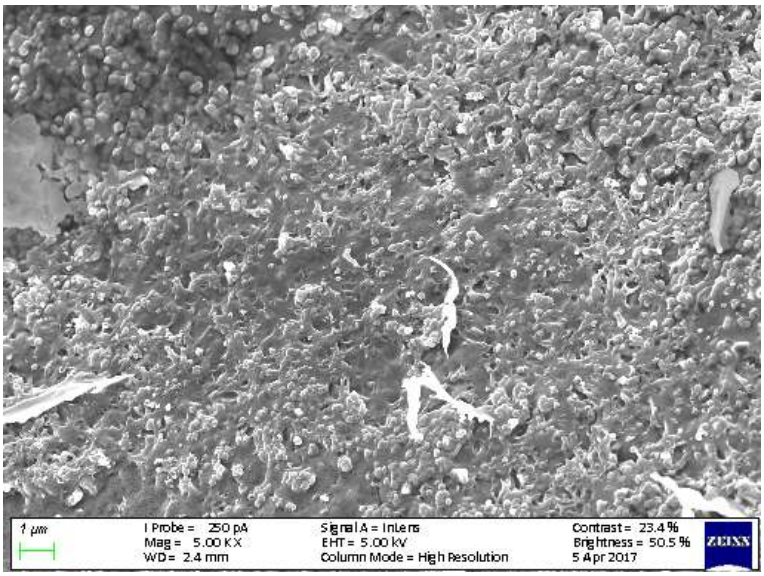

Fig. 14. After cold storage of ‘Laetitia’ the tubules were significantly flattened in some areas, almost as if melted into the crust. Yet some platelets were still visible, and the tubule clusters were also still present in smaller areas.

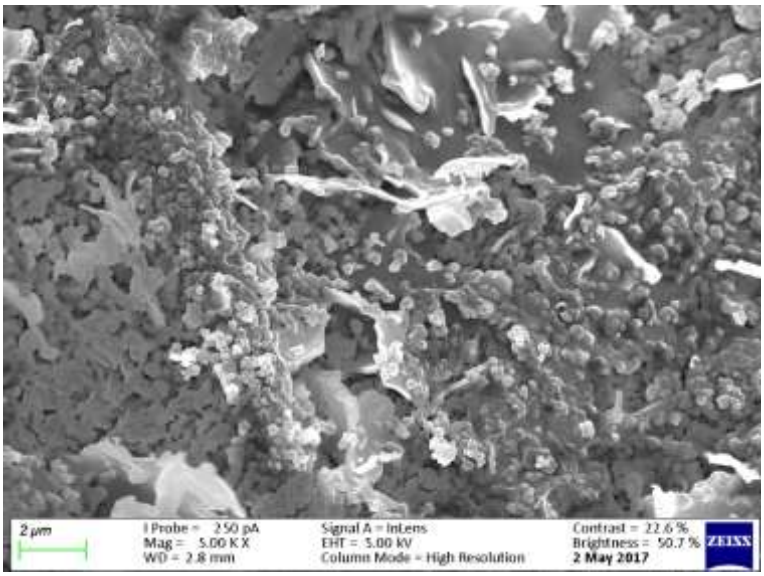

Fig. 15. After cold storage, the cuticular ultrastructure of ‘Songold’ looked slightly less ‘melted’ compared to ‘Laetitia’.

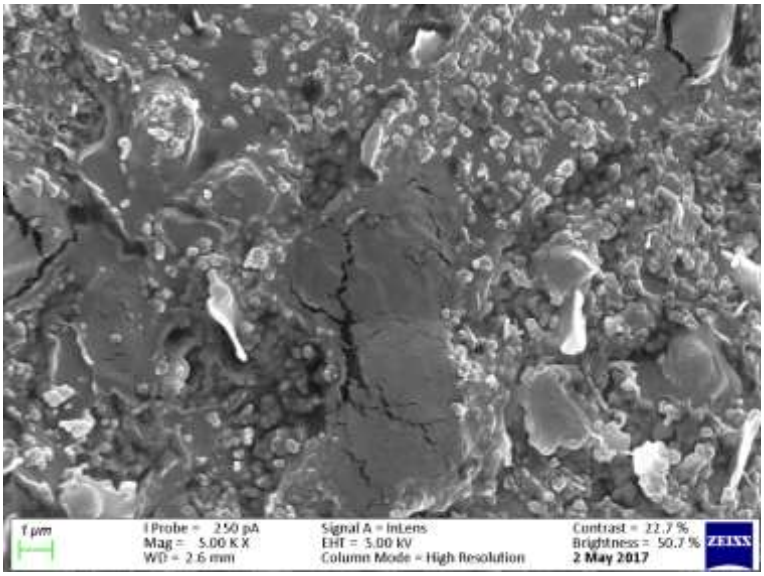

Fig. 16. After cold storage of ‘Songold’, tubule density was lower compared to at harvest and extensive cracks were visible in the cuticle.

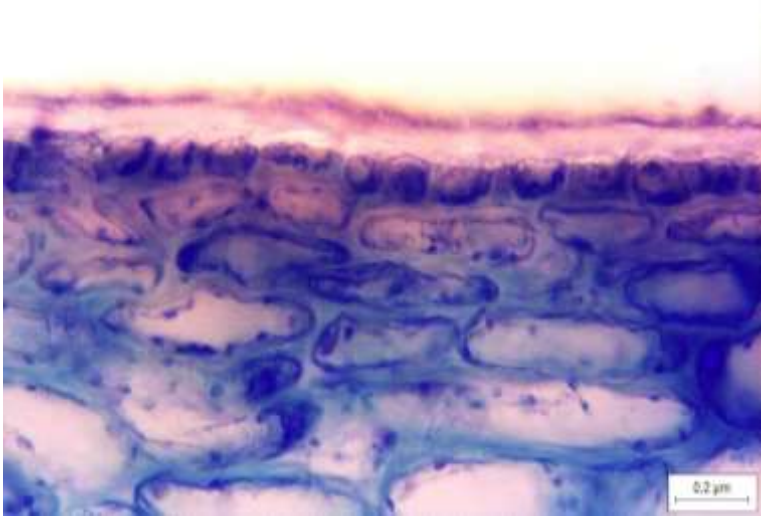

Fig. 17. ‘Micrograph of ‘Laetitia’ peel section at 40 x magnification, stained with Toluidine blue to visualise the epidermal and hypodermal cells.

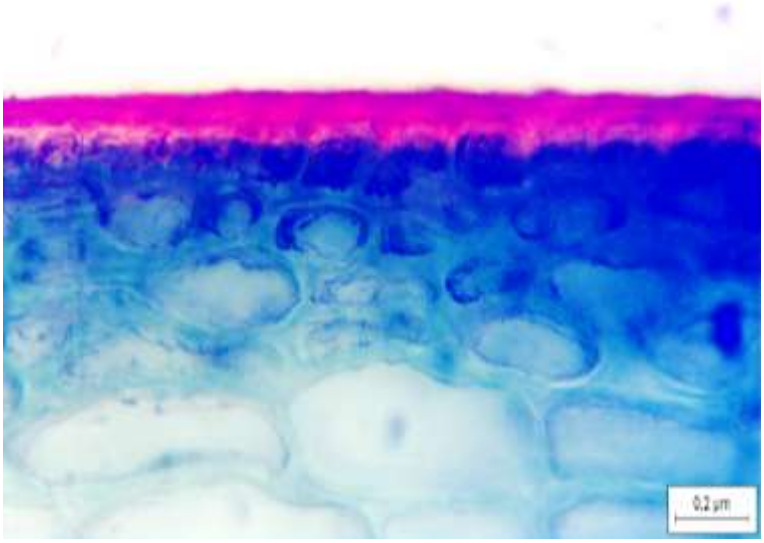

Fig. 18. Micrograph of ‘Laetitia’ cuticle at 40 x magnification, stained bright orange by Sudan III.

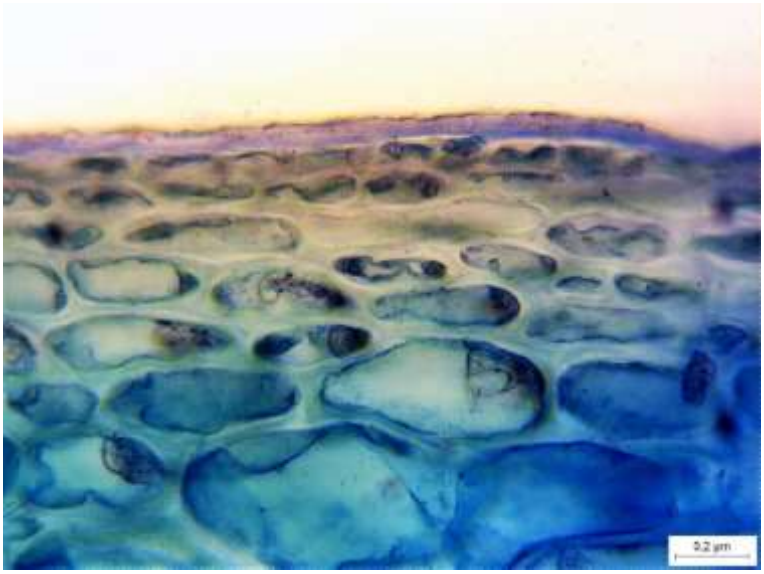

Fig. 19. Micrograph of ‘Songold’ peel section at 40 x magnification, stained with Toluidine blue to visualise the epidermal and hypodermal cells.

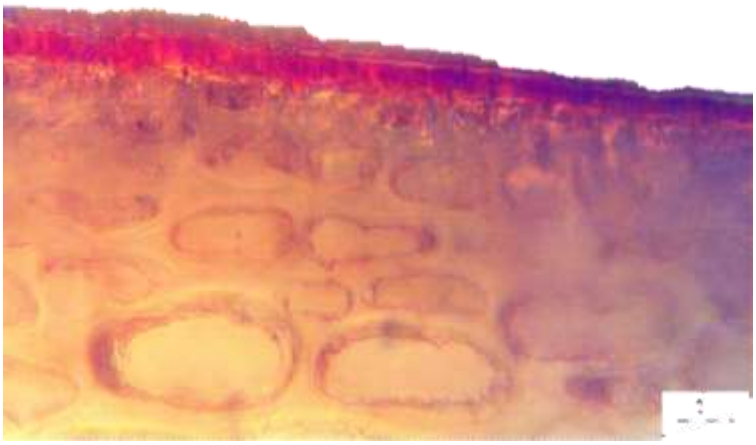

Fig. 20. Micrograph of ‘Songold’ cuticle at 40 x magnification, stained bright orange by Sudan III.

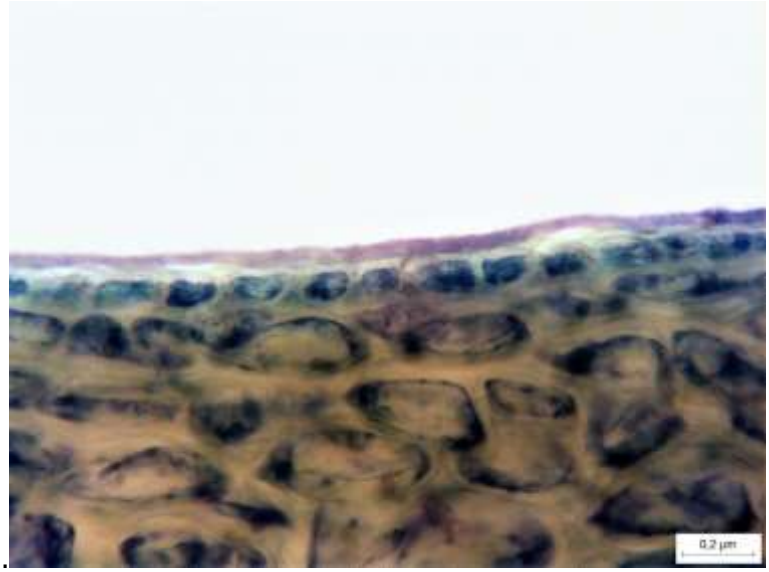

Fig. 21. Micrograph of ‘African Delight™’ peel section at 40 x magnification, stained with Toluidine blue to visualise the epidermal and hypodermal cells.

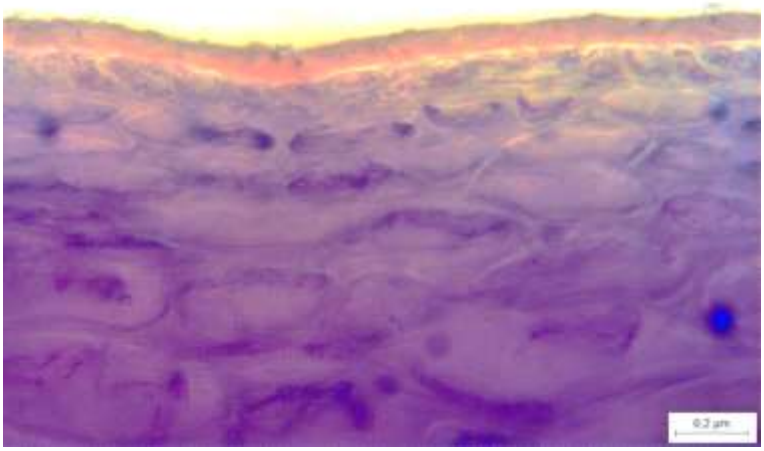

Fig. 22. Micrograph of ‘African Delight™’ cuticle at 40 x magnification, stained bright orange by Sudan III.

PAPER 5

Evaluation of perforated low-density polyethylene bags for reduction of post-harvest moisture loss and shrivelling in Japanese plums (*Prunus salacina* Lindl.)

Abstract

Japanese plums (*Prunus salacina* Lindl.) produced in South Africa spend up to eight weeks in shipping to reach overseas markets. Significant mass loss and fruit with a shrivelled appearance is often a consequence of this extended shipping period. This leads to consignments being rejected, or requiring costly repacking, leading to significant financial losses for the industry. A range of commercially available high-density polyethylene (HDPE) and low-density polyethylene (LDPE) bags with different numbers of macro- and micro-perforations were compared to the commercially used HDPE macro-perforated shrivel sheet for their efficacy to reduce moisture loss and shrivelling of 'Laetitia', 'Songold', 'Sapphire' and 'African Delight™' plums during two consecutive seasons (2015/16 and 2016/17). Five packaging treatments with five replicates/boxes per treatment, were evaluated per cultivar. Compared to the HDPE macro-perforated shrivel sheet used currently, LDPE bags with micro-perforations (92, 72 and 4) performed better in all cultivars. LDPE micro-perforated bags reduced shrivel incidence in 'Laetitia' with ± 50 % and 'African Delight™', with ± 93 %, to less than the 10 % cut-off maximum for export in 2015/16. 'Laetitia' and 'Sapphire' had very low shrivel incidence in 2016/17 (3.54 % and 6.82 %), but the LDPE bags tended to still lower shrivel incidence. 'African Delight™' had a higher shrivel incidence in season two (24 %) and, while the packaging treatments lowered shrivel percentage by 22 %, it was not reduced below the threshold value of 10 %. LDPE packaging with fewer and smaller perforations than the HDPE packaging currently in use, can reduce moisture loss and shrivel in plums, though the reduction is not statistically significant. Further

investigation of LDPE packaging is required to fine-tune the bags specifically for plums.

Key words: Cold-storage, Macro-perforation, Micro-perforation, Packaging, Post-harvest

1. Introduction

Stone fruit are climacteric and deteriorate rapidly after ripening, manifesting as softening, dehydration and decay. This becomes relevant for export, as Japanese plums (*Prunus salacina* Lindl.) produced in South Africa spend almost eight weeks in shipping freight to reach overseas markets. Like other stone fruit, plums have a limited post-harvest life (Cantin et al., 2008). Therefore, commercial storage conditions (0-5 °C and 80 % to 95 % relative humidity (RH)) were adopted to delay the softening process. However, these low temperatures can cause chilling injury. To further address this challenge, plums are packaged in “shrivel sheets” and, in some cases, in high density polyethylene (HDPE) bags to prevent moisture loss, but significant mass loss and shrivelling still occur during cold-storage and shipping (Stone Fruit Packaging Work Group, 2016). This can lead to fruit being rejected upon arrival at overseas markets or requiring costly repacking.

Even slight moisture loss in fruit and vegetables can cause changes in fruit colour and texture. When a critical moisture loss threshold is reached, more obvious changes, such as changes in turgidity, firmness, flavour and nutritional value can occur (Nunes, CN; Edmond et al., 2007). To prevent this, fresh produce is generally packaged in plastic bags in boxes, with the bag acting as a barrier between fruit and the surrounding ‘drier’ atmosphere. Close packing of the produce itself restricts the movement of air around individual fruit, which further reduces moisture loss (Wills et al., 2007). In addition, the degree to which moisture loss is reduced by packaging depends largely on the permeability of the package/ packing material to water vapour transfer.

The vapour pressure deficit (VPD) between the fruit and the surrounding atmosphere, as well as fruit type and cultivar, play a significant role in determining

moisture loss from fruit during storage (Whitelock et al., 1994). At harvest, the water content of fruit is very high and produce has a fresh appearance (Holcroft, 2015). Harvesting removes fruit from its only water supply and imminently creates a water potential gradient for fruit to lose water to surrounding air, thus causing mass loss. Continued water loss causes wilting and shrivelling of the fruit. The point at which water loss affects fruit quality, differs between commodities. In addition, mass loss of individual fruit within a carton also varies, with some fruit appearing shrivelled, while others are still acceptable for sale and consumption. This is likely due to maturity differences or differences in cuticle permeability and other cuticle properties (thickness, compositions, wax layer etc.) between the individual fruit in the carton (Holcroft, 2015; Maguire et al., 2003).

Plastic packaging generally increases the relative humidity RH of the air around the fruit in the package (Crouch, 1998; Opara et al., 2015), by preventing the moisture lost from the fruit from being released into the surrounding atmosphere. Water vapour then accumulates in the packaging until it reaches a state close to saturation (90-100 %) (Crouch, 1998; Sastry, 1985a). This decreases the VPD between the fruit and surrounding atmosphere and thus, there is a decreased potential for moisture loss.

Changing the number of perforations per packaging wrap (bag) changes the transmission capability of the film (Fishman et al., 1996). This alters the VPD between the fruit and the surrounding atmosphere, thus changing the driving force for water vapour permeation through the film. Therefore, bags with larger holes and/or a higher number of holes will result in more moisture loss compared to bags with fewer and/or smaller perforations. Importantly, fruit types/cultivars differ in terms of their tolerance limits to low O_2 and elevated CO_2 levels (Fishman et al., 1996). Therefore, the permeability of a chosen packaging material to O_2, CO_2 and water vapour must be considered when designing packaging solutions.

Low-density polyethylene (LDPE) is the most commonly used packaging film (Mangaraj et al., 2009). LDPE is a good barrier to water vapour, but a poor barrier to oxygen and carbon dioxide. Because LDPE is relatively transparent, it is predominantly used in film applications. HDPE films are stiffer than LDPE films,

though still flexible, and are less transparent. Their water vapour barrier and gas barrier capabilities are also better.

In this study a range of HDPE bags and the HDPE shrivel sheet, currently used in the South African fruit industry, were evaluated and compared to LDPE bags, also currently used for fruit / vegetable packaging in South Africa. Treatment bags with different numbers and/or sizes of perforations were evaluated on 'Sapphire', 'Laetitia', 'Songold' and 'African Delight™' plums. These cultivars are very prone to moisture loss and shrivelling and therefore packaging solutions which could reduce moisture loss, while still maintaining optimal CO_2 and O_2 levels, were evaluated over two consecutive seasons.

2. Materials and methods

2.1 Trial layout and site selection

During 2015/16, 'Laetitia', 'Songold' and 'African Delight™' (cv. ARC PR00-29) were evaluated, while 'Songold' was replaced with 'Sapphire' in 2016/17. Export-quality, size A fruit (50 – 55 mm diameter) were collected from a commercial pack-house in Franschhoek, South Africa (33°54'24.4"S; 19°06'47.0"E), shortly after the respective cultivar's harvest dates. A completely randomised design was used as experimental layout, with six replicates per treatment. One replicate consisted of one carton of fruit, containing approximately 60 fruits.

Upon arrival at the Department of Horticultural Science, Stellenbosch, fruit were repacked into the respective packaging treatments. During 2015/16 the following treatments were evaluated: HDPE Shrivel sheet (a sheet of plastic with ± 48 perforations with 5 mm diameter) (Control), HDPE 54 x 2 mm (54 perforations of 2mm diameter), HDPE 36 x 4 mm, HDPE 60 x 5 mm, HDPE 48 x 4 mm, HDPE Micro-perforation (a series of very small perforations across the entire bag), LDPE 92 (micro-perforation) and LDPE 72 (micro-perforation). In 2016/17, the treatments were refined to only micro-perforated packaging, as these had performed better during the previous season. Therefore, treatments in 2016/17 were: HDPE Shrivel sheet (Control), HDPE Micro-perforation, LDPE 92 (micro-perforation), LDPE 72 (micro-perforation) and LDPE 4 (micro-perforation). All bags were 30 µm thick. The

size of the LDPE bags was 1 m^2 and had an O_2 transmission rate (before perforation) of 7265 ml air·m^2.day at 23 ˚C and a CO_2 transmission rate of 217 955 ml air·m^2.day at 23 ˚C. The HDPE bag size was 0.12 m^2 and the shrivel sheets were 0.4 m^2.

Fruit were packed by inserting the bag/sheet into the carton (38.5 x 30 x 10.5 cm open-top) and then inserting two layers of fruit, packed in count-30 pulp trays. In the case of the shrivel sheets, they were neatly folded over the fruit after packing and a corrugated paper sheet was placed on top to prevent the sheets from moving or opening during cooling.

Fruit maturity was determined upon arrival at the Department of Horticultural Science by performing the following measurements on 10 fruit per replicate. Hue angle (h°) was determined with a Minolta CR-400 Chroma meter (Konica Minolta, Japan), on both cheeks of 10 fruit per treatment per replicate. H° was calculated from the coordinates obtained from the chroma meter (arctangent of b^*/a^*), where 0° = red, 90° = yellow, 180° = green and 270° = blue (McGuire, 1992). Flesh firmness (N) was determined on both paired cheeks of 10 fruit per treatment per replicate, using an electronic fruit texture analyser (GÜSS GS-20, Strand, South Africa) fitted with an 11 mm tip. Total soluble solids (TSS) were measured by pooling and juicing wedges of 10 fruit per replicate, with three replicates in total, measuring the % Brix using a digital refractometer (Palette PR-32 ATAGO, Bellevue, USA). Titratable acidity (TA), expressed as the percentage malic acid present, was measured using 10 g of the pooled juice sample which was then titrated with 0.1 M NaOH to an end-point of pH 8.2, using a 719 S Titrino automated titrator, fitted with a Metrohm AG 760 sample changer (Herisau, Switzerland). All the fruit except for one (±29 fruit) from the bottom tray of three replicates per treatment were weighed on a balance accurate to 0.001g (XB 320M, Precisa Instruments Ltd., Switzerland) upon arrival and again after the shelf life period, to determine mass loss (%) during the storage period. Atmospheric RH and temperature were recorded inside the carton using a HygrochronTM iButton (CST electronics, Sandton), stuck to the inside of the treatment bag or shrivel sheet,

using double sided tape. Pulp temperature was recorded by inserting a Thermocron® iButton into the one un-weighed fruit per tray.

'Sapphire', 'Laetitia' and 'Songold' were stored according to commercial intermittent warming regimes as summarised in Fig. 1 (PPECB, 2017). 'African Delight™' was stored at a commercially used single-temperature regime of 56 days at -0.5 °C. For all cultivars, cold-storage was followed by a simulated shelf life of 7 days at 10 °C. All plastic bags were removed from the treatments after cold-storage, before the commencement of the simulated shelf life period.

2.2 Evaluation after cold-storage and shelf-life

After cold-storage and shelf-life, hue angle and flesh firmness were determined using the same methodology as described above. In addition, twenty fruit per replicate, per treatment, were inspected for shrivel (%) and decay (%), while the other 10 fruit in the tray were used to determine hue angle and flesh firmness. Shrivel was counted when shrivelled skin extended over the shoulder of the fruit.

Chilling injury (%) was determined for 20 fruit per replicate, per treatment, by cutting the fruit around the equatorial axis and separating the two halves of the fruit. A gelatinous breakdown (soft and translucent) of the inner mesocarp tissue surrounding the stone, while the outer mesocarp tissue had a healthy appearance, was classified as gel breakdown (GB) (Crisosto et al., 2004). A brown discolouration of the mesocarp tissue, associated with a loss in juiciness, was classified as internal browning (IB). To obtain the total chilling injury (CI), the sum of the percentage GB and IB per replicate per treatment was calculated.

Fruit were classified as overripe when abnormally soft to the touch, with excessive amounts of free juice and/or when cut around the equatorial axis, and the two halves of the fruit were twisted in opposite direction, the skin and sub-epidermal layers of the mesocarp separated from the inner mesocarp, which remained attached to the stone.

2.3 Respiration rate and ethylene evolution rate

Respiration rate and the rate of ethylene evolution was determined upon arrival at the laboratory, after cold-storage and after the simulated shelf life period.

Rate of ethylene (C_2H_4) evolution (µL kg^{-1} h^{-1}) and respiration rate ($mgCO_2$ kg^{-1} h^{-1}) was measured on two fruit per replicate, per treatment. The fruit were sealed in airtight 0.75 L glass jars for 1 h. A headspace gas sample was taken from each jar, with a 10 mL airtight syringe, and injected into a Varian GC system (Model 3300, Varian Instrument Group, Palo Alto, California, USA) fitted with a flame ionisation detector (FID) and 2 m PoropakQ column. The carrier gas was nitrogen. The oven temperature was programmed from 60 °C (isothermal for 2 min) to 70 °C, at 1 °C min^{-1}. The injector temperature was 65 °C and the detector temperature, 250 °C. A 1 mg/L standard gas was used to identify and quantify C_2H_4 and a 7.7 % standard gas was used to identify and quantify CO_2.

After cold-storage, before the treatment bags were removed, gas samples were taken from the headspace of the LDPE bags to determine whether the bags modified the atmosphere surrounding the fruit. A needle and syringe were used to obtain gas samples from the bags by introducing the needle into the package through a stick-on rubber septum (Ø15 mm grey Septum, Dansenor). The septum was used to prevent tearing of the bag and atmospheric air from entering the bag through the needle hole (Fig. 2).

2.3 Water vapour transfer rates of packaging

To determine the potential of fruit moisture loss through the different plastic bags, the permeability of the bags to water loss was determined using a modification of the Modified Cup Method (Bourtoom and Chinnan, 2008; Moyls, 1998). Plastic containers (5 replicates per treatment) with “mouth” diameter of 74 mm were filled with distilled water to a final mass of 500 g, close to the rim of the container. Instead of covering the mouth of the container with a piece of each plastic bag, one container per replicate was placed in a plum carton, lined with the treatment bag, to simulate the conditions of fruit in a carton. The bags were folded over the water-filled containers according to general packaging practice and a white corrugated paper sheet was placed on top according to standard practice when packaging fruit. The cartons were then placed in cold-storage at -0.5 °C for 1 week, after which they were weighed again. Bag permeability was expressed as mass loss across the area of the bag per day.

2.4 Statistical analysis

A mixed model repeated measures analysis of variance (ANOVA) was performed for h° and flesh firmness, determined on the three evaluation dates (at harvest, after cold-storage, and after shelf life). For the analysis of shrivel, decay and chilling injury incidence, a mixed model repeated measures ANOVA was performed over the two evaluation dates (after cold-storage and after shelf life). The effect of treatment on fruit mass loss was expressed as means with standard error, since only three replicates were used. ANOVA generated p-values and the significant differences between means were determined using Fisher's least significant differences (LSD) test with a 95 % confidence interval.

3. Results

3.1 Laetitia

Maturity indexing performed shortly after harvest (data not shown) indicated that the TSS (Brix %) was 13.62 ± 0.85 (SE), the TA (%) was 1.44 ± 0.08 and the firmness (N), was 66.6 ± 10.78 (2015/16). In 2016/17, TSS (Brix %) was 9.47 ± 0.47, TA (%) was 1.75 ± 0.04 and firmness was 53.9 ± 3.92. Standards for export are a min. TSS of 11 % and firmness between 83.3 – 39.2 N. Fruit sourced from the pack-house showed a high incidence of uneven ripeness in 2016/17.

In 2015/16, no significant differences were found between the packaging treatments for h°, fruit firmness and shrivel or respiration rate, while significant differences were observed between evaluation dates in all the parameters (Table 1). Both h° and fruit firmness declined over time. Fruit peel colour changed from an orange/red colour to a redder colour during storage and shelf-life. Shrivel incidence was only evaluated after cold storage and after the simulated shelf-life period and it increased significantly during shelf-life. No significant packaging treatment differences were observed for shrivel incidence, although the micro-perforated bag almost halved the incidence of shrivel compared to the shrivel sheet control, lowering it to below the 10 % cut-off maximum for export. The highest respiration rates were measured at harvest. Respiration rate then declined during cold storage and increased again after the simulated shelf-life period at 10 °C. No C_2H_4 was

detected in fruit at the optimal harvest date or after cold-storage, but only after shelf-life at 10 °C, but no significant treatment differences were observed. Moisture loss (%) over the total storage time of 56 days, was expressed as the average with SE. All treatments showed a higher percentage of fruit moisture loss compared to the control, though only the HDPE 54 x 2mm, HDPE 36 x 4mm and HDPE 60 x 5mm differed significantly. There was a significant interaction between treatment and cold storage duration for CI (Fig. 3). The incidence of chilling injury did not increase significantly in the control or the HDPE micro-perforated packaging treatment during the simulated shelf-life period at 10°C. However, there was a significant increase in chilling injury during the shelf-life period for the other packaging treatments.

In 2016/17, no significant packaging treatment differences were found for h˚, shrivel, CI, respiration rate or ethylene production (Table 2). Shrivel incidence was very low for all treatments, but a trend was observed that the LDPE-72 and LDPE-4 bags resulted in less shrivel than the control, albeit not significantly so. Fruit from the control were significantly firmer than HDPE micro-perforation and LDPE 72 packaged fruit and fruit packaged in HDPE micro-perforated bags the least firm, but not significantly less firm than LDPE 72 bagged fruit. The HDPE micro-perforated bags also resulted in the lowest respiration rate, albeit not significantly so.

Hue angle decreased during cold storage, but no further significant decrease occurred during shelf-life (Table 2). Firmness decreased during cold storage and shelf-life, while chilling injury increased after cold storage while fruit were exposed to 10 °C simulated shelf-life. Respiration rate increased from the harvest date until after the shelf-life period as the fruit ripened. Ethylene production did not differ between the harvest date and the end of the cold-storage period but increased significantly during the seven days shelf-life at 10 °C. Moisture loss per carton was significantly lower than the control in all treatments.

3.2 Songold

Maturity indexing performed shortly after harvest (data not shown) indicated that the TSS (Brix %) of the fruit was 13.90 ± 0.58, TA (%) was 1.13 ± 0.06 and firmness

(N) was 53.9 ± 2.9 (2015/16). Standards for export are a min. TSS of 11 % and firmness between 88.2 – 49.0 N.

No significant packaging treatment differences were observed for h°, flesh firmness, shrivel, CI, respiration rate or ethylene production, while significant differences were observed between evaluation dates in all the parameters (Table 3). Hue angle and flesh firmness decreased over time. Fruit peel colour changed from green to light yellow during storage and shelf-life. Shrivel and CI were only quantified after cold-storage and after the simulated shelf-life period at 10°C and both increased significantly during shelf-life.

Although no significant treatment differences were seen for shrivel incidence, the LDPE 72 bags almost halved shrivel percentage compared to the control. The respiration rate of 'Songold' followed the same trend as 'Laetitia'. The highest values were seen at harvest, respiration rate then declined significantly during cold-storage and increased during the shelf-life period at 10 °C. Ethylene was not detected at harvest or after cold-storage. However, C_2H_4 production occurred during the shelf-life period, but no significant treatment differences were observed. Moisture loss was significantly lower in the LDPE 72, LDPE 92, and HDPE 36 x 4mm bags.

3.3 African Delight™

Maturity indexing (data not shown) performed shortly after the harvest date in 2015/16 (data not shown) indicated that the TSS (Brix %) of the fruit was 16.23 ± 0.59, TA (%) was 1.13 ± 0.12 and firmness (N) was 73.5 ± 5.9. In 2016/2017 TSS was 15.55 ± 0.62, TA was 1.10 ± 0.13 and firmness was 76.4 ± 4.9. Standards for export are min. TSS of 15% and firmness between 88.2 – 53.9 N.

No significant differences were observed between packaging treatments for h° or flesh firmness in 2015/2016 (Table 4). Shrivel, decay and CI incidence was only quantified after the cold-storage and shelf-life period. Significant differences in shrivel incidence were observed between packaging treatments, the LDPE bags having the lowest incidence. No decay or CI was observed on either of the evaluation dates. Due to technical problems with the GC-FID, respiration and C_2H_4

could not be quantified. Hue angle decreased significantly during cold storage at -0.5 °C and then increased significantly during the shelf-life period. Flesh firmness declined significantly between each evaluation date. Moisture loss was significantly lower in all packaging treatments compared to the control.

In 2016/17 there were no significant packaging treatment differences between shrivel, respiration rate, or ethylene production (Table 5). However, firmness, shrivel and ethylene production differed significantly between storage durations. Firmness and shrivel decreased during storage and shelf-life while ethylene production increased during this period. Moisture loss was higher than in the previous season and significantly lower in the LDPE-72 and LDPE-4 bags, compared to the other treatments. There was a significant interaction between treatment and storage duration for h° (Fig. 4). At harvest, differences occurred between the treatments. This was likely due to mixed maturity within cartons, which can be difficult to avoid. Hue angle declined significantly during cold storage at -0.5 °C in all packaging treatments. No further significant changes occurred in h° during shelf-life. The decline in h° during storage and shelf-life was less in the LDPE 4 bags compared to the other treatments.

3.4 Sapphire

Maturity indexing performed shortly after the harvest date (data not shown) in 2016/17 indicated that the TSS (Brix %) of the fruit was 10.0 ± 0.5, TA (%) was 1.5 ± 0.01 and firmness (N) was 48.0 ± 6.9. Standards for export are min. TSS of 11% and firmness between 93.1 – 49 N.

No significant packaging treatment differences were found for flesh firmness, but storage duration had a significant effect (Table 6). Flesh firmness declined significantly during the cold storage and shelf-life period. A significant treatment effect was observed for CI and the LDPE 4 bags had significantly more CI than any of the treatments and control. Chilling injury also increased significantly during shelf-life at 10 °C. Due to technical problems with the GC-FID, respiration rate and ethylene could not be measured. Moisture loss was significantly higher in the control. There was a significant interaction between storage duration and treatment on h˚ (Fig. 5). At harvest, fruit designated to be packed in the HDPE micro-

perforated bags had a significantly higher hue angle compared to the other treatments. This must have been due to sampling error and mixed maturity in the cartons because no treatments had been applied at that time. All packaging treatments, except LDPE 4 showed a decline in h˚ between harvest and the end of cold storage. No further significant decrease in h˚ occurred during shelf-life. After cold-storage and shelf-life, there were no significant differences between treatments. A significant interaction was also found between treatment and storage duration for shrivel (Fig. 6). With the HDPE shrivel sheet (control), shrivel incidence increased dramatically during shelf-life, while all the other treatments remained the same.

3.5 Bag permeability

There were no statistical differences in permeability to water vapour between the treatment bags (Table 1). However, the LDPE bags tended to have lower moisture loss compared to the control and HDPE micro-perforated bags in all cultivars evaluated.

4. Discussion

Quality of plums and other stone fruit rapidly deteriorate after harvest. Since South African plums take between three to eight weeks to reach overseas markets by sea freight, this short post-harvest life can have a large economic impact. Some of these quality problems include softening, dehydration and decay (Cantin et al., 2008). To extend post-harvest shelf life, a high-density polyethylene shrivel sheet or other type of perforated bag is currently included in plum cartons, but moisture loss and fruit shrivelling remain a problem in the South African stone fruit industry. Better packaging solutions are required to maintain fruit quality, decrease moisture loss and shrivelling, and limit decay. However, there is a trade-off between increasing the RH inside the bag to such an extent that moisture loss is reduced, while keeping it low enough to discourage decay. This study set out to determine whether micro-perforated LDPE bags could serve as an alternative to the HDPE shrivel sheets and/or micro-perforated HDPE bags currently in use to reduce moisture loss in plums.

The packaging treatments had no effect on fruit peel colour. The changes in h° values during cold-storage, observed for all cultivars, were expected and agreed with the findings of other plum studies (Abdi et al., 1997; Jooste, 2012; Singh et al., 2009), as fruit colour changes during ripening (Chen and Ramaswamy, 2002). At higher temperatures, such as those experienced during shelf-life at 10°C, colour changes associated with fruit ripening tend to happen faster, explaining why h° differed significantly between storage durations.

During fruit ripening, ethylene affects the enzymes involved in the degradation of cell walls, and there is a direct relationship between ethylene concentration and fruit softening (Pretel et al., 1993). Since ethylene concentrations did not differ between treatments, it explains why fruit firmness also did not differ between treatments. The decrease in flesh firmness during storage was expected as loss of fruit firmness is associated with the ripening process (Cantin et al., 2008; Díaz-Mula et al., 2011; Khan and Singh, 2007). All fruit were considered to be at the same maturity level before being repacked into different treatment bags. The changes in ethylene production and respiration rate during the cold storage and shelf-life period were expected. Ethylene production declines substantially at low temperatures (Zamorano et al., 1994). Fruit are harvested while still in the pre-climacteric phase, when the steep rise in ethylene production has not yet occurred. Ethylene levels then remain low during cold-storage and only increase after being moved to the simulated shelf life conditions, where temperatures are higher. Temperature and respiration rate are directly linked (Akbudak and Eris, 2004). Warm fruit, taken from the field at harvest, have a higher respiration rate compared to fruit stored at lower temperatures (Wang and Qi, 1997). The respiration rate of climacteric fruit follows a general pattern during fruit ripening (Zamorano et al., 1994). During the climacteric, respiration rate increases, and then decreases again. Low temperatures during cold-storage, lower the rate of respiration.

In 2015/16 shrivel incidence was above the cut-off 10 % maximum level in ‘Laetitia’ (Table 1). Although not significant, the HDPE micro-perforated bags decreased shrivel incidence by almost 50 % compared to the HDPE shrivel sheets used commercially, lowering it to less than 10 %. In 2016/17, shrivel incidence in

‘Laetitia’ was very low (Table 2). The LDPE bags lowered shrivel incidence when compared to the shrivel sheets (not significantly), but since shrivel was very low overall, using these bags would not have a significant impact on export during this season.

Although not significant, the LDPE- 4 bag had more than double the percentage of CI compared to the control in 2016/17 (Table 2). The LDPE 72 bags decreased shrivel by more than 50 % in ‘Songold’ but did not have a substantial effect on CI compared to ‘Laetitia’ (Table 3). In 2016/17, the LDPE bags lowered shrivel incidence by 25 % in ‘African Delight™’, albeit not significantly. No CI was observed with any of the treatments. In ‘Sapphire’, the use of micro-perforated bags had no effect on shrivel incidence after cold-storage, compared to the control (Fig. 4). Shrivel was below 2 % in all treatments but increased above 10 % in the HDPE shrivel sheets during shelf-life. The LDPE-4 bags had a negative effect on CI in ‘Sapphire’ as well, increasing it by 10 % more than the HDPE shrivel sheets (Table 6). This corroborates findings by Crouch (1998) that ‘Laetitia’ packed in micro-perforated bags (160 or 210 micro-perforations), had a lower shrivel incidence and less mass loss compared to fruit packed in macro-perforated or no bags. The use of micro-perforated LDPE bags have also shown positive results in ‘Angeleno’ plums (Kayna et al., 2009). These bags delayed fruit softening, prevented flesh browning and internal breakdown, as well as mass loss up to 90 days of storage. Our study shows that simply using a shrivel sheet in a carton does not reduce moisture loss as much as using perforated bags do. In seasons where higher moisture loss is experienced, or in cultivars that are more prone to moisture loss, this difference could lead to consignments being rejected due to excessive mass loss. For all cultivars, LDPE-72 seemed to perform the best, though not always significant. Similarly, in grapes, mass loss is significantly higher when carton liners with macro-perforations are used, compared to micro-perforated and non-perforated liners, because these liners are unable to maintain the RH above 95 % (Ngcobo et al., 2012).

Smaller perforations in a package increases the RH and CO_2 inside the package, due to a restriction of gas exchange (Macnish et al., 1997). At higher RH, the rate

of mass loss is significantly lower than at lower RHs. The rate of gas movement through a perforated film is a sum of gas diffusion through the perforation and gas permeation through the polymeric film. Generally, total gas flow through the perforations is much greater than gas movement through the film.

The increase in CI observed with the LDPE-4 bags might have been due to the build-up of high CO_2 concentrations, due to the lower gas permeability of bags with fewer perforations (Cantin et al., 2008; Ding et al., 2002). The use of LDPE-4 bags would therefore not be a viable option to reduce moisture loss while still retaining fruit quality. CO_2 levels higher than 4 % during cold-storage periods longer than 45 days, may enhance the development of CI symptoms, especially in cultivars with a high ripening rate and short market life (Cantin et al., 2008). In both 'Sapphire' and 'Laetitia', some of the replicates of LDPE-72 and LDPE-4 had CO_2 concentrations above 4 % (data not shown).

5. Conclusions

Post-harvest moisture loss in Japanese plums remains a significant problem. Optimized packaging solutions need to be found for individual cultivars since there can be a lot of variance between cultivars in terms of susceptibility to moisture loss and shrivel. This study showed that packaging solutions that are currently used, do not reduce moisture loss and shrivelling effectively. Under ideal conditions, the use of LDPE-92 and LDPE-72 micro-perforated bags may be a viable option to reduce moisture loss, while still maintaining fruit quality by preventing excessive in-package humidity, decay and chilling injury. However, this will have to be tested on a commercial level to verify the results. In seasons when high rates of moisture loss are experienced, the use of these bags can reduce the amount of consignments rejected at overseas markets, thus preventing significant financial losses. However, in seasons with low moisture loss, the use of these bags may provide no additional benefit. Currently, there is no reliable way to predict what the incidence of moisture loss and/or shrivel will be in a particular season. Further research needs to be done to evaluate the economic feasibility of using these micro-perforated bags, because they are more expensive compared to the shrivel sheets currently used.

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Table 1

Quality and maturity measurements for ‘Laetitia’ plums as measured shortly after harvest in 2015/16, after 49 days of dual-temperature storage and a 7-day simulated shelf-life period at 10˚C. Ethylene production was only detectible after shelf-life. Moisture loss shown as mean with standard error

Treatment*	Hue angle (h°)	Firmness (N)	Shrivel (%)	Respiration rate ($mgCO_2$/ kg/h)	Ethylene (µL/kg/h)	Moisture loss (%)
HDPE Shrivel sheet (Control)	34.62 ns	41.85 ns	16.25 ns	20.96 ns	5.31 ns	4.2± 1.90
HDPE 54 x 2	33.33	39.69	18.75	18.12	3.14	7.7± 0.07
HDPE 36 x 4	32.33	37.44	11.33	21.06	4.33	7.4± 0.20
HDPE 60 x 5	32.27	40.87	10.83	20.29	4.17	8.1± 0.13
HDPE 48 x 4	31.62	42.04	11.25	23.63	3.84	6.5± 1.23
HDPE Micro-perforation	31.37	42.92	8.33	18.93	4.60	5.3± 1.36
Storage duration (days)						
0	41.84 c	67.13 c	-	27.65 c	-	
0 + 49	29.62 b	33.61 b	1.83 a	12.22 a	-	
0 + 49 + 7	26.31 a	21.66 a	23.75 b	21.76 b	-	
Pr>F						
Treatment	0.424	0.176	0.106	0.372	0.613	
Storage duration	<0.001	<0.001	<0.001	<0.001		
Treatment x Storage	0.056	0.132	0.197	0.968		

Significant differences are indicated by lower case letters.

*Numbers next to treatment bags indicate the number of perforations x size (mm) of perforation

Table 2

Quality and maturity measurements for ‘Laetitia’ plums as measured shortly after harvest in 2016/17, after 49 days of dual-temperature cold-storage and a 7-day simulated shelf-life period at 10˚C. Ethylene production was only measurable after shelf life. Moisture loss shown as mean with standard error.

Treatment *	Hue angle (h°)	Firmness (N)	Shrivel (%)	CI (%)	Respiration rate ($mgCO_2/kg/h$)	Ethylene (µL/kg/h)	Moisture loss (%)
HDPE Shrivel sheet (Control)	31.65 ns	34.64 b	3.54 ns	5.02 ns	49.38 ns	5.63 ns	1.88± 0.06
HDPE Micro-perforation	28.84	30.34 c	1.71	8.45	41.76	1.62	1.15± 0.03
LDPE 92	28.56	32.95 ab	1.96	4.77	52.84	1.84	0.82± 0.05
LDPE 72	29.51	31.35 ac	0.96	4.56	45.43	0.17	0.80± 0.04
LDPE 4	32.41	33.54 ab	0.67	12.90	50.83	3.28	0.88± 0.39
Storage duration							
0	43.01 b	53.84 c	-	-	32.40 a	0.32 a	
0 + 49	24.33 a	24.04 b	1.14 ns	1.08 a	49.75 b	0.31 a	
0 + 49 + 7	23.07 a	19.80 a	2.39	13.20 b	63.53 c	6.91 b	
Pr>F							
Treatment	0.183	0.014	0.259	0.153	0.657	0.208	
Storage duration	<0.001	<0.001	0.137	<0.001	<0.001	<0.001	
Treatment x Storage	0.0500	0.081	0.188	0.096	0.818	0.129	

Significant differences are indicated by lower case letters.

*Numbers next to treatment bags indicate the number of micro-perforations

Table 3

Quality and maturity measurements for ‘Songold’ plums as measured shortly after harvest in 2015/16, after 42 days of dual-temperature cold-storage and a 7-day simulated shelf-life period at 10˚C. Ethylene production was only measurable after shelf life. Moisture loss shown as mean with standard error.

Treatment *	Hue angle (h°)	Firmness (N)	Shrivel (%)	CI (%)	Respiration rate ($mgCO_2/kg/h$)	Ethylene (µL/kg/h)	Moisture loss (%)
HDPE Shrivel sheet (Control)	98.63 ns	44.10 ns	8.66 ns	5.83 ns	14.07 ns	2.31 ns	10.80 ± 0.46
HDPE 54 x 2	101.59	42.92	7.08	4.58	13.76	1.32	9.19 ± 0.32
HDPE 36 x 4	99.23	42.34	7.78	4.42	14.68	1.23	6.62 ± 0.18
HDPE 60 x 5	100.24	42.92	6.92	7.5	12.89	2.10	10.94 ± 0.67
HDPE 48 x 4	99.92	43.90	9.80	9.58	11.63	1.30	9.34 ± 0.44
HDPE Micro-perforation	99.25	43.90	9.08	10.83	9.91	3.15	9.39 ± 1.24
LDPE 92	101.10	42.73	9.18	9.17	12.37	2.00	4.87 ± 4.72
LDPE 72	100.95	43.02	3.78	4.58	17.10	0.87	6.45 ± 2.57
Storage duration							
0	108.67 c	53.61 c	-	-	20.02 c		
0 + 42	97.74 b	42.04 b	5.67 a	0.104 a	6.25 a		
0 + 42 + 7	93.87 a	32.14 a	9.90 b	15.52 b	13.63 b		
Pr>F							
Treatment	0.533	0.323	0.322	0.233	0.148	0.139	
Storage duration	<0.001	<0.001	<0.001	<0.001	<0.001		
Treatment x Storage	0.906	0.175	0.268	0.260	0.160		

Significant differences are indicated by lower case letters.

*Numbers next to treatment bags indicate the number of perforations x size (mm) of perforation.

Table 4

Quality and maturity measurements for 'African Delight™' plums as measured shortly after harvest, after 56 days of single-temperature cold-storage at -0.5˚C and a 7-day simulated shelf-life period at 10˚C, in 2015/16. Moisture loss shown as mean with standard error.

Treatment *	Hue angle (h°)	Firmness (N)	Shrivel (%)	Moisture loss (%)
HDPE Shrivel sheet (Control)	77.18 ns	64.876 ns	30.00 b	2.96 ± 0.03
HDPE 54 x 2	78.24	64.092	11.25 a	1.59 ± 0.09
HDPE 36 x 4	77.77	61.446	11.67 a	1.38 ± 0.07
HDPE 60 x 5	78.96	61.544	27.92 b	1.86 ± 0.17
HDPE 48 x 4	79.05	66.248	8.33 a	1.71 ± 0.12
HDPE Micro-perforation	78.51	64.386	4.17 a	1.25 ± 0.05
LDPE 92	80.76	67.718	5.00 a	1.12 ± 0.03
LDPE 72	78.61	65.66	2.08 a	1.02 ± 0.05
Storage duration				
0	32.48 b	7.47 b	-	
0 + 56	26.74 a	6.16 a	9.69 a	
0 + 56 +7	176.68 c	6.11 c	15.42 b	
Pr>F				
Treatment	0.405	0.095	<0.001	
Storage duration	<0.001	<0.001	<0.001	
Treatment x Storage duration	0.178	0.117	0.378	

Significant differences are indicated by lower case letters.

*Numbers next to treatment bags indicate the number of perforations x size (mm) of perforation.

Table 5

Quality and maturity measurements for 'African Delight™' plums as measured shortly after harvest, after 56 days of single-temperature cold-storage at -0.5˚C and a 7-day simulated shelf-life period at 10˚C in 2016/17. No levels were detected on the harvest date, so ethylene and respiration were only analysed after cold-storage and after shelf-life. Moisture loss shown as mean with standard error.

Treatment *	Firmness (N)	Shrivel (%)	Respiration rate (mgCO2/kg/h)	Ethylene (µL/kg/h)	Moisture loss (%)
HDPE Shrivel sheet (control)	67.72 ns	23.61 ns	38.52 ns	1.58 ns	4.20 ± .08
HDPE Micro-perforation	64.97	15.83	33.59	1.00	4.30 ± 0.14
LDPE 92	62.13	16.38	43.11	0.84	4.91 ± 0.75
LDPE 72	33.03	18.33	41.38	0.40	3.79 ± 0.09
LDPE 4	62.72	16.11	32.82	0.31	3.86 ± 0.16
Storage duration					
0	76.83 b				
0 + 56	57.53 a	22.44 b	43.05 ns	0.27 a	
0 + 56 + 7	57.62 a	13.67 a	32.72	1.37 b	
Pr>F					
Treatment	0.15	0.27	0.881	0.378	
Storage duration	<0.001	0.007	0.157	0.003	
Treatment x Storage duration	0.47	0.944	0.317	0.702	

*Numbers next to treatment bags indicate the number of micro-perforations

Table 6

Quality and maturity measurements for ‘Sapphire’ plums as measured shortly after harvest, after 56 days of single-temperature cold-storage and a 7-day simulated shelf-life period at -0.5˚C in 2016/17. No levels were detected on the harvest date, so ethylene and respiration was only analysed after cold-storage and after shelf-life. Moisture loss shown as mean with standard error.

Treatment *	Firmness (N)	CI (%)	Moisture loss (%)
HDPE Shrivel sheet (control)	32.24 ns	34.25 a	3.61 ± 0.25
HDPE Micro-perforation	33.91	30.52 a	1.31 ± 0.06
LDPE 92	29.20	32.76 a	0.93 ± 0.12
LDPE 72	28.32	30.90 a	1.07 ± 0.09
LDPE 4	32.44	49.39 b	0.97 ± 0.08
Storage duration			
0	47.92 c		
0 + 42	24.60 b	26.69 a	
0 + 42 + 7	21.17 a	44.44 b	
Pr>F			
Treatment	0.067	0.043	
Storage duration	<0.001	<0.001	
Treatment x Storage duration	0.214	0.280	

*Numbers next to treatment bags indicate the number of micro-perforations

Table 7

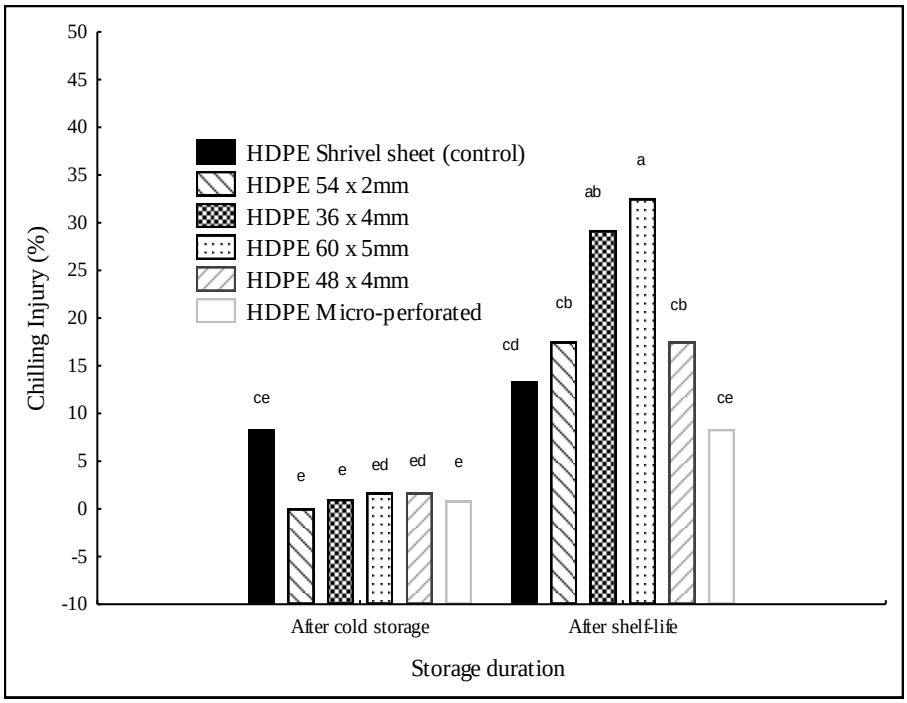
Water vapour transmission rate of the control and HDPE and LDPE treatment bags used in 2016/17.

Treatment *	Permeability (mg H_2O/day/m^2)
HDPE Shrivel sheet (control)	1.625 ns
HDPE Micro-perforation	1.016
LDPE 92	0.682
LDPE 72	0.535
LDPE 4	0.385
Pr > F	0.1166

*Numbers next to treatment bags indicate the number of micro-perforations

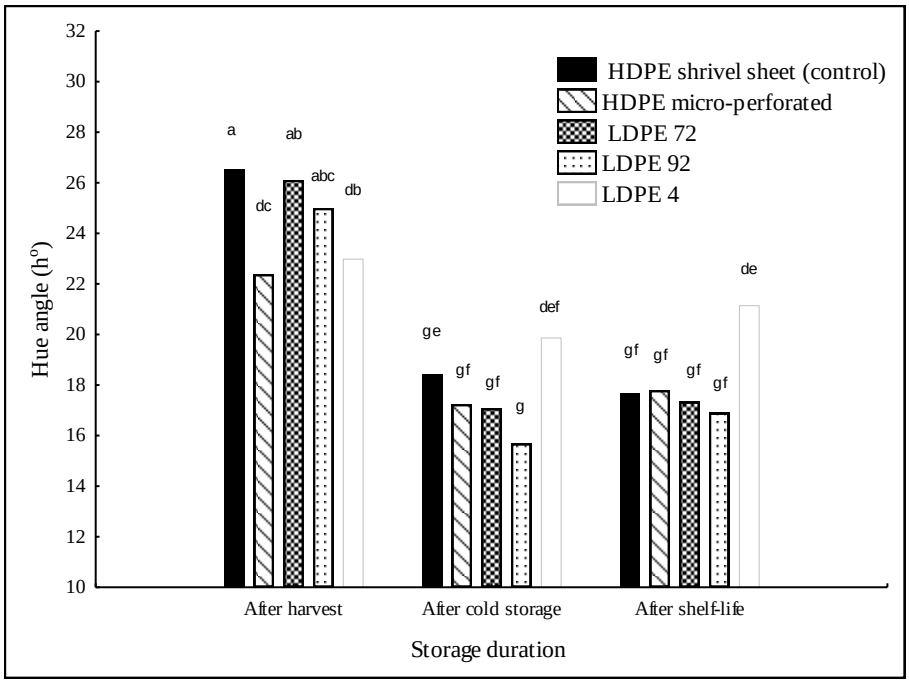
Fig. 1. Summary of dual-temperature storage regimes at which individual cultivars were stored.

Fig. 2. The stick-on rubber septum applied to the treatment bags to obtain gas samples from the headspace of the LDPE bags.



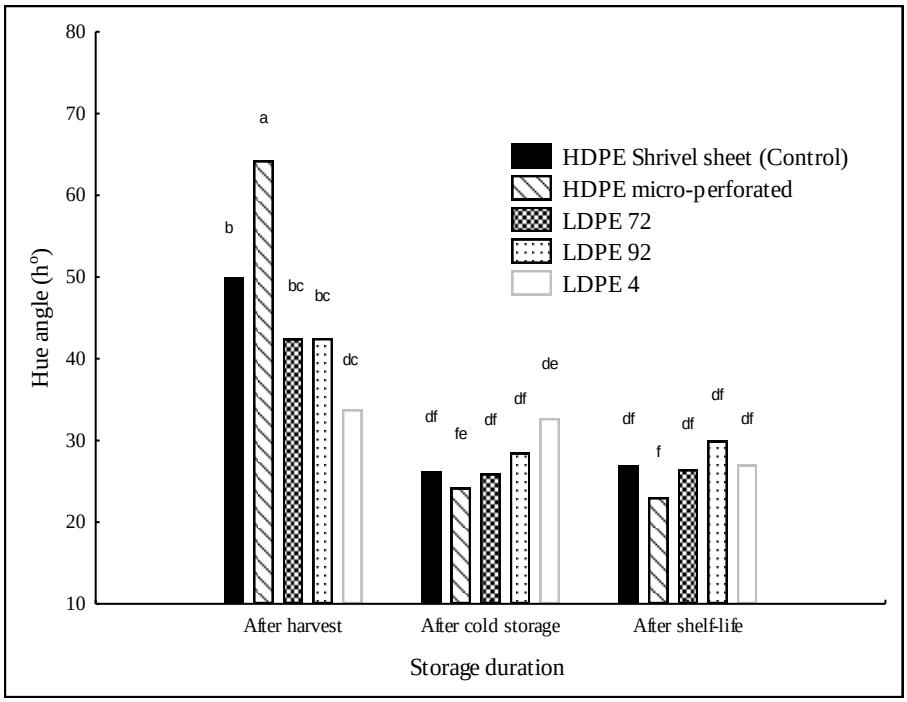
Effect	F	P
Treatment	1.55	0.2050
Storage duration	86.36	< 0.0001
Treatment x storage duration	5.19	0.0015

Figure 3. Interaction between treatment and storage duration on chilling injury of ‘Laetitia’ in 2015/16. Significant differences are indicated in lowercase letters.



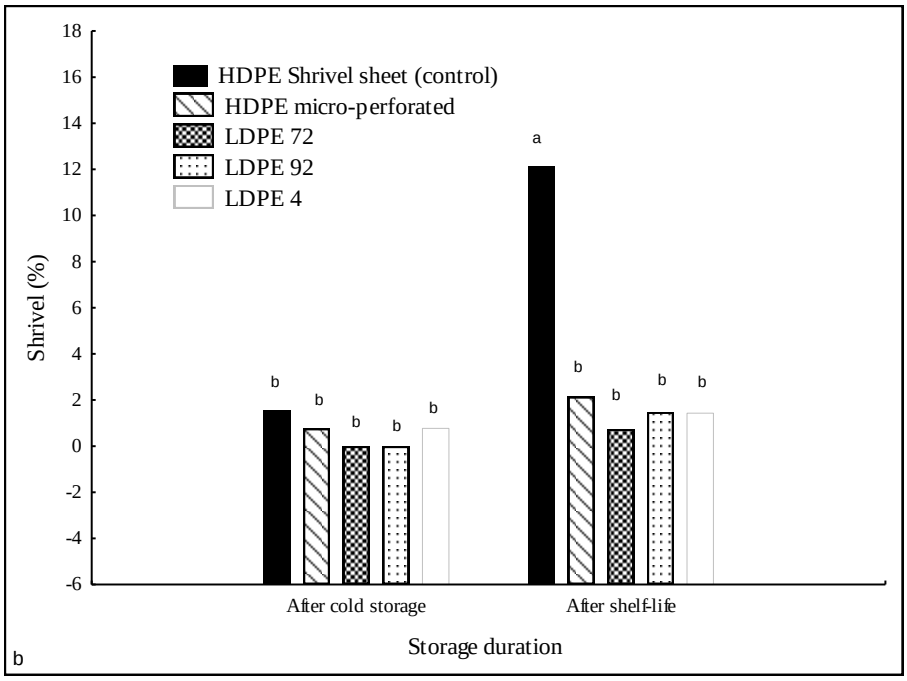
Effect	F	P
Treatment	1.92	0.1463
Storage duration	59.29	< 0.0001
Treatment x storage duration	2.33	0.0373

Figure 4. Interaction between treatment and storage duration on the hue angle of ‘African Delight™’ in 2016/17. Significant differences are indicated in lowercase letters.



Effect	F	P
Treatment	1.60	0.2129
Storage duration	57.10	< 0.0001
Treatment x storage duration	5.84	0.0001

Figure 5. Interaction between treatment and storage duration on the hue angle of ‘Sapphire’ in 2016/17. Significant differences are indicated in lowercase letters.



Effect	F	p
Treatment	6.92	0.0011
Storage duration	11.43	0.0030
Treatment x storage duration	4.74	0.0075

Figure 6. Interaction between treatment and storage duration on shrivel of ‘Sapphire’ in 2016/17. Significant differences are indicated in lowercase letters.

GENERAL DISCUSSION AND CONCLUSIONS

Plums exported from South Africa have to travel to overseas markets by ship, which entails a relatively long sea freight period. Yet consumers still expect fruit to be in perfect condition on the supermarket shelves. Even though great care is taken to limit moisture loss throughout the handling chain, fruit still show the negative effects thereof. Mass loss, textural changes, wilting, shrivel and reduced glossiness are all symptoms of moisture loss (Mahajan et al., 2008; Sastry, 1985a, 1985b; Wilson et al., 1995).This reduction in fruit quality leads to rejection of export consignments at overseas markets, causing major financial losses for South African stone fruit producers.

Some fruit or even cultivars of the same fruit, are more prone to moisture loss than others. This can be due to internal factors such as the surface area to fresh mass ratio of the fruit, (Díaz-Pérez et al., 2007; Konarska, 2013), cuticle composition and the presence of open stomata, lenticels, cracks or wounds (Sastry, 1985a) which all determine the water vapour permeance (P_{H2O}) of the peel.

The driving force of postharvest moisture loss is determined by the vapour pressure deficit (VPD) between the fruit and the environment (Mitchell and Crisosto, 1995). VPD is controlled by the temperature and relative humidity (RH) of the air surrounding the fruit, the temperature of the fruit and velocity of the air moving over the fruit. Water vapour diffuses along the concentration gradient from the area of high vapour pressure in the intercellular spaces to the surrounding atmosphere which is at a lower vapour pressure. The fruit peel and cuticle are the main barriers against moisture loss (Dietz et al., 1985; Konarska, 2013; Wills et al., 1989). This is especially important after harvest, when the fruit do not receive additional moisture from the tree to replace moisture lost to the atmosphere.

The aim of Paper 1 was to determine whether the peel water vapour permeance of Japanese plums is an indicator of susceptibility to postharvest shrivelling. Peel permeability differed between farms, seasons, cultivars, orchards and developmental stage. Apple cultivars with higher peel water vapour permeance, are more prone to shrivel development during storage (Maguire et al., 2000) and it was expected that plums would behave similarly. In general, the P_{H20} of the cultivars Angeleno, Fortune

and Songold, not prone to shrivel remained lower than the other cultivars. The cultivars that are prone to shrivel had higher $P_{H20.}$ However, 'Laetitia' and 'African Delight™' (shrivel prone) performed like the cultivars that are not prone to shrivel development. The lack of a strong relationship between peel water vapour permeance shows that moisture loss during storage is not the only factor that causes shrivel. Measuring pre-harvest P_{H2O} to predict whether a cultivar is shrivel-prone is only successful in some cultivars.

The next step was to investigate whether differences in the number of lenticels in the fruit determine peel permeability. 'Songold' had significantly more open lenticels and higher peel permeability compared to 'Laetitia' in 2015/16 and 2016/17, which was unexpected, since 'Laetitia' is considered more prone to postharvest moisture loss and shrivelling. Lenticel numbers differed significantly between seasons and cultivars, confirming previous findings in apple (Turketti et al., 2012). The lenticels clearly contribute to moisture loss, but this contribution is higher in 'Songold' and 'Sapphire' where significant positive relationships between peel water vapour permeance and the number of open lenticels were seen on some sampling dates. Since the number of open lenticels could not explain all the variation in peel permeability between cultivars, cuticle composition must play an important role in determining peel permeability.

In Paper 3, cuticle composition was compared between 'Laetitia' and 'Songold'. Wax and cutin amounts differed between seasons, cultivars and developmental stage. The extent to which postharvest modification can take place seems to depend on the availability of wax and cutin precursors, though this requires further investigation. One of the main wax components in 'Laetitia' and 'Songold' was the secondary alcohol, nonacosan-10-ol. According to Barthlott et al., (1998), this compound is not generally present in cuticular waxes, but when it does occur, it occurs in high concentrations, as shown in our results. Although low concentrations of nonacosan-10-ol in apples indicates susceptibility of a cultivar to moisture loss, it did not explain the variation in shrivel incidence of Japanese plums.

Interestingly, a compound not before identified in plums, was present in high concentration in both cultivars, in the cutin and wax fraction, in both seasons. This compound, 2,4-bis (dimethyl benzyl) phenol has been identified in other fruit, including Brazilian cherries, Natsugumi fruit, the stems of castor plants and leaf extracts of bitter

melon (Lee et al., 2007; Malaman et al., 2011; Panlilio et al., 2012; Salem et al., 2017), where it is considered as an anti-oxidant. Still, literature on this compound and its function is limited and requires further investigation before conclusions about its role in Japanese plum cuticles can be drawn. However, we speculate that the content of this phenol increases in 'Laetitia' during cold storage in order to make the cuticle more rigid and thus prevent deformation and shrivelling due to excessive moisture loss.

In pepper cultivars, a negative relationship exists between moisture loss and primary alcohol content (Parsons et al., 2013). In our study, the relative concentration of primary alcohols increased significantly during cold storage in the season when shrivel incidence was low and remained constant but low during storage in the season when shrivel incidence was high. Thus, primary alcohol content at harvest may indicate potential shrivel incidence in Japanese plums. This possibility is worth further investigation and validation.

We hypothesise that, due to a combination of different components in the cutin and wax fractions, the cuticles of 'Laetitia' are more flexible compared to 'Songold'. 'Laetitia' has a higher tri-hydroxy acid content in its cutin fraction, which is known to increase cuticle elasticity (Marga et al., 2001). The flexibility of 'Laetitia' advances deformation and therefore the fruit may develop a shrivelled appearance if the hypodermal cells shrink due to moisture loss in storage. The rigid cuticles of 'Songold' at harvest may prevent deformation and consequent shrivelling, even though cuticle flexibility increases during cold storage. Cold storage leads to even higher flexibility of the cuticles, because water binds to the cutin matrix at high relative humidity, increasing its permeability and flexibility (Domínguez et al., 2011, 2009; Knoche and Peschel, 2006; Matas et al., 2005; Round et al., 2000). Thus, in 2017/18 when the amount of wax decreased during cold storage in both cultivars, their cuticles became more flexible. At low temperatures, the diffusion rate within the fruit tissue is reduced, while moisture loss from the surface continues (Nguyen et al., 2006; Sastry, 1985a). Water vapour lost from the epidermal cells during cold storage cannot be replaced by the underlying cells at the same rate, which leads to the dehydration and shrinkage of the hypodermal cells (Díaz-Pérez et al., 2007; Nguyen et al., 2006). These cells they eventually collapse (Fanta et al., 2014) and when the cuticle if flexible, it may deform, resulting in shrivel development.

The high concentrations of nonacosan-10-ol identified in the cuticular waxes of ‘Laetitia’ and ‘Songold’ were confirmed in Paper 4, when characteristic nonacosan-10-ol tubules were identified on scanning electron micrographs of the fruit cuticle.

A significantly higher number of hypodermal cells were identified in ‘Songold’ compared to ‘Laetitia’ and ‘African Delight™’, indicatingthat its intercellular spaces are much smaller in ‘Songold’ compared to the other cultivars. As moisture loss is driven by the vapour pressure deficit between the intercellular spaces in fruit and the surrounding atmosphere (Lara et al., 2014; Maguire et al., 2001; Wills et al., 1989), the smaller intercellular spaces of ‘Songold’ may slow down moisture loss and dehydration of the hypodermal cells, explaining why ‘Songold’ is less prone to moisture loss than the other cultivars.

The combination of a rigid cuticle, due to high phenol content, fewer tri-hydroxy acids, and high primary alcohol content, and the smaller intercellular spaces reduces the chances of ‘Songold’ cuticles to deform due to excessive postharvest moisture loss. Since the hypodermal cells of ‘Songold’ are closer together, their dehydration and collapse do not result in substantial shrinkage observed in the other cultivars. The cuticle is also more rigid, which means that it is less likely to collapse when the supporting cells underneath it shrink and collapse due to moisture loss. These findings indicate why ‘Songold’ is less prone to postharvest shrivel development compared to ‘Laetitia’ and ‘African Delight™’.

This study showed the complex interplay of different cuticle characteristics in response to or as a result of, moisture loss. It would be interesting to investigate how environmental signals lead to a certain cuticular response – which genes are involved, how are these genes activated and so forth. Elucidating some of the mechanisms involved in the functioning and response of this complex biopolymer might enable manipulation of the cuticle to improve fruit quality and extend shelf life or to select and breed cultivars that are not prone to cuticular defects.

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APPENDIX A

Poster 11

VII INTERNATIONAL CONFERENCE ON MANAGING QUALITY IN CHAINS (MQUIC)

Evaluation of perforated bags for reduction of post-harvest moisture loss and shrivelling in Japanese plums

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1. Introduction

- South African plums spend up to eight weeks in shipping to reach overseas markets.
- Results in significant weight loss and shrivelled fruit
- Consignments can be rejected, or require costly repacking, leading to significant financial losses
- HDPE and LDPE bags with different numbers of macro- and micro-perforations were evaluated for reduction of moisture loss and shrivelling in 'Laetitia', 'Sapphire' and 'African Delight' plums.

2. Materials & Methods

- Fruit collected from a commercial pack-house at harvest and repacked into treatment bags (Table 1)
- Commercial cold storage & shelf-life
- Evaluated for moisture loss, shrivel and internal quality

Table 1: Summary of treatments

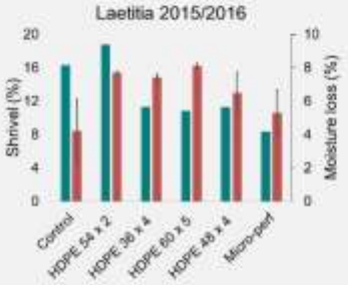
Treatment	
HDPE Shrivel sheet (Control)	Season 1 & 2
HDPE 54 x 2 mm	Season 1
HDPE 36 x 4 mm	Season 1
HDPE 60 x 5 mm	Season 1
HDPE 48 x 4 mm	Season 1
HDPE Micro-perforation	Season 1 & 2
LDPE 92 Micro-perforation	Season 1 & 2
LDPE 72 Micro-perforation	Season 1 & 2
LDPE 4 Micro-perforation	Season 1 & 2

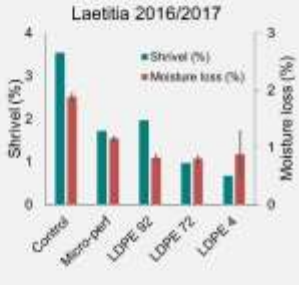
3. Results

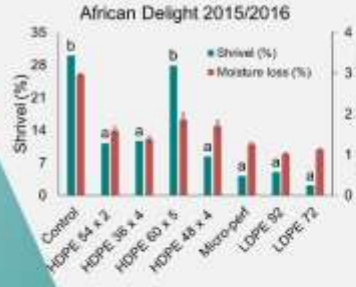
- Treatment differences in shrivel incidence and moisture loss (± s.e) of each cultivar in the two seasons.
- Sapphire showed no shrivel after storage - graph only shows shrivel and moisture loss after shelf-life.
- All other graphs show a combination of storage duration.

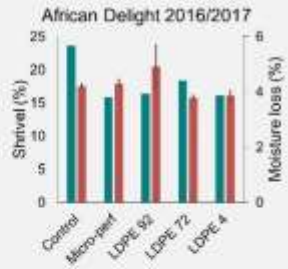
Table 2: Significant differences in shrivel incidence between storage dates

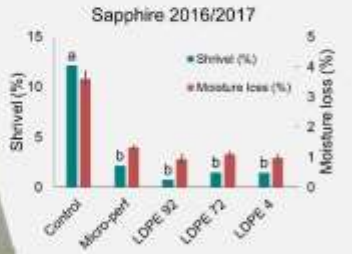
Cultivar	Season	Storage	Shelf-life	P-value
Laetitia	2015/2016	1.83a	23.75b	<0.001
	2016/2017	1.14	2.39	0.137
African Delight	2015/2016	9.69a	15.42b	<0.001
	2016/2017	22.44b	13.67a	<0.001
Sapphire	2016/2017	0.61	1.45	-











4. Conclusions

- Optimized packaging solutions need to be found for individual cultivars, since cultivars vary in terms of susceptibility to moisture loss and shrivel.
- Currently used packaging do not reduce moisture loss and shrivelling optimally.
- The use of LDPE-92 and LDPE-72 micro-perforated bags is effective at reducing moisture loss and shrivel while still maintaining fruit quality.
- LDPE-4 bags lead to a high incidence of chilling injury

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Shrivelled African Delight plum versus un-shrivelled plums