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Occurrence and implications for postharvest quality of intercellular callus hair growth in the outer cortex of apples of 'Fuji' and 'Fuji' sports

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Abstract

Mature apples of 'Fuji' and 'Fuji' sports sourced from around the world were found to possess clumps of multicellular, branched callus hairs in the outer 17 mm of the outer cortex. The clumps of callus hairs were particularly well developed in samples of 'Fuji Suprema' from Brazil, moderately developed in 'Fuji KIKU' from Italy and in 'Fuji' from South Africa, Chile, New Zealand and the USA, and were consistently least developed in 'Fuji' from China. These previously unreported callus hairs grow in the intercellular air spaces between the parenchyma cells and also in larger air lacunae in the apple flesh. In both of these locations they have the potential to reduce the efficiency of gas exchange into and out of the fruit during storage. Initial observations suggest that the callus hairs within a clump do not continue to develop after harvest. In ripe apples, the cells of the callus hairs contain chlorophyll and starch and under UV light the contents of the vacuoles autofluoresce an intense blue colour. The outer surface of each callus hair cell is covered with characteristic globular protuberances which attach the hairs to each other and to the surrounding parenchyma. After a period of storage, many of the clumps of callus hairs remain packed with starch granules even though starch has been metabolised from the surrounding parenchyma.

Keywords: 'Fuji' apples; Callus; Cell proliferation; Outer cortex; Gas exchange; Internal browning; Storage

1. Introduction

'Fuji' apples (*Malus domestica*, Borkh.) are widely grown in the USA, New Zealand, Japan, China and Australia, and increasingly in Italy, France, South America and South Africa. 'Fuji' is a late-ripening cultivar valued for its attractive appearance, flavour, crispness, sweetness and good keeping qualities (Yoshida et al., 1995). Bred in Japan, 'Fuji' was selected as the best of many crosses between the two American varieties 'Ralls Janet' and 'Red Delicious', and has itself been used as a parent in apple breeding. The recent identification of numerous 'Fuji' sports with improved red colour and an earlier ripening period has led to widespread planting of commercial orchards and there is a strong demand on the world market for good quality fruit. 'Fuji' apples are available all the year round from harvests in October/November in the Northern hemisphere and April/May in the Southern hemisphere.

Good storage properties are essential in apples grown for export. It is known that in some years 'Fuji' apples, particularly large, late-harvested fruit, are vulnerable to internal browning during controlled atmosphere storage (Grant et al., 1996). Consequently the interrelationship between pre-harvest factors, fruit maturity, temperature and the concentration of oxygen and carbon dioxide during storage regimes has been much investigated in order to establish optimal storage conditions for this cultivar (Park and Lee, 1991; Grant et al., 1996; Kupferman, 1997; Park et al., 1997; Volz et al., 1998; Argenta et al., 2000, 2002; Chung et al., 2005).

There are several factors or combination of factors, other than the composition of gases in the external atmosphere, which have the potential to influence carbon dioxide levels within stored apples, or restrict the diffusion of gases into and out of the fruit. These factors include the respiration rate of the tissue, the density and diffusivity of the outer cortex and hypodermis, the thickness and waxiness of the skin and the distribution and porosity of the lenticels (Schotsmans et al., 2004). Furthermore the limiting factor, or factors, may not be the same in different apple cultivars.

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During a recent comparison of fruit morphology of several apple cultivars, we coincidently observed multicellular hair-like growth in the intercellular spaces of the outer cortex of ripe organic 'Fuji' apples grown in the USA. These hairs resemble in some respects the 'tufts' which develop in the seed locules of certain apple cultivars (Sorauer, 1909; Carpenter, 1924; Winton and Winton, 1935) including 'Fuji', but their presence in the outer cortex of apple fruit appears not to have been previously documented. The presence and distribution of these hairs may be of great relevance to postharvest quality as they are positioned in the main gas diffusion pathway of the fruit where they have the potential to restrict or modify the local gas composition during storage.

To establish whether these cells are a characteristic component of all mature apples of 'Fuji' and 'Fuji' sports, fruit (conventionally grown and organic) imported from North and South America, New Zealand, China, South Africa and Europe was sourced over a period of 3 years from retail outlets in the UK, and screened for the location, extent and morphology of hair growth. Although 'Fuji' apples are not grown commercially in the UK, freshly harvested fruit were also obtained directly from the UK National Apple Collection. Fruit of the sport 'Fuji KIKU' were sampled at intervals over a storage period of 4 months to investigate whether the hairs continued to develop after harvest. The effect that the presence and development of this multicellular growth might have on storage properties and other aspects of apple physiology is discussed.

2. Materials and methods

2.1. Commercially grown 'Fuji'

'Fuji' apples (*M. domestica*, Borkh.), conventionally grown and organically grown when available, from USA, New Zealand (NZ), China, South Africa (SA), Chile, Brazil, Italy and France were purchased from UK supermarkets during 2004–2006. The growth conditions, rootstock and storage history of these commercially grown apples were unknown.

2.2. Mature unstored 'Fuji'

Ripe 'Fuji' apples were picked in October 2004 and 2005 from trees (identification codes 2/39 and 19/59) grown on M9 rootstock at the UK National Fruit Collection (The Brogdale Horticultural Trust, Faversham, Kent, UK).

2.3. 'Fuji' sports (clones)

Fruit of 'Sun Fuji', 'Raku Raku', 'Heisei Fuji', 'Fuji Nagafu 12', 'Fuji Hou Fu 3A' (irradiated strain), 'Gunfu', 'Fuji 2001', 'KIKU 4', 'KIKU 7' and 'KIKU 8' growing at Laimburg or Frangart OGS in the South Tyrol, Italy, were harvested in September ('Heisei Fuji' only) or October 2004 and stored in air at 2 °C and 90% humidity. The fruit were sent to IFR from Italy for examination in February 2005. Samples of imported 'Fuji Suprema' grown in 2006 at an altitude of 1400 m in Santa Catarina State, Brazil, were purchased locally in the UK.

2.4. Storage effects

Fruit of 'KIKU 8' picked on 21st or 25th October 2005 at Vinschgau, South Tyrol, Italy, were stored at Laimburg in air at 2 °C and 90% humidity and sent to IFR for examination in mid-November 2005, and in mid-January and mid-February 2006.

2.5. Light microscopy

Apples were cut in half lengthwise. Thin radial sections of the skin and cortex were cut with a razor blade from the equatorial region midway between stalk and calyx. Sections were vacuum-infiltrated with water and examined unstained, or after staining for starch in iodine/potassium iodine (I/KI Lugol solution, Sigma), with an Olympus BX60 microscope (Olympus, Japan) with Acquis software (Syncroscopy, Cambridge, UK). The autofluorescence in unstained sections was recorded using the UV filter cube (U-MWU, exciter filter BP330-385, barrier filter BA420) of the microscope.

Thicker sections were stored in CDTA (50 mM Na_3H_1 CDTA, 5 mM $Na_2S_2O_5$, pH 7) to soften the tissue. After a minimum of 7 days, parenchyma cells in these sections separated under light pressure, and were examined either unstained or stained with I/KI.

2.6. Scanning electron microscopy

Sections, about 3 mm thick, were cut as described above from 'Fuji' apples grown in New Zealand. Sections were fixed overnight in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), then dehydrated in a series of ethanol solutions (10, 20, 30, 40, 50, 60, 70, 80, 90, 3 × 100%) followed by three changes in 100% acetone. Tissue sections were critical-point dried (Polaron E3000, Quorum Technologies Ltd., Ringmer, UK) using liquid carbon dioxide as the transition fluid, mounted on aluminium SEM stubs (Agar Scientific Ltd., Stansted, UK) with conductive silver paint (Agar Scientific Ltd., Stansted, UK), and sputter coated with gold (K550, Emitech, Ashford, UK). Scanning electron microscopy was carried out using a Leica Stereoscan 360 SEM (Carl Zeiss SMT Ltd., Cambridge) operating at an accelerating voltage of 10 kV.

3. Results

The general features of the outer flesh of all the 'Fuji' and 'Fuji' sports examined were similar to those previously described for other apples. In thin sections in which the internal air has been replaced with water (Fig. 1a), the pigmented epidermis, the underlying hypodermal cells, and the parenchyma of the outer cortex interspersed by vascular strands are readily identifiable. However, in all the 'Fuji' and 'Fuji' sports examined in this investigation, an additional cell type was present, appearing at low magnification as dense white or pale green clumps (Fig. 1a, arrows) of variable size within the parenchyma tissue of the outer cortex. These cell clumps are most abundant in the outer 10 mm of the apple, but are also found up to

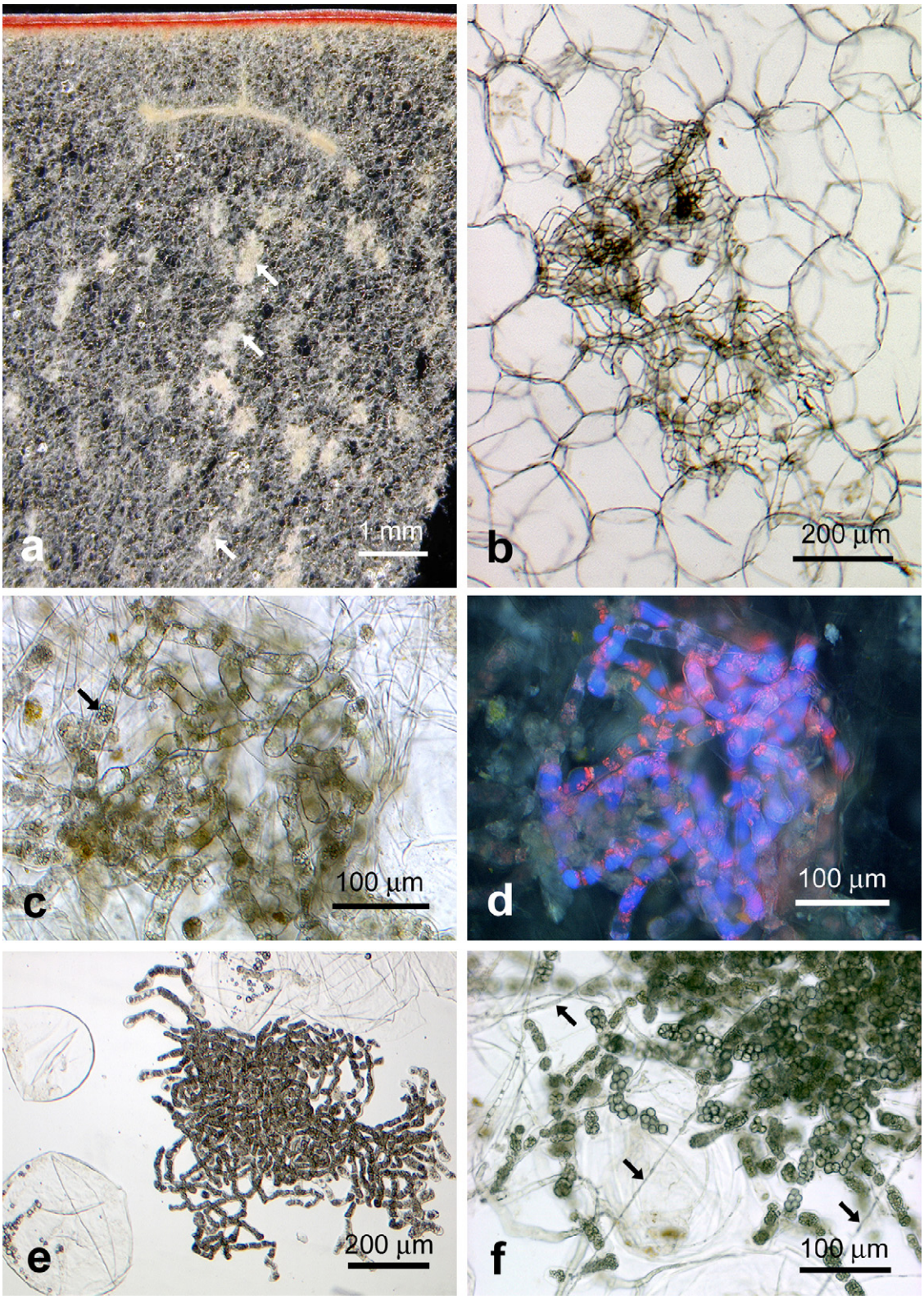

Fig. 1. Light microscopy of callus hairs in mature 'Fuji' apples. (a) Transverse section of skin and outer cortex with clumps of callus hairs arrowed ('Fuji Suprema' 2006 harvest, Brazil). (b) Detail of a clump of callus hairs surrounded by parenchyma (2005 harvest, New Zealand organic). (c) Callus hairs contain chlorophyll and starch granules (arrowed) (2006 harvest, South Africa). (d) Callus hairs viewed by UV light: chlorophyll autofluoresces red, vacuole contents blue and damaged parenchyma green (2006 harvest, South Africa). (e) Starch in a clump of callus hairs isolated in CDTA stains heavily with iodine, but the individual callus cells do not separate ('KIKU 8' 2004 harvest, Italy). (f) The callus hairs are distinguishable from fungal hyphae (arrows) in decayed flesh of 'Fuji Suprema' by their internal starch content and a larger filament diameter.

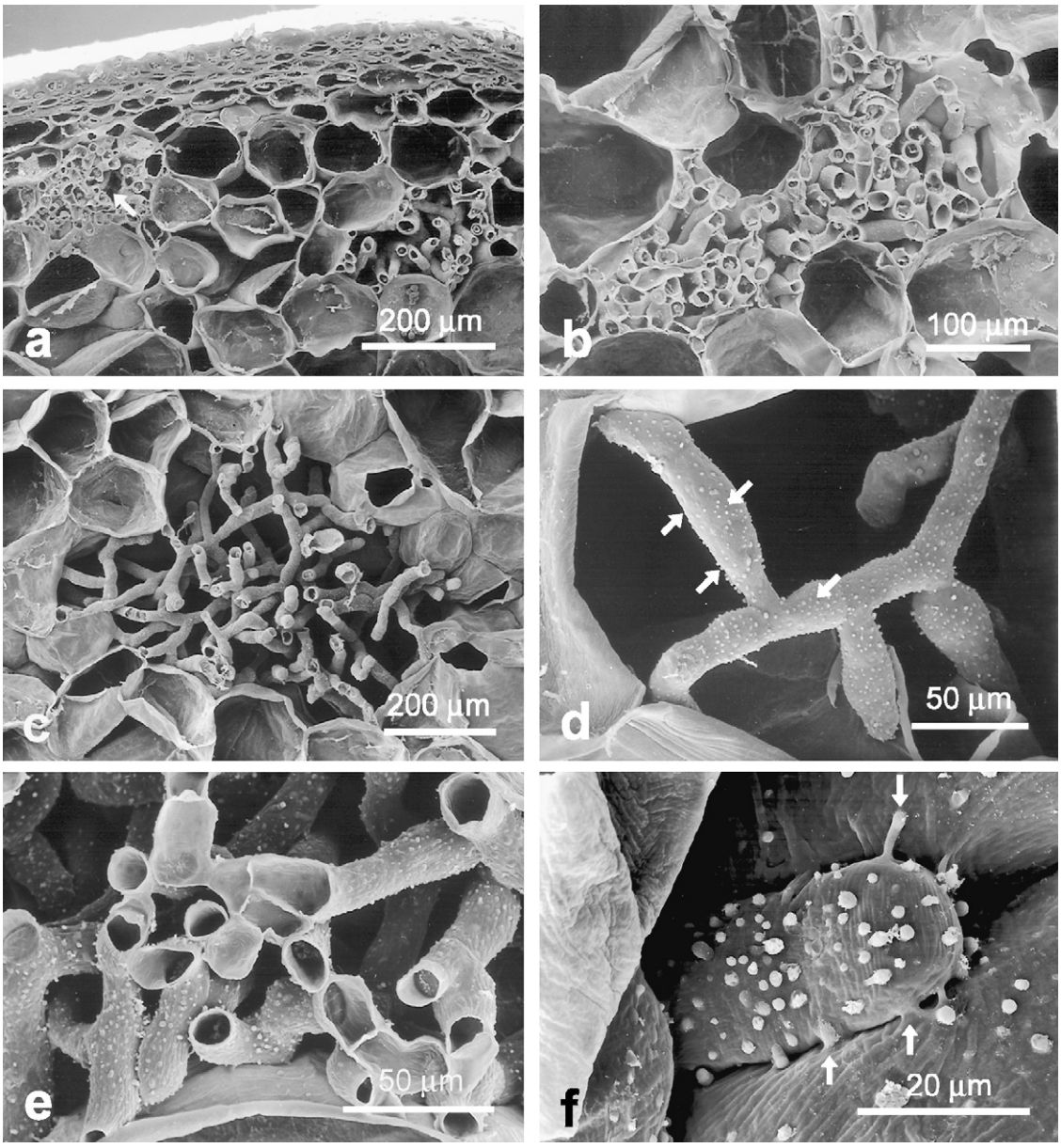

Fig. 2. Scanning electron micrographs of 'Fuji apples' (2004 harvest, New Zealand). (a) Skin, hypodermis and underlying parenchyma showing two clumps of intercellular callus hairs, one (arrowed) adjacent to the hypodermis. (b) Intercellular space in the outer cortex filled with a dense proliferation of callus hairs. (c) An intercellular cavity or lacuna showing the extensive branching of the callus hairs. (d) Callus hairs showing details of the branching pattern and surface protuberances (arrows). (e) Callus hairs cut open during specimen preparation showing the internal cross walls of component cells. (f) Detail of an apical callus hair cell showing the points of attachments (arrows) between the surface protuberances and the adjacent parenchyma cells.

17 mm from the skin. There is no obvious correlation between the position of cell clumps and the proximity of vascular bundles. The clumps tend to be elongated with their long axis in a radial direction. Initial observations suggest that the clumps are most numerous in the equatorial third of the apple, but there is a considerable variation in the frequency and size of the clumps. In this initial investigation, the cell clumps appeared to be consistently abundant and well developed in 'Fuji Suprema' from Brazil (Fig. 1a and f), moderately developed in 'Fuji KIKU' from North Italy (Fig. 1e) and 'Fuji' from South Africa (Fig. 1c and d), Chile, USA (organic and conventionally grown fruit), and New Zealand (Figs. 1b and 2a–f). The least developed clumps were found in commercially grown 'Fuji' from China.

At higher magnification (Fig. 1b), the morphology of a sectioned cell clump can be seen in detail. The clump consists of a mass of small, elongated and branched cells growing in an air cavity within the parenchyma tissue. Because of their resemblance to the hair-like callus cells which develop in the air space around the seeds in some apples, including 'Fuji', these cells will be referred to as callus hairs. Less well-developed clumps of callus hairs are found in the narrow intercellular airspaces between parenchyma cells, and these can proliferate between the parenchyma cells if the extending tips of the callus hairs

approach a suitable opening. Unlike the larger clumps (Fig. 1a) the small complexes, typical of Chinese and UK-grown 'Fuji', cannot be resolved at low magnification.

The cells of the callus hairs, particularly those just below the skin, usually have a green colouration (Fig. 1c) due to the presence of chlorophyll within their plastids. The callus hairs frequently contain small starch granules (Fig. 1c (arrow), Fig. 1e and f) which gives them a dense appearance distinct from the surrounding parenchyma. The distribution of the plastids and vacuoles within the individual cells of the callus hairs is most clearly seen using UV light (Fig. 1d) due to the red autofluorescence of the chlorophyll and the intense blue autofluorescence of the contents of the vacuole or vacuoles. This contrasts with the greenish-yellow autofluorescence of the surrounding parenchyma cells which have been damaged during sectioning, and gives an indication that the callus hairs are robust and able to maintain their integrity independent of the parenchyma.

The full extent of callus hair development within the clumps in 'Fuji' apples is demonstrated in apple tissue that has been immersed in CDTA for at least 7 days (Fig. 1e). During this time, calcium ions involved in the cross-linking of pectin in the middle lamella between parenchyma cells are chelated so that the cells separate under light pressure and the clumps of callus hairs are released in their entirety (Fig. 1e). The callus hairs rarely separate into individual component cells even after long periods of immersion in CDTA, suggesting that the composition of the cell walls differs from that of the neighbouring parenchyma. Infiltration with iodine reveals numerous black-staining starch grains which persist in many clumps of callus hair cells in stored 'Fuji' apples (Fig. 1e), even after starch has been metabolised from the bulk of the apple. These starch-filled clumps, when present on the surface of iodine-stained slices of stored 'Fuji', can be seen with the naked eye.

The appearance of the callus hairs may superficially resemble that of fungal hyphae, but the presence of starch within the hairs (Fig. 1e and f) confirms that they are not of fungal origin. The considerable size difference between the callus hairs and fungal hyphae (Fig. 1f, arrows) is evident in flesh of a decaying 'Fuji Suprema'.

The morphology and location of the callus hairs are revealed in further detail by scanning electron microscopy. The skin, underlying hypodermis and part of the outer cortex of a typical 'Fuji' apple is illustrated in Fig. 2a. The hypodermal layer, just below the pigmented epidermis, is approximately 200 μm thick and consists of 4 or 5 layers of tightly packed, flattened cells with small intercellular spaces. Beneath the hypodermal layer, the outer cortex tissue consists of spherical parenchyma cells 100–150 μm in diameter with thin cell walls. Deeper in the outer cortex, the cells are radially elongated, up to 200 μm long and less-tightly arranged so that the network of intercellular spaces through which gases can diffuse is more extensive than in the hypodermis. Callus hairs which develop in intercellular spaces immediately below the hypodermis (Fig. 2a, arrow) are easier to identify in the SEM than in fresh sections (Fig. 1a) where they tend to be obscured by the contents of the sub-hypodermal cells. However, only callus hairs exposed on the cut surface of the apple tissue can be seen by SEM, whereas in fresh apples all the callus hairs in the thickness of the section can be observed because of the transparency of the tissue. In the SEM, the callus hairs are immediately recognisable as groups of small cells in the airspaces between parenchyma cells (Fig. 2a–c). During specimen processing, the exposed starch and other components of cut parenchyma cells are generally lost. At higher magnification (Fig. 2b), the extent to which the callus cells fill the intercellular spaces is illustrated and demonstrates how the presence of a well-developed clump such as this might obstruct the diffusion of gases into and out of the fruit. Also apparent is the comparative size of the parenchyma cells and the component cells of the callus hairs. Cell contents required for chemical analysis are readily released from the large-celled parenchyma, but the contents of the callus hairs are less easy to sample because of the small diameter and multicellular organisation of the hairs.

In 'Fuji' as in many apple cultivars, there are in addition to the network of intercellular spaces, radially elongated air-filled cavities or lacunae which may reach 900 μm in length and around 500 μm in width. They are surrounded by parenchyma cells packed so tightly together that some air cavities may effectively be isolated from the rest of the intercellular air network. In fresh 'Fuji' tissue slices held under water, air bubbles trapped in these isolated cavities are difficult to remove even under vacuum. An example of a lacunae filled with multicellular, branched callus tissue is shown in Fig. 2c. Where there is no continuity with other intercellular spaces, the continued ramifying growth of the callus hairs within the isolated lacuna can be extensive forming a dense well-developed clump.

Typically, the callus hairs are 20 μm in diameter (Fig. 2d–f), variable in length and have numerous lateral branches (Fig. 2d). The surface of each component cell, particularly at the tip of the callus hair, is covered with characteristic small protuberances (Fig. 2d and f, arrows). Callus hairs cut open during specimen preparation show the internal cross walls delineating the individual cells (Fig. 2e) but cell contents are typically lost during processing. The surface protuberances adhere to the surrounding parenchyma (Fig. 2f, arrows) and to adjacent hairs. Treatment with CDTA loosens these points of attachment so that individual clumps of hairs can readily be separated from the parenchyma cells (Fig. 1e).

A small number of 'KIKU 8' apples harvested in October 2005 were examined after being stored for 1 month, 3 months and 4 months in air at 2 °C and 90% humidity in an attempt to ascertain whether callus hairs continued to grow during storage. Although this limited observation using CDTA-separated apple tissue was on a small scale and difficult to quantify, the overall impression was that the callus hairs did not continue to develop during storage.

4. Discussion

The internal structure of apples of 'Fuji' and 'Fuji' sports is similar to that previously described for other apples (Khan and Vincent, 1990). However, all the fruit of 'Fuji' and 'Fuji' sports examined in this study contained numerous clumps of multi-

cellular, branched callus hairs in the outer cortex. There was considerable variation in the extent of the growth within individual clumps. The clumps were particularly well developed in 'Fuji Suprema' from Brazil and were least well developed in 'Fuji' grown in China. The internal anatomy of 'Fuji' apples has been the subject of several recent investigations (Alandes et al., 2006; Quiles et al., 2007), but the presence of this distinctive callus growth has not, to our knowledge, been previously documented.

The callus hairs are found in the outer cortex of 'Fuji', to a depth of approximately 17 mm from the skin. They are similar in many respects to the callus hairs forming white tufts in the seed locules of some cultivars of apples (Sorauer, 1909; Carpenter, 1924; Winton and Winton, 1935; Tukey and Young, 1942) including 'Fuji'. The tufts, which are considered to be a varietal characteristic (Hedrick, 1922), develop when the lignified epicarp cells which line the seed locules split apart during the cell expansion phase of the developing apple. The underlying tissue is stimulated to divide, probably as a wound response, and the newly formed cells grow through the splits into the seed locule where the humid conditions support callus growth. This locule callus consists of multicellular, branched hairs which are also covered with protuberances and contain varying amounts of starch. Because the hairs in the outer cortex of 'Fuji' are morphologically similar to those in the locule tufts, the term 'callus hairs' has been used in this investigation to describe the growth in the flesh of 'Fuji'. Some of the cells of callus hairs in locule tufts develop into sclereids with thickened, lignified walls (Sorauer, 1909; Winton and Winton, 1935) but those found in the outer cortex do not. This may be a reflection of the tissue type in which they arise as the former develop in the pericarp tissue where sclereids are frequently present, whereas the cortex callus hairs arise in the hypanthium or floral cup (Esau, 1965) which tends to be sclereid-free in domesticated apple cultivars.

The retention of starch in the flesh hairs during storage, and their ability to maintain membrane integrity despite damage to adjacent parenchyma cells suggests that these metabolically active cells are, to some degree, independent of the surrounding parenchyma. The trigger for the onset of callus hair growth in the outer cortex of 'Fuji' is not yet known, but our ongoing observations indicate that this type of growth also occurs in close relatives of 'Fuji' as well as some other cultivars suggesting that it may be an inherited response.

The maintenance of an unrestricted gas flow through the fruit is vital for successful long-term storage of apples, particularly during modified atmosphere storage. Comparison of the internal anatomy and gas diffusion properties of two apple cultivars, 'Jonica' and 'Braeburn', with contrasting susceptibility to storage disorders (Schotsmans et al., 2004) has shown differences in diffusivity of oxygen and carbon dioxide. These differences were found to be related to intercellular volume and cell size. In addition, in both cultivars a reduction in gas diffusivity in the skin and outer cortex was considered a consequence of the dense tissue of the outer cortex and skin. 'Fuji' is a relatively dense apple which is known to be susceptible to internal browning, a gas-transport-related storage disorder. Given the commercial importance of this cultivar, it is perhaps not surprising that considerable research effort has been made to understand the basis of internal browning during storage (e.g. Argenta et al., 2002) and in detecting (Gonzalez et al., 2001) and preventing it (e.g. Kupferman, 1997; Chung et al., 2005).

It is suggested that the presence of additional callus hair growth with its own oxygen requirement and carbon dioxide output may further reduce the efficiency of gas transport through the intercellular spaces of the outer part of apples. Although the volume of the intercellular air network in apples is estimated to be between 20% and 35% in ripe apples (Bain and Roberson, 1951; Reeve, 1953; Goffinet et al., 1995; Drazeta et al., 2004), continuity of the intercellular network between cells in plant tissue is also an important factor in gas transport (Burton, 1982). For this reason, the local composition of the air around callus hair growth in the isolated lacunae of 'Fuji' may differ significantly from that measured in the bulk of the fruit. Heterogeneity of the internal atmosphere in 'Braeburn' apples may be a factor affecting the dynamics of gas diffusion within the fruit of this cultivar (Drazeta et al., 2004).

Developing fruit of 'Fuji' are not easily sourced in the UK as they are not grown commercially, but our preliminary unpublished observations on an unrelated cultivar, 'Lord Derby' which also exhibits callus hairs, have indicated that the initial stages of hair development are visible when the developing apple is approximately 25 mm in diameter and the proliferation of hairs within the clumps continues up until harvest. Callus hairs do not appear to proliferate in storage in this cultivar, as also seems to be the case for 'Fuji'. Thus the common practice of 'Fuji' growers to prefer the first-picked fruit for long-term storage may, in effect, favour fruit with less internal callus hair growth.

In future investigations on 'Fuji', the contribution of the outer cortex callus hairs and their contents to storage disorders and other areas of current interest such as phytonutrient content (Imeh and Khokhar, 2002; Boyer and Liu, 2004), allergen distribution (Marzban et al., 2005), starch content (Brookfield et al., 1997) and gene expression during development (Harada et al., 2005) should be considered. Compared to the parenchyma cells, the callus cells are small and the proportion of wall to cell volume is higher. Preparation methods currently used for releasing cell contents from apple tissue for analysis may not be optimal for callus hair cells and as a consequence, their contribution to these areas may not be fully represented.

Further in-depth investigations on fully replicated samples of fruit of known origin are necessary to quantify the development of callus hair growth in apples of 'Fuji' and 'Fuji' sports and its possible contribution to storage disorders. Factors such as the cultivar, position on the tree, geographical location and altitude of the orchard, type of rootstock and/or interstem, agronomic management of orchards including practices such as bagging, and harvesting and storage regimes will all need to be taken into consideration.

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References

Alandes, L., Hernando, I., Quiles, A., Perez-Munuera, I., Lluch, M.A., 2006. Cell wall stability of fresh-cut Fuji apples treated with calcium lactate. J. Food Sci. 71, 615–620.

Argenta, L.C., Fan, X., Mattheis, J.P., 2000. Delaying establishment of controlled atmosphere or CO_2 exposure reduces 'Fuji' apple CO_2 injury without excessive fruit quality loss. Postharvest Biol. Technol. 20, 221–229.

Argenta, L.C., Fan, X., Mattheis, J.P., 2002. Responses of 'Fuji' apples to short and long duration exposure to elevated CO_2 concentration. Postharvest Biol. Technol. 24, 13–24.

Bain, J.M., Roberson, R.N., 1951. The physiology of growth in apple fruit. 1. Cell size, cell number and fruit development. Aust. J. Sci. Res. B4, 75–91.

Boyer, J., Liu, R.H., 2004. Apple phytochemicals and their health benefits. Nutr. J. 3, 5.

Brookfield, P., Murphy, P., Harker, R., MacRae, E., 1997. Starch degradation and starch pattern indices; interpretation and relationship to maturity. Postharvest Biol. Technol. 11, 23–30.

Burton, W.G., 1982. Post-harvest Physiology of Food Crops. Longman Scientific and Technical, London.

Carpenter, C.C., 1924. Apple tufts. Bot. Gaz. 78, 414–423.

Chung, H.-S., Moon, K.-D., Chung, S.-K., Choi, J.-U., 2005. Control of internal browning and quality improvement of 'Fuji' apples by stepwise increase of CO_2 level during controlled atmosphere storage. J. Sci. Food Agric. 85, 883–888.

Drazeta, L., Lang, A., Hall, A.J., Volz, R.K., Jameson, P.E., 2004. Air volume measurement of 'Braeburn' apple fruit. J. Exp. Bot. 55, 1061–1069.

Esau, K., 1965. Plant Anatomy, 2nd ed. John Wiley & Sons, London.

Goffinet, M.C., Robinson, T.L., Lakso, A.N., 1995. A comparison of 'Empire' apple fruit size and anatomy in unthinned and hand-thinned trees. J. Hortic. Sci. 70, 375–387.

Gonzalez, J.J., Valle, R.C., Bobroff, S., Biasi, W.V., Mitcham, E.J., McCarthy, M.J., 2001. Detection and monitoring of internal browning development in 'Fuji' apples using MRI. Postharvest Biol. Technol. 22, 179–188.

Grant, J., Mitcham, B., Biasi, B., Chinchiolo, S., 1996. Late harvest, high CO_2 storage increases internal browning of 'Fuji' apples. Calif. Agric. 50, 26–29.

Harada, T., Kurahashi, W., Yanai, M., Wakasa, Y., Satoh, T., 2005. Involvement of cell proliferation and cell enlargement in increasing the fruit size of *Malus* species. Sci. Hortic-Amsterdam 105, 447–456.

Hedrick, U.P., 1922. Cyclopedia of Hardy Fruits. Macmillan Co., New York, pp. 15–71.

Imeh, U., Khokhar, S., 2002. Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. J. Agric. Food Chem. 50, 6301–6306.

Khan, A.A., Vincent, J.F.V., 1990. Anisotropy of apple parenchyma. J. Sci. Food Agric. 52, 455–466.

Kupferman, E., 1997. Controlled atmosphere storage of apples. In: Mitcham, E.J. (Ed.), Proceedings of Seventh International Controlled Atmosphere Conference, Postharvest Outreach Program, vol. 2. University of California, Davis, CA, pp. 1–30.

Marzban, G., Puehringer, H., Dey, R., Brynda, S., Ma, Y., Martinelli, A., Zaccarini, M., van der Weg, E., Housley, Z., Kolarich, D., Altmann, F., Laimer, M., 2005. Localisation and distribution of the major allergens in apple fruits. Plant Sci. 169, 387–394.

Park, Y.M., Lee, S.K., 1991. Susceptibility of 'Fuji' apples to low-oxygen injury and high-carbon dioxide injury during CA storage. J. Korean Soc. Hortic. Sci. 33, 38–43.

Park, Y.M., Kweon, H.J., Kim, H.Y., Ryu, O.H., 1997. Preharvest factors affecting the incidence of physiological disorders during CA storage of 'Fuji' apples. J. Korean Soc. Hortic. Sci. 38, 725–729.

Quiles, A., Hernando, I., Perez-Munuera, I., Lluch, M.A., 2007. Effect of calcium propionate on the microstructure and pectin methyl-esterase in the parenchyma of fresh-cut Fuji apples. J. Sci. Food Agric. 87, 511–519.

Reeve, R.M., 1953. Histological investigations of texture in apples. II. Structure and intercellular spaces. Food Res. 18, 604–617.

Schotsmans, W., Verlinden, B.E., Lammertyn, J., Nicolai, B.M., 2004. The relationship between gas transport properties and the histology of apple. J. Sci. Food Agric. 84, 1131–1140.

Sorauer, P., 1909. Handbuch der Pflanzenkrankheiten. Paul Parey, Berlin, pp. 324–326.

Tukey, H.B., Young, J.O., 1942. Gross morphology and histology of developing fruit of the apple. Bot. Gaz. 104, 3–25.

Volz, R.K., Biasi, W.V., Grant, J.A., Mitcham, E.J., 1998. Prediction of controlled atmosphere-induced flesh browning in 'Fuji' apple. Postharvest Biol. Technol. 13, 97–107.

Winton, A.L., Winton, K.B., 1935. The Structure and Composition of Foods. Vol II: Vegetables, Legumes and Fruits. John Wiley & Sons, New York.

Yoshida, Y., Fan, X., Patterson, M., 1995. 'Fuji' apple. Fruit Varieties J. 49, 194–197.