21

# Development of a strategy for the prevention of black cold injury in South African export avocados

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### ABSTRACT

Black cold injury, like greypulp, is initiated by a range of factors. However, certain of the causative factors manifest in a reverse fashion. For instance, more mature fruit are most susceptible to grey pulp while less mature fruit, especially from later sets, are more affected by black cold injury. In addition to maturity, aspects such as orchard temperature and the orientation of the fruit with regard to the sun are also important. Unfortunately, it is not possible to manipulate the latter two factors so as to reduce the risk of black cold injury. At present, it would seem that the most promising pre-harvest approach would be to improve the nutritional status, especially regarding the nitrogen and calcium content of the fruit. However, appropriate chemical content parameters are considerably narrower for black cold injury than for grey pulp. The best postharvest approach is to increase the storage temperature. In this regard, a number of techniques aimed at retaining fruit firmness at higher storage temperatures, are presently being evaluated. These include storage in an ozone enriched atmosphere, ethylene scrubbing, CO, shock applications and 1-methyl cyclopropene (1-MCP) treatments. Of these, 1-MCP shows the most promise. The elimination of inappropriate packhouse procedures, that may increase the incidence of black cold injury, is also important. It is envisaged that future research on black cold injury will include the refinement of the above strategies while novel approaches should continuously be developed.

#### INTRODUCTION

Black cold injury is a physiological disorder effecting the rind of cold-stored export avocados. It is annually responsible for substantial losses to the South African avocado export industry and repacking of the fruit upon arrival in Europe is often required.

An intensive research effort aimed at rectifying the quality problems encountered with the Pinkerton cultivar was conducted during the last three seasons (Kruger *et al.*, 2000; Kruger *et al.*, 2001 & Snijder *et al.*, 2002). This substantially benefited the industry and resulted in a significant reduction in grey pulp. In contrast with black cold injury, grey pulp is only detected upon ripening, and consignments can not be repacked and have to be discarded. Our initial research efforts therefore concentrated on this ailment and less attention was paid to black cold injury. However, during the last season we have conducted a number of experiments aimed at characterising black cold injury and determining the causative factors. Preliminary experiments were also conducted, aimed at developing a control strategy.

#### **CHARACTERISATION**

It is important to correctly define black cold injury so as to avoid confusion with other physiological and pathological disorders associated with the avocado rind.

Although it is unlikely that black cold injury might be confused with pre-harvest infections such as cercospera or pepper spot, it has frequently happened that researchers confuse the damage caused by postharvest anthracnose infections with black cold injury. Black cold injury can further be confused with advanced lenticel damage. However, we are of the opinion that this type of damage is caused by desiccation of the thinner skin tissue around the lenticels and the damage is usually located on the side of the fruit with the most prominent lenticels. The lesions may coalesce and ultimately have a somewhat similar appearance to black cold injury. This type of damage is usually mistaken for black cold injury in experiments where a substantial period has passed from packing until the fruit is placed into storage.

Another disorder that may be confused with black cold injury is anaerobiosis. These lesions are caused by inappropriately high  $CO_2$ or low  $O_2$  levels. However, in contrast with black cold injury, these lesions are more diffuse, they have a creased appearance and are more prevalent on the neck of the fruit.

Black cold injury lesions are caused by the death of epidermal cell patches in specific areas, especially at the distal end of the fruit. The contents of the dead cells within the lesions would seem to have been absorbed by the live tissue surrounding the lesions. For this reason, the cell walls are compacted and the lesions have a sunken appearance. We have, as yet, found no visual clues as to why such a clear cut distinction exists between the dead cells on the inside of the black cold lesion and the live cells on the outside of the lesion.

#### **CAUSATIVE FACTORS**

The most important postharvest causative factor remains a sub optimal storage temperature. However, unlike certain other subtropical fruit crops, there does not seem to be a specific threshold value below which all fruit become injured and above which all fruit stay healthy. It is more a case of a temperature range within which the disorder starts to emerge. During the last two seasons, we have conducted a number of experiments aimed at establishing whether relative humidity and the speed at which cold air is circulated over the fruit also contribute towards black cold injury. As yet, we have failed to establish a significant link between relative humidity and air speed on the one hand and black cold injury on the other.

A number of pre-harvest factors have been implicated in the manifestation of black cold injury. Swarts (1982) compared the effect of orchard temperature and fruit maturity on the incidence of black cold injury in the Fuerte cultivar. He found the susceptibility of the fruit to decrease as orchard temperatures dropped during autumn. In contrast, our own experiments indicated maturity to probably be more important than orchard temperature. With all export cultivars, we found the incidence of black cold injury to decrease as they matured. This happened during autumn, when the orchard temperatures decreased as well as during late winter when the temperatures increased. Interestingly, an increase in the saturated : unsaturated fatty acid profile of the fruit pulp was not necessarily associated with lower orchard temperatures as this ratio was found to increase in cultivars that mature during late winter. This observation would therefore seem to corroborate the maturity hypothesis. In addition, Snijder, et al. (2002), found earlier sets to be less susceptible to black cold injury than later sets. The earlier sets are more mature but are subjected to the same orchard temperatures as the less mature later sets. It can therefore be inferred that maturity probably plays a more important role than orchard temperature.

Another factor that has been implicated as a role player is the amount of direct sunlight the fruit receive while hanging on the tree. Most higher organisms have a molecular mechanism to protect the proteins in their cells from high temperatures. This is done by socalled heat shock proteins that chaperone protein molecules after the transcription and translation processes have taken place. Agglutination is hereby prevented and the molecules are allowed to fold into secondary and tertiary configurations. Interestingly, the heat shock proteins would also seem to protect avocado fruit from lower temperatures during subsequent storage (Woolf, et al., 2000). We have repeated certain of the experiments performed by Woolf et al. (2000) and have found outer fruit to be less susceptible to chilling injury than shaded fruit. Unfortunately, we found the unexposed side of outer fruit to be just as susceptible to black cold injury as fruit inside the tree. It would therefore not be a solution to harvest outside fruit during the early season and inside fruit later. However, in terms of maturity this may be an option. Fruit from a warmer part of the tree is known to mature faster than fruit from a cooler part.

It has been shown that postharvest heat treatments may also elicit the production of heat shock proteins. Experiments conducted in New Zealand have shown postharvest heat shock treatments to effectively protect Hass against chilling injury (Woolf *et al.*, 1995, Woolf *et al.*, 1996). Unfortunately, extensive trials conducted in South Africa by Kritzinger & Kruger (1997), Kritzinger *et al.* (1998), Weller *et al.* (1998), Kremer-Köhne (1999) and Grové, *et al.* (2000) have shown this technique to be unreliable and impractical.

#### **PRELIMINARY RECOMMENDATIONS**

At present, the available results seem to indicate that the upgrading of procedures related to plant nutrition, storage temperature and packhouse practices hold the greatest potential for the reduction of black cold injury.

Kruger et al, (2000, 2001), formulated preliminary fruit mineral content recommendations for the Pinkerton cultivar and these were upgraded by Snijder, et al, (2002). The recommendations are primarily aimed at the reduction of grey pulp as this disorder was found to be the single most important factor affecting the Pinkerton cultivar. It was recommended that the fruit nitrogen content be less than 1% by March if an effective reduction in grey pulp is to be attained. It was further recommended that the calcium content be above 1000 ppm during November when the fruit are between 50 g and 100 g in size. Fruit nitrogen and calcium content surveys conducted with the Pinkerton cultivar during the last two years indicate the required specifications to be considerably more strict for black cold injury than for grey pulp. If black cold injury is to be reduced, the N level should already be below 1% by January. In terms of Ca, more than 1200 ppm should be present in the fruit during November. Also, this level should not drop below 800 ppm by January and 600 ppm by March. With regard to the calcium nitrogen ratio, our recommendation with regard to grey pulp was that the ratio should be above 500 ppm/% by February and that it should not decrease by more than 100 points per month during the subsequent three months. It would seem that, in terms of black cold injury, the ratio for January should be increased to 700 ppm/% if the incidence of the disorder is to be reduced.

There seems to be a positive correlation between grey pulp incidence and fruit N content and a negative correlation between incidence and fruit Ca content. In the case of black cold injury, however, increased fruit Ca and decreased fruit N levels would not seem to have much of an effect until a specific threshold value is reached. The above specifications should therefore be interpreted as threshold values. Our present research results indicate that the required values are more readily attainable in older orchards, while it is doubtful whether they will be achievable in orchards younger than 5 years of age.

The second important field of intervention concerns storage temperature. The results generated in this and previous studies, indicate black cold injury to be considerably reduced, but not eliminated, by storage temperatures approaching 10°C. When exporting fruit at these temperatures, additional mechanisms are required to prevent softening during transport. Presently, controlled atmosphere (CA) and specific versions of modified atmosphere (MA) storage are used for this purpose. During the last two seasons, we have conducted elaborate tests on the ethylene inhibitor, 1-methyl cyclopropene (1-MCP). The results were very encouraging and it is envisaged that this product will be extensively used by the South African avocado export industry in future.

The third area of intervention concerns packhouse procedures. In this case, however, our recommendations are not based on appropriately designed trials and epidemiological surveys, but rather on casual observations. The first aspect that we would like to stress is that packhouse managers should refrain from introducing untested products into their packing operation. This is especially relevant with regard to cleaning agents and waxes. A range of new cleaning agents has recently become available and these are promoted by representatives who visit the various packhouses. It is important to note that the possibility exists that certain of these disinfectants may cause damage to the skin of the fruit that may increase the chances of contracting black cold injury during subsequent storage. It is therefore advised that packhouses stay with chlorine and that they ensure that it is used at the correct pH (Kremer-Köhne, 1996). The same applies to waxes. Rather stick to a well known wax than opting for a cheaper formulation that has not been tested under South African conditions.

The final recommendation concerns the water stressing of fruit before harvest. Inappropriate withholding of irrigation before harvest may increase the chances of black cold injury occurring during storage. Packhouse managers should ensure that producers are aware of this aspect.

It is envisaged that future research on black cold injury will involve the refinement of the above mentioned control strategies. However, novel approaches to the problem should continuously be contemplated and developed.

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