# Systemic resistance inducers applied pre-harvest for anthracnose control in 'Fuerte' avocados

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**ABSTRACT**

World avocado production has consistently increased over the past decade. Long shipping periods result in extended cold storage which often results in poor fruit quality and early softening. This may enhance post-harvest disease incidence, such as by *Colletotrichum gloeosporioides*, occurring first as a latent infection, and later post-storage, causing considerable losses. The objective of this study was to use systemic resistance inducers to enhance the concentration of antifungal compounds and decrease post-harvest disease incidence. Thereby the need for other chemical control measures could be decreased. Pre-harvest application of phosphorous acid (500 ppm a.i.) to 'Fuerte' avocado fruit trees were carried out. Mature fruit were harvested on the day of treatment application, as well as 7, 14, and 21 days thereafter. Fruit were either ripened immediately at room temperature or stored for 28 days at either 5.5°C or 2°C before ripening. The general fruit appearance was evaluated and exocarp samples were analysed for potential antifungal compounds and phenylalanine ammonia-lyase (PAL) activity. Phosphorous acid application decreased anthracnose development with optimum results obtained when fruit were harvested 14 days after application.

## INTRODUCTION

Avocados are commonly transported over long distances and are placed under low temperatures for an extended period of time. During this time, the fruit is prone to attack by several pathogenic fungi. Since many products currently used to control decay of fruit are regarded as environmentally hazardous or leave undesirable residues on the fruit, new techniques need to be implemented to control the disease (Eckert & Brown, 1986).

Anthracnose infects unripe fruit and once infected, the fungus remains dormant until ripening begins (Crane, 2001). Unripe fruit are typically free of post-harvest rots or other internal disorders, but when ripe enough for consumption, the fruit can be severely affected by disease (Darvas *et al*., 1990).

Antifungal compounds are concentrated in the outer layers of the fruit and act as a "first line of defence". When the concentrations of these compounds drop below fungistatic levels during the ripening process, latent infections are activated and hyphae invade the fruit (Prusky *et al*., 1983). Once the concentration of antifungals has declined past a certain level, anthracnose is able to resume development and symptoms are produced (Prusky *et al*., 1991). Anthracnose is usually found in conjunction with other fungi and, therefore, overall disease severity is high (De Villiers, 2001).

Although there are many strategies to control post-harvest diseases, the activation of inducible defence mechanisms is one of the most environmentally friendly ones (Terry & Joyce, 2004; Tian *et al*., 2006). Phosphorous acid has been used to control Phytophthora root rot in avocados, inducing plant defence mechanisms. The chemical acts as a fungal growth inhibitor at high concentrations, while at low concentrations it acts as a defence elicitor (Guest *et al*., 1995).

Phenylalanine ammonia-lyase (PAL) has been aligned with the increased disease resistance to anthracnose (Yakoby *et al*., 2002). Phenylalanine is the central amino acid in this pathway, with many derivatives of antifungal nature produced from this compound (Hahlbrock & Scheel, 1989).

The aim of this investigation was to use phosphorous acid as a pre-harvest application spray on 'Fuerte' avocado trees in order to enhance the concentration of antifungal compounds by increasing PAL activity to delay anthracnose symptom development.

## MATERIALS AND METHODS

### Fruit

Fruit were obtained from a commercial farm in Wartburg, KwaZulu-Natal. Pre-harvest applications of

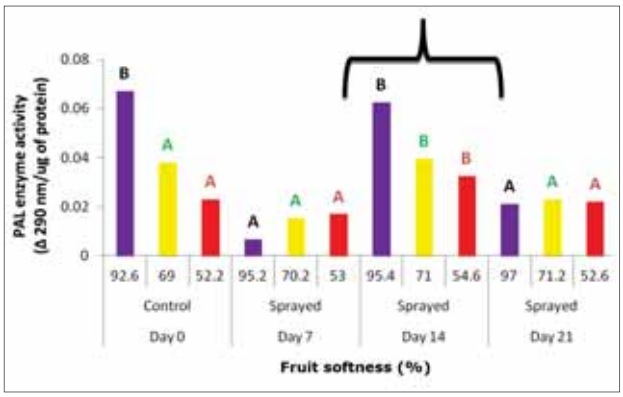


**Figure 1. Phenylalanine ammonia-lyase activity at specific fruit softness of 'Fuerte' avocados stored at room temperature (22°C). Trees were sprayed pre-harvest with phosphorous acid (500 ppm a.i.) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at p<0.05 for a specific softness across evaluation days.**

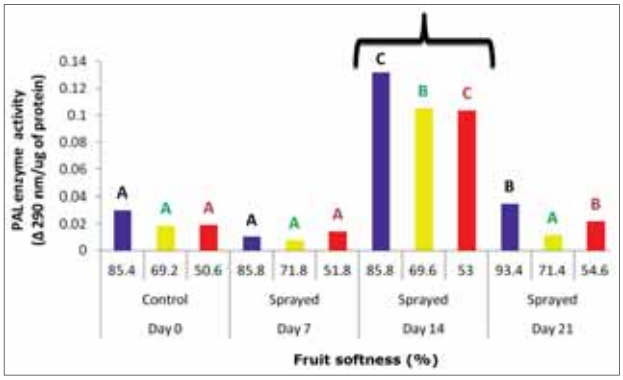


**Figure 2. Phenylalanine ammonia-lyase activity at specific fruit softness of 'Fuerte' avocados stored at 5.5°C. Trees were sprayed pre-harvest with phosphorous acid (500 ppm a.i.) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at p<0.05 for a specific softness across evaluation days.**

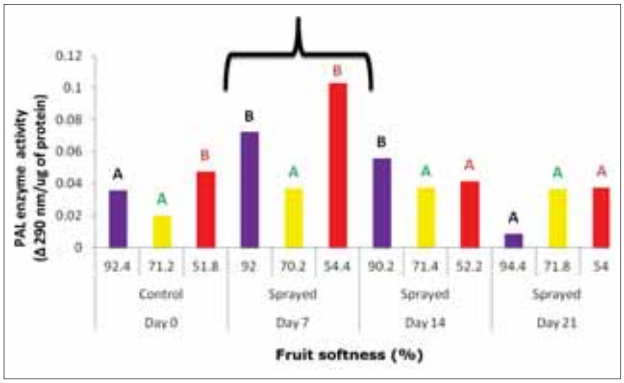


**Figure 3. Phenylalanine ammonia-lyase activity at specific fruit softness of 'Fuerte' avocados stored at 2°C. Trees were sprayed pre-harvest with phosphorous acid (500 ppm a.i.) and harvested after 0, 7, 14 and 21 days after application. Different letters above columns represent statistical differences at p<0.05 for a specific evaluation day.**

phosphorous acid (500 ppm) were administered to drip off to trees and mature avocados were harvested prior to phosphorous acid application as well as 7, 14 and 21 days thereafter. Fruit were then stored either at 5.5°C or 2°C for 28 days or ripened at room temperature without storage.

**Fruit softness**

Fruit softness was determined using a densimeter (Bareiss, Germany) with a 5 mm diameter and 4 mm long conic probe (Eaks, 1966). Fruit softness was measured on a scale from 90-100 (hard), 68-75 (medium soft) and 50-55 (soft).

**Fruit sampling**

Exocarp tissue was sampled at five stages of fruit softness. These samples were later analysed for potential antifungal compounds and PAL activity.

**Extraction and analysis of PAL**

PAL was extracted and determined according to the methods of Lister *et al*. (1996) and Jiang and Joyce (2003) with slight modifications.

**Statistical analysis**

Data were analysed as a factorial design with each treatment having 20 fruit replicates. A general analysis of variance was performed using GenStat 12th edition.

**RESULTS**

Pre-harvest application of phosphorous acid showed that harvesting fruit 14 days after phosphorous acid application resulted in the highest PAL activity, when fruit were stored at room temperature and at 5.5°C (Figure 1 and 2). Fruit stored at 2°C showed best results when fruit were harvested seven days after spraying (Figure 3).

**DISCUSSION**

The maturity of fruit is an important parameter in the sensitivity towards fungal attack as it influences the natural resistance of fruit (Prusky, 1996). It has been shown that the presence of antifungal dienes in the peel of avocados is adequate to prevent the growth of anthracnose in immature fruit (Prusky *et al*., 1983). Prusky *et al*. (1990) found that diene concentrations in fruit peel decreased between the first and third day after harvest but initial levels were regained thereafter, a process that occurred faster in more mature fruit.

Due to the increased levels of PAL, it can be assumed that phosphorous acid is capable of inducing resistance in avocado, possibly by increasing compounds derived from the pathway, such as the antifungal dienes. The different pattern of PAL activity in the exocarp of fruit stored at 2°C, compared with those stored at room temperature or at 5.5°C, could be due to the specific storage temperature. Lutge (2012) recently reported that 2°C is a storage temperature at which no chilling injury is incurred by 'Fuerte' avocados, indicating that certain physiologi-

cal reactions occur, or do not occur, at this specific temperature that happens at 5.5°C. Storing fruit at low temperatures affects membrane function and activity and the position of membrane-associated enzymes (Lyons & Raison, 1970). As a plant is exposed to lower temperatures, the composition of its membrane lipids may change in such a way that lipids with a lower freezing point become more dominant (Lyons *et al.*, 1980). This may, therefore, affect the reaction time of phosphorous acid to induce a high PAL activity.

Antifungal activity following application of phosphorous acid needs to be determined in order to verify these findings; if antifungal compounds are indeed elevated following the application, a promising tool for reducing the traditionally used copper fungicides to control anthracnose could be available in the near future.

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