Interaction of storage, ethylene and ethylene inhibitors on post harvest quality of 'Maluma'

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Abstract

'Maluma' is a precocious and productive early bearing 'Hass'-like cultivar, commercially released towards the end of 2007. 'Maluma' currently enjoys full plant protection in South Africa (ZA 20043215) and the USA (US PP21,099 P3). Plant Breeder's Rights are pending in other avocado producing countries. 'Maluma' is an approved South African export cultivar. The responses of 'Maluma' fruit to different postharvest (pre-storage) fruit treatments with interactions under storage ($6^{\circ}C$) and non-storage (ambient) conditions were examined. The aim of this study was to determine the effect of ethylene on postharvest fruit decays and physiological disorders. The effective management of the negative effects of ethylene through the use of ethylene inhibitors (2,4-dichlorophenoxy acetic acid (2,4-D) and 1-methylcyclopropene (1-MCP)) was also examined. The fruit were ripened at ambient ($20 - 22\ ^{\circ}C$) while the days to ripen were recorded. Fruit were evaluated for the presence of stem end rots, body rots (anthracnose), vascular browning, red/pink vascular staining and greypulp. The impact of storage on the rate of ripening was evident, with the least days to ripen with ethylene treated fruit and the most with the 1-MCP treatment. 1-MCP acts as an effective ethylene inhibitor. Less mature fruit are more prone to fungal diseases. Vascular browning is associated with fruit rots. Stored fruit shows less disease related disorders which relates to the faster ripening of the fruit. The frequency and severity of vascular staining significantly increases amongst fruit treated with ethylene prior to storage. This phenomenon, which is ethylene induced, can be efficiently controlled by a 2,4-D (Deccomone®) stem treatment (dip) during picking together with a 1-MCP (SmartFresh™) treatment prior to cold storage.

Key words: 'Maluma', postharvest, physiological disorders, ethylene, inhibitor, 2,4-D, 1-MCP, rots, vascular browning, vascular staining, greypulp

Interacción del Almacenamiento, Inhibidores de Etileno y Etileno en Calidad Post-cosecha de 'Maluma'

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Resumen

'Maluma' es un cultivar precoz y productivo, tipo 'Hass', fructífero, lanzado comercialmente a fines de 2007. 'Maluma' actualmente goza de la completa protección de plantas en Sudáfrica (ZA 20043215) y en EE. UU. (US PP21,099 P3). Los derechos de productores de plantas están pendientes en otros países productores de palta. 'Maluma' es un cultivar de exportación sudafricano aprobado. Se analizaron las respuestas de la fruta de 'Maluma' a diferentes tratamientos de fruta post-cosecha (pre-almacenamiento) con interacciones bajo condiciones de almacenamiento ($6^{\circ}C$) y no almacenamiento (ambiente). El objetivo de este estudio fue determinar el efecto del etileno en los deterioros de la fruta post-cosecha y trastornos fisiológicos. El efectivo manejo de los efectos negativos del etileno a través del uso de inhibidores de etileno (2,4-ácido diclorofenoxiacético (2,4-D) y 1-metilciclopropeno (1-MCP)) también se analizó. La fruta maduró a temperatura ambiente ($20-22^{\circ}\ C$) mientras se registraron los días para la maduración. La fruta se evaluó en presencia de putrefacción de tallos, el cuerpo putrefacciones (antracnosis), dorado vascular, manchas vasculares rojas/ rosadas y pulpa gris. El impacto del almacenamiento en la velocidad de maduración fue evidente, siendo menor la cantidad de días para la maduración con la fruta tratada con etileno y mayor con el tratamiento con 1-MCP. El 1-MCP actúa como

un inhibidor efectivo de etileno. La fruta menos madura tiene más tendencia a enfermedades de hongos. El dorado vascular se asocia con la putrefacción de la fruta. La fruta almacenada muestra menos trastornos relacionados con enfermedad que se relacione con la maduración más rápida de la fruta. La frecuencia y gravedad de las manchas vasculares aumenta significativamente en las frutas tratadas con etileno antes del almacenamiento. Este fenómeno, inducido por etileno, puede controlarse en forma eficiente con un tratamiento (inmersión) del tallo en 2,4-D (Deccomone®) durante la recolección, junto con un tratamiento de 1-MCP (SmartFresh™) antes del almacenamiento en frío.

Palabras clave: 'Maluma', post-cosecha, trastornos fisiológicos, etileno, inhibidor, 2,4-D, 1-MCP, putrefacción, dorado vascular, manchas vasculares, pulpa gris.

1. Introduction

'Maluma' is a precocious and productive early bearing Hass-like cultivar, commercially released at the VI World Avocado Congress held in Viña del Mar, Chile during November 2007 (Ernst, 2007). 'Maluma' currently enjoys full plant protection in South Africa (ZA 20043215) and the USA (US PP21,099 P3). Plant Breeder's Rights are pending in the other avocado producing countries. 'Maluma' is an approved South African export cultivar**.**

The avocado (*Persea americana* Mill.) is a typical climacteric fruit that exhibits a sharp rise in ethylene production during ripening, which occurs after harvest; mature avocado fruits will not ripen while attached to the tree (Blumenfeld & Gazit, 1974). Rhodes (1981) defines the climacteric as "a period in the ontogeny (morphogenesis) of certain fruits during which a series of biochemical changes is initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and leading to ripening". Therefore ethylene and its autocatalytic production are of vital importance to normal avocado ripening. Tingwa and Young (1975) postulated that some substance, possibly an anion, acts as a ripening regulator and moves either to or from the fruit pedicel once detached from the tree.

Adato and Gazit (1974) found that the faster avocado fruit loses water after picking, the faster they ripen. The ethylene peak occurs earlier if fruits were allowed to become dehydrated, whereas the reverse is true if harvested fruits were infiltrated with water.

Apart from ethylene, abscisic acid (ABA) appears to be a ripening promoter (Bower, 1985). Lieberman, Baker and Sloger (1977) showed an increase in ethylene and ripening following the application of ABA before the climacteric peak. Bruinsma (1981) considered the likely role of ABA to be the stimulation of ethylene biosynthesis once ripening inhibitors of the tree is no longer available after picking. According to Bower (1985) stress is a major factor affecting ABA levels. Once picked, considerable loss of water from the avocado fruit by transpiration normally takes place. This could create stress conditions in the fruit, leading to ABA accumulation once the fruit water potential approaches zero turgor. Factors other than water stress can also affect ABA synthesis, the most notable in avocado fruits being temperature.

Hershkovitz, Friedman, Goldschmidt and Pesis (2009) illustrated the inducing effect of ethylene on mesocarp discoloration located at the base of the seed in avocado fruit. 1-Methylcyclopropene (1-MCP), the ethylene inhibitor, effectively prevented mesocarp discoloration. Auxins, cytokinins and gibberellins, are also inhibitors of ethylene resulting in fruit ripening (Rhodes, 1981). To increase the days to ripen, with consequential improvement of shelf life, Lemmer *et al.* (2008) recommend a 1-MCP dosage rate of 500 parts per billion (ppb), for open market 'Maluma'.

Abscission is an active developmental process occurring at the abscission zone of the fruit pedicel. It is accepted that the increase in ethylene production in the fruit is followed by increased sensitivity of the target cells in the abscission zone to ethylene, which would lead to abscission. In the event to repress postharvest decay of citrus, the auxin, 2,4-dichlorophenoxy acetic acid (2,4-D), is used as a postharvest packhouse treatment to retard calyx abscission. Commercially the sodium salt of 2,4-D (Deccomone®) is applied to the fruit in a dip treatment at 500 parts per million (ppm) (Cronjé *et al.* 2005). Deccomone® is registered for the

use on citrus in South Africa at a dosage rate of 2 litre / 100 litre water (Vermeulen, Grobler, & van Zyl, 2000).

The changes in colour of the fruit skin, as indicator of ripeness, from green to purple/black, is visually more intense with 'Maluma' compared to 'Hass' (Ernst, 2007). The change in pigment is due to an increase in the anthocyanin concentration in the skin tissue during ripening (3-6 days postharvest) and is almost entirely due to a single anthocyanin: cyaniding 3-O-glucoside (Cox, McGhie, White & Woolf, 2004).

On examining the interaction of ethylene and temperature on postharvest quality of 'Hass' avocado, Arpaia (2006) reported on the incidence of pink vascular staining following ripening at 20ºC after pre-storage ethylene treatments, subsequently stored at respectively 4 and 12ºC (95% RH) for 0, 4 and 14 days. Pink vascular staining was most prevalent in ethylene treated fruit held at 12ºC for 14 days. It has been suggested that the occurrence of this disorder is directly linked to the treatment of ethylene in combination with storage, particularly at intermediate temperatures. In relation to storage duration, fruit which did not receive any ethylene displayed a dramatic reduction in days to ripen. A trend of decreasing decay incidence with storage duration was observed, which is believed to be due to the protracted time of ripening of fruit stored for 0 (zero) days.

The aim of this study is to determine the effect of ethylene on postharvest fruit decays and physiological disorders and to investigate methods to decrease the incidence thereof.

2. <u>Material and methods</u>

The response of 'Maluma' fruit to different postharvest (pre-storage) fruit treatments with interactions was examined. The treatments included ethylene and ethylene inhibitors under different storage conditions. Fruit treatment commenced within 4 hours after picking. Three different experiments were conducted namely:

Experiment 1:

The experiment was a 2x2x2 factorial design, laid out on 30 March 2010 and consisted of the following treatments:

- 2 x Storage temperatures: control (0 storage at ambient: 20 to 22ºC) and 6ºC for 7 days.
- 2 x 1-MCP (SmartFresh^{TM}) treatments at 0 and 500 parts per billion (ppb) at 7ºC for 16 hours.
- 2 x Ethylene treatments at 0 and 100 part per million (ppm) at 20ºC for 16 hours prior to storage.

  The dry matter content of the fruit was 23% (77% moisture)

Experiment 2:

The experiment was a 2x2x2 factorial design, laid out on 2 August 2010 and consisted of the following treatments:

- 2 x Storage temperatures: control (0 storage at ambient: 20 to 22ºC) and 6ºC for 7 days.
- 2 x 2,4-D (Deccomone$^{®}$) stem dip treatments at 0 and 2 litres / 100 litres (2%) water at harvest.
- 2 x Ethylene treatments at 0 and 100 part per million (ppm) at 20ºC for 16 hours prior to storage.

  The dry matter content of the fruit was 33% (67% moisture).

Experiment 3:

The experiment was a 2x2x2x2 factorial design, laid out on 9 May 2011 and consisted of the following treatments:

- 2 x Storage temperatures: control (0 storage at ambient: 20 to 22ºC) and 6ºC for 7 days.
- 2 x 2,4-D (Deccomone®) stem dip treatments at 0 and 2 litres / 100 litres (2%) water at harvest.
- 2 x 1-MCP (SmartFresh^TM) treatments at 0 and 500 parts per billion (ppb) at 7ºC for 16 hours.
- 2 x Ethylene treatments at 0 and 100 part per million (ppm) at 20ºC for 16 hours prior to storage. . The dry matter content of the fruit was 23% (77% moisture).

All fruit were treated with Westfalia Biocote^TM (92ml / 1 litres water, stem dip) at harvest and prochloraz (1,8 ml / litres water, fruit dip) during packing. Ripening occurred at ambient (20 to 22ºC). Days to ripen for each treatment were recorded. Fruit were evaluated for the presence of stem end rots, other rots (anthracnose), vascular browning, red/pink vascular staining and greypulp. Data was statistically analysed with the Chi-square % defective test through the aid of the computer software package Minitab 16.

3. Results and discussion

The results as illustrated in Figure 1 to 5 are from Experiment 1: 'Maluma' typically displays the characteristics of a climacteric fruit as described by Blumenfeld and Gazit (1974). 'Maluma' exhibits a strong autocatalytic production of ethylene which is clearly illustrated (Figure 1) by the days to ripen of the
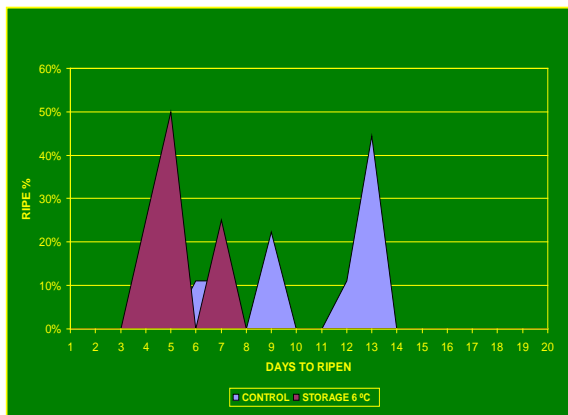

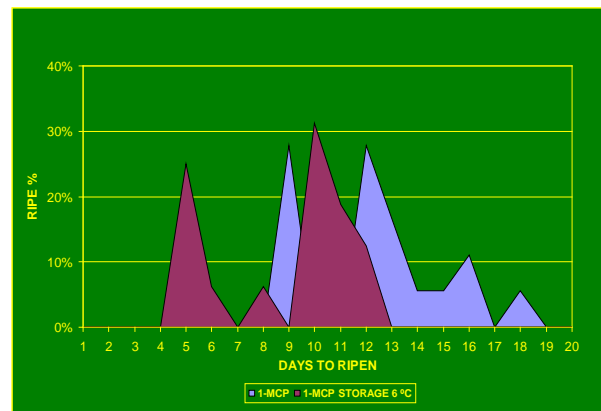**Figure 1.** The effect of storage (7 days at 6ºC) on days to ripen of 'Maluma'

Figure 2. The effect of 1-MCP (500 ppb; SmartFresh) on days to ripen of stored (7 days at 6ºC) and non-stored 'Maluma'
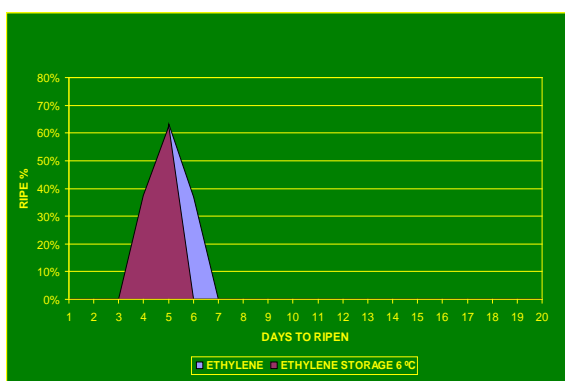

Figure 3. The effect of ethylene (100 ppm) on days to ripen of stored (7 days at 6ºC) and non-stored 'Maluma'
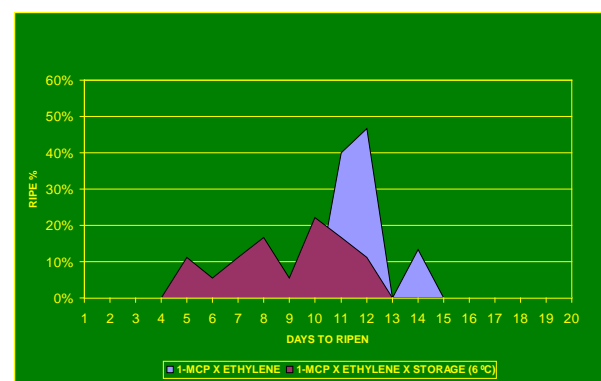

Figure 4. The effect of 1-MCP (500 ppb) and ethylene (100 ppm) on the days to ripen of stored (7 days at 6ºC) and non-stored 'Maluma'

control compared to stored (6ºC) fruit, in the absence of applied ethylene, where a substantial decrease in the average time to ripen was visible. This is supported by the fact that the experiment was conducted with fruit only reaching the onset of maturity at a 23% dry matter level.

The shortest days to ripen with the least difference between the control and stored fruit were evident with the ethylene treated fruit (Figure 3). As suggested by Lemmer *et al.* (2008) 1-MCP (500 ppb) effectively increased the average days to ripen of stored and non-stored fruit (Figure 2) and also acts as an effective ethylene inhibitor, resulting in comparable average days to ripen if compared with non-ethylene treated fruit (Figure 4). The dramatic reduction in days to ripen of stored fruit which did not receive any ethylene correlates with the findings of Arpaia (2006).
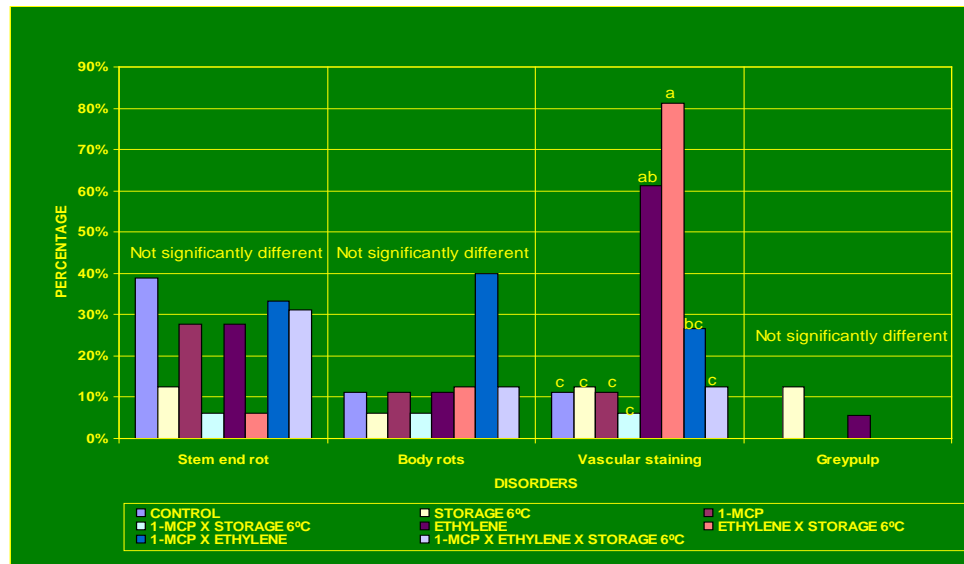

Figure 5. The effect of 1-MCP (500 ppb) and ethylene (100 ppm), individually and in combination, on the intensity of different disorders after ripening of stored (7 days at 6ºC) and non-stored 'Maluma'. All values with different superscript letters are significantly different ($P \leq 0,05$).

As far as the different fruit rots (stem end and body rots) and greypulp are concerned no significant statistical difference could be found between the different treatment for a particular variable. However a clear tendency towards a lower percentage of rots is visible among the stored fruit compared with the control (Figure 5 and 7). This supports the observations of Arpaia (2006) who reported on a trend of decreasing decay incidence with an increase in storage duration. It is explained by the protracted time of ripening of fruit stored for 0 (zero) days. Figures 5 and 7 respectively illustrates a typical decrease in the occurrence of stem end rots and an increase in body rots (mainly anthracnose) associated with the increase in dry matter content of the fruit from 23 to 33% (moisture decrease from 77 to 67%).

The incidence of greypulp can be explained due to the fact that the fruit collected for the experiment was from young trees (3,5 years old) with relatively high nitrogen levels. 1-MCP effectively suppressed greypulp under storage conditions even with ethylene pre-storage treated fruit (Figure 5). As illustrated in Figure 7 and 9, greypulp is of no concern in older orchards (9 and 10 years old) were the nitrogen and calcium levels are within the prescribed norms.

According to Figure 5 a statistically significant difference ($P \leq 0,05$) exists between the ethylene treated and non-treated fruit with regards to red/pink vascular staining (Figure 6), with the highest incidence among the pre-stored ethylene (100 ppm) treated fruit stored for 7 days at 6ºC and ripened at 20ºC. 1-MCP (500 ppb) significantly suppressed vascular staining of ethylene treated and stored 'Maluma' fruit, to a level similar to that of non-ethylene treated fruit. The inhibition of ethylene with 1-MCP confirms the
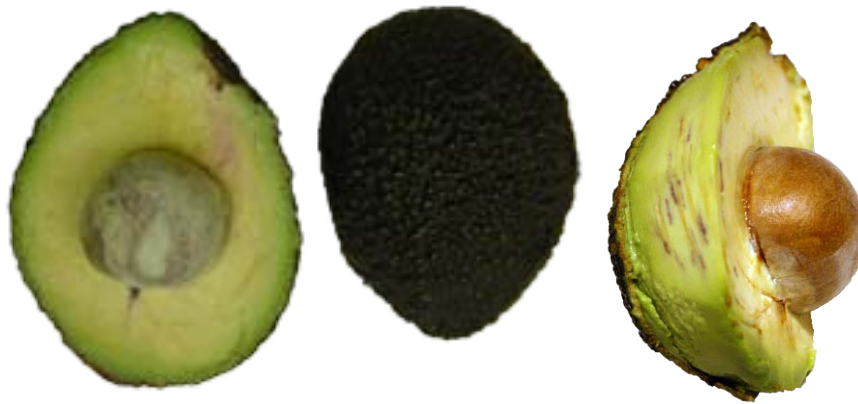
Figure 6. Incidence of pink / red vascular staining following ripening at 20⁰C after pre-storage (7 days at 6⁰C) ethylene (100 ppm) treatment of 'Maluma'

findings of Hershkovitz *et al.* (2009). The inducing effect of ethylene on the occurrence of this phenomenon supports the findings of Arpaia (2006) who suggested that this disorder is directly linked to the treatment of ethylene in combination with storage, particularly at intermediate temperatures.
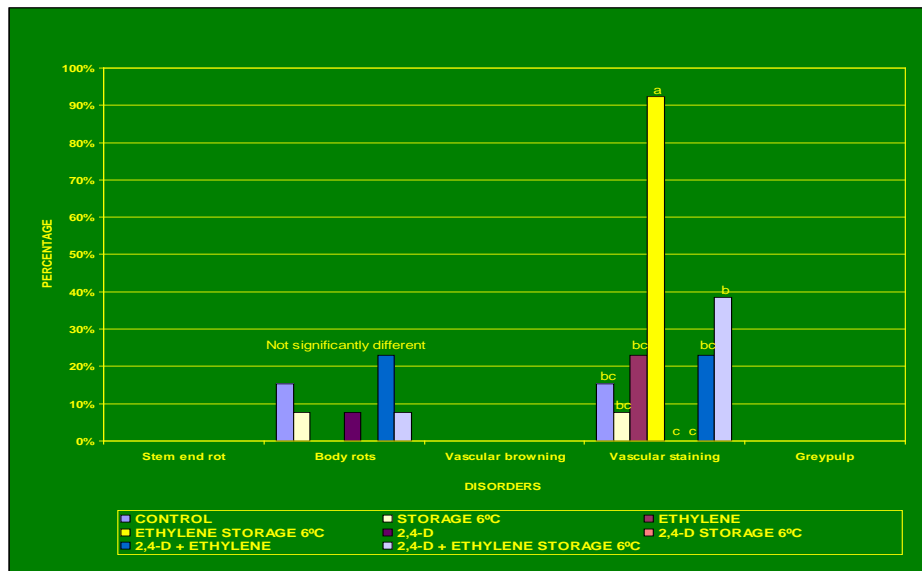

Figure 7. The effect of 2,4-D (2%) and Ethylene (100 ppm), individually and in combination, on the intensity of different disorders after ripening of stored (7 days at 6⁰C) and non-stored 'Maluma' fruit. All values with different superscript letters are significantly different ($P ≤ 0,05$).

In Figure 7 (Experiment 2) Maluma displays a visible lower intensity of stem end rots and vascular browning at a dry matter content of 33%. Manageable levels of anthracnose (body rot) are observed. 2,4-D effectively reduces the incidence level of vascular staining to zero with non-ethylene treated fruit. With pre-stored ethylene treated fruit, 2,4-D as a stem dip treatment during harvest successfully inhibits ethylene production resulting in an significant reduction (55%) of vascular staining. In Figure 8 (Experiment 3) this inhibition was not significant; however a clear tendency of ethylene inhibition was illustrated. From the results it is clear that the suppressive effect of 2,4-D is not sustainable during
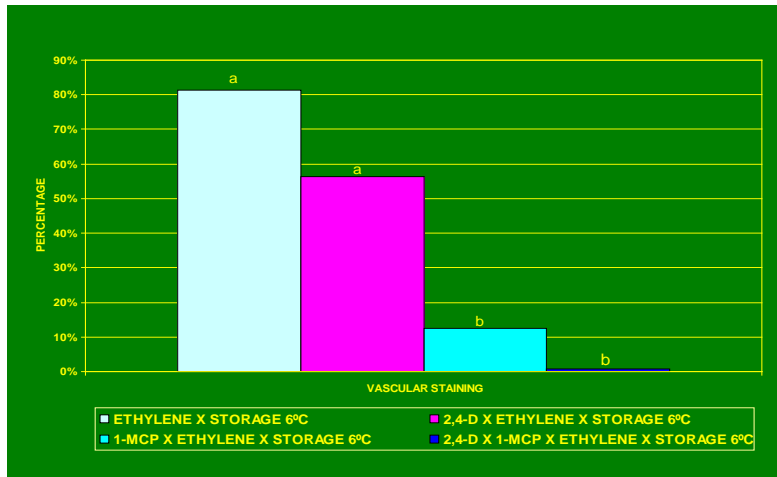
Figure 8. The effect of ethylene and ethylene inhibitors on vascular staining of stored (7 days at 6⁰C) and subsequently ripened 'Maluma' fruit. All values with different superscript letters are significantly different ($P \leq 0.05$).

storage however it effectively reduces pre-storage ethylene by inhibiting post harvest stress related ethylene production. During storage 1-MCP effectively and significantly inhibits the effect of pre-storage exposure to ethylene, while in combination with 2,4-D it significantly and fully prevents the development of red/pink vascular staining
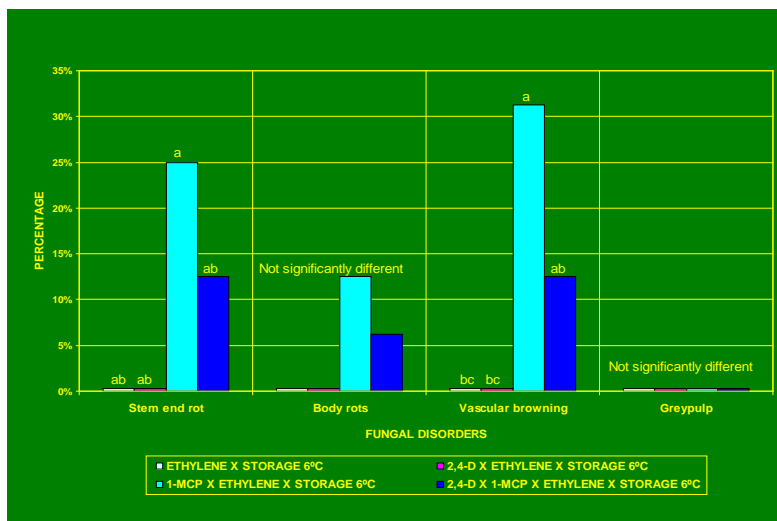

Figure 9. The effect of ethylene and ethylene inhibitors on fungal disorders of stored (7 days at 6⁰C) and subsequently ripened 'Maluma' fruit. All values with different superscript letters are significantly different ($P \leq 0.05$).

As far as fungal disorders are concerned no significant difference existed between the different treatments of a particular disorder except for vascular browning, where the stored fruit which were pre-treated with ethylene and 1-MCP had a significantly higher incidence. In all instances the intensity of the disorder was the lowest with the shortest days to ripen. The combination of 1-MCP and 2,4-D effectively reduced the intensity of the particular fungal disorder (Figure 9).

4. Conclusions

'Maluma' is a typical climacteric fruit displaying a sharp rise in ethylene production during ripening, which occurs directly after harvest. The autocatalytic production of ethylene is evident as a sharp decrease in days to ripen occurs with stored fruit, even in the absence of applied ethylene. The dramatic reduction in days to ripen of stored fruit which did not receive any ethylene correlates with the findings of Arpaia (2006).

A clear tendency towards a lower percentage of rots is visible among stored fruit compared with non-stored fruit. This supports the observations of Arpaia (2006) who reported a trend of decreasing decay incidence with an increase in storage duration. It is explained by the protracted time of ripening of non-stored fruit. A decrease in the occurrence of stem end rots together with an increase in body rots (mainly anthracnose) is typically associated with an increase in the dry matter content of the fruit.

As suggested by Lemmer *et al.* (2008) 1-MCP (500 ppb) effectively increased the average days to ripen of stored fruit and acts as an effective ethylene inhibitor. 1-MCP effectively suppressed greypulp. Greypulp with 'Maluma' is of no postharvest concern particularly in older orchards where the nitrogen and calcium levels are within the prescribed norms.

The changes in colour of the fruit skin, as indicator of ripeness, from green to purple/black, is visually more intense with 'Maluma' compared to 'Hass' (Ernst, 2007). The change in pigment is due to an increase in the anthocyanin concentration in the skin tissue during ripening (3-6 days postharvest) (Cox *et al.*, 2004). The red/pink vascular staining relates to an excessive anthocyanin production as a result of a rapid increase in respiration due to a stress related over exposure to ethylene. To prevent this disorder it is essential to manage stress related ethylene production with consequential increase in respiration particularly during picking and packing of the fruit.

From the results it is clear that red/pink vascular staining is induced by postharvest/pre-storage exposure of 'Maluma' fruit to ethylene. The inducing effect of ethylene on the occurrence of this phenomenon supports the findings of Arpaia (2006) who suggested that this disorder is directly linked to the treatment of ethylene in combination with storage, particularly at intermediate temperatures.   As an effective ethylene inhibitor 1-MCP (500 ppb) significantly suppressed vascular staining. The inhibition of ethylene with 1-MCP confirms the findings of Hershkovitz *et al.* (2009) regarding the inhibition of mesocarp decolouration with green fruit.

ABA acts as a stimulant for ethylene biosynthesis once ripening inhibitors of the tree are no longer available after picking (Bruinsma, 1981) According to Bower (1985) stress is a major factor affecting ABA levels. Considerable loss of water from the avocado fruit through transpiration, as well as high temperature exposure after picking, creates stress conditions in the fruit, leading to ABA accumulation (Bouwer, 1985), which in turn stimulates the autocatalytic production of ethylene. As a stem dip treatment during harvest 2,4-D successfully inhibits ethylene production resulting in a significant reduction of vascular staining. The suppressive effect of 2,4-D is not sustainable during storage, however it effectively reduced pre-storage ethylene by inhibiting post harvest stress related autocatalytic ethylene. During storage 1-MCP effectively and significantly inhibits the effect of pre-storage exposure to ethylene, while in combination with 2,4-D it significantly and fully prevents the development of red/pink vascular staining.

It can therefore be concluded that the impact of storage on the rate of ripening is evident, with the least days to ripen with ethylene treated fruit and the most with the 1-MCP treatment. 1-MCP acts as an effective ethylene inhibitor. Less mature fruit are more prone to fungal diseases. Vascular browning is associated with fruit rots. Stored fruit shows less disease related disorders which relates to the faster ripening of the fruit. The frequency and severity of vascular staining significantly increases amongst fruit treated with ethylene prior to storage. This phenomenon, which is ethylene induced, can be efficiently controlled by a 2,4-D (Deccomone®) stem treatment (dip) during picking together with a 1-MCP (SmartFresh[TM]) treatment prior to cold storage.

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