

## Postharvest Calyx Retention of Citrus Fruit

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### Abstract

**Abscission is an active developmental process occurring at the abscission zone of the fruit peduncle. The target cells in the abscission zone, which are involved in the process of separation, are located in the separation layer, which is organized in a few cellular layers. In general, it is accepted that the increase in ethylene production in the fruit is followed by increased sensitivity of these cells to ethylene, which would lead to abscission. The abscission process is divided into two phases with respect to sensitivity to auxin and ethylene. The auxin, 2,4-D (2,4-dichlorophenoxy acetic acid), has been used as a postharvest packhouse treatment to retard calyx abscission (to repress postharvest decay). Commercially the sodium salt 2,4-D (Deccomone<sup>®</sup>) is applied to the fruit in a dip treatment at 500 ppm. The aim of this experiment was to test three agrochemicals (aminoethoxyvinylglycine (AVG), 1-naphthylacetic acid (NAA) and 1-methylcyclopropene (1-MCP)) that could possibly replace the postharvest application of the auxin 2,4-D. AVG had a fruit firming effect but no significant effect on calyx retention. NAA resulted in a high percentage of abscission, probably due to auxin overdose. The 1-MCP at low concentrations of 100 ppb and 250 ppb resulted in calyx retention but had a desiccating effect on the calyx whereas at a high concentration of 500 ppb there was a significant increase in calyx abscission. There was no loss of internal quality or colour. To conclude, according to what is known about the role of ethylene in abscission, 1-MCP and AVG should have prevented abscission of the calyx. Unfortunately, the results show that this complex plant mechanism is not so readily manipulated and 2,4-D remains the best product to inhibit calyx abscission of citrus fruit.**

### INTRODUCTION

Abscission is an active developmental process, which occurs at a specific zone, the abscission zone. The target cells in the abscission zone, which are involved in the process of separation, are located in a specific layer, the separation layer, which is organized in a few cellular layers. These cells are not active until the mature or senescing organ start to abscise (Salisbury and Ross, 1992). Three major physiological stages can be identified in the process of abscission. The first is the stimulus by either natural senescence or an external factor such as heat stress or plant growth regulator treatments. The second signal is characterized by several internal factors, such as a decrease in the endogenous auxin at the abscission zone, protein degradation, chlorophyll breakdown, increased ethylene production and other phytoogerontological changes. The third and last stage in the response is characterized by specific nucleic acid and protein synthesis. This synthesis is responsible for the *de novo* synthesis of the hydrolytic enzymes, cellulase and polygalacturonase, which are responsible for the degradation of the cell walls. In general, it is accepted that the increase in ethylene production during this stage is followed by increased sensitivity of cells to ethylene (Goren, 1998). The major plant hormones which are involved in the control of the abscission process are auxin, ethylene and, more seldom, abscisic acid (ABA). The abscission process is divided into two phases with respect to sensitivity to auxin and ethylene (Addicott, 1982). In the first phase auxin is responsible for the elongation of the cells, which precedes the following cell wall degradation. During this phase, the cells are insensitive to ethylene and auxin can delay

the separation process. Besides cell division, the major anatomical change is cell elongation, which is induced by auxin. During the second phase, when the level of auxin decreases below a certain threshold thereby losing its delaying effect, the cells react to ethylene, and auxin can even stimulate the process by inducing greater ethylene synthesis (Goren, 1996).

The auxin 2,4-D (2,4-dichlorophenoxy acetic acid) is a plant growth regulator that has been widely used in citriculture around the world since the 1950's (Steward et al., 1952). When used as a pre-harvest spray, 2,4-D reduces fruit drop. As a post harvest packhouse treatment it is used as a dip, drench or in a wax to retard calyx abscission. Retention of the calyx reduces the fungal decay that was a serious storage problem prior to the introduction of the technology involving the isopropyl ester of 2,4-D (Eiset and Lyon, 1981; Dewolfe et al., 1959). This effect is ascribed to repression of entry of mycelium of *Alternaria* stem-end rot into fruits. Commercially the 2,4-D sodium salt (Deccomone) is applied to the fruit in a dip treatment of 500 ppm.

During 2003 European Union authorities implemented new legislation regarding the use of 2,4-D, in which the level of detection was decreased from 2 ppm to 0.05 ppm. This unexpected development had a negative impact on citrus exports from South Africa to the traditional markets in the EU. As a suitable replacement strategy doesn't exist, the aim of this trial was to test several agrochemicals that could possibly replace the post-harvest application of the auxin 2,4-D in order to prevent abscission of the calyx.

## MATERIALS AND METHODS

'Navelate' oranges (*Citrus sinensis*) were harvested on a commercial farm during week 25 in the Western Cape, South Africa. Thirty randomly selected fruit were packed by hand into a plastic tray (eighty-four trays were packed) representing a single replication. After sorting the fruit into the trays, they were drenched with a combination of fungicides (Ortho-Phenylphenol, Thiabendazole, Imazilil). For each of the thirteen treatments there were six replications. The fruit were treated with four different agrochemicals: aminoethoxy vinylglycine hydrochloride (AVG, 85 and 166 g/100L), 1-naphthylacetic acid (NAA, 22 mL/100 L) and 1-methylcyclopropene (1-MCP, 500 and 1000 ppb). AVG is a natural amino acid that forms during fermentation. It competitively inhibits the development/production of ACC synthase thereby blocking ethylene biosynthesis in the plant tissue (Sisler et al., 1999). NAA is an auxin used for fruit thinning and prevention of premature fruit drop of certain apple cultivars. 1-MCP is used to significantly extend the storage and shelf-life of fruit and flowers by blocking the action of ethylene. 1-MCP attaches to the receptor proteins so that they no longer recognise ethylene, resulting in an inhibition of synthesis of internal ethylene and a lack of perception of external ethylene (Sisler et al., 1999). After the fungicide treatment the fruit were dipped in a 2,4-D, AVG or NAA solution for five minutes. Those fruit selected for the 1-MCP gas treatment, were transported to the Capespan Technology Development facility in Stellenbosch where the treatments were administered. These treatments took place in an airtight container with a small electric fan for air circulation and took 24 hours. Those treatments that received a degreening treatment (gassing with ethylene to hasten chlorophyll degradation and improve the colour development of citrus fruit) were put into the degreening room for three days (20-25°C, RH>90% and ethylene at 300 ppm). After the various treatments, all the trays were stored at ambient temperature on the packhouse premises. The first evaluation was done three weeks after the treatments were completed and repeated one-week thereafter. Evaluation consisted of testing each fruit for a loose calyx by rubbing over the calyx with the hand. After the second evaluation internal quality analysis was conducted to determine % juice, % acid and %TSS. Fruit firmness at the equatorial position was quantified by using a densimeter with a 5 mm diameter tip (0-100 scale, high value = firmer fruit); three measurements per fruit were made.

A second trial was conducted to test the efficacy of 1-MCP on calyx retention of 'Midnight Valencia' oranges. The fruit were from the same region in the Western Cape and picked on the 1<sup>st</sup> of September. Fruit were selected by hand and packed into cartons,

with six treatments of 25 fruit per replication and six replications. Cartons were randomly assigned to the following treatments: 1-MCP at 100, 250 and 500 ppb, the commercial 2,4-D treatment, and fruit that received no chemical treatment or wax. The 1-MCP treatment lasted for 24 hours. All the fruit were drenched with normal fungicides (ortho-phenylphenol, thiabendazole, imazilil), waxed and packed back into the cartons. The cartons were held at 4°C for three weeks to simulate shipping conditions to Europe, followed by a further three weeks at 15°C. The fruit evaluations were conducted in the same manner as for Trial 1, described earlier. In addition, three peel colour measurements were done with a chromameter (Minolta NR 4000, Osaka, Japan) on the equatorial region.

## RESULTS

Regardless of degreening, 2,4-D resulted in the lowest percentage calyx abscission in 'Navelate' oranges. 1-MCP treated fruit was not significantly different to untreated fruit with regards to incidence of abscission, irrespective of 1-MCP concentration. However, 1000 ppb 1-MCP following degreening resulted in significantly more calyx abscission. AVG with degreening and NAA also significantly increased percentage calyx abscission (Fig. 1). AVG treated fruit retained their firmness compared to the 2,4-D treated fruit (Fig. 2). The second application of 1-MCP was done at lower concentrations after observing a desiccating effect of the rind near the stem and a higher percentage abscission at 1000 ppb vs. 500 ppb. The higher concentrations of 1-MCP (500 ppb vs. 250 ppb or 100 ppb) resulted in more calyx abscission of 'Midnight Valencia' oranges (Fig. 3). Calyx abscission increased with higher concentrations of 1-MCP used, but was only significant for fruit treated with 500 ppb. As in the first treatment of the 'Navelate' oranges, 2,4-D resulted in the least percentage calyx abscission in 'Midnight Valencia'. The chromameter measurements on the 'Midnight Valencia' oranges revealed no significant colour differences between fruit treated with 1-MCP or 2,4-D and fruit that were only waxed. Only the non-waxed fruit differed significantly. Neither 'Navelate' nor 'Midnight Valencia' showed any significant internal quality differences between treatments and no off flavours were detected (data not shown). Ethylene production rates of fruit that received 500 ppb 1-MCP and those receiving only wax, as the control, were measured after the storage period. The ethylene production rates of the 1-MCP treated fruit were  $1.52 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  and the control fruit had no detectable levels. The total average ACC concentrations in the rind of the 1-MCP and control fruit were  $0.0975$  and  $0.1925 \text{ nmol}\cdot\text{g}^{-1}$  fresh weight, respectively.

The 1-MCP treatment after the ethylene degreening resulted in a peel symptom resembling stem end browning. Although the calyxes from the 1-MCP were not abscised, they were desiccated. Application of NAA resulted in very high abscission, confirming the complex relationship between auxin concentration and abscission. High auxin concentrations are known to cause epinasty, premature leaf abscission, inhibition of root and shoot growth and stimulation of ethylene synthesis. Application of high auxin concentrations forms the basis of its use as a herbicide (Grossmann and Hansen, 2001; Sterling and Hall, 1997). The interplay of ethylene and auxin in the regulation of abscission is complex, including effects of ethylene on auxin transport and conjugation. The hormonal control of abscission probably involves gene activation but the details await elucidation (Spiegel-Roy and Goldschmidt, 1996).

## CONCLUSIONS

Abscission of the calyx was reduced with the 1-MCP and, to a lesser extent, with AVG, but the application of the 2,4-D cannot be substituted at this stage with any known agrochemical.

NAA application resulted in the highest percentage calyx abscission and illustrates the complex nature of the ethylene/auxin balance in the regulation of this plant process.

AVG, without the degreening treatment, had a positive effect on the calyx retention and was the only treatment that retained fruit firmness similarly to non-treated

fruit. Fruit firmness is a problem during high rainfall seasons and leads to an increased postharvest loss of produce. Our results did not concur with previous findings (Einset and Løyen, 1981) that AVG and AOAA - a related compound - inhibit the abscission of the button.

Results from the 1-MCP applications on the 'Navelate' and 'Midnight Valencia' oranges indicate that the abscission process can be positively manipulated but its efficacy depends on factors such as degreening treatments, timing and concentration of 1-MCP application. The results of the 1-MCP applications on abscission do not support previous findings on citrus abscission (Porat et al., 1999) or colour development (Sisler et al., 1999). In contrast to what has been found on flowers where 1-MCP delayed exogenous ethylene-induced abscission (Cameron and Reid, 2000; Serek et al., 1994, 1995) the 1-MCP seems to enhance abscission, especially at high concentrations.

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## Figures

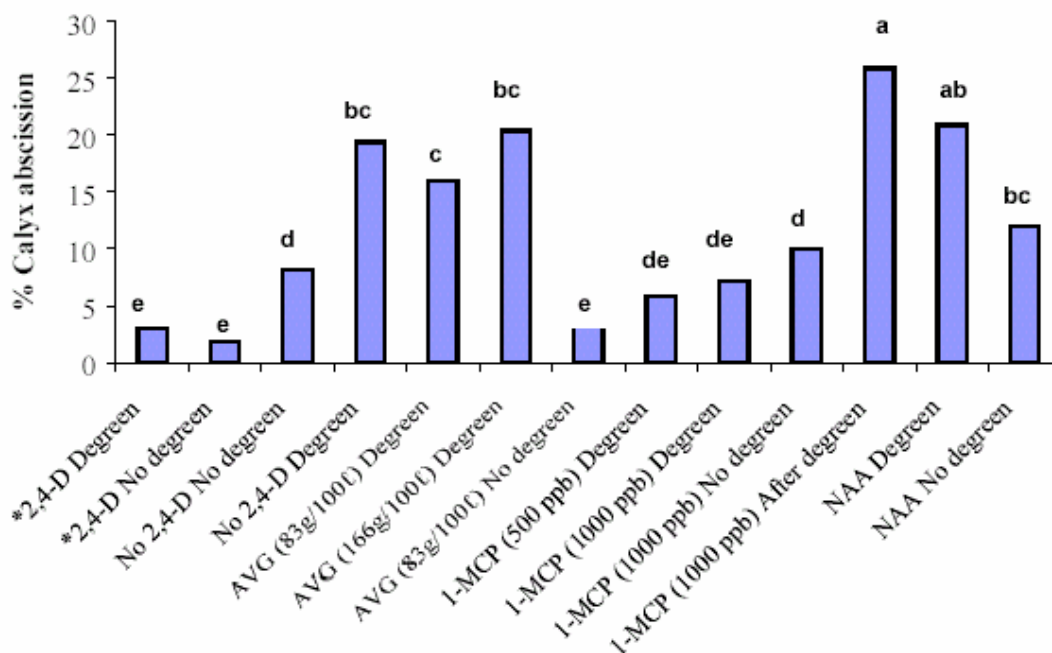


Fig. 1. Percentage calyx abscission of 'Navelate' orange after treatment with three chemicals that influence ethylene production. Columns sharing the same letter are not significantly different ( $p < 0.05$ ) by Student t-test. \*Commercial control.

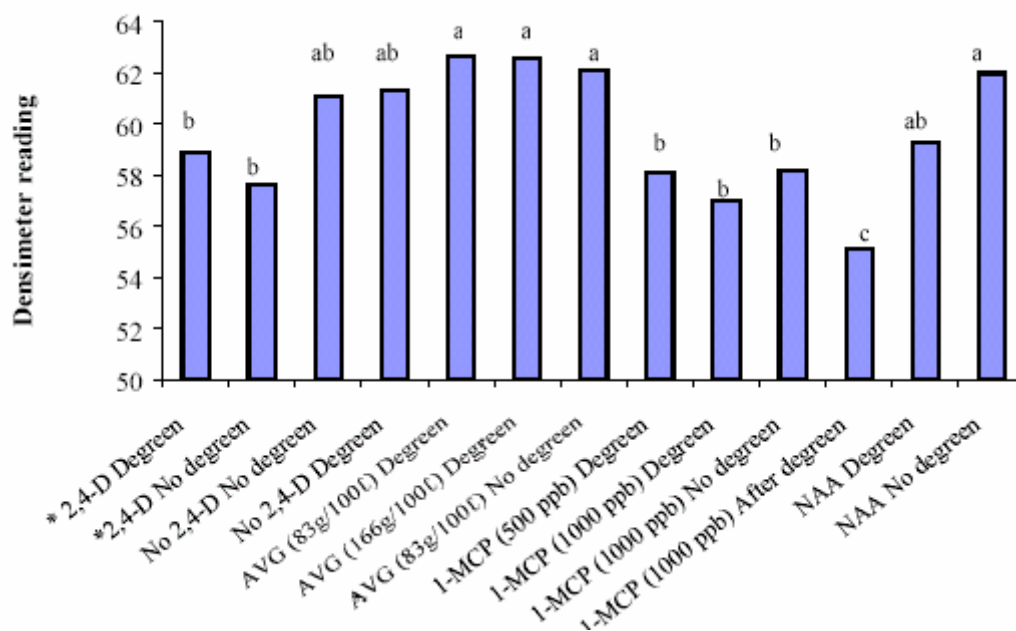


Fig. 2. Fruit firmness measurements of 'Navelate' orange after application of various chemicals influencing ethylene production. Columns sharing the same letter are not significantly different ( $p < 0.05$ ) by Student t-test. \*Commercial control.

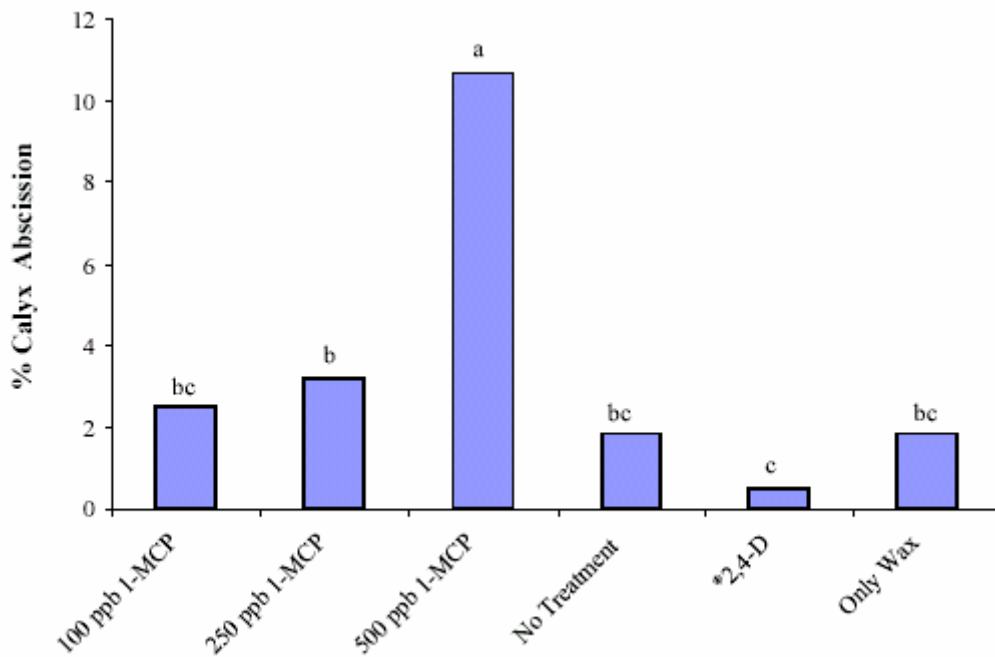


Fig. 3. Abscission percentage of ‘Midnight Valencia’ orange calyxes after treatment. Columns sharing the same letter are not significantly different ( $p < 0.05$ ) by Student t-test. \*Commercial control.

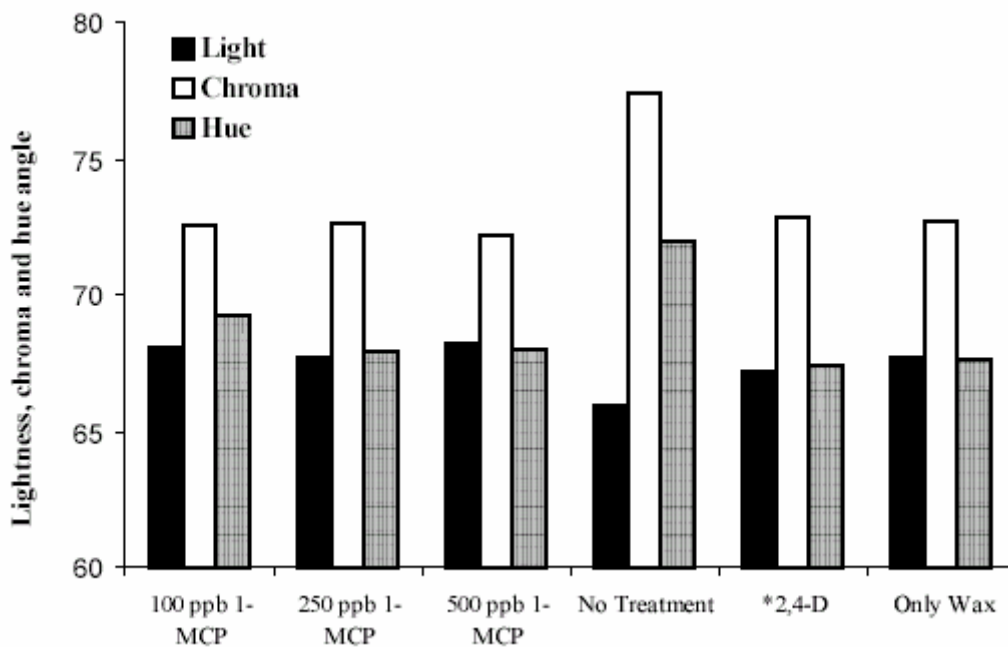


Fig. 4. Effect of 1-MCP, 2,4-D and wax alone on colour of ‘Midnight Valencia’ oranges. All three variables differ significantly between No Treatment and all other treatments, but there were no significant differences between the 1-MCP, 2,4-D and wax alone. \*Commercial control.