

# LENTICEL DAMAGE

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## ABSTRACT

Upon arrival in the USA market, New Zealand avocado fruit has been prone to the development of black spots and fungal rots on the skin of green fruit ('measles'). These symptoms appeared to be associated with lenticels and were worse in fruit harvested following a period of rain. To investigate the effect of mechanical damage on imbibed and on dehydrated avocados, detached fruit were artificially imbibed with water by inserting the cut stem of the fruit into a flask filled with reverse osmosis water, and then covering the fruit with a plastic bag. Fruit in flasks were placed in a high airflow in a chamber fitted with a row of fans. Water uptake and loss from the fruit was measured by changes in fruit weight and weight of water in the flask. Lenticels were then damaged using a standardized technique. Spots on the peel surface with diffusible browning were counted after leaving fruit for two days in a coolstore (5.5°C). Fruit were removed from flasks and plastic bags after up to 24 hours of water uptake, and placed directly in the airstream to dehydrate. Fruit were damaged and lesions counted as for water-imbibed fruit. After 2 hours of water uptake, the average number of lesions on fruit caused by jostling increased markedly. When fruit was taken out of the water and placed in the air stream, they became less susceptible to damage after 2 hours drying, as evidenced by a decrease in the number of lesions after handling. There was a morphological change in lenticels associated with water uptake. In normal fruit, there were intercellular spaces between cells under the lenticels. When the fruit had taken up water, the cells below the lenticels became turgid and the intercellular spaces were no longer evident. These turgid cells were more susceptible to damage, resulting in a brown discolouration. *Colletotrichum acutatum* and *Phomopsis* were the fungi isolated most frequently from 'measles' symptoms.

**Keywords:** peel damage, imbibition, lenticel structure

## INTRODUCTION

Upon arrival in the American market during the 1998 and 1999 export seasons, New Zealand avocados have been prone to the development of black spots and fuzzy patches on the skin of green fruit ('measles'). These symptoms appeared to be worse in fruit harvested following a period of rain in November, and appeared to be associated with lenticels (John White *pers. comm.*). In support of the first of these observations, Duvenhage (1993) found that lenticel damage in both Hass and Fuerte fruit in South Africa was worse when fruit were harvested following a period of rain.

## METHODS

### **Water uptake, dehydration, and susceptibility to lenticel damage**

Fruit were harvested with long stalks and transported to the Horticulture and Food Research Institute laboratories at Mt Albert Research Centre as rapidly as possible within two hours of harvest. Fruit stems were then recut and immediately inserted into flasks containing water. The flask was sealed except for a small hole through which the stalk was inserted. Fruit in flasks were covered with plastic bags and placed in a high airflow. After commencement of water uptake, 5 fruit per treatment were sampled, damaged by jostling (rolling from one end to another of a plastic crate 10 times) and placed in a coolstore at 5.5°C after the following times: 5 mins, 10 mins, 15 mins, 30 mins, 1 hour, 2 hours, 4 hours, 8 hours and 24 hours.

In a second series of experiments, fruit were imbibed for 24 hours, which was the point in time where fruit had taken up the maximum amount of water (determined by graphing weight gain), after which fruit were removed from the water source and placed in the airstream. Fruit were sampled at the time periods specified above. Fruit were sampled, jostled and placed in a coolstore after those times.

### **Fungal isolations**

Isolations were made from symptoms of lenticel damage, and from adjacent spots on the surface of the avocado fruit following swabbing with 70% ethanol and placing tissue pieces on Difco Potato Dextrose Agar (PDA) after 2 days coolstorage.

Fruit that had been jostled were left in the coolstore for an extended period of time (4 - 5 weeks) to enable 'measles' symptoms to develop. Fungi associated with 'measles' were isolated and identified, following swabbing with 70% ethanol and placing tissue pieces on PDA. Adjacent tissue showing no apparent symptoms was also swabbed and placed on agar.

### **Microscopic observations**

Firm avocado fruit were examined after the following treatments;

1. No previous treatment,
2. Imbibition with water for 5 - 24 hours,
3. Imbibition and subsequent drying for 5 - 8 hours.

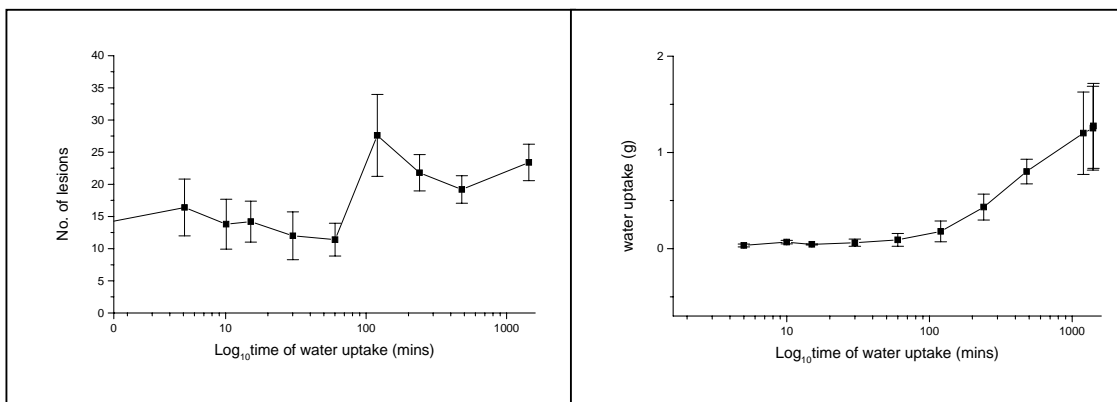
In each case material was chosen that contained numerous lenticels, visible as pale yellow spots on the green skin.

After each of the above three treatments fruit were 'jostled' 50 - 60 times in a plastic crate. For untreated fruit that had been imbibed then dried, representative samples containing lenticels were taken. In the case of imbibed fruit, where brown patches soon occurred, these areas were sampled at intervals between 15 - 30 minutes and 2 hours after jostling, and at 24 hours after jostling.

## RESULTS

### Water uptake, dehydration, and susceptibility to lenticel damage

After 120 minutes of water uptake, the number of lesions caused by jostling increased from an average of 15 per fruit to an average of 20-25 lesions per fruit (Fig. 1a). This change was concomitant with an increase in the rate of water uptake by the fruit (Fig. 1b).



a)

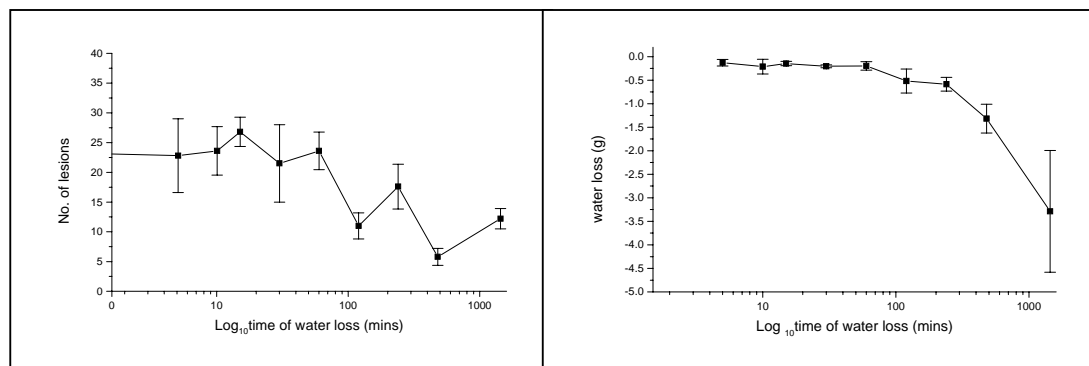
b)

**Figure 1:** Change in susceptibility to lenticel damage after increasing time of water uptake. Values are means  $\pm$  standard error.

When fruit was taken out of the water and placed in the air stream, the fruit became less susceptible to damage 120 minutes after removal from water. (Fig. 2 a,b). There was more variation in the response of individual fruit to water loss than to water uptake, and individual fruit could take up to 24 hours to return to their original weight (water content).

a)

b)



**Figure 2:** Change in susceptibility to lenticel damage after increasing time of water loss. Values are means  $\pm$  standard error.

### Fungal isolations

A range of saprophytic fungi and bacteria (*Bacillus subtilis*, *Cryptosporiopsis* sp., *Cladosporium* sp., unidentified fungi) were isolated from damaged lenticels (Table 1), but no pathogenic fungi. However, once 'measles' symptoms had been allowed to develop after 4-5 weeks in the coolstore isolations showed that *Colletotrichum acutatum* and *Phomopsis* were the fungi isolated most commonly from 'measles' symptoms compared with symptomless regions (Table 2).

**Table 1:** Isolations from damaged lenticels on green avocado fruit.

Organism	Isolations from Lenticel damage
<i>Bacillus subtilis</i>	3
<i>Cryptosporiopsis</i>	1
<i>Cladosporium</i>	4
<i>Botryosphaeria</i> <sup>1</sup>	1
Unidentified fungi	2
No organism isolated	6
Total	17

<sup>1</sup>. A *Botryosphaeria*-like fungus that was dissimilar to the rot pathogens.

**Table 2:** Isolations from 100 fruit that had been jostled and placed in the coolstore for 4 - 5 weeks until 'measles' symptoms developed. Four samples of skin swabbed with 70% ethanol were taken from each fruit, two from 'measles' symptoms and two from symptomless regions (control).

symptoms	Fungi isolated							total
	C.a.	C.g.	B.p.	B.d.	P.	No isolation	sap.	
Control	6	24	2	0	0	163	5	200
'measles'	50	20	4	0	29	85	12	200

C.a.= *Colletotrichum acutatum*, C.g.= *Colletotrichum gloeosporioides*, B.p.= *Botryosphaeria parva*, B.d.= *Botryosphaeria dothidea*, P.= *Phomopsis*. sap= saprophytic fungi.

**Figures 20 and 22:** Lenticel structure in imbibed and imbibed then dried fruit.

**Figure 27:** Areas of diffuse browning.

14A. Bars = 0.1mm.

## **Microscopic observations**

### **Lenticel structure.**

Lenticels are breathing pores through which the fruit can lose water vapour and take in gases needed for ripening and growth. Preliminary studies showed that water uptake when fruit become imbibed is not through lenticels, but through the stalk. Lenticels are visible by the naked eye as small yellow dots (Fig. xx). Lenticels form all over the fruit surface, not just on the lumps. When viewed at a high magnification by a microscope, lenticels are seen as small holes on the fruit surface surrounded by a depressed circle, similar to a crater on the surface of the moon (Fig. xx). When a cross section (sideways slice) of this 'crater' is looked at, the lenticel is seen to be a small hole in the fruit surface guarded by skin cells, but under this small hole there is a larger cavity lined by loosely packed cells (Fig. xx).

### **Lenticel structure in imbibed and imbibed then dried fruit.**

As fruit take up water the loosely packed cells within the lenticel expands and becomes rounded (Fig. 20). Eventually they occupy the whole space under the skin (Fig. 22). As the fruit is allowed to dry out the cells contract and the cavity under the skin is recovered (as in Fig. 32- browning figure in composite figure)

### **Areas of diffuse browning.**

Diffuse browning appears as a mid to deep brown discolouration of portions of the surface usually up to only a few millimetres in diameter. Jostling water-imbibed fruit to simulate grading/handling damage always caused the occurrence of diffuse browning. These could be seen as little as 30 minutes after rolling as slightly discoloured areas of the skin. More extensive browning and discernible cellular collapse were visible in the region of the lenticels. After two hours more extensive browning could be seen. Discolouration and cellular damage was intense around lenticels and extended to the surface (Fig. 4).

Observations of imbibed damaged fruit that were inoculated with fungal spores failed to show any evidence of extensive fungal growth through the damaged areas of the tissue.

## DISCUSSION

Two methodologies were successfully developed. These were; 1) artificial imbibition and dehydration of avocado fruit, and 2) a standardized technique for artificially and rapidly causing areas of diffuse browning on the surface of avocado fruit. In addition, morphological changes of lenticels associated with water uptake were described.

The use of these techniques demonstrated that;

- fruit become more susceptible to lenticel damage after approximately two hours of taking up water,
- hydrated fruit become less susceptible to lenticel damage after approximately two hours of losing water, and
- skin cells in hydrated fruit become more turgid and more susceptible to mechanical damage.

Further work is needed in order to relate these results to field conditions and the time after rain when fruit in the field becomes susceptible to lenticel damage. An indirect measure of lenticel status was developed based on susceptibility to handling damage, this measure will be a good aid in future field studies. In addition, this technique can be used to ascertain how long after a period of rain fruit in the field become less susceptible to lenticel damage.

When fruit take up large amounts of water, either artificially by imbibition or on the tree during and immediately after a period of rain, it is likely that all cells of the fruit increase in water content. Expansion of most cells within the fruit is limited by the dense cellular packing. The loosely packed cells around and in the lenticellular cavity are able to expand and fill the entire cavity. The expansion of these cells appears to be completely reversible when the fruit is allowed to lose water.

It is likely diffuse browning results from the breakdown of cellular membranes caused by impact and compression forces on cells that have already been stressed by increased turgor pressure. Membrane rupture destroys compartmentalisation in the cells and results in, amongst other things, the production of brown phenolic products. It is likely that the greater effect seen in the lenticel regions is due to greater fragility. This is caused by these cells being less constrained by adjacent cells as in the internal fruit tissues, and able to expand to a greater extent, increasing internal stress. The frequent absence of browning in cell layers adjacent to the epidermis is probably due to the surface cells being able to deform whilst as the force is transmitted deeper into the tissue the cells are less able to. In non-imbibed tissue where diffuse browning is less frequently formed the less turgid cells are more easily able to absorb the forces.

The role of lenticel damage (diffuse browning) in the development of rots ('measles') was not definitively proven. Isolations from lenticels showing diffuse browning failed to yield pathogenic fungi, but saprophytic fungi were found. Indeed, isolations showed that the fungi *Colletotrichum acutatum* and *Phomopsis* appeared to be most closely associated with 'measles' symptoms. The other fungi usually associated with avocado postharvest rots (Hartill 1992) were either not or rarely isolated (*Botryosphaeria dothidea* and *Botryosphaeria parva*), or isolated in equal numbers from apparently symptomless tissue (*Colletotrichum gloeosporioides*).

In conclusion, it is recommended that in order to avoid lenticel damage fruit should not be harvested immediately after or during a heavy period of rain. Results of this study suggest



that following a period of heavy rain, approximately two hours is required for fruit to return to a 'dry' or unimbibed state. Actual drying times in the field will depend on ambient conditions of relative humidity, temperature and windspeed. Before these results can be applied directly to the field situation, the effect of ambient conditions on fruit water loss needs to be determined.

Because preliminary studies showed that water uptake to imbibe fruit is primarily through the stalk, not through lenticels, then surface wetness of the fruit during a period of rain may not be responsible for hydrating the lenticels. Instead surface wetness may have the secondary effect of preventing the lenticels from losing water, and thereby increase turgor pressure within the fruit. However, most of the increased turgor pressure is by uptake of water through the plant root system and through the stalk into the fruit. This suggests that high soil water potential is likely to be the most important factor leading to lenticel damage. Therefore rainfall probably needs to be sufficient to wet soil before causing increased fruit turgor and consequent increased lenticel damage.

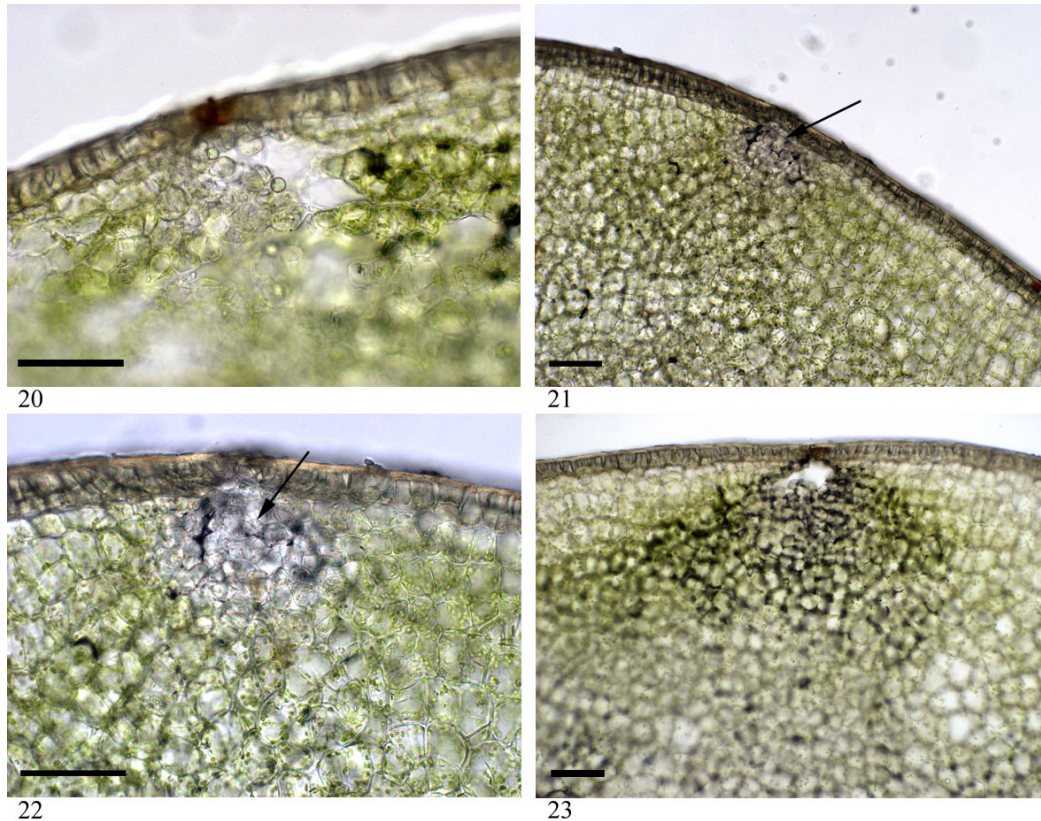
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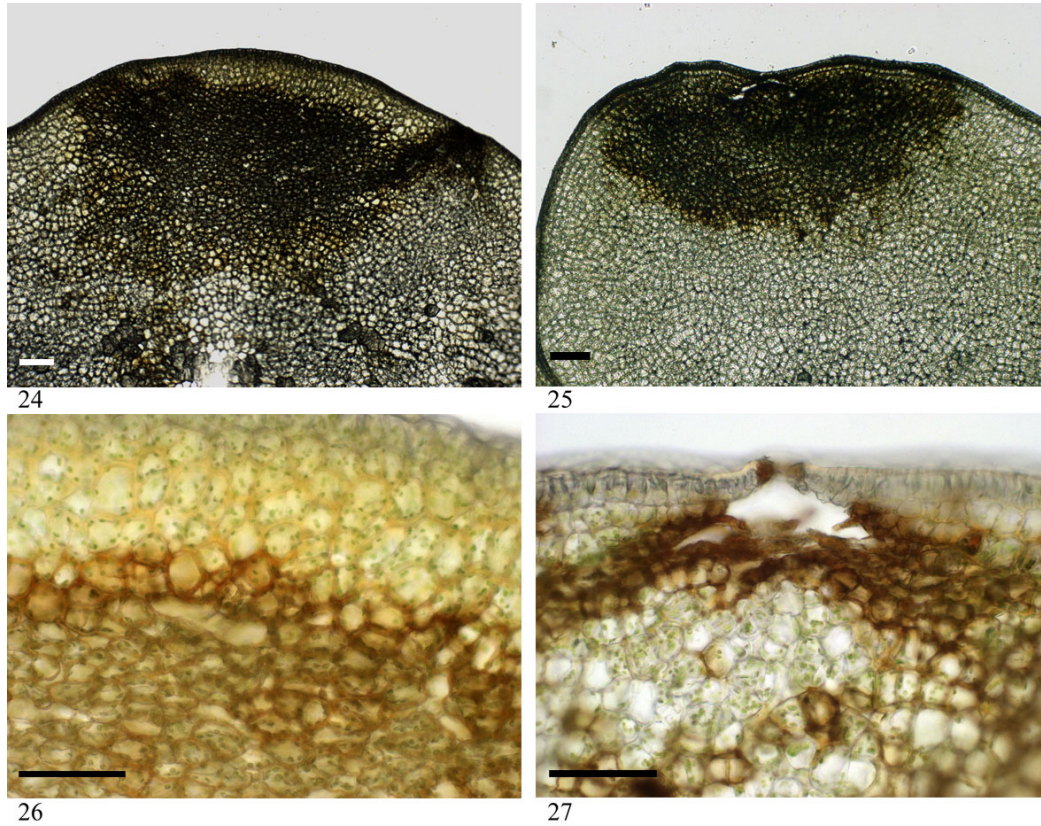
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**Figure 4:** Lenticel structure in imbibed and imbibed then dried fruit. Bars = 0.1mm.

- A) Lenticel from fruit after 4 hours imbibing water. Lenticel cavity is reduced in size and loosely packed cells have started to round.
- B) Lenticel from fruit after 12 hours imbibing water. Lenticel cavity has been completely occluded by swollen loosely packed cells (arrow).
- C) Detail of B) showing swollen rounded loosely packed cells occluding the cavity (arrowed). These have few chloroplasts resulting in the region below the lenticel pore remaining a less intense green than other parts of the skin.
- D) Lenticel cavity that has re-formed in fruit imbibed then allowed to dry. All light microscope – unstained vibratome section.



**Figure 5:** Areas of diffuse browning. Bars = 0.1mm.

- A) Typical example of severe diffuse browning damage showing much of the browning is in cell layers beneath the immediate epidermal and sub-epidermal layers. Browning extends to the surface in the region of lenticels.
- B) Diffuse browning damage with more extensive browning at the surface.
- C) Detail of brown region underlying an apparently undamaged region just under the epidermis. Damaged cells show extensive browning to the wall and cells are distorted.
- D) Lenticel region showing extension of browning to the surface and damage to cells bounding the lenticel cavity. All light microscope – unstained vibratome section.

