CSIRO PUBLISHING

Functional Plant Biology, 2020, **47**, 1019–1031
https://doi.org/10.1071/FP19135

Review

A review of current knowledge about the formation of native peridermal exocarp in fruit

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Abstract. The outer skin layer in any plant is essential in offering a protective barrier against water loss and pathogen attack. Within fleshy fruit, the skin supports internal cell layers and can provide the initial cues in attracting seed-dispersing animals. The skin of a fruit, termed the exocarp, is a key element of consumer preference and a target for many breeding programs. Across fruiting species there is a huge diversity of exocarp types and these range from a simple single living cell layer (epidermis) often covered with a waxy layer, to complex multicellular suberised and dead cell layers (periderm), with various intermediate russet forms in between. Each exocarp can be interspersed with other structures such as hairs or spines. The epidermis has been well characterised and remains pluripotent with the help of the cells immediately under the epidermis. The periderm, in contrast, is the result of secondary meristematic activity, which replaces the epidermal layers, and is not well characterised in fruits. In this review we explore the structure, composition and mechanisms that control the development of a periderm type fruit exocarp. We draw upon literature from non-fleshy fruit species that form periderm tissue, from which a considerable amount of research has been undertaken.

Additional keywords: exocarp, fruit skin, periderm.

Received 21 May 2019, accepted 29 May 2020, published online 23 June 2020

Fleshy fruit skin types

The epidermal layer in any terrestrial plant is essential in providing a protective barrier against water loss and pathogen attack. In fleshy fruit, there exists a continuum of exocarp types ranging from an epidermal exocarp (e.g. tomato, epidermal type kiwifruit: Fig. 1*a*) through various degrees of russeting, to being completely covered with a native peridermal exocarp (e.g. completely russeted pear, peridermal type kiwifruit: Fig. 1*b*). Both native and russet periderms have highly suberised cell layers that collapse and die to create a protective barrier of suberised phellem, and are treated as points on a continuum in this review. Although peridermal exocarps protect fruit against external stresses such as pathogens and environmental conditions, they may alter permeability of water vapour. For example, the periderm directly beneath the micro cracked primary fruit exocarp has a higher rate of water loss compared with live exocarp covered with cuticle (Khanal *et al*. 2019).

The main difference between native and intermediate sporadically russeted periderms is that a native periderm appears to be developmentally programmed, and covers the entire organ surface whereas sporadic russet periderms can occur at any stage of development. The intermediate russet periderm types also produce tissue that is structurally more disorganised and callus like (Schreiber *et al*. 2005), and can vary greatly in morphology. Russet periderm can arise after wound healing as well as after formation of certain lenticel types found in apple, pear and cherry. Research in citrus has generally been focussed on scab formation in direct response to pathogens such as *Elsinoë fawcettii*, whereby peridermal layers restrict hyphae growth (Kim *et al*. 2004); however, russeting can occur on citrus fruit. In the fruit of *Actinidia* species (kiwifruit) there is a wide variation of exocarp type and for most readers kiwifruit invokes the typical dead cell type of structure (green and yellow fleshed varieties), although epidermal exocarp ‘Kiwiberry’ types are becoming more

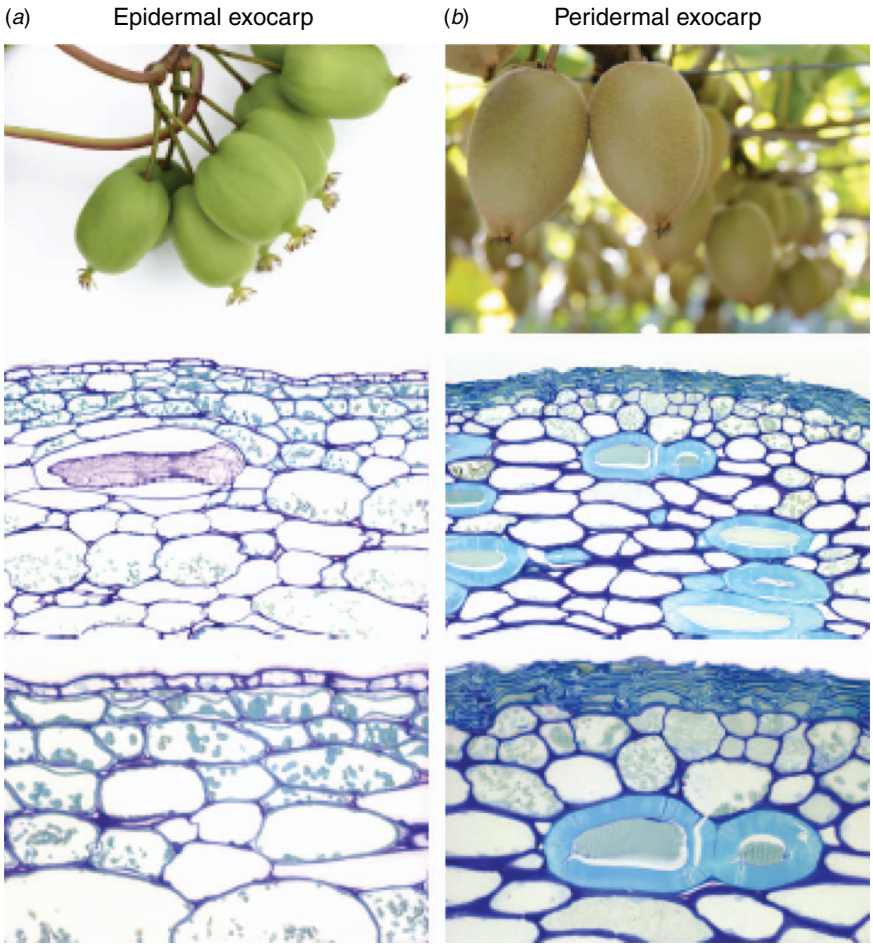

Fig. 1. Whole fruit from two different species of kiwifruit. (*A*) *Actinidia arguta* showing live (epidermal) skin. (*b*) *A. chinensis* with peridermal (dead skin). Corresponding brightfield microscopy images of sectioned fruit skin stained with toluidine blue are displayed beneath. Bottom images are magnified portions of the corresponding middle image.

widely available in different markets. Within the germplasm and breeding populations, we also observe genotypes with differing propensities to form sporadic russet periderms: an example being differing sensitivities to wind rub induced epidermal damage. At the opposite end of the exocarp continuum, epidermal exocarps typically consist of a simpler structure (Figs 1*a*, 2*a*) and generally retain much of their structure throughout development- although they are dynamic metabolically (Lara *et al*. 2019).

In the case of peridermal kiwifruit, the ovaries start out with a live epidermis but within a few weeks following fruit set, phellem, phellogen and phelloderm cell layers become apparent underneath it. Later these layers and the epidermis suberise, and the phellem and epidermis appear to undergo programmed cell death (PCD) and collapse to form the peridermal exocarp seen on mature fruit (Figs 1*b*, 2*b*). The death of the epidermis can occur only in mature non-expanding fruit because fruit exocarp regulates fruit expansion (Thompson *et al*. 1998).

The exocarp can also be interspersed with structures including hairs and spines (trichomes). It appears that hairiness and russet are unlinked because epidermal pericarp type genotypes can have hairs and different peridermal genotypes vary in their degree of hairiness and types of trichomes present (simple uniseriate and complex multiseriate) (Hallett and Sutherland 2005).

This review ignores details for some of the intermediate skin types possible, for example varying degree of lignification, netted periderm formation in melon, or the rind of citrus fruit, to focus on the peridermal skin type. For the remainder of this review we will focus on peridermal exocarp formation. Currently there is little literature on native periderm exocarp formation; most work has focussed on russet. However, there is considerable knowledge in non-fruit tissue types, as a native periderm develops to form the bark of gymnosperms and eudicotyledons and on subterranean organs such as potato tubers (*Solanum tuberosum* L.). Here we shall draw upon this work, together with the work on russet, because it is likely that the formation of these structures is similarly controlled.

Periderm formation

Commonly, the periderm is a tissue layer that protects vasculature against biotic and abiotic stress (Fischer *et al*.

2019). The periderm comprises an outer layer of cork cells, also known as phellem, that arise through the periclinal division of cork meristem phellogen, with the inner growing cells termed phelloderm. Periderm formation involves significant rearrangement of the subepidermal cells leading to a functional cork meristem. The corky layer produced is distinctive due to its cuboid shaped cells (rectangular in profile), ranging from the traditional cells in the cork oak to the much more compressed cells in other examples such as kiwifruit. In thin-sectioned material, cork cells have thin walls and reduced cellular content (under light microscopy). These radially flattened cells lie outside on the phloem and inside the epidermis (Bernards 2002). The cork meristem is a secondary meristem, distinct from vascular cambium and much less documented. The pool of meristematic cells that generate periderm arise from the pericycle in most roots but in aerial tissue it derives from epidermis, hypodermis or phloem (Esau 1977). For a native periderm exocarp to form there are a series of developmental steps that need to occur (visualised using kiwifruit as an example in Fig. 2*b*). In summary, it begins with dedifferentiation of hypodermal cells and meristem induction, then the cork meristem creates new thin-walled cells, then these thinly layered cells are suberised and then undergo PCD and collapse to form the periderm. The

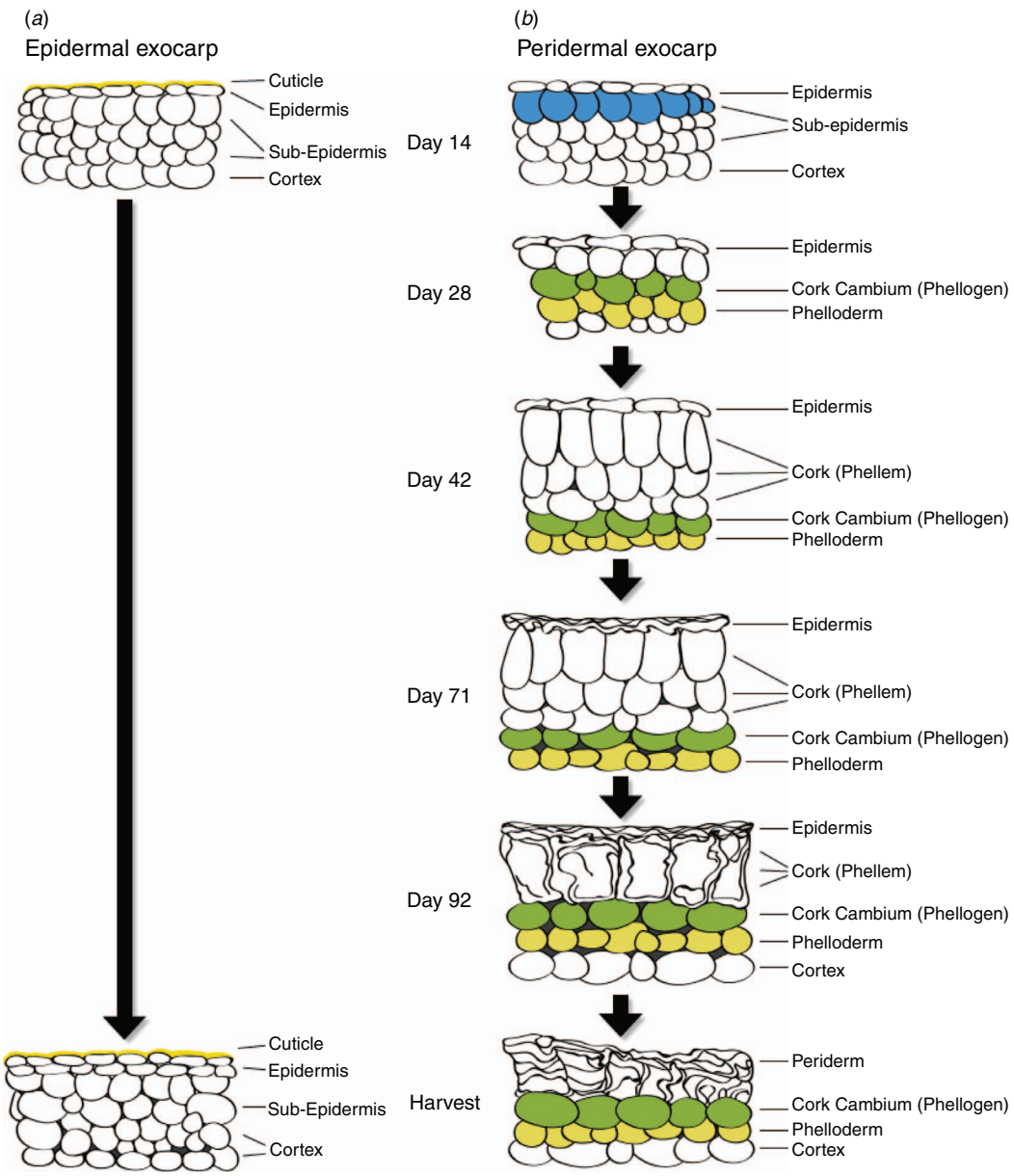

Fig. 2. A model for exocarp formation using kiwifruit as an example. (*a*) Epidermal exocarp maintains an epidermis throughout development. (*b*) Peridermal exocarp involves the formation of secondary cork meristem that goes through suberisation and programmed cell death to form mature periderm. (Based on, unpubl. data of kiwifruit (*Actinidia* sp.) R. Rebstock).

epidermal cells die during the process and the peridermis becomes the outermost protective layer. The resulting native peridermis consists of three main tissue layers: the phellem (cork), the phellogen (cork meristem) and the phelloderm; a supportive layer beneath the meristematic layers.

Dedifferentiation

The transition from a somatic cell to a meristematic cell is termed dedifferentiation, and involves the ability to re-enter the cell cycle, trans/re-differentiate or initiate PCD (Grafi 2004; Jiang *et al*. 2015). Lateral cell division begins within the outer pericarp through the development of the meristematic cork layer. In all meristems, the identity of stem cells and the subsequent developmental fate of their derivatives is determined primarily by positional cues rather than cell lineage (Laux 2003). The periderm that develops in kiwifruit exocarp has unresolved origins but features cell wall modifications and extensive cell proliferation (Hallett and Sutherland 2005). It appears to vary from that described for root and hypocotyl periderm in *Arabidopsis* where the endodermis undergoes PCD and then the epidermis and cortex detach leaving the periderm as the outermost layer (Wunderling *et al*. 2018). In order for a new cell layer to form under the epidermis, the underlying hypodermal cells must either be or become phellogen. If it is the latter then the cells must dedifferentiate, i.e. re-enter the cell cycle and modify chromatin, analogous to other processes that involve programmed cell death (Grafi 2004; Florentin *et al*. 2013; Grafi and Barak 2015). Chromatin (DNA packaged with histone proteins) is usually condensed but following stress events, the peri-centromeric regions in the chromosomes are known to decondense, allowing acquisition of meristematic cell properties before assuming a new fate (Florentin *et al*. 2013). Studies about dedifferentiation and vascular patterning have found similar factors are required to stimulate dedifferentiation, namely phytohormones (specifically auxins/ethylene/cytokinins), transcription factors and additional epigenetic signals (Tuominen *et al*. 1997; Mattsson *et al*. 2003; Li *et al*. 2011; Krogan *et al*. 2012; Zhang *et al*. 2014). As they do with vascular cells, it is possible that phytohormones also play a similar role in fruit hypodermal cell dedifferentiation.

Cork oak periderm (tree bark) was recently used as a model for traumatic wounding (referred to in this review as a russet periderm), and as the cork cells differentiated their chromatin condensed with a noticeable increase in DNA methylation. They also observed distinct gene expression between native and russet periderm which suggests there are specific genetic regulatory pathways to native and russet periderm formation (Inácio *et al*. 2018).

Meristem induction

Once a cell or group of cells have been dedifferentiated, further instructions are required before proceeding to form meristematic tissue. Primarily these instructions typically depend on the physical location of the cell, and then secondarily on chemical gradients that lead to changes in cell polarity and subsequent cell fates within the meristem. In the shoot apical meristem, transcription factor activities combine with auxin, cytokinins and small peptides to control proliferation and cell fate (Barton 2010). A study using 3D confocal imaging determined quiescent centre initiation within post embryonic roots required the AP2 domain gene *PLETHORA* (*PLT*), in addition to GRAS transcription factors SHORT-ROOT (*SHR*) and downstream SHR-regulated SCARECROW (*SCR*) (Goh *et al*. 2016). The PLETHORA genes pattern the *Arabidopsis* meristematic cell niche (Aida *et al*. 2004), and act as dose-dependent master regulators of root development (Galinha *et al*. 2007).

The SCARECROW peptide forms tissue specific higher-order transcription factor complexes with *SHR* being a key binding partner in relation to endodermal differentiation (Motte *et al*. 2019). The SHR peptide moves from the stele symplastically through plasmodesmata into the SCR expression domain, whereby it interacts with *WOX5*, *CYCD6*;*1* and *CASP1* (Long *et al*. 2015). The development and regulation of plasmodesmata is thus a breaking point for meristem induction, at least in *Arabidopsis* roots. The presence of iron leads to callose deposition at the plasmodesmata, functionally limiting the intercellular movement of small peptide signals including SHR, iron can furthermore induce ROS signalling within the elongation zone resulting in the stiffening of cell walls (Motte *et al*. 2019). Although these observations might not directly relate to fruit exocarp, it illustrates potential mechanisms that may also be occurring in fruit exocarp. For a periderm related example, transgenic hybrid aspen overexpressing *SHR*-like *PtSHRB* showed an overall reduction in growth and the proportion of bark increased relative to wood. In conjunction with increased bark growth there was a marked increase in cytokinin concentration within bark tissue compared with wood tissue (Miguel *et al*. 2016). This suggests that the *PtSHR2B* gene is a positive regulator of periderm development and it is possible that similar gene(s)/process could be at work in fruit.

The transition from stem cell to axillary meristematic tissue has been described as bi-phasic in the initiation of branching (Shi *et al*. 2016). During shoot branching in *Arabidopsis* the stem cell population was found to express *SHOOT MERISTEMLESS* (*STM*; a class 1 KNOX homeodomain containing transcription factor), which depends on local auxin minimum. In this model, *STM* expressed at low levels maintains stem cell competence whereas increased expression of *STM* marks the beginning of meristem initiation. Overexpression of maize *KNOTTED1* (*STM* orthologue) and *STM* causes formation of ectopic meristems (Sinha *et al*. 1993; Williams *et al*. 1997). In mature leaves, the homeodomain-leucine zipper protein REVOLUTA, which regulates meristematic initiation at lateral positions (Talbert *et al*. 1995) was found to bind STM only in leaf axil meristematic cells, subsequently causing epigenetic modifications (Shi *et al*. 2016). The highly conserved sequence of *STM* and its concurrence in meristematic tissue in many plants, suggest Class 1 KNOX factors, in combination with long distance signals, may initiate secondary meristematic cell fate, and furthermore the growth of secondary tissue layers and organs (Barton 2010). The *ARBORKNOX2* gene (*ARK1*; orthologous to *STM*) regulates cell differentiation during secondary growth (Du *et al*. 2009),

and was previously found expressed not only within the shoot apical meristem but also within the cambial zone (Groover 2005). Furthermore, ARK1 was found to bind to an array of evolutionarily conserved target genes of diverse function, similar to maize *KNOTTED1* (Liu *et al*. 2015).

In summary several papers report class 1 KNOX genes and phytohormones can induce secondary meristems, however only when auxin is at a minimum, thereby meristem induction during secondary growth is likely initiated by the degradation of auxin (Chae *et al*. 2012; Spartz *et al*. 2012; Spartz *et al*. 2014).

Cork cell formation (phellem) and secondary growth of periderm

The next stage of periderm formation is the formation and proliferation of cork cells. The formation of cork cells has conserved regulatory elements common to other axillary meristems and periderm formation generally is similar to the formation of secondary tissue in other plants. In *Arabidopsis*, polar auxin transport together with the transcriptional regulators AINTEGUMENTA, which regulates ovule development (Klucher *et al*. 1996), and REVOLUTA coordinate early gynoecium development (Nole-Wilson *et al*. 2010). The expression of *REVOLUTA* marks functional changes in cellular polarity that allow for growth in a lateral direction (Otsuga *et al*. 2001).

Once cell polarity is specified, another set of genes is needed to develop and proliferate the newly established meristematic layer. Cork cells as described previously are distinctive based on their size and shape, and radial cell division. It is unknown what genes are required for this proliferation in fruit tissues, but in potato tubers, periderm formation occurs during normal development. Genes identified with potato tuber periderm development have a general association with the organs protective function, the secondary cell wall and stress response (Vulavala *et al*. 2019). A potentially relevant class of proteins named 'no apical meristem/cup shaded cotyledon' (NAM/CUC), are highly conserved and are essential for the establishment and function of boundaries. These genes are 'NAC' transcription factors (characterised by a conserved 'NAC' DNA binding domain, ~160 amino acids in length) that are able to specify shoot organ boundaries (Hibara *et al*. 2006).

Secondary meristems grow laterally through cell division and expansion and phytohormones such as auxin, ethylene, cytokinins and gibberellins influence these processes. Phytohormones and transcription factors often have conserved functions, with key genes consistently active in meristematic regions. Auxin is the major shoot signal that regulates vascular differentiation and is common to secondary growth causing developmental changes via auxin response factors (Mattsson *et al*. 2003). The polar flow of auxin such as indole 3 acetic acid (IAA) has been visualised along epidermis-phellogen, like the flow in the procambium-cambium (Aloni 2013). In hybrid Aspen (*Populus tremulua* L. × *Populus tremuloides* Michx) there is a radial distribution of IAA across the developing tissues of the cambial region in the stem and the concentration of IAA was found to be at peak level within the cambium zone and is thought to be a positional cue during xylem development (Tuominen *et al*. 1997).

Auxin directly affects cell expansion by inducing short-lived SMALL AUXIN UP RNA (SAUR; SAUR19-24 subfamily) proteins which then activate plasma membrane H^+ ATPases by promoting phosphorylation of the *C*-terminal auto inhibitory domain (Spartz *et al*. 2014). H^+ ATPases promote proton efflux to acidify the apoplast and facilitate the uptake of solutes and water to drive plant cell expansion (Chae *et al*. 2012; Spartz *et al*. 2012, 2014). SAUR proteins were also found to interact with the H^+ ATPases inhibitor PP2C-D subfamily of type 2C protein phosphatases, which are inhibited upon SAUR binding (Spartz *et al*. 2014; Wong *et al*. 2019).

Suberisation of the phellem layer

Cutin and suberin are two non-carbohydrate cell wall biopolymers (lignin is the other) that share a high degree of compositional similarity, being polyesters of predominantly 16 and 18 carbon fatty acid monomers, as well as sharing biosynthetic and regulatory genes. Suberin is the core component of natural and russet periderms, in addition to other internal barrier layers. It is deposited in wounded plant surfaces or under normal growth within organs such as endodermis, bark, potato tubers and seed coats (Lashbrooke *et al*. 2016). The process of suberisation involves deposition of polymeric material between the plasma membrane and cell wall and within the cell wall itself. In many cases, suberin lamellae appear within the cell walls facing towards the plasma membrane. In heavily suberised cells, the entire wall may have a lamellar appearance (Kolattukudy 1984). The deposition of suberin first requires the biosynthesis of aliphatic, phenolic and glycerol monomers which are then transported to the cell wall in order to form an insoluble macromolecular assembly (Vishwanath *et al*. 2015). Many of the building blocks are shared with cutin but suberin is distinct from cutin in that it is an assembly of two polymeric domains; one polyphenolic and the other poly aliphatic (Bernards 2002). Another distinction is that cutin is deposited on the outside of the polysaccharide cell wall of epidermal cells (Fig. 3), whereas suberin is mostly deposited on the inner face of primary walls of internal cell layers (Fig. 4) (Beisson *et al*. 2012; Fich *et al*. 2016). In addition to the difference in spacial deposition, suberin has more hydroxy cinnamic acids (predominantly ferulate (phenolic)), generally more polyhydroxy α,ω-dicarboxylic acids (DCAs; aliphatic), less polyhydroxy-fatty acids (aliphatic), more glycerol conjugates (aliphatic) and very-long-chain aliphatics (≥ C20) (Franke *et al*. 2005; Pollard *et al*. 2008). The combination of DCAs with glycerol can provide cross-linking, rendering the suberised layer rigid and insoluble (Pollard *et al*. 2008). Reverse genetic studies have linked both ferulic acid and aliphatic suberin to be important for the water barrier function and correct periderm maturation (Serra *et al*. 2010; Serra *et al*. 2009*a*, 2009*b*). Natural variation in these components could therefore have important fruit post-harvest implications in terms of fruit quality and storability. Lignin is another component in peridermal exocarp structure and can occur in combination with suberin or on its own.

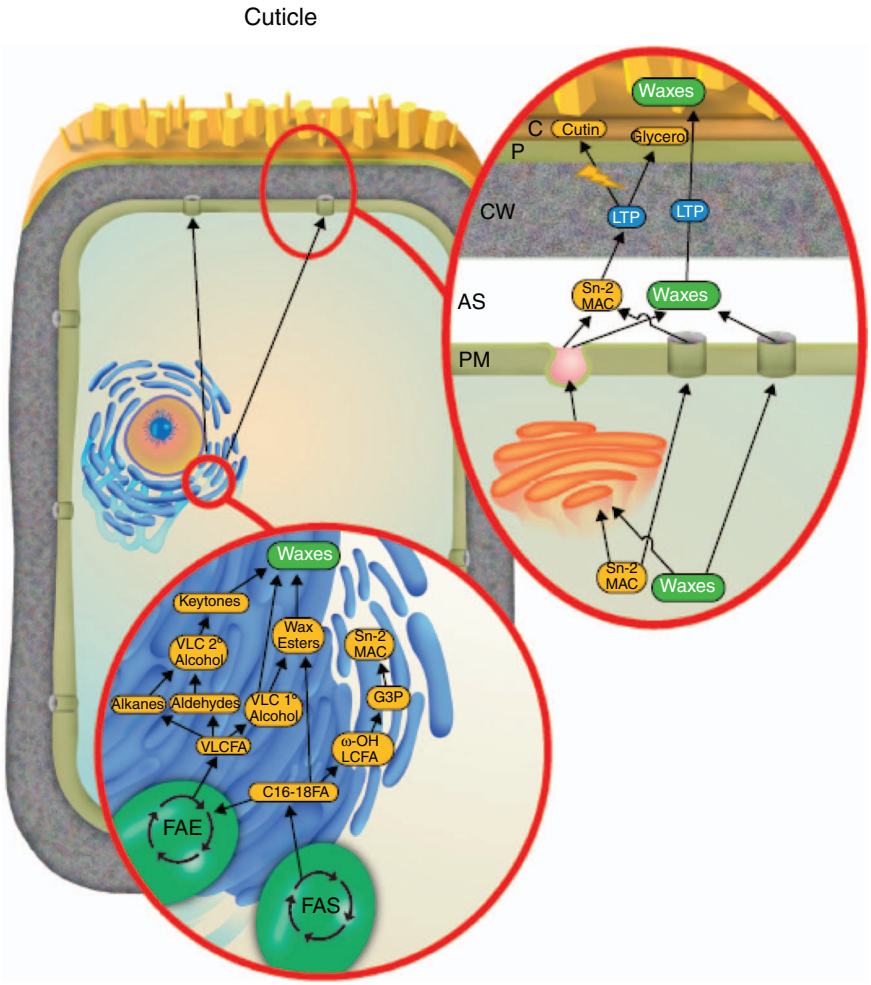

Fig. 3. Cuticle synthesis begins with fatty acids (FA) produced by plastids (green) via the fatty acid synthase (FAS) cycle. The FAs are then transported to the endoplasmic reticulum where they may be lengthened by the fatty acid elongation (FAE) cycle or retained as C16-18 fatty acids. FAs and very long chain fatty acids (VLCFAs) are also converted to other products involving various processes within the endoplasmic reticulum. The generation of cutin monomers and wax components occur on two distinct pathways. Export through the plasma membrane (PM) could occur through transport by ABC transporters (green channel) or by secretion via vesicles, generally produced by the Golgi apparatus (orange organelle). Cutin monomers are polymerised outside the PM before embedding and interlocking with pectin (P) with help from lipid transfer proteins (LTPs) in the cell wall (CW). Wax components are delivered to various locations including the cuticle proper (epicuticular wax) and intracellular wax within the cutin matrix. Abbreviations: LCFA, long chain fatty acid; DCA, α,ω-dicarboxylic acid; MAG, monoacylglycerol; G3P, glycerol-3-phosphate.

To date, the number of suberin synthesis genes identified is relatively few for a presumably complex process and include: β-ketoacyl-CoA synthases, fatty acyl reductases, long-chain acyl-CoA synthetases, cytochrome P450 monooxygenases, glycerol 3-phosphate acyltransferases and phenolic acyl-transferases (Ranathunge and Schreiber 2011; Beisson Li-Beisson and Pollard 2012).

The compositional continuum concept: the case of suberin and cutin

The classification of cutin and suberin into separate classes could be misleading. Instead, is has been suggested that they are different types of a single compositional continuum (Fich *et al*. 2016). The same authors also speculated, 'that suberin arose as a result of ectopic expression of a master regulator of cutin biosynthesis that proved to be beneficial'. This idea of a 'compositional continuum' is extendable to the exocarp as a whole. In a segregating population of kiwifruit (*Actinidia* spp.; smooth exocarp × peridermal exocarp backcrossed to peridermal exocarp; populations bred by Ron Beatson, unpubl. data, Plant and Food Research Ltd), the spectrum of phenotypes observed lends support to this continuum hypothesis. Within this segregating population, phenotypes range from heavily suberised to cuticle dominant, with large variation in the degree of lignification and russet.

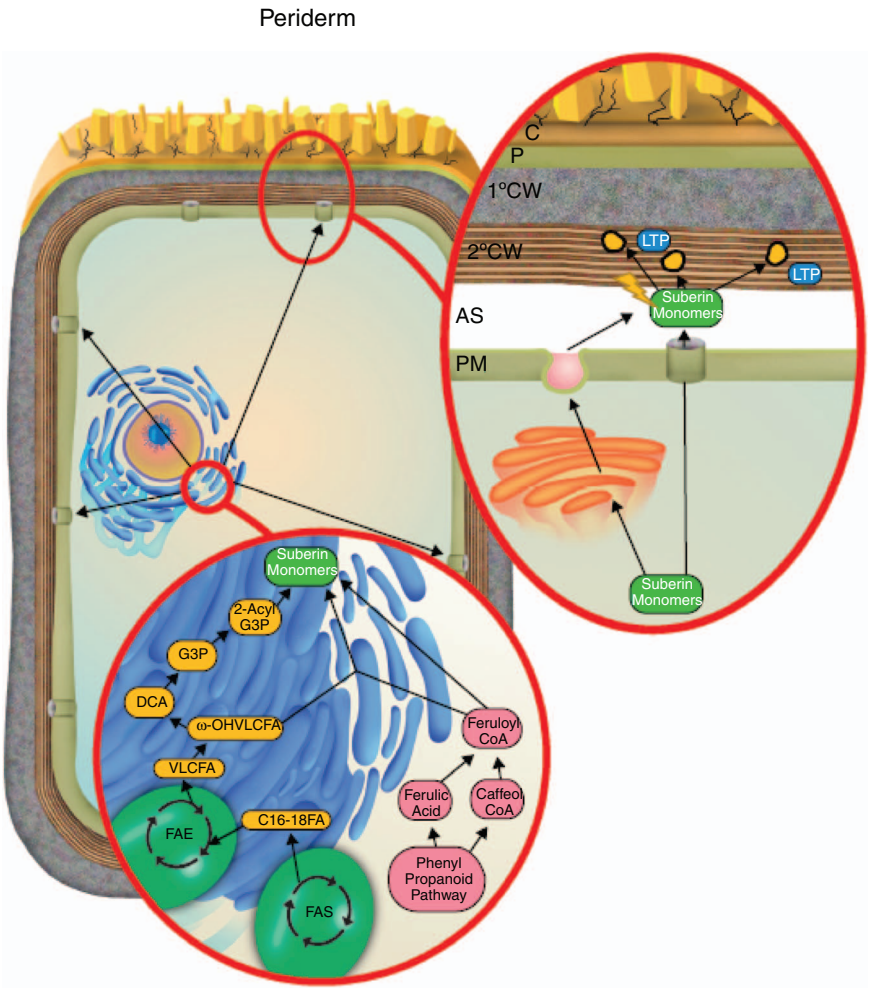

Fig. 4. Suberin synthesis begins with fatty acids produced by plastids (green circle) via the fatty acid synthase (FAS) cycle. The fatty acids (FA) are then transported to the endoplasmic reticulum where they may be lengthened by fatty acid elongation (FAE) cycle or retained as C16-18 FA. FA and very long chain fatty acids (VLCFAs) are also converted to other products involving various processes within the endoplasmic reticulum with additional components from the phenylpropanoid pathway. Suberin monomers are exported through the plasma membrane (PM), via ABC transporters (green membrane), lipid transfers proteins (LTP) or by secretion via vesicles, generally produced by the Golgi apparatus (orange organelle). Suberin monomers are polymerised in the apoplastic space before being embedded into the primary cell wall as suberin lamellae (brown alternating layers) guided by enzymes (yellow and black circles). Abbreviations: LCFA, long chain fatty acid; DCA, α,ω-dicarboxylic acid; G3P, glycerol-3-phosphate; CW, cell wall; P, Pectin.

Programmed cell death (PCD) and compression of periderm

The systematic senescence of outer pericarp layers of fruit has many unresolved questions. Although the studies of native systems such as kiwifruit and melon are few in number, there is evidence that many cell fate and programmed cell death genes are conserved. A recent paper has provided an elegant framework for the study of periderm formation using *Arabidopsis* root and hypocotyl (Wunderling *et al*. 2018). The study demonstrated that periderm formation in *Arabidopsis* roots and hypocotyls share many characteristics and features with woody and tuberous periderms and defined six stages of periderm development. Stage 1 is the anticlinal division of the pericycle and flattening of endodermal cells. Stage 2 is characterised by reduction in endodermal cells and pericycle proliferation (now classed as phellogen). Stages 3 and 4 are where 'the cortex and the epidermis break and the periderm is the outmost tissue'. At stage 5 'the endodermis is no longer present and a ring of phellem cells is visible' and at stage six the periderm is mature and is the outside tissue (see fig 2 in Wunderling *et al*. 2018). Endodermal PCD was preceded by a reduction in cell length and suberin deposition and the first sign of PCD was observed in some endodermal cells at stage 2 and PCD marker expression was

observed in endodermal cells from stage 1 (the inner cortex in hypocotyls also underwent PCD).

Aside from the differences in meristem origin and cortex and epidermal detachment, periderm formation in kiwifruit exocarp appears generally analogous to *Arabidopsis* roots and hypocotyls (Fig. 2). The result for *Arabidopsis* periderm is cell death as it is with kiwifruit peridermal exocarp. PCD must be a component of exocarp periderm formation but the onset of PCD in peridermal exocarp is less defined based on available information. In kiwifruit peridermal exocarp, as with *Arabidopsis* root and hypocotyl potato tuber skin and tree bark, PCD is involved in maturation of the periderm although as the work in *Arabidopsis* highlighted PCD associated processes happen at earlier stages and that endodermal PCD was a gradual event (Wunderling *et al*. 2018). The final maturation process is the collapse of the cork layers to form a compressed external barrier (Fig. 1*b*).

Intermediate exocarp structures: russet formation

In general, a (sporadic) russet is considered a defect, and is considered a major problem by many fleshy fruit breeders (Tafolla-Arellano *et al*. 2018). Environmental variation plays a role in triggering sporadic russet formation, for example, russet occurrence was observed to be highest at the bottom of valleys compared with valley tops (Faust and Shear 1972). Russeting has also been observed in the response to certain chemical sprays (Sánchez *et al*. 2001). A plants propensity to russet has unresolved factors but it is thought that it is linked to mechanical failure derived from excessive growth strain during early fruit development (Maguire 1998), and/or from surface moisture over an extended period (Knoche and Grimm 2008). It is generally considered to be produced in response to fine cuticular cracks (microcracks) (Faust and Shear 1972).

The exocarp of each fruit type and genotypes within fruit types are different, and although some fruit like tomato continuously synthesise a thick waxy cuticle, other fruit exocarp types are prone to surface strain and microcracks. Apples (*Malus* × *domestica*) and pears (*Pyrus communis* L.) may undergo russeting, whereby microscopic cracks form in the cuticle, leading to periderm formation (Khanal *et al*. 2013*a*). There are overlying patterns of microcracking on the cuticle in apple (and likely fleshy fruit generally), whereby microcracks generally follow the outlines of major ridges (encloses two to four epidermal cells) (Knoche *et al*. 2018). Microcracks in the cuticle are usually aligned with the anticlinal walls of the underlying cells, resulting from increased skin strain in those areas (Knoche *et al*. 2018). Furthermore, increased skin strain is associated with increased skin transpiration, such that high strain in the skin causing irreversible changes. Hence, although russet formation has genetic origins, it can often be highly influenced by the environment. A study of sweet cherry suggests water uptake from the roots may cause microcracks by raising internal fruit pressure (as opposed to common view of rain induced cracking) (Measham *et al*. 2010). This supports recent findings that russet periderm increases water loss from aerial plant surfaces, when they cannot otherwise release the water (Khanal *et al*. 2019). This abiotic factor is worth considering since many recent studies have concluded growth strain is the key determinant of microcracks, yet they have not delved into what causes that growth strain in the first place. Variation in exocarp formation impacts fruit development and has lasting consequences on postharvest potential, and cuticle composition appears to be a major factor (Lara *et al*. 2014). However, the component that is important remains unclear because a study of 22 apple cultivars having widely different russeting susceptibilities found there was no relationship between the mechanical properties of the cuticle at maturity and russet onset, suggesting the measured properties do not influence susceptibility to russet, or are relevant at other stages of development (Khanal *et al*. 2013*b*).

Russeting can be mitigated with hormone application

Russeting is a major issue faced by the horticultural industry because russeted fruit has reduced value (Faust and Shear 1972). Various treatments involving plant growth regulators (PGR) have been tested on fruit skin, traditionally with the aim of fruit thinning (Davis *et al*. 2004) but more recently to reduce russeting (Ginzberg and Stern 2016). Commonly tested PGRs include IAA and gibberellins (GAs), which stimulate cell and organ growth, and cytokinins that stimulate cell division. Fleshy fruits including tomato, pear, persimmon, apricot, grape, mandarin and kiwiberry had similar responses to PGR, including increased cell density, improved mechanical strength and as a result economically important reductions in russeting (Ginzberg and Stern 2016).

The application of GA_{4+7} + BA onto apple during early fruit development mitigated cracking by increasing epidermal cell density, in a way that does not alter developmental cues but instead seems to enhance them (Joshi *et al*. 2018). It was found that GA_{4+7} + BA application upregulated the transcription of genes previously reported to be associated with epidermal cell patterning and cuticle formation. The gene *MdSHN3*, previously linked with regulating the cutin and wax synthesis pathway in apple (Lashbrooke *et al*. 2015) was significantly upregulated, which could explain how the cracking resistance was achieved (Joshi *et al*. 2018).

Genes that regulate native periderm formation: clues from potato tuber and bark

The first genetic suberin mutants identified were *eld1* (elongation defective 1) (Cheng *et al*. 2000) and *GPAT5* (glycerol-3-P acyltransferase) (Beisson *et al*. 2007). Since then additional studies have revealed other genes including an acyl-CoA dependent acyltransferase (*At5 g41041*), BAHD genes (Gou *et al*. 2009) and the transcription factor *AtMYB41* (Kosma *et al*. 2014).

Genes involved in the formation of potato tuber periderm have been determined through RNA interference studies (Serra *et al*. 2009*a*, 2009*b*). Knocking out *CYP86A-33* (cytochrome P450) caused a 70–90% reduction of 18:1 ω-hydroxy acids, 60% less suberin and altered suberin lamellae which were 3–5 times more permeable (Serra *et al*. 2009*b*). Plants silenced for *StKCS6* (3-ketoacyl-CoA synthase) showed a reduction in the

composition of monomers with chain length >28, whereas C20 and C22 monomers had increased. The result was the deposition of a disorganised cell layer in tuber periderm and root epidermis, a 2-fold loss in tuber weight and a 1.5 times higher rate of transpiration (Serra *et al*. 2009*a*).

In potato, the transporter StABCG1 has been associated with suberin barrier formation through its function of exporting suberin components (Landgraf *et al*. 2014). *ABCG1* expression was localised to the plasma membrane and highly expressed in roots and tuber skin. Transgenic *ABCG1*-silenced (using RNAi) potato plants had unorganised tissue layers and reduced suberin staining, whereas the aerial parts of the plant were normal. The tubers from these silenced lines had a reduction in esterified suberin components, whereas putative suberin precursors hyperacculumated, resulting in twice as much water loss (Landgraf *et al*. 2014). Indeed an ABCG family transporter has been suggested as causal gene for the mapped major determinant locus (*Ru*) for apple russet development (Falginella *et al*. 2015).

Developing potato tubers were analysed with a range of visual techniques during tuber development (Boher *et al*. 2013). In potato tubers, suberin feruloyl transferase (*FHT*) is expressed specifically in phellogen cells, and in lines transformed with *FHT* promoter driving β-glucuronidase-green fluorescent protein fusions (GUS-GFP), it was found that FHT accumulates first near lenticels (Boher *et al*. 2013). Suberisation of potato tubers was shown to begin first at the basal end when tubers enter the growth stage, followed by a progressive spread until the signal covers the whole tuber surface, suggesting that periderm formation is developmentally controlled (Boher *et al*. 2013). Within the potato tuber skin *FHT* expression was localised to phellogen derivative cells with phellem identity and downregulation of the *FHT* gene induced alterations of the periderm anatomy, modifying sealing properties and maturation (Serra *et al*. 2010). Gene expression of *FHT* is maximal during tuber maturation and remains at high levels several months after harvest, suggesting the cells retain the capacity to synthesise ferulated esters (Serra *et al*. 2010).

Cork oak has also been extensively studied for suberisation. It differs from other Mediterranean oaks due to its thick and highly organised bark. Suberin biosynthesis genes identified in Cork oak include cytochromes P450, ABC transporters, acyltransferases and fatty acid elongases (Soler *et al*. 2007). Early summer is crucial for cork development and coincides with the upregulation of structural genes including *CYP86A1*, *GPAT* and *HCBT* in addition to regulatory genes of the *NAM* and *WRKY* families. The cork structural genes including *FAT* and *F5H* were also found to have a significant correlation with temperature and relative humidity (Soler *et al*. 2008). Additionally upregulated factors include developmental/meristematic regulators such as *NAM*, *MYB*, *HD-ZIPIII*, *KNOX* and *KANADI* transcription factors. *QsMYB1* is hypothesised to regulate cork biosynthesis but with its alternative splicing mechanism may also have a regulatory function during early periderm development (Almeida *et al*. 2013). Indeed a high degree of conservation of similar gene expression was found in *Arabidopsis* periderm formation which included the *QsMYB1* orthologue *MYB84*/*RAX3*, *ANAC78* and the suberin/wax biosynthesis genes *GPAT5*, *KCR1*, *HORST*, *DAISY*, *RALPH* and *ASFT* (Wunderling *et al*. 2018).

Genes that have been associated with exocarp russet periderm formation

As in native periderm development, in russet fruit, the stiff cuticular membrane is replaced by a more plastic periderm membrane (Khanal *et al*. 2013*a*) with an accumulation of suberin on the inner part of the cell wall of outer epidermal cells (Wang *et al*. 2016). Gene expression studies of russet fruit show a reduction in cuticle biosynthesis genes whereas stress-responsive genes and suberin deposition genes are upregulated (Legay *et al*. 2015; Wang *et al*. 2016). In contrast fruit that have thick cuticles also have higher expression of genes related to cuticle synthesis, and varieties that decrease cuticle synthesis during development are generally the varieties that are prone to russeting (Legay *et al*. 2015). As mentioned previously, a quantitative trait locus (QTL) controlling apple peel russet on LG12 of the russet cultivar ‘Renetta Grigia di Torriana’ has been identified, and within this QTL resides an ABC transporter likely to be involved in cuticle organisation (Falginella *et al*. 2015).

The gene *Defective in cuticular ridges* (*DCR*) is a member of the BAHD family of acyltransferases responsible for incorporation of the most abundant monomer into the polymeric structure of the *Arabidopsis* flower cutin (Panikashvili *et al*. 2009). In both tomato and apple, silencing of *DCR* led to russeting, characterised by an intensive suberisation of the exocarp surface (Lashbrooke *et al*. 2016). The phenotype involves major cracking and browning, resembling the surface of potato tubers. *SlDCR* silenced tomato lines showed reduced cuticle deposition under light microscopy, and scanning electron microscopy showed microscopic cracks between cells and larger fissures across the surface. Transmission electron microscopy showed lipid inclusion bodies in the cytosol (Lashbrooke *et al*. 2016). *SlDCR* is highly expressed during early stages of tomato fruit development and decreases sharply with maturation and ripening, and the same pattern is typical for fruit cuticle biosynthesis (Mintz-Oron *et al*. 2008).

A study comparing waxy (cuticle covered) and russet apples found a large number of transcription factors were differentially expressed and included bHLH, AP2/EREBP, C2H2, NAC-domain and R2R3-MYB and MYB-related genes (Legay *et al*. 2015). Candidate genes likely to be involved in determining pericarp type were *MYB52*, *MYB42* and *ANAC073*/*SND2*, which are known to function in the regulation of secondary cell wall biogenesis and are likely to be regulated by the master regulator SND1 (Zhong *et al*. 2008). The transcription factor *MYB52* is co-expressed with *SND2* and may repress lignin biosynthesis in addition to lignin polymerisation (Cassan-Wang *et al*. 2013). SND2 is another master regulator able to regulate cellulose, xylan and mannan synthesis, as well as lignin polymerisation (Hussey *et al*. 2011).

The MYB transcription factors appear to be important players in apple pericarp determination and differentially

expressed MYBs between russet and waxy apples were *MYB5*, *MYB42*, *MYB52*, *MYB67*, *MYB84*, *MYB94*, *MYB102* and *MYB93* (with *MYB102* thought to be involved in cell expansion). *MYB5* has a gene ontology (GO) classification as a negative regulator of trichome branching and required for correct formation of the seed coat and possibly underlying endosperm layers. *MYB42* has the GO classification of cell differentiation and regulation of secondary cell wall biogenesis. *MYB52* has the GO classification of regulation of secondary cell wall biogenesis and the function of *MYB67* is unknown. *MYB84* regulates axillary (cork) meristem formation (and is orthologue of *QsMYB1* discussed earlier) and *MYB102* is involved in wounding and osmotic stress response. For *MYB93*, quantitative polymerase chain reaction (qPCR) data from the apple study related *MYB93* expression with suberin/wax biosynthetic genes, *GAPT5*, *CYP86A1* and *CYP86B1* (R^2 = 0.987, 0.976 and 0.976) (Legay *et al*. 2015). *AtMYB93* acts as a repressor of lateral root development in *Arabidopsis* and it was confirmed that *MdMYB93* regulates suberin deposition in russeted apple exocarps (Legay *et al*. 2016).

Discussion

The epidermis of fruit is typically a layer of live skin cells, unless it becomes compromised, then it develops into russet periderm. Less common is a native peridermal fruit that undergoes programmed cell division beneath the epidermis to create new cells that undergo suberisation and cell death to create a corky native periderm. Fruit with an epidermis are often considered more aesthetically pleasing and easier to consume, but peridermal exocarps are often more resistant to damage. The range of possible fruit exocarps is a continuum between live skin cells or heavily suberised dead skin cells with variation in the extent of lignification. Wounding studies involving apple and pear support the hypothesis that microcracks in the cuticle lead to replacement by a plastic periderm tissue layer. Kiwifruit with peridermal exocarp have thin cuticles in the first phase of development (N. Macnee, unpubl. data), which suggests a developmental program whereby the periderm functionally replaces the cuticle, as observed in the russet of apples and pears, but in this case would apply to the whole organ as seen in potato. This theory is supported by studies of completely russeted pear varieties that downregulate cuticle synthesis before depositing suberin in their outer epidermal cell walls (Legay *et al*. 2015; Wang *et al*. 2014, 2016).

The process of cork meristem initiation and its subsequent programmed cell death has unresolved regulation in fruit exocarp. However, as discussed in this review there are significant correlations between meristematic regulation and periderm development in diverse plants such as *Arabidopsis*, potato and cork oak. Studies on fruit russet have also identified several associated genes. Combining all this information will be useful to understand the underlying genetics.

The formation of plant surfaces is an integral component of plant development and the continued characterisation of genetic pathways related to exocarp regulation will be vital for horticultural breeding. Advances in the field of cuticle biology have been significant in recent years but the interplay between the cuticle and periderm development has many knowledge gaps. The analysis of meristematic development and senescence in model plants has led to many discoveries. The next step is to extend these findings to important horticultural varieties. Considering fruit particularly, there is a need for a model that develops a native periderm in order to disentangle russeting from developmentally controlled periderm formation. Kiwifruit is one such fruit and could be a useful model for characterising fruit exocarp in general.

Conflicts of interests

The authors declare no conflicts of interest.

Acknowledgements

BayWa AG for providing financial assistance to NM in the form of a studentship. This work was funded by the Plant and Food Research Kiwifruit Royalty Investment Program: Fruit Characterisation. The authors would like to thank Tony Corbett for help in producing figures for this publication, particularly Figs 3 and 4.

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Handling Editor: Sergey Shabala