Development of a more effective post-harvest treatment for the control of post-harvest diseases of avocado fruit

ZS Mavuso and JM van Niekerk

Westfalia Technological Services
PO Box 1103, Tzaneen 0850, South Africa
E-mail: zilungiselem@westfalia.co.za

ABSTRACT

Research done in Israel on mangoes indicated that, by lowering the pH of prochloraz solutions applied post-harvest, more effective control of post-harvest diseases was obtained. To test this principle in the control of post-harvest diseases of avocado, two trials were conducted. The first trial was done to determine the combined hydrochloric acid (H*Cl*) and prochloraz concentrations effective in inhibiting *in vitro* growth of post-harvest pathogens of avocado. Three H*Cl* concentrations (25, 50 and 75 mM) in combination with five prochloraz concentrations (0, 50, 100, 150 and 200 ppm) were tested for efficacy in inhibiting the growth of three *Colletotrichum gloeosporioides* and three Botryosphaeriaceae isolates using the infiltrated paper disc method. Results indicated that most combinations tested were less effective in inhibiting *in vitro* growth of the pathogens, compared to the standard concentration of 810 ppm prochloraz. The only exception was 200 ppm prochloraz combined with 50 mM *HCl* which was just as effective in inhibiting pathogen growth as the standard 810 ppm prochloraz. The second trial was to determine the efficacy of 200 ppm prochloraz combined with 50 mM H*Cl* applied post-harvest as either a spray or dip treatment compared to the standard post-harvest treatment of a dip in a solution of 810 ppm prochloraz. Fruit from cultivars 'Fuerte', 'Mexican 2' and 'Mexican 3' (Hass-like cultivars) were subjected to the following treatments: (1) water treated fruit, (2) 810 ppm prochloraz, (3) 200 ppm prochloraz, (4) 200 ppm prochloraz + 50 mM H*Cl*, (5) 50 mM H*Cl* alone. These treatments were applied as a 15 s dip treatment to one set of fruit and as a spray-on treatment on another set of fruit. After treatment fruit were stored for 28 days at 5.5°C before being ripened and evaluated for post-harvest disease incidence. Results from the treated fruit indicated that general application of the treatments as a dip resulted in more fruit free from anthracnose and stem-end rot than when the treatments was applied as a spray. In comparing the different treatments, it was shown that when applying 200 ppm prochloraz + 50 mM H*Cl* as a dip, it was just as effective as applying 810 ppm prochloraz as a dip in controlling post-harvest diseases. These results indicate that with further research, the amount of prochloraz used for the post-harvest treatment of avocados can potentially be reduced drastically.

INTRODUCTION

In South Africa anthracnose and stem-end rot as post-harvest diseases are major limiting factors in the production and export of avocados (Le Roux *et al.*, 1985). The fungi causing these diseases include *Colletotrichum gloeosporioides* (anthracnose) and various species in the Botryosphaeriaceae (stem-end rot) (Darvas, 1977; Darvas & Kotze, 1979). They occur as latent infections in the fruit and are therefore rather difficult to control with fungicides (Le Roux *et al.*, 1985). Currently these diseases are controlled by a combination of pre-harvest fungicide applications and a post-harvest prochloraz dip in the packhouse (Darvas, 1984). Despite the use of large amounts of fungicide, quality control results from the 2008 avocado season indicated that a substantial percentage of fruit were still lost due to anthracnose, stem-end rot or other body rots occurring on export fruit. This could indicate that the post-harvest application of prochloraz currently being used is not optimally effective.

Research has shown that during ripening, the pH of avocado fruit increases from pH 5.2 to pH 6.0 (Yakoby *et al.*, 2000). It was furthermore found that under these pH values *pelB*, one of the virulence genes of *C. gloeosporioides*, was expressed more actively and that the pathogen enhances this process by excreting ammonia in the infected host tissue (Prusky *et al.*, 2001; Yakoby *et al.*, 2000; 2001). This change in the ambient pH of the host tissue at the infection site is therefore regarded as the cause for the activation of the latent *C. gloeosporioides* infections to cause necrotic lesions in the fruit (Prusky & Yakoby, 2003).

Alternaria alternata, an important post-harvest pathogen of mango, acts in the same manner as *C. gloeosporioides* described previously (Prusky *et al.*, 2006). This characteristic of the pathogen was used in Israel to develop a more effective post-harvest treatment of mango fruit. It was found that by adding 50 mM hy-

drochloric acid to the prochloraz solution in the packhouse, post-harvest decay caused by *A. alternata* was controlled significantly better (Prusky *et al.*, 2006). This effect of the acidified prochloraz is due to (1) the pH directly affecting the germination of the pathogen conidia (Pelser & Eckert, 1977), (2) influencing the virulence of the pathogen (Prusky *et al.*, 2004) and (3) affecting the toxicity of the fungicides used (Smilanick *et al.*, 2005). Prusky *et al.* (2006) showed that by adding hydrochloric acid to the prochloraz solution, the solubility of the prochloraz is increased significantly. This means that in an acidified prochloraz solution a significantly lower concentration of prochloraz can be used, while the disease control obtained by this solution is significantly better.

The aim of this study is therefore to develop the use of acidified prochloraz for use in avocado packhouses to potentially control post-harvest diseases better while using much less prochloraz compared to the currently used post-harvest practices.

MATERIALS AND METHODS

In vitro screening

The three *Colletotrichum gloeosporioides* and three Botryosphaeriaceae isolates used in the *in vitro* study were obtained from infected avocado fruit at Westfalia Fruit Estates and cultured for 1 to 2 weeks on potato detrox agar (PDA).

Poison agar method

Potato dextrose agar (PDA) was prepared and allowed to cool to 50°C. Three H*Cl* concentrations (25, 50 and 75 mM) in combination with five prochloraz concentrations (0, 50, 100, 150 and 200 ppm) were mixed with pre-cooled PDA. PDA containing the different concentrations of prochloraz and H*Cl* were then poured into 90 mm Petri dishes and allowed to cool. The cultures of *Colletotrichum gloeosporioides* and Botryosphaeriaceae were cut into 5 mm x 5 mm blocks. Each block was placed in the centre of each plate. Each treatment included four replicates, and each test was repeated three times. Plates were incubated at 22-24°C for three days. Growth diameter was measured around each block and results were recorded.

Paper disc method

Botryosphaeriaceae mycelia were harvested by adding 3-4 ml of sterile distilled water to the Petri dish. The mycelia were rubbed with a sterile glass rod to free it from the PDA medium. The mycelium suspension was then passed through a gauze cloth to filter it and diluted to 250 ml solution for further use in the experiments. In the case of *C. gloeosporioides*, spores were collected using sterile cotton swabs rolled gently over the culture, the collected spores were then shaken off into 50 ml sterile distilled water. The spore suspension was also passed through a gauze cloth to filter it. The spore suspension was diluted to 1.2 x 10^4 with sterile distilled water to obtain the concentrations needed for the experiments.

PDA dishes were seeded with 0.1 ml (100 µl) of the *Colletotrichum gloeosporioides* spore suspension and others with 0.1 ml (100 µl) of the Botryosphaeriaceae mycelial suspension. A sterile hockey stick was used to spread the suspensions on the dishes before dishes were allowed to dry in a laminar flow cabinet. Three H*Cl* concentrations (25, 50 and 75 mM) in combination with five prochloraz concentrations (0, 50, 100, 150 and 200 ppm) were prepared, using sterile distilled water. A total of 15 solutions (3 H*Cl* concentrations x 5 prochloraz concentrations) were prepared that represented the different treatments in the test. Sterile paper discs were allowed to float on each solution and then placed in the centre of each dish seeded with the different pathogens. Each treatment included four replicates, and each test was repeated three times. Plates were incubated at 22-24°C for 3 days. The zone of mycelial inhibition was measured around each disc and results were recorded.

Due to better results, the paper disc method was repeated using a concentration of 50 mM H*Cl* in combination with the 5 prochloraz concentrations (**Table 1**). The experiment was repeated three times.

Table 1. Prochloraz and H*Cl* concentration combinations (treatments) tested *in vitro* for the inhibition of *Colletotrichum gloeosporioides* and Botryosphaeriaceae mycelial growth using the paper disc method.

Treatment	Prochloraz and H*Cl* concentrations
1	50 ppm prochloraz + 50 mM H*Cl*
2	100 ppm prochloraz + 50 mM H*Cl*
3	150 ppm prochloraz + 50 mM H*Cl*
4	200 ppm prochloraz + 50 mM H*Cl*
5	810 ppm prochloraz only
6	H*Cl* only
7	Water (control)

Post-harvest trial

Fruit from cultivars 'Fuerte', 'Mexican 2' and 'Mexican 3' (Hass-like cultivars) were subjected to different treatments (**Table 2**). A hundred fruit were used for each treatment. The treatments were applied as a 15 s dip treatment to one set of fruit and as a spray-on treatment on another set of fruit. The spray-on treatments were applied using a custom built mini-packline with wax applicator at Westfalia Technological Services. After treatment, fruit were stored for 28 days at 5.5°C before being ripened and evaluated for post-harvest disease incidence. All results were statistically analyzed using STATISTICA Version 6 (StatSoft, Inc., Tulsa, USA).

RESULTS

In vitro screening

Results from the *in vitro* poison agar method did not

Table 2. Prochloraz and H*Cl* concentrations applied post-harvest as either a dip or spray-on treatment for the control of post-harvest diseases to fruit of cultivars 'Fuerte', 'Mexican 1' and 'Mexican 2'.

Treatment	Prochloraz and H*Cl* concentrations
1	Water treated fruit (Control)
2	50 mM H*CL* only
3	200 ppm prochloraz only
4	200 ppm prochloraz + 50 mM H*CL*
5	810 ppm prochloraz only

clearly differentiate the efficacy of the different H*Cl* and prochloraz concentrations. However, the results from the paper disc method indicated that the most combinations tested were less effective in inhibiting the *in vitro* growth of the pathogens compared to prochloraz at a concentration of 810 ppm. The only exception was 200 ppm prochloraz in combination with 50 mM H*Cl* which was just as effective as 810 ppm prochloraz alone (**Figure 1**).

Post-harvest trials

Results of the statistical analysis indicated that for all cultivars there were no significant statistical difference between the treatments in terms of percentage fruit free from anthracnose and stem-end rot. Therefore, the mean percentage of fruit free from anthracnose and stem-end rot was calculated across all fruit and cultivars with no distinction made between cultivars. The means were subsequently used to compare the different treatments applied as a dip or a spray. Comparing the means indicated that in general application of the treatments as a dip resulted in more fruit free from anthracnose and stem-end rot than when the treatments was applied as a spray (**Figure 2**). The observed results might be due to the wax applicator which applied the treatments as a low pressure mist that possibly did not cover the fruit as well as the dip application. In comparing the different treatments, it was shown that when applying 200 ppm prochloraz + 50 mM H*Cl* as a dip, it was just as effective as applying 810 ppm prochloraz as a dip (**Figure 2**). However, some treated 'Fuerte' fruit showed minor phytotoxic damage that might indicate chemical burn, which could not have been foreseen from the *in vitro* testing. This problem can possibly be rectified using lower concentrations of prochloraz in combination with 50 mM H*Cl* that might be just as effective on fruit as the 200 ppm prochloraz + 50 mM H*Cl*. The trial will be repeated in the 2010 season using early and late season fruit. Prochloraz residue analysis will also be done on the most effective treatment.

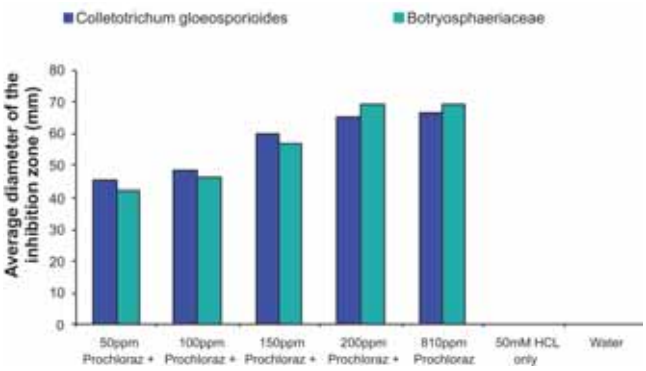

Figure 1. Average diameter of the inhibition zone in the mycelial growth of *Colletotrichum gloeosporioides* and Botryosphaeriaceae caused by the different prochloraz and H*Cl* concentrations.

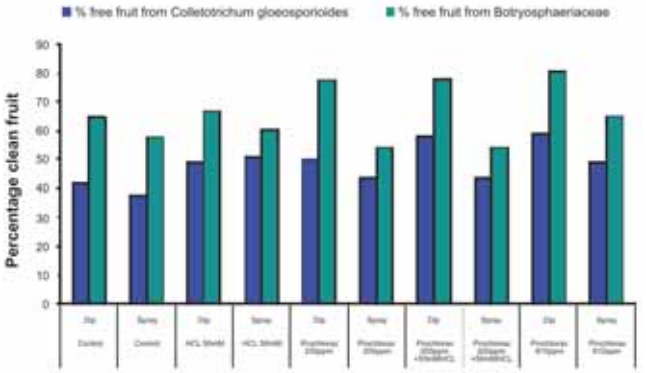

Figure 2. Percentage fruit, after storage and ripening, of cultivars 'Fuerte', 'Mexican 1' and 'Mexican 2' free from post-harvest anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (Botryosphaeriaceae) when the different prochloraz and H*Cl* concentrations were applied as either a dip or spray-on post-harvest treatment.

ACKNOWLEDGEMENTS

The authors wish to thank SAAGA for financial support and Westfalia Technological Services for materials, equipment, technical assistance and funding.

LITERATURE CITED

DARVAS, J.M. 1977. Fungi associated with postharvest diseases of avocados. *South African Avocado Growers' Association Proceedings of the Technical Committee*, 1: 9-10.

DARVAS, J.M. & KOTZE, J.M. 1979. Stem-end rot and other postharvest diseases. *South African Avocado Growers' Association Research Report*, 3: 41-43.

DARVAS, J.M. 1984. The control of postharvest avocado diseases with prochloraz. *South African Avocado Growers' Association Yearbook*, 7: 57-58.

LE ROUX, A.W.G., WENTZEL, R.C. & ROOSE, C. 1985. Efficacy of prochloraz treatments for post harvest disease control in avocados. *South African Avocado Growers' Association Yearbook*, 8: 44-45.

PELSER, P. DU T. & ECKERT, J.W. 1977. Constituents of orange juice that stimulate the germination of conidia of *Penicillium digitatum*. *Phytopathology*, 67: 747-754.

PRUSKY, D., MCEVOY, J.L., LEVERENTZ, B. & CONWAY, W.S. 2001. Local modulation of host pH by *Colletotrichum* species as a mechanism to increase virulence. *Molecular Plant-Microbe Interaction*, 14: 1105-1113.

PRUSKY, D. & YAKOBY, N. 2003. Pathogenic fungi: leading or led by ambient pH? *Molecular Plant Pathology*, 4: 509-516.

PRUSKY, D., MCEVOY, J.L., SAFTNER, R., CONWAY, W.S. & JONES, R. 2004. Relationship between host acidification and virulence of *Penicillium* spp. on apple and citrus fruits. Phytopathology, 94: 44-51.

PRUSKY, D., KOBILER, I., AKERMAN, M. & MIYARA, I. 2006. Effect of acidic solutions and acidic prochloraz on the control of postharvest decay caused by *Alternaria alternate* in mango and persimmon fruit. *Postharvest Biology and Technology*, 42: 134-141.

SMILANICK, J.L., MANSOUR, M.F., MARGOSAN, D.A. & MLIKOTA BABLER, F. 2005. Influence of pH and $NaHCO_3$ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. *Plant Disease*, 89: 640-648.

YAKOBY, N., KOBILER, I., DINOOR, A. & PRUSKY, D. 2000. pH regulation of pectate lyase secretion modulates the attack of *Colletotrichum gloeosporioides* on avocado fruits. *Applied Environmental Microbiology*, 66: 1026-1030.

YAKOBY, N., BENO-MOUALEM, D., KEEN, N.T., DINOOR, A., PINES, O. & PRUSKY, D. 2001. *Colletotrichum gloeosporioides pelB*, is an important factor in avocado fruit infection. *Molecular Plant-Microbe Interaction*, 14: 988-995.