

**Growth Rate and Surface Wetness are Critical Factors in Microcracking,
Loss of Water Vapour and Russeting of the Apple Fruit Surface**

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ABSTRACT

The study was carried out at the Leibniz Universität Hannover Germany. The objectives were to quantify microcracking of the cuticle, fruit surface permeability and fruit surface russeting in apple (*Malus x domestica* Borkh. Cultivar 'Pinova'). Samples were obtained from the experimental orchards of the Leibniz Universität Hannover Germany. Selected portion of the fruit surface was exposed to 1-(2-chloro-4-pyridinyl)-3-phenylurea (CPPU) solution (20 ml l⁻¹) or liquid water for two days and 12 days, respectively. After the termination of each treatment, assessment of the indices above-mentioned was carried out four times. Microcracking of the fruit surface was quantified using acridine orange infiltration. Permeability was quantified by water loss using excised epidermal segments (ES). Calibrated photographs of the russeted fruit were taken and russeted area quantified by image analysis using Cell P[^]. At 21 days after full bloom (DAFB), acridine orange (AO) infiltrated area of the CPPU treated and moisture treated surface of the fruit were respectively 4-folds (8 %) and 8-folds (8 %) more than that of the untreated surface (2 % and 1 % respectively). Permeability of CPPU treated and moisture treated surface of the fruit were respectively 15 x 10⁻⁵ m s⁻¹ and 40 x 10⁻⁵ m s⁻¹ when compared to the untreated surface (10 x 10⁻⁵ m s⁻¹ and 10 x 10⁻⁵ m s⁻¹ respectively) at 21 DAFB. There was 10 % (10.3 ± 2.6) and 37 % (37.1 ± 7.3) russeting when the fruit surface was treated with CPPU and moisture respectively at 21 DAFB. Therefore, both factors are critical in microcracking, loss of water vapour and russeting of the apple fruit surface.

INTRODUCTION

Russeting is a fruit skin defect of economic concern worldwide in many horticultural crops including apple. In russeting, periderm replaces the primary fruit skin when the cuticle is wounded (Faust and Shear, 1972). The periderm results in brownish and rough appearance of the fruit skin. Commercial apple cultivars differ considerably in their susceptibility to russeting. Some cultivars such as 'Egremont Russet' and 'Karmijn' are highly sensitive to russeting (Khanal *et al.*, 2013a). This is considered by consumers as normal feature of the fruit. Another group of apple cultivars ('Granny Smith', 'Idared', 'Braeburn' etc.) are non-susceptible to russeting (Khanal *et al.*, 2013b) and remain almost russet free even in adverse

climate conditions. Cultivars such as ‘Elstar’, ‘Golden Delicious’, ‘Pinova’ etc (Khanal *et al.*, 2013b) are considered to be medium russet susceptible. In these cultivars, adverse climate (high humidity) increases the severity of russetting (Faust and Shear, 1972). Formation of microcracks in the cuticle is the first event in the sequence of phenological events of russetting (Faust and Shear, 1972). When the functional barrier of the fruit skin breaks, epidermis and hypodermis are exposed to the external environment. This leads to stress on the cellular layers, thus the fruit has to repair or re-establish the barrier loss. Previous works (Curry, 2009; Roy *et al.*, 1999) reported the deposition of wax into cracks thereby healing microcracks. Periderm develops underneath the cracks and repairs the injury only when wax deposition is not sufficient to heal the wounds properly. Growth stress has been reported as the primary cause of microcracking (Skene, 1982). The growth stress on the surface is high when the fruit surface growth rate is high. Consequently, it manifests as microscopic cracks in the fruit skin and considered as symptoms of russet formation (Simons and Chu, 1978; Hatch, 1975).

In commercial apple fruit production, different kinds of plant growth regulators are used. The role of these plant growth regulators included increasing the fruit volume or changing the fruit shape. An example of such growth regulators is, 1-(2-chloro-4-pyridinyl)-3-phenylurea-CPPU), commonly known as Forchlorfenuron); a synthetic cytokinin of the phenylurea group (Costa, 1999). Application of CPPU on the fruit skin increases fruit growth rate and surface expansion rate (Zhang and Whiting, 2011). As the fruit growth rate increases, it generates higher strain in the cuticular membrane (CM) thereby increasing the severity of microcracks (Iwahory *et al.*, 1988). Furthermore, environmental factors such as fruit surface wetness or high humidity are reported to increase microcracking in the cuticle and subsequently russetting of the fruit surface (Winkler *et al.*, 2014; Knoche and Grimm, 2008; Elfving and Allen, 1987; Faust and Shear, 1972). However, there is no comparative study between fruit surface growth rate and surface moisture in causing russetting of apple. Therefore, in this study we monitored the role of fruit surface growth rate and surface wetness in microcracking of the cuticle, water vapour permeance of the cuticle and fruit surface russetting.

MATERIALS AND METHODS

Apple (*Malus x domestica* Borkh. Cultivar 'Pinova') samples were obtained from the experimental orchards of the Leibniz Universität Hannover Germany. Selected portion of the fruit surface was exposed to 1-(2-chloro-4-pyridinyl)-3-phenylurea (CPPU) solution (20 ml l⁻¹) or liquid water for two days and 12 days respectively. Application of CPPU solution (20 ml l⁻¹) was done by selecting the fruit inflorescence and trimmed to only one fruit. A small piece of cotton pad was then attached to the cheek region of the fruit by wrapping with a parafilm and the pad soaked with CPPU using a fine syringe. The CPPU was left to dry for two days and the treatment terminated. Moisture treatment was done by selecting the fruit inflorescence and trimmed leaving only one fruit per inflorescence. Eppendorf tubes (8 mm in diameter) were then fixed on the cheek region of the fruit surface using fast curing silicone glue. After curing of the glue, tubes were filled with water by injecting using a fine syringe via the minute hole prepared on the tip of the tube and sealed with the silicone glue. The tubes were regularly monitored to make corrections where necessary, and after 12 days the treatment was terminated. CPPU and moisture treated fruits were harvested at four different stages: 21, 44, 73 and 100 days after full bloom (DAFB) of fruit growth. Fruits were processed immediately by removing the flower remnants and microcracking as well as permeability experiments carried out on the same day of sampling or stored in conventional cold storage (2 °C; 90 – 94 % humidity) for not more than 24 h.

Microcracks on the surface of CPPU treated and moisture treated and non-treated fruits were monitored by acridine orange infiltration assay. Whole fruits were incubated for 10 minutes in 0.1 % acridine orange solution in 50 mM citric acid buffer solution. Fruits were then rinsed in deionized water and cautiously blotted using soft tissue paper. The fruits were then transferred to the fluorescence dissecting microscope (Model MZ 10F; Leica Microsysteme GmbH, Germany; camera DP73, Olympus, Hamburg, Germany; Software Cell Sens, Bensheim, Germany) and portions (2 mm²) of the CPPU and moisture treated as well as the non-treated surface of the fruit were observed in transmitted and incident blue light (330 to 385 nm excitation wavelength). Three representative images of randomly selected area

of the CPPU treated, moisture treated and non-treated surface of the fruit were prepared using software cell sens. The non-treated surface served as control. The images were then calibrated and the acridine infiltrated area quantified using software Cell P[^]. The acridine infiltrated area was then expressed as a percentage of the area observed under the microscope. The number of replication was 10.

Transpiration from excised epidermal segment (ES) of the CPPU treated, moisture treated and non-treated surface were observed using diffusion cells. The ES from the CPPU treated, moisture treated and non-treated surface of the fruits were excised using a sharp razor blade and mounted on greased stainless steel diffusion cells such that the outer part of the fruit skin were exposed in the 7 mm diameter orifice (Geyer and Schönherr, 1988). The diffusion cells were then filled with deionized water through a minute port in the base and sealed using clear tape, placed upside down in a closed box containing dry silica gel at low humidity (0 %) and stabilised for 1 h as reported by Grimm *et al.* (2012). Water loss was quantified by repeatedly weighing the diffusion cells using a digital balance (Sartorius CPA225D) at regular intervals (0, 2, 4, 6 and 8 h) during the incubation. The amount of water loss with time (transpiration) was computed as the decrease in the weight of the diffusion cell with time. The rate of water loss was calculated from the slope of a linear regression line by plotting cumulative water loss against time. Permeance (P, m s⁻¹) of the ES to water vapour was then calculated using the formula $P = F / (A \cdot \Delta C)$. Where F (kg s⁻¹) is the rate of water loss; A (m²) is defined as the area of the orifice of the diffusion cells or the transpiring surface; ΔC (kg m⁻³) = C_i - C_o is the water vapour concentration between the inside of the diffusion cell (C_i) and the atmosphere in the box (C_o). The saturation concentration of water vapour (100 % RH) at the respective temperature of 24°C (from climate data loggers) was 21.8 g m⁻³ (Nobel, 1999). The number of experimental replication was 20.

CPPU treated and moisture treated fruits were allowed to grow on the trees till maturity. Fruits that were treated at 21, 44, 73 and 100 DAFB were sampled for russeting analysis at 159 DAFB. Photographs of the russeted area of the fruits were taken and calibrated and russeting quantified by image analysis using software Cell P[^]. The percentage of russeted area of the fruits were calculated by dividing the russeted area by the treated fruit surface area at the time of sampling and expressed as a percentage.

Data were subjected to one-way analysis of variance using SigmaPlot (version 12.5) at 5 % level of probability and presented in figures and tables as means \pm standard errors of the means.

RESULTS

Application of CPPU significantly increased acridine orange infiltrated area of the treated fruit surface only when applied during early developmental stage (21 DAFB; Fig. 1A). At 21 DAFB, AO infiltrated area of CPPU treated surface was four times (8 %) higher than that of the untreated surface (2 %). AO infiltrated area of the CPPU treated surface of the fruit was not significantly different from the AO infiltrated area of the untreated surface when CPPU was applied at 44, 73 and 100 DAFB (Fig. 1A). Application of moisture increased AO infiltrated area of the treated surface of the fruit only when applied during early developmental stage (21 DAFB; Fig. 1B). At 21 DAFB, AO infiltrated area of the moisture treated surface was 8-folds (8 %) higher than that of the untreated surface (1 %). AO infiltrated area of the moisture treated surface of the fruit was similar to the AO infiltrated area of the untreated surface of the fruit when moisture was applied at 44, 73 and 100 DAFB (Fig. 1B).

Application of CPPU increased permeability of the treated fruit surface only during early developmental stage (21 DAFB; Fig. 2A). The permeability at 21 DAFB of the CPPU treated surface was about 1.5 times ($15 \times 10^{-5} \text{ m s}^{-1}$) higher than that of the control surface ($10 \times 10^{-5} \text{ m s}^{-1}$). The permeability of the CPPU treated surface of the fruit decreased and became similar to the permeability of the control surface when CPPU was applied at 44, 73 and 100 DAFB (Fig. 2A). The application of moisture increased permeability of the treated surface of the fruit when applied during early developmental stage (21 DAFB; Fig. 2B). At 21 DAFB, the permeability of the moisture treated surface was four times ($40 \times 10^{-5} \text{ m s}^{-1}$) significantly higher than that of the control surface ($10 \times 10^{-5} \text{ m s}^{-1}$). Permeability of the moisture treated surface of the fruit decreased and was similar to the permeability of the control surface of the fruit at 44, 73 and 100 DAFB (Fig. 2B).

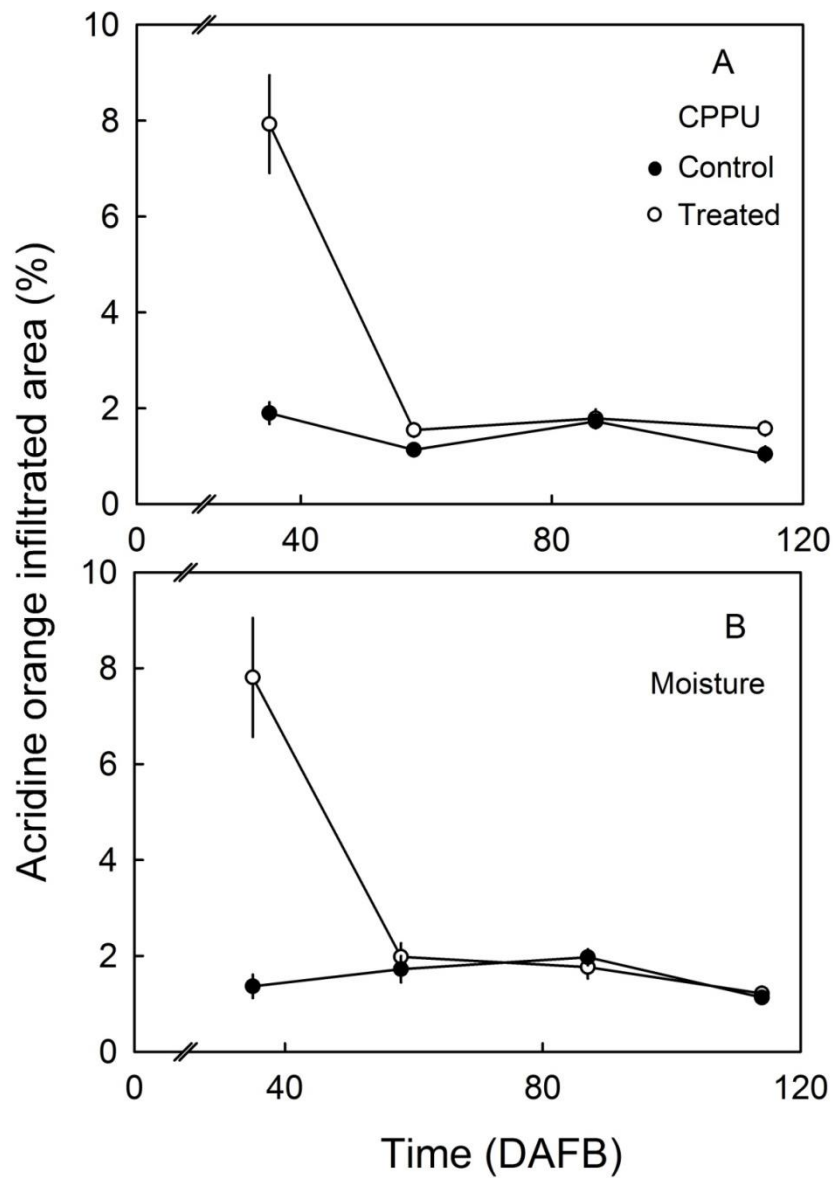


Fig. 1: Effect of CPPU (A) and surface moisture (B) on the acridine orange infiltrated area of the fruit surface as affected by the developmental stages of the fruit. Values represent mean \pm SEM ($n = 10$).

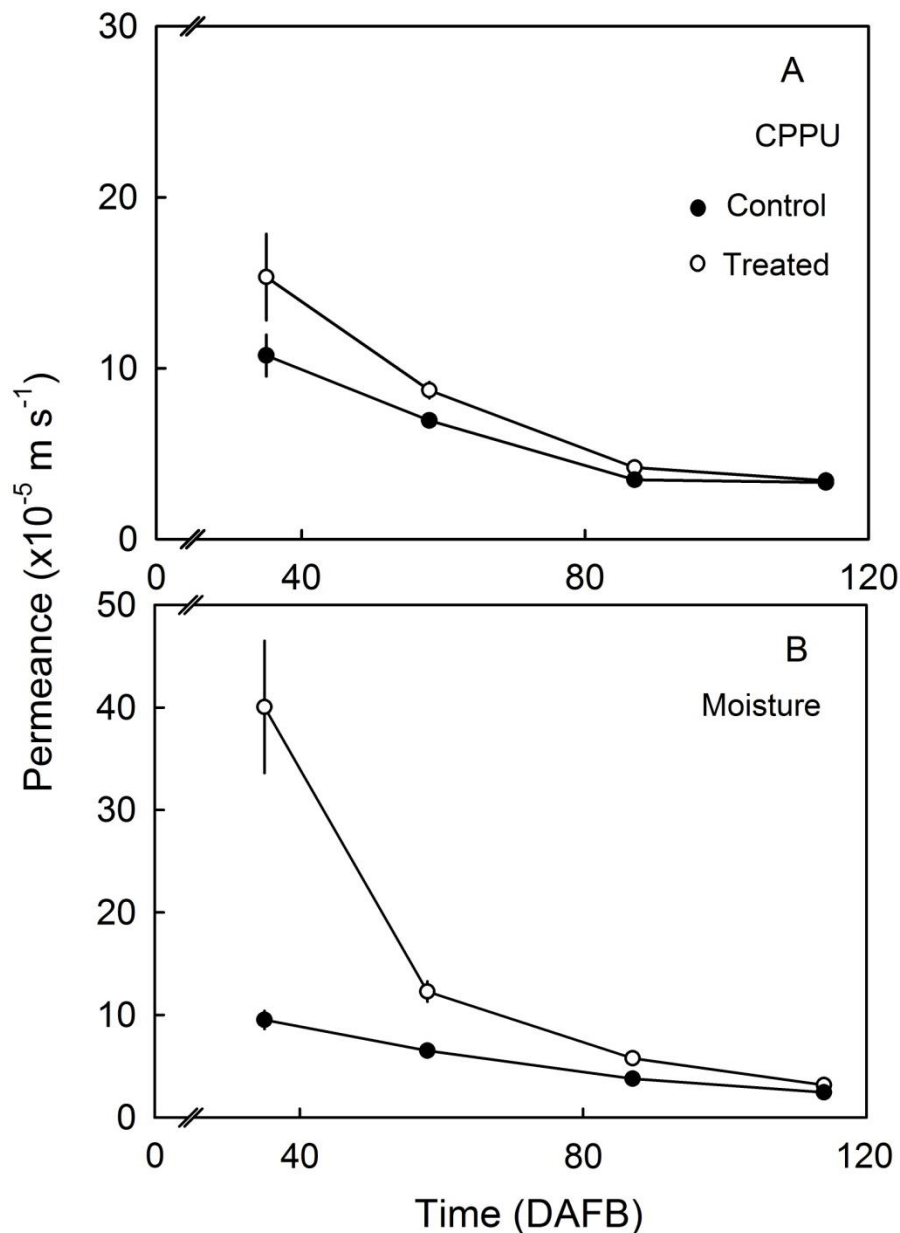


Fig. 2: Effect of CPPU (A) and surface moisture (B) on the permeance of the fruit surface as affected by the developmental stages of the fruit. Values represent mean \pm SEM ($n = 20$).

Application of CPPU on the growing fruit surface induced fruit russetting when treatment was applied at 21 DAFB (Table 1). Thus, at 21 DAFB, percentage of russeted area of the treated fruit surface when russetting was analysed at 159 DAFB was 10 %. However, there was no effect of the CPPU in inducing fruit russetting when the treatment was applied at 44, 73 and 100 DAFB.

Application of moisture on the growing fruit surface induced fruit russetting when treatment was applied at 21 DAFB (Table 2). At 21 DAFB, percentage of russeted

area of the treated fruit surface when russetting was analysed at 159 DAFB was 37 %. But, there was no effect of the moisture in inducing fruit russetting when the treatment was applied at 44, 73 or 100 DAFB.

Table 1: Effect of fruit development in CPPU induced russetting in 'Pinova' apple.

Treatment time (DAFB)	Russetting analysis (DAFB)	No. of reps (n)	% of fruit with russet	Russetting area (%)	
				Treated (Mean \pm SE)	Control (Mean \pm SE)
21	159	26	92	10.3 \pm 2.6	0
44	159	26	0	0	0
73	159	26	0	0	0
100	159	26	0	0	0

Table 2: Effect of fruit development in moisture induced russetting in 'Pinova' apple.

Treatment time (DAFB)	Russetting analysis (DAFB)	No. of reps (n)	% of fruit with russet	Russetting area (%)	
				Treated (Mean \pm SE)	Control (Mean \pm SE)
21	159	21	100	37.1 \pm 7.3	0
44	159	21	0	0	0
73	159	21	0	0	0
100	159	21	0	0	0

DISCUSSION

We observed that application of CPPU on the fruit surface enhanced fruit growth rate by increasing fruit surface area expansion rate due to rapid cell division in the early stages of development. This subject the fruit surface to strain resulting in microcracking (Cruz-Castillo *et al.*, 2014; Costa, 1999; Famiani *et al.*, 1997). The findings agree with those of Grimm *et al.* (2012), who reported that AO infiltration is indicator of mass flow through microcracks. We also found that when the fruit surface was treated with moisture at 21 DAFB, AO infiltrated area on microcracking was significantly higher than in the untreated surface (Fig. 1B). Surface moisture increased the severity of microcracks as depicted in the acridine orange infiltrated area (Fig. 1B). This finding supports previous studies (Khanal *et al.*, 2013b;

Considine and Brown, 1981; Creasy, 1980; Faust and Shear 1972) that application of surface moisture promotes microcracking during young fruit development.

It may be argued that CPPU had an effect on the relative growth rate on the fruit surface area particularly in early developmental stages (Ginzberg *et al.*, 2014; Iwahory *et al.*, 1988). This is in tandem with our findings that suggested significant increase in microcracking and permeability of the CPPU treated surface of the fruit than the untreated (Fig. 1A). We presumed that the cuticular membrane of the fruit was not sufficiently deposited or developed to restrict moisture uptake/loss, as reported in previous study (Kerstiens, 1996) during early developmental stages (Fig 2B). This could have contributed to altering the physical integrity of the moisture treated fruit surface culminating in increased permeability of the fruit surface.

In apple, application of CPPU in the early stages of fruit growth triggers cell division which enhances surface expansion rate thereby making the fruit sensitive to russetting (Famiani *et al.*, 1997). This agrees with our findings that CPPU induced russetting in the treated fruit surface in the early stage of fruit growth. Additionally, we observed that the moisture treated fruit surface induced russetting probably because the moisture applied on the fruit surface prevented the deposition of wax to fix the microcracks and prevent russetting on the fruit surface during the early developmental stage. Our findings are consistent with earlier works (Faust and Shear, 1972; Turkey, 1969), who reported that moisture plays a significant role in inducing russetting during early stage of fruit growth and development.

CONCLUSION

Growth rate and surface wetness increased microcracking, permeability and russetting of the apple fruit surface. Surface moisture had a more pronounced effect in microcracking, permeability and russetting of the apple fruit surface than that of growth rate during early fruit development, even though both factors are critical. There was no effect of surface moisture at late developmental stage where the fruit surface growth rate is low.

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