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Cuticle of 'Gala' and 'Galaxy' Apples Cultivars under Different Environmental Conditions

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ABSTRACT

This study aimed to analyze the cuticle thickness and pattern of epicuticular wax deposition in 'Gala' and 'Galaxy' apple cultivars (Malus domestica Borkh.) from three Brazilian producing areas: Vacaria (RS), Fraiburgo (SC) and São Joaquim (SC) with altitudes of 971, 1,048 and 1,353m, respectively. Harvested fruit were kept under two storage conditions: regular atmosphere (RA) (0 °C and 90% RH) and controlled atmosphere (CA) (1.5% O₂, 2.5% CO₂, 0°C and 90% RH). Cuticle thickness measurements were made using LM and the deposition pattern of epicuticular wax observed with a SEM. Altitude among the apple producing areas was not a factor in deposition pattern of waxes between the cultivars but at higher altitudes, the cuticle was thicker in both the cultivars. In the freshly-harvested fruits, waxes deposition in the form of platelets and the mechanism of "tear and repair" were observed. Severity of microcracks in the cuticle was more evident on the fruits from CA.

Key words: *Malus domestica* Borkh, altitude, cold storage, controlled atmosphere, light microscope

INTRODUCTION

The southern region of Brazil accounts for more than 95% of the national production of apples. During the 2008-09 season, it produced 1184.3 tons of apples that comprised primarily of two cultivars, 'Gala' (46%) and 'Fuji' (45%) (IBGE 2009). However, the 'Gala' cultivar is gradually being replaced by the clones of red ones, such as 'Galaxy' (Boneti et al. 2002).

Epicuticular waxes are a major component of the cuticle that covers the surface of apple, protecting it against the environmental stresses, such as wind, solar radiation, dehydration and entry of pathogens causing rot in the fruit (Kolattukudy 1984; Belding et al. 1998). Wax biosynthesis occurs from the beginning of apple fruit development. Once the

bud begins to develop, exposing the tissue to the water vapor deficit, the pro-cuticle begins to cover the surface of the tissue with an overlay network of lipid-basis microtubules produced by the epidermal cells and linked to them. The elongation process of wax microtubules, aggregation, crystallization and polymerization occur simultaneously during the fruit growth, causing the deposit of wax platelets on the cuticle and on the microcracks formed in it, a procedure known as "Tear and Repair" (Curry 2009). Wax production continues during the storage (Belding et al. 1998), until the origin cells become biochemically inhibited, necrotic or without substrate (Curry 2005). The fruits of different apple cultivars vary in terms of epicuticular waxes morphology, quantity and chemical composition during

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development, as well as during the storage and subsequent life (Veraverbeke et al. 2001). Changes in the process of wax formation and deposition can cause damage, reducing the quality of the fruit.

The aim of the present work was to study the cuticle thickness and pattern of epicuticular wax deposition in 'Gala' and 'Galaxy' apple cultivars from three Brazilian producing areas with different altitudes and kept under two storage conditions.

MATERIAL AND METHODS

Fruits collection and storage

'Gala' and 'Galaxy' apple fruits harvested in three commercial orchards in the 2008/09 season, Vacaria (RS), Fraiburgo (SC) and São Joaquim (SC) located at 971; 1,048 and 1,353m, respectively, were studied. The producing areas were selected according to the cultivar, rootstock and orchard age, aiming to reduce the heterogeneity deriving from the interaction among these factors. The fruits were harvested randomly at commercial maturity, at the medium height of the plant. They were packed in cardboard trays, placed in plastic boxes lined with bubble wrap, and then transported to the Estação Experimental de Fruticultura Temperada, Embrapa Uva e Vinho, Vacaria, RS.

After the harvest, the initial assessment was carried out to characterize the cuticle and epicuticular waxes. Ten fruits of each variety and location were placed in screened bags and labeled. These samples were stored under regular atmosphere (RA) at 0 °C and 90 % relative humidity (R.H.) and controlled atmosphere (CA) at 1.5 % O₂, 2.5 % CO₂, 0 °C and 90 % R.H. and evaluated after 120 days of storage.

Preparation of the samples, capturing images and measurements

For the cuticle analysis, four samples were collected in the equatorial region of freshly harvested fruits. Samples were fixed in Karnovsky solution (Karnovsky 1965). For better fixation, the samples were placed in the vacuum pump to remove the air from the intercellular spaces. The samples were dehydrated in a graded ethanol series and embedded in Leica historesin® (Heraeus Kulzer GmbH and Co. KG, Hanau, Wehrheim, Germany). Serial sections (5-10 µm thick) were cut on a rotary microtome and stained with Sudan IV to detect the lipophilic substances

(Jensen 1962). The images were digitally captured through a Leica DM LB microscope with a video camera attached to a PC, using the IM50 image analysis software. For the thickness of the cuticle, the captured images from the areas without cuticular flanges were measured using the Image Tool software. Three replicate measurements were made in four different fruits.

For surface examination using the scanning electron microscope, portions of the fruit surface in the equatorial region were taken at harvest and after 120 days of storage. Four fruits of each cultivar and location were sampled for the assessment. The samples were attached by their ends on slides with adhesive tape and kept in a desiccator containing silica gel for dehydration. After the minimum period of 72 h, fragments were removed (0.2 x 0.2 mm) and attached on aluminum stubs and coated with gold (30-40 nm). Then, the samples were examined under a LEO VP-435 SEM (Electron Microscopy Ltd, Cambridge, UK) at 20 kV.

Statistical analysis

The experiment was arranged in a completely randomized design in a 3 x 2 (producing areas x cultivars) factorial scheme. Data were submitted to the analysis of variance (ANOVA) and means were compared by Tukey's test at $P < 0.05\%$ using Sisvar Program (Sisvar 2000).

RESULTS AND DISCUSSION

The cuticle of freshly harvested fruits of both the cultivars from the three producing areas was thick and had conspicuous flanges (Fig. 1 A and B).

The cuticle thickness was different between the cultivars and among the producing areas ($P < 0.05\%$) (Table 1). There was no difference between the cultivars of Vacaria. Thicker cuticles were observed in 'Gala' apples grown at the highest altitude (São Joaquim -1,353 m), and in 'Galaxy' apples from Fraiburgo, the producing area of intermediate altitude (1,048 m).

Zhang (1983) and Xing-Jun et al. (2004) investigated the effect of altitude on apple fruit and they found that the cuticle became thicker with the increase of altitude. According to Curry (2009), the thickness of the cuticle is a function of the genetic background of cultivar and environmental growth of the fruit.

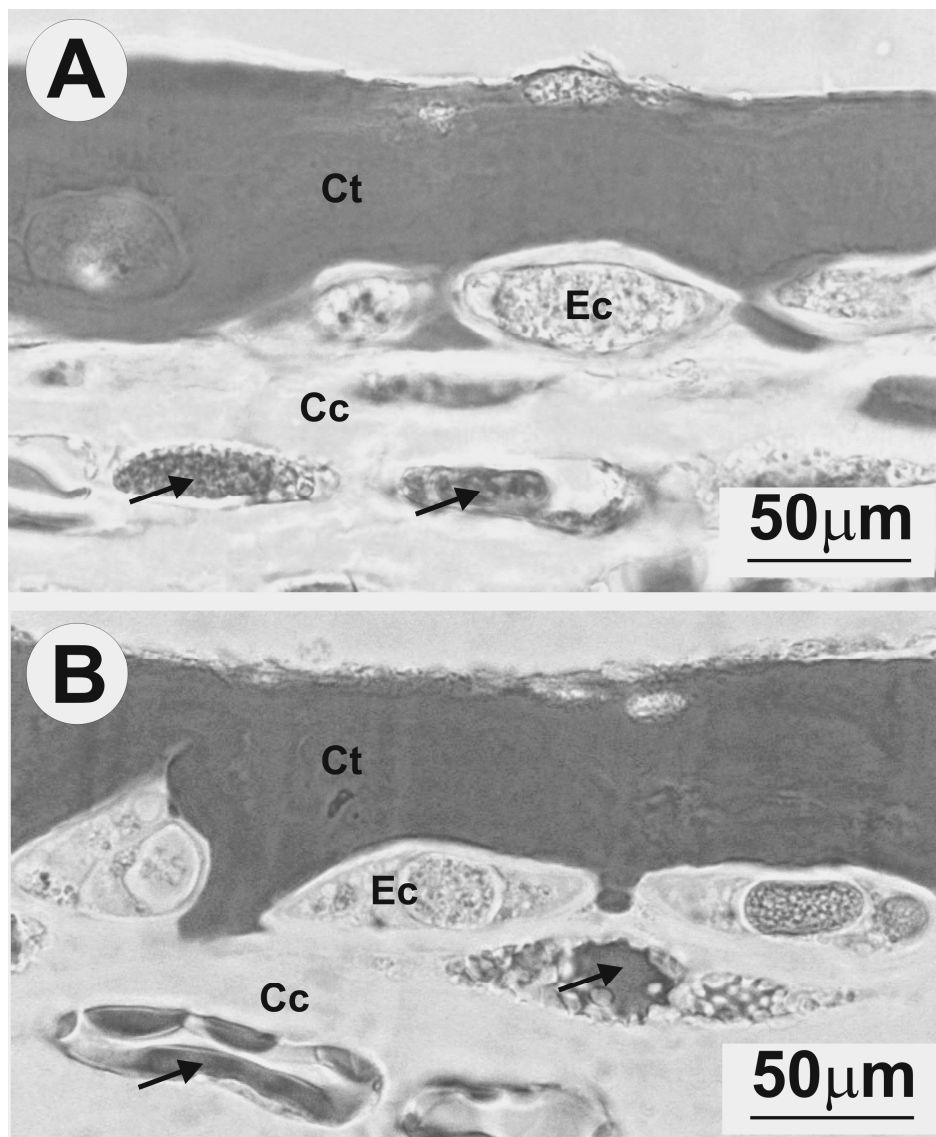


Figure 1 - Cross sections of the freshly harvested fruits in Fraiburgo-SC. A: 'Gala', B: 'Galaxy'. Cuticle (Ct), Epidermal cell (Ec), Collenchyma cell (Cc), Phenolic compounds (arrows).

Table 1 - Cuticle thickness of apple fruits from three different Brazilian producing areas.

Producing area (altitude m)	Cuticle thickness (μm)	
	Gala	Galaxy
Vacaria/RS (971)	17.77 \pm 2.04 Ab	18.12 \pm 2.08 Ab
Fraiburgo/SC (1,048)	18.49 \pm 3.19 Bb	21.99 \pm 2.31 Aa
São Joaquim/SC (1,353)	20.41 \pm 2.41 Aa	18.74 \pm 2.28 Bb

Means followed by the same capital letter within a row and by the same lower-case letter within a column are not significantly different at $P \leq 0.05$. Means followed by standard deviation.

The cuticle is a dynamic system that expands in proportion to the growth of the fruit. As the parenchyma cells elongate during the expansion of

the fruit, the cuticle begins to stretch causing microcracks in the wax platelets (Fig. 2 A and E). Under favorable environmental conditions, this

process occurs gradually, beginning the repair of microcracks through the parallel deposition of wax platelets before the complete disruption and exposure of underlying epidermal cells in a procedure, known as "tear and repair" (Curry 2005).

This pattern was observed in both the cultivars (Fig. 2 A-C and 3 A-F). Other wax patterns were also found, such as granules (Fig. 2 D and F), plates (Fig. 3 A), platelets (Fig. 2 B) as also

reported by Barthlott et al. (1998). There was no difference in the shape and distribution of epicuticular waxes among the growing areas.

After 120 days of storage, there was an increase of microcracks in the cuticle, which were more extensive and deeper than in the freshly-harvested fruits. There were also changes in the shape of the epicuticular waxes from the platelets to spherical or amorphous clusters (Fig. 2 C-F, I-M, R-S and 3 C-F, I-M, P-S).

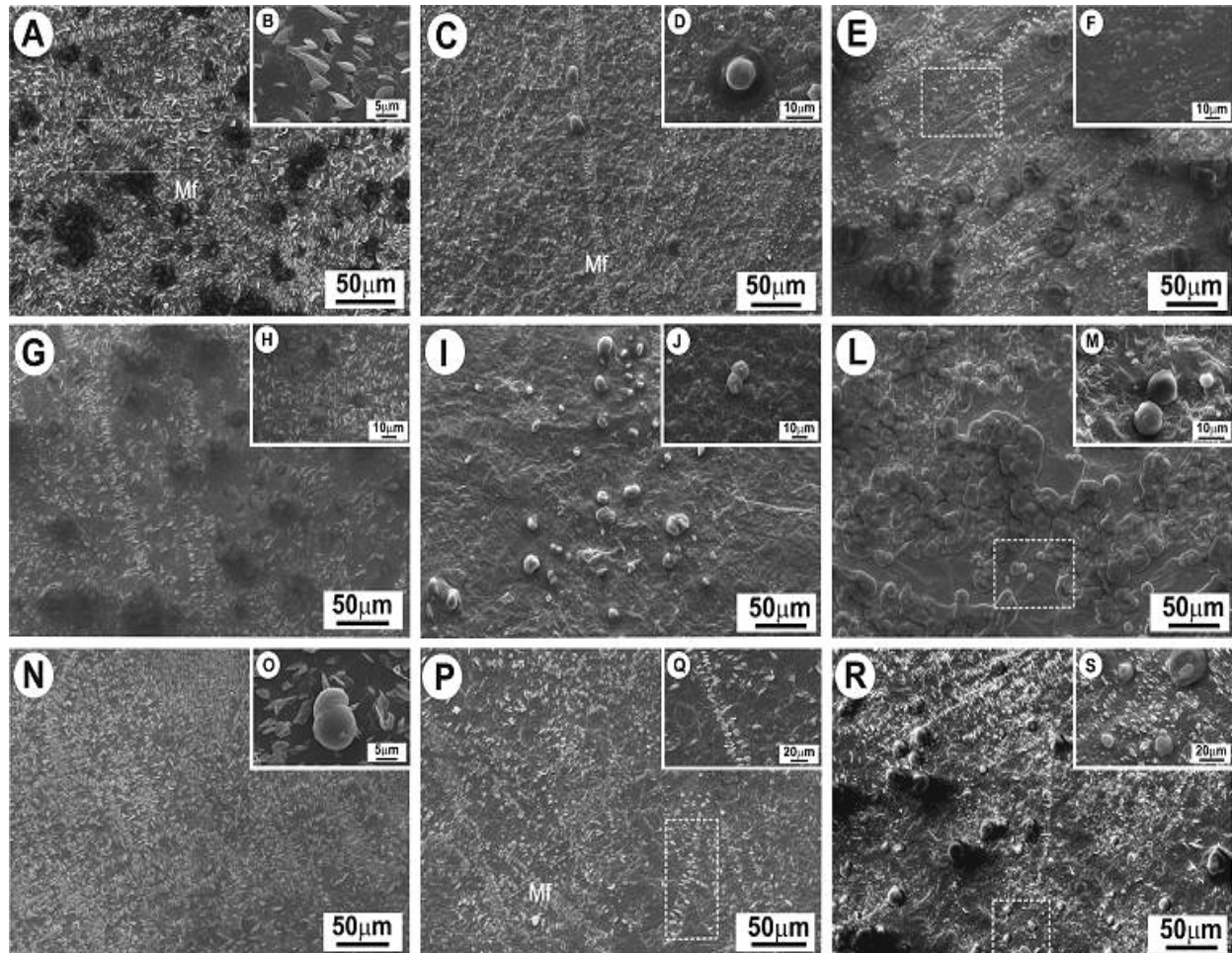


Figure 2 - Scanning electron micrographs of epicuticular wax of 'Gala' apple fruits from three producing areas: Vacaria-RS (A-F), Fraiburgo-SC (G-M) and São Joaquim-SC (N-S) after harvest (A-B, G-H, N-O), stored under regular atmosphere (RA) (C-D, I-J, P-Q) and controlled atmosphere (CA) (E-F, L-M, R-S). There is no difference in shape and distribution of epicuticular waxes among producing areas but under storage there are changes in the shape of the epicuticular waxes from platelets (A-B, C, G-H, N-O, P-Q, R-S), globules (Q) to amorphous clusters (C-D, E-F, L-M, R-S). Under CA also occur waxes melting (E-F, L-M). Microcracks (MF) and "tear and repair" mechanism is observed in all conditions (A-B, C, P-Q).

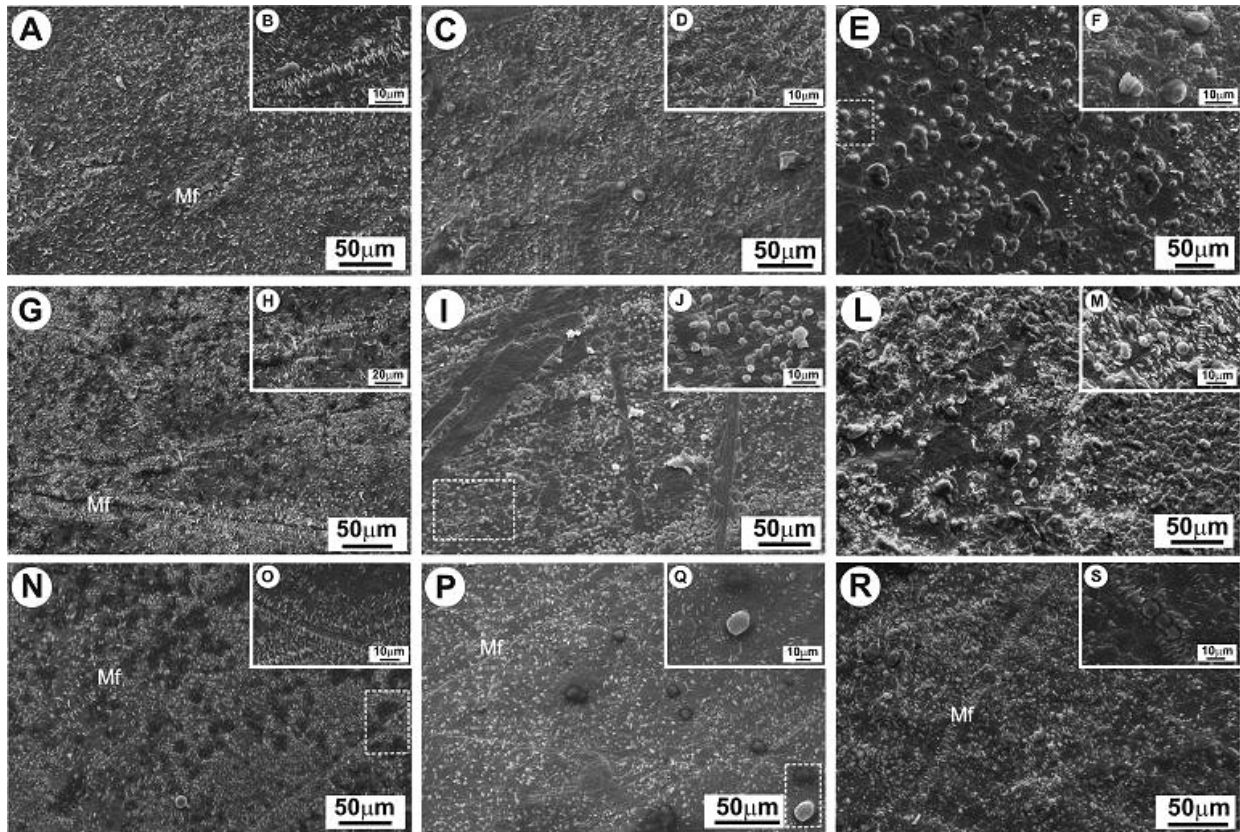


Figure 3 - Scanning electron micrographs of epicuticular wax of 'Galaxy' apple fruits from three producing areas: Vacaria-RS (A-F), Fraiburgo-SC (G-M) and São Joaquim-SC (N-S) after harvest (A-B, G-H, N-O), stored under regular atmosphere (RA) (C-D, I-J, P-Q) and controlled atmosphere (CA) (E-F, L-M, R-S). There is no difference in shape and distribution of epicuticular waxes among producing areas but under storage there are changes in the shape of the epicuticular waxes from platelets (A-B, C-D, G-H, L-M, N-O, P-Q, R-S), globules (N-seta) to amorphous clusters (E-F, I-J, L-M, R-S). Under CA also occur waxes melting (E-F, L-M). Microcracks (MF) and "tear and repair" mechanism is observed in all conditions (A-B, G-H, N-O, R).

These changes were observed in both the cultivars and in all the producing areas, but they were more evident in the fruits stored under the controlled atmosphere. This was probably due to the reduction of the respiratory process, by changing the gas composition of the chamber and reduced production of ethylene during the storage. The synthesis of the major wax components occurs via sequential elongation of a C_2 primer from acetyl-CoA with C_2 units derived from malonyl-CoA (Shepherd and Griffiths 2006). The lower respiratory activity reduces the production of acetyl-CoA, causing the reduction in the formation of waxes. The controlled atmosphere has the ability to delay the onset of autocatalytic ethylene production during the storage of apples (Lau et al. 1984) and decrease the amount of

ethylene produced (Bufler and Streif 1986). Ju and Bramlage (2001) studied the developmental changes of cuticular constituents and their association with ethylene during the ripening of 'Delicious' apples, indicating that the increase in the waxes production coincided with the climacteric rise of ethylene. During the process of ripening, the composition of the cuticle is altered due to the accumulation of unsaturated fatty acids, which have lower freezing point. The accumulation of these acids results in changes in the physical properties of the cuticle (Argenta 2002). Because of this change in compound formation, the crystalloids begin to change their appearance, crystallizing into fatty clusters (Fig. 2 C-F, I-M, R-S and 3 C-F, I-M, P-S). According to Barthlott et al. (1998), the predominant

components of waxes are generally regarded as responsible for the crystalloids shapes. Tests carried out by Monteiro et al. (2007) with heat treatment and subsequent cold storage of 'Gala' and 'Fuji' apples showed partial or complete fusion of wax crystall.

CONCLUSIONS

The altitude of the growing regions influenced the thickness of the cuticle, appearing thicker in places of higher altitude. The deposition pattern of the waxes was the same in 'Gala' and 'Galaxy' cultivars, but after 120 days of storage, an increase of microcracks in the cuticle and changes in the shape of the epicuticular wax, especially in controlled atmosphere were observed.

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