Alternatives to synthetic fungicides to control postharvest diseases of avocados – Commercial trials with thyme oil vapours

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ABSTRACT

Anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (i.e. *Lasidiodiplodia theobromae*) are the predominant postharvest diseases resulting in severe losses on the local and export markets. For postharvest control of anthracnose, a synthetic non-systemic fungicide, prochloraz, is used. However, an increase in consumer concern regarding food safety on the export markets has necessitated the development of alternative disease control measures. In our previous investigations, the volatile phase of thyme oil (TO) was found a suitable natural alternative to control anthracnose in avocados. Therefore, semi commercial fumigation trials using TO were done on 'Hass' avocado from the Letaba region during the early (June), mid (July) and late (August) seasons. Fruit were evaluated under local (early, mid and late season) and export conditions to the UK (late season) market. The effect of TO impregnated sachets was also evaluated. Sachets incorporated with 10% TO packed in trays reduced anthracnose incidence measured after five days storage at 15 °C compared to the untreated control treatment. In early and late seasonal fruit, the 10% TO sachet treatment reduced anthracnose similar to the prochloraz treatments when measured after five days storage. The 10% TO sachet treatment also reduced the stem-end rot incidence throughout the season compared to the untreated control, while late seasonal fruit were less effectively controlled compared to the prochloraz treatment. Vascular browning was not observed in both the early and mid-season fruit and in late season fruit, the incidence was reduced in both the prochloraz and 10% TO sachet treatments. The exposure of 'Hass' avocado fruit to TO vapour treatments (packhouse temperature – PFT) prior to cold storage (at 5.5 °C for 30 days) and ripening at room temperature reduced the incidence of anthracnose throughout the season. Compared to the untreated control treatment, TO vapours effectively reduced the incidence of anthracnose by 30%, 28% and 4% in early, mid- and late seasonal fruit respectively. Similarly, the TO vapours reduced the incidence of stem-end compared to both the untreated control and prochloraz treatments. No differences were noticed in the firmness of the fruit during analysis in all the treatments. No vascular browning incidence was noticed in the early and mid-season fruit. The overseas trial indicated that the TO vapour treatments had a positive effect on quality retention of the fruit compared to the other treatments. The TO treatments also improved the sensory qualities of the fruit. These results therefore suggest that TO vapours can be used as an alternative natural treatment to control anthracnose and stem-end rot on avocado. However, the same work has to be repeated on other avocado cultivars ('Fuerte' and 'Pinkerton') and from other growing areas or packers.

INTRODUCTION

Anthracnose (*Colletotrichum gloeosporioides* Penz.) and stem-end rot (caused by several pathogens, depending on the season) are the major postharvest diseases of avocado that affect the quality and hence it's marketability (Pegg *et al*., 2002; Sanders and Korsten, 2003). Preharvest copper based field sprays and postharvest prochloraz dip or spray applications are currently being used to control anthracnose (Smith *et al*., 2011). An increase in consumer concern regarding food safety especially for USA and EU markets has necessitated the development of safer methods to control postharvest decay (Bill *et al*., 2014). In our previous investigations, the volatile

phase of thyme oil (TO) was selected as a suitable natural volatile to control anthracnose in avocados during postharvest storage. Also, prochloraz may be phased out by 2020 (Z. van Rooyen, personal communication) prompting the need to find an alternative. Therefore, based on our previous findings, TO was administered during commercial application in the currently used tray-packs in the volatile phase by using TO impregnated polylactic acid sachets at 5 and 10% concentrations (Bill *et al*., 2018). The TO (10%) polylactic acid sachets significantly reduced the anthracnose incidence and severity in artificially inoculated fruit (cv. Hass) after five days at 10 °C and thereafter at 20 °C. However, in naturally infected fruit, anthracnose disease was controlled completely with TO (10%) polylactic acid sachets after 15 days at 10 °C and then 5 days storage at 20 °C (ready to eat programme). The TO (10%) polylactic acid sachets treatment retained the appropriate firmness for ready to eat fruit (Bill *et al.,* 2018).

With a view to controlling anthracnose during large volume shipment, 20 cartons of naturally infected cv. Ryan staked in a pallet were exposed to TO vapours (78.9 mL) at 7.5 °C (packhouse holding temperature) and also at 25 °C for 24 h under a pallet cover to determine the effectiveness of the treatment during the laboratory stages (Bill, 2016). Fruit were removed from the pallet and stored at 7.5 °C and 85% RH for 21 days and also at 15 °C for 5 days to simulate market shelf conditions. Fruit exposed to TO vapours at 7.5 °C for 24 h showed significant control of anthracnose. The sensory properties of the fruit were not altered due to the exposure to TO vapours. To the best of our knowledge, this work will be the first report on the use of TO in controlling postharvest diseases in fruit at a commercial scale.

Therefore, under the 2017-2018 research programme, the following objectives were investigated: i) Semi commercial fumigation trials with TO on avocado cultivars to assess effectiveness of postharvest disease control at different packhouses, using two popular counts and during early, mid- and late seasons; ii) Assess the overall quality including the incidence of anthracnose, stem-end rot, physiological disorders and fruit firmness on fruit shipped to the UK (Overseas trial) and, iii) Investigate the effect of sachets incorporated with TO on the control of anthracnose and stem-end rot in popular avocado cultivars in a commercial trial at different packhouses.

MATERIALS AND METHODS

Study site: Plant and Soil Sciences UP – Plant Pathology laboratories, Westfalia Technological Services and Tzaneen Fruit Packers (TFP).

Local semi-commercial trial: Freshly harvested healthy fruit ('Hass') count 18 were obtained from the TFP packhouse during early (June), mid- (July) and late (August) seasons. The semi-commercial trial was carried out with the TO vapour treatment at specified concentrations (96 µL L^{-1}) according to Bill *et al*. (2017) with 15 replicate cartons of fruit kept at 8 °C and 15 replicate cartons at ~15 °C for 24 h. Fruit were exposed to TO vapours under the pallet covers. Standard prochloraz and untreated control fruit were used for comparison. Thereafter, 'Hass' avocados were stored under cold storage (5.5 °C) for 30 days. After completion of the low temperature storage, fruit were ripened at 20 °C (eating ripe, densimeter reading: 55-60). Incidences of anthracnose or stem-end rot were determined according to Sellamuthu *et al.* (2013). Fruit quality parameters such as firmness, sensory properties, smell, flavour and taste were also assessed according to Bill *et al*. (2017).

***TO sachet trial*:** Freshly harvested healthy fruit ('Hass') count 18 were obtained from Tzaneen Fruit Packers (TFP) during early (mid-June), mid- (mid-July) and late (mid-August) season with two popular counts. Fruit were cold stored at 6.5 °C for 14 days to simulate current supply chain conditions for domestic markets. Thereafter, fruit were removed from the low temperature storage, ripened (20 °C) for 3-4 days, packed in currently used avocado punnets with poly lactic acid sachets incorporated with TO (10%) according to Bill (2016) and held at the market shelf for 5 days (15 °C). At completion of postharvest storage, the fruit were industry assessed jointly with Westfalia: Fruit quality parameters such as firmness, sensory properties, smell, flavour and taste were also assessed. Standard prochloraz and untreated control fruit were used for comparison. Each treatment had ten replicate punnets (containing 4 fruit) for each treatment.

Statistical analysis

A completely randomised design was adopted for the postharvest treatments and storage trials. All variables measured were subjected to analysis of variance (ANOVA). Means of significant effects were separated using Fisher's protected t-LSD (least significant differences) at a 5% significance level. Data were analysed using the statistical programme SAS, SAS Enterprise Guide 4.0; SAS Institute, 2006, Cary, NC.

RESULTS AND DISCUSSION

The exposure of 'Hass' avocado fruit to TO vapour treatments (packhouse temperature) prior to cold storage (at 5.5 °C for 30 days) significantly ($P<0.05$) reduced the incidence of anthracnose in all three seasons (Fig. 1). This observation confirms the findings of our previous study (Bill *et al.,* 2017). Thyme oil either controls anthracnose by directly affecting the germination of the *C. gloeosporioides* spores or by up regulating the fruit's defense mechanism (Bill, 2016). Compared to the untreated control treatment, TO vapours effectively reduced the incidence of anthracnose by over 30%, 28% and 4% in the early, mid- and late season fruit respectively. It was however noticed that the exposure of the fruit to TO vapours at the packhouse temperature (~15 °C) prior to cold storage was more effective in controlling anthracnose compared to exposure at 8 °C.

The TO vapours at packhouse temperatures were more effective in the control of anthracnose in both early and late seasonal fruit compared to the commercial prochloraz treatment. It is noteworthy to mention that the TO vapours at the packhouse temperature consistently reduced the incidence of anthracnose in all three seasons in large-scale trials. Similarly, the TO vapours at the packhouse temperature significantly ($P<0.05$) reduced the incidence of stem-end compared to both the untreated control fruit and fruit exposed to prochloraz (Fig. 2).

Compared to untreated fruit, the incidence of stem-end rot was approximately five times less in fruit exposed to TO vapours at the packhouse temperature. Vascular browning is also one of the major postharvest physiological disorders of avocados (Duvenhage, 1993). Findings of this semi-commercial trial showed that no vascular browning incidence occurred in the early and mid-seasonal fruit (Fig. 3). The incidences of vascular browning was also reported to increase significantly in 'Hass' avocado picked during wet conditions (late in the season) compared to the fruit picked during dry weather conditions (early in the season) (Duvenhage, 1993). Both the TO vapour treatments and the prochloraz treatments significantly ($P<0.05$) reduced the vascular browning incidence by half (~20%) compared to the untreated control treatment (~40% in the late season fruit). No significant ($P<0.05$) differences were noticed in the firmness of the fruit (data not shown) in all the treatments. This confirms that fruit evaluations were done at the same stage of ripeness. The TO vapour treatments were also noticed to improve the sensory qualities of the fruit (data not shown).

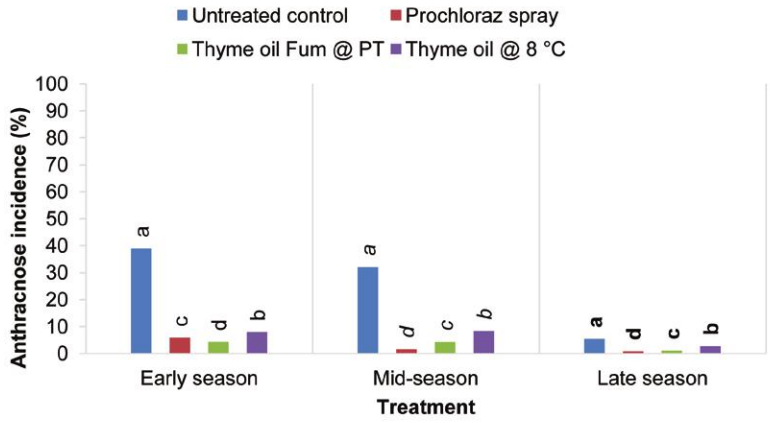

Figure 1: The effect of Thyme oil vapour treatments on anthracnose incidence in naturally infected 'Hass' avocado fruit.

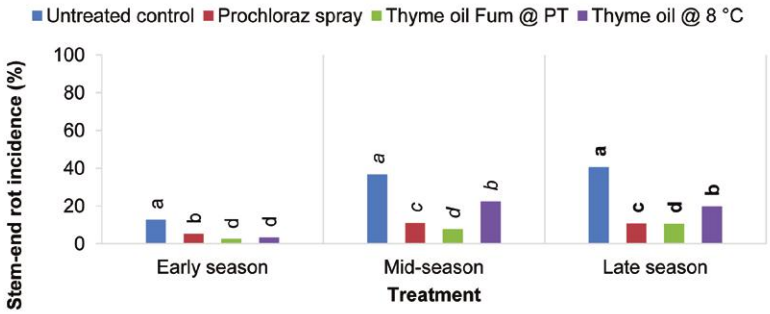

Figure 2: The effect of Thyme oil vapour treatments on stem-end rot incidence in naturally infected 'Hass' avocado fruit.

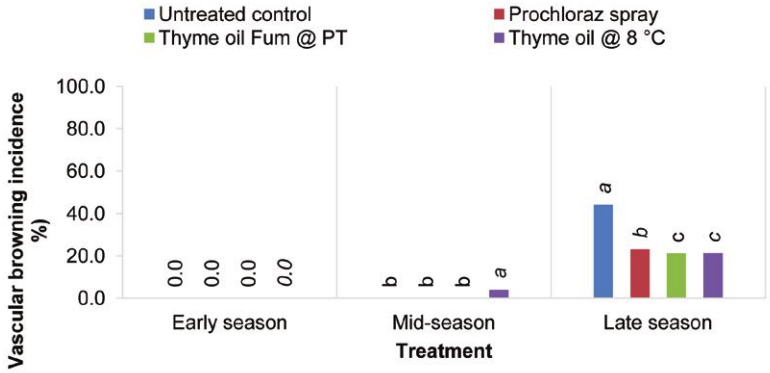

Figure 3: The effect of Thyme oil vapours treatments on vascular browning incidence in naturally infected 'Hass' avocado fruit.

Sachets incorporated with the fruit volatile 10% TO (10% TO sachet) in tray packs significantly ($P<0.05$) reduced anthracnose incidence after 5 days storage at 15 °C compared to the untreated control treatment. In early and late season fruit the 10% TO sachet treatment reduced the anthracnose and was similar to the prochloraz treatment after 5 days storage (Fig. 4). These results also confirm our findings of the previous work (Bill, 2016). It was also noticed that the 10% TO sachet reduced the stem-end rot incidence (Fig. 5) across all seasons compared to the untreated control treatment, while in the late seasonal fruit it was less effective compared to the prochloraz treatment. Vascular browning was not observed in both the early and mid-season fruit (Fig. 6). For late season fruit, the vascular browning incidence was significantly ($P<0.05$) reduced in fruit exposed to prochloraz and 10% TO sachet treatments.

In conclusion, the TO vapours and sachets treatments reduced the anthracnose and stem-end rot disease incidences. Therefore, exposure of avocado fruit to TO vapours and sachets prior to cold storage and in punnets respectively provides a promising alternative to commercial fungicide prochloraz treatment. We recommend that the same work be repeated in other commercially exported

avocado cultivars ('Fuerte' and 'Pinkerton'), including 'Hass' sourced from growing areas of high disease pressure. We also recommend the adoption of a more holistic approach at the preharvest stage for effective control of stem-end rot.

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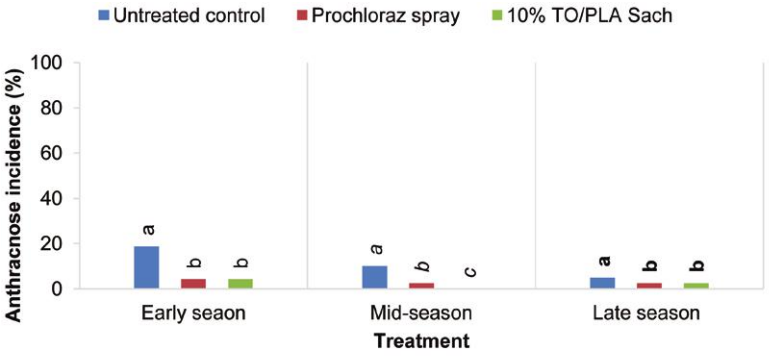

Figure 4: The effect of sachets incorporated with Thyme oil on anthracnose incidence in naturally infected 'Hass' avocado fruit.

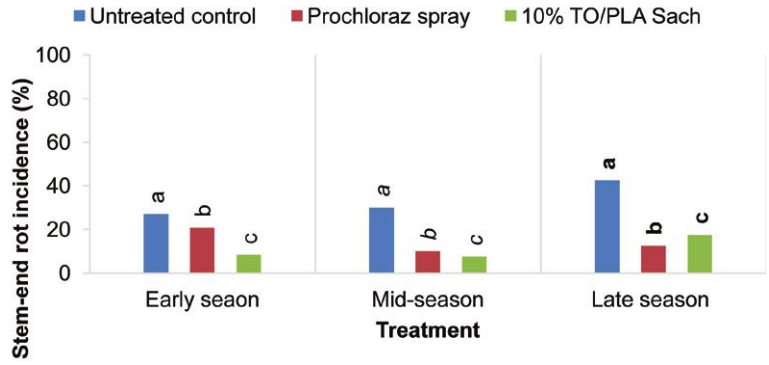

Figure 5: The effect of sachets incorporated with Thyme oil on stem-end rot incidence in naturally infected 'Hass' avocado fruit.

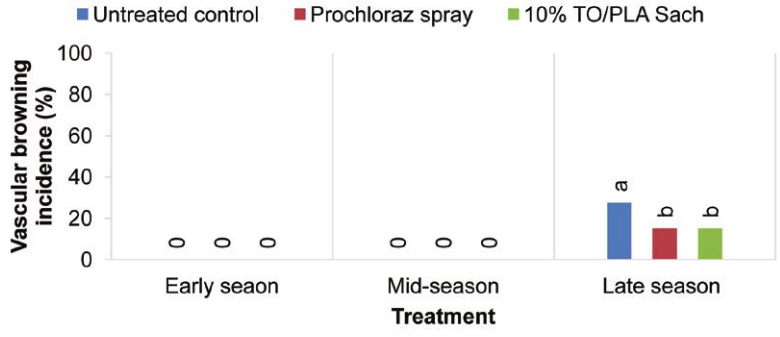

Figure 6: The effect of sachets incorporated with Thyme oil on vascular browning incidence in naturally infected 'Hass' avocado fruit.

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