Prochloraz – a look into factors affecting the variability of residue levels on avocado fruit

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ABSTRACT

The main aim of the study was to determine reasons for significant variation in the residues on fruit when using prochloraz as a postharvest treatment. The impact of fruit size, cultivars ('Hass', 'Ryan' and 'Pinkerton') and standing over of fruit were evaluated, following an improved protocol based on last years' results. Results showed the highest residue levels were found on small size fruit, cultivars varied in residue levels with 'Hass' having the highest residue level and standing over had little effect on residue levels, although it was slightly higher on fruit packed one day after harvest. A sanitation agent, Agrigold, was tested in combination with prochloraz to determine whether efficacy could be improved. Results showed that prochloraz residue levels were higher when Agrigold was added.

Several postharvest products were tested to determine the effectiveness against anthracnose. Products included imazalil sulphate, fludioxonil, propiconazole and pyrmethanil. Results showed that prochloraz seemed to be the most effective postharvest product tested against anthracnose, while imazalil sulphate gave the best control of stem-end rot.

Pack houses were visited and water samples were collected to test the potential of the turbidity meter in measuring concentrations of prochloraz. Due to the detected level of impurities in the water, it is recommended to use a misting system for the application of prochloraz as the concentration is made up fresh every day and is not diluted throughout the day while a considerable lower amount of chemical is used.

INTRODUCTION

Prochloraz (Chronos 450 g/L SC – Adama) is registered as a postharvest treatment to control anthracnose (*Colletotrichum gloeosporoides*) at a concentration of 180 mL/100 L water + 0.2% HCl, while the EC formulation is registered as 1100 mL/100 L water as a spray-on treatment using a 1.6 L spray mixture per ton fruit applied with a low volume applicator (Van Zyl, 2011).

Although the allowed export default MRL for prochloraz is 2 ppm (DAFF, 2007) some importers in Europe require a lower MRL. When using the recommended concentrations, this lower MRL might be exceeded.

Additionally, discrepancies in residue analyses of fruit treated similarly throughout the season are sometimes observed for no apparent reason. Last season, the study was initiated to determine reasons for these inconsistencies but we also ended with serious variation in the results. It was realised that the application and sampling protocol had to be improved since both were part of the high variation in residue levels encountered (Daneel *et al*., 2016).

An aim of the study was thus to investigate factors that could possibly have an effect on residue levels of prochloraz on the fruit, factors such as fruit size, cultivar and storage of fruit before packing. Another aim was to investigate the use of a sanitising agent in combination with prochloraz with the prospect of reducing the concentration of prochloraz, thus reducing the risk of excessive residue levels on the fruit.

Finally, pack houses were visited to observe the current protocols used and present alternative protocols regarding prochloraz applications.

MATERIALS AND METHODS

Factors affecting residue levels on avocado fruit

Avocado fruit was collected in several pack houses from April until October 2016, including Halls (Mbombela area), Koeltehof (Hazyview area), Westfalia and Letaba Packers (Tzaneen area). The cultivars used included 'Fuerte', 'Hass', 'Ryan' and 'Pinkerton'.

Fruit were brought to the ARC-Tropical and Subtropical Crops (ARC-TSC) campus in Mbombela where tests were carried out in the postharvest laboratory. Tests were conducted the day following collection from the pack house (unless otherwise stated). Fruit were properly mixed after collection from the pack house while it was divided among the different treatments. This was done to ensure that factors like origin of orchard, position in orchard and tree, fruit size, etc. would not play a role in the results obtained.

Among the treatments, fruit were further divided into three containers for the three replicates used.

Experiments consisted of dipping the containers with fruit in a water solution for 1 min while shaking the container to simulate the washing process. Then fruit were left to drip a short while, after which the fruit were dipped for 30 sec in a separate container containing the prochloraz solution. The concentrations of prochloraz (Chronos® 450 SC – Prochloraz zinc complex [imidazole] 530 g/L and prochloraz equivalent 450 g/L) tested were 180, 90, 60 mL or 45 mL + HCl per 100 L water. An untreated control with only water was also included. The prochloraz bath was filled with 60 L of water using a flow meter. Two liters of the water was poured into another container where the correct concentration of prochloraz was added. This mixture was properly stirred, after which it was added to the rest of the water where it was again thoroughly mixed. Each concentration was prepared separately ensuring that in each prochloraz solution a maximum of three containers (for the three replicates) were dipped. When more than one cultivar was tested on a specific day, a new solution was prepared for each cultivar. The untreated control was dipped in water for 30 sec.

Thereafter the fruit was left to dry. After drying, fruit residue samples were collected and placed in a freezer at -20°C until delivery to Hortec for further analysis, while the remaining fruit were packed in the cold room for 28 days following the standard procedure: 7.5°C for 2 days, 6.5°C for 2 days and 5.5°C for 24 days, after which it was removed from cold storage and brought to room temperature and evaluated.

a. Determining the effect of different sizes of avocado fruit on residue levels of prochloraz

For this trial, after treating with the different prochloraz concentrations, fruit (cv. 'Hass' – obtained from Hazyview area) from each container was sorted according to size using small, medium and large classes. Small consisted of class 22-24 fruit, medium of class 16-18 fruit and large of class 12-14 fruit. Ten fruit per replicate per size class was packed in separate bags using three replicates.

b. Determining the effect of cultivar on residue levels of prochloraz

For this trial, the same procedure as discussed above was followed while 45 mL/100 L water + HCl was used as the lower concentration. Cultivars compared were 'Hass' and 'Ryan' (from Tzaneen area – August) and 'Fuerte' (from Nelspruit area – June).

c. Determining the effect of storage period of fruit on residue levels of prochloraz

Another trial was aimed at determining the effect of waiting period of fruit in the pack house before packing. Fruit were collected from the pack house and left for 1, 2 and 3 days respectively after harvest before it was washed and dipped in the prochloraz concentrations. In this test, only one prochloraz concentration was used (180 mL/100 L water).

d. Determining the effect of a sanitizing agent on the residue levels of prochloraz

In this trial the same procedure as above was followed, but four additional treatments were included: each concentration of prochloraz was compared with a similar concentration of prochloraz with the addition of Agrigold. Agrigold was shown previously to enhance the efficacy of prochloraz (Daneel, 2010).

Determining the effect of alternative postharvest chemicals for the control of anthracnose and stem-end rot

In this trial the same procedure as above was followed but instead of prochloraz, different postharvest solutions were used. The trial was conducted on 'Pinkerton' and 'Hass' fruit. The products and concentrations used can be seen in Table 1.

Table 1. Different postharvest treatments on avocado fruit, indicating formulations, concentration per 100 L water, as well as active ingredient (ppm).

	Treatments	Concentration/ 100 L water	Active ingredient (ppm)
1	Untreated control	n/a	n/a
2	Imazalil Sulphate (750 SG)	40	300
3	Imazalil Sulphate (750 SG)	67	500
4	Propiconazole (250 EC)	145	360
5	Propiconazole (250 EC)	240	600
6	Pyrimethanil (400 SC)	150	600
7	Pyrimethanil (400 SC)	250	1000
8	Fludioxonil (200 WDG)	100	200
9	Fludioxonil (200 WDG)	170	340
10	Prochloraz (Chronos) (450 SC)	180 mL & 0.2% HCL	810

Pack house visits

Pack houses in the Tzaneen and Hazyview area were visited and water samples were collected over a 2-day period (3-4 samples) to determine the prochloraz concentration. Water samples were analysed using the turbidity meter method described by Daneel & Botha (2013).

Residue analysis

The samples were analysed for prochloraz residue levels by Hortec, a SANAS accredited laboratory.

RESULTS

Factors affecting residue levels on avocado fruit

a. The effect of different sizes of avocado fruit on residue levels of prochloraz

Table 2 presents the residue levels (ppm) of different treatments on the different sizes of fruit treated. The smaller fruit seemed to have a tendency to have the higher residue levels.

Table 2. Residue levels (ppm or mg/kg) of 'Hass' fruit for the different fruit sizes treated per treatment.

Treatments	Large	Medium	Small
Untreated	0.03	0.04	0.07
60 mL	0.31	0.54	0.37
90 mL	0.31	0.40	0.44
180 mL	0.62	0.78	0.91

b. The effect of different cultivars on residue levels of prochloraz

Three cultivars were tested and results can be seen in Table 3. Cultivar 'Hass' had the higher residue levels compared to the other two cultivars. This was observed for all the treatments. The 45 mL + HCl treatment for 'Hass' showed a high residue level (mean of 0.72 ppm) caused by high residue levels ranging from 0.36-0.98 ppm. This was significantly higher than the residue levels for the other two cultivars where the residue levels varied between 0.2-0.3 ppm. It is clear that 'Hass' is more prone to higher prochloraz residue levels and maybe the addition of the acid makes the fruit of 'Hass' even more prone to increased residue levels, much more so than other cultivars.

Table 3. Prochloraz residue levels (mg/kg or ppm) of three avocado cultivars per treatment.

Cultivars	'Hass'	'Ryan'	'Fuerte'
Untreated	0.005	0.004	0.025
45 mL + HCl	0.72*	0.24	0.21
90 mL	0.48	0.27	0.38
180 mL	0.67	0.38	0.55

*two of the three replicates had a high ppm

c. Determining the effect of storage period of fruit on residue levels of prochloraz

Table 4 shows the residues of fruit left in storage for several days. There is little difference between fruit collected on consecutive days. However, the fruit sampled at day 1 had a slightly higher residue level.

Table 4. Prochloraz residue levels (mg/kg or ppm) on 'Fuerte' avocado fruit when left for 1, 2 and 3 days in storage before packing.

	180 mL
Day 1	0.55
Day 2	0.45
Day 3	0.44

d. The effect of a sanitising agent (Agrigold) on the residue levels of prochloraz

Table 5 shows the effect of Agrigold on the residue levels of prochloraz. It can be seen that residue levels were considerably higher when Agrigold was added to the solution. This could indicate that prochloraz is more efficiently taken up by the fruit when Agrigold is added. This was observed in all treatments. The anthracnose incidence as well as percentage edible fruit can also be seen in Table 5. Both confirm an increase of prochloraz efficacy with the addition of Agrigold in the fungicide bath. Anthracnose levels were low, but especially in the lower prochloraz concentration fruit showed a lower anthracnose incidence when Agrigold was added.

Table 5. Prochloraz residue levels (mg/kg or ppm) on 'Pinkerton' avocado fruit for the different treatments.

	Residue levels	% Anthracnose	% Edible fruit
Untreated	0.04	23.1	71.8
180 mL + Agrigold	1.18	8.3	83.3
180 mL	0.48	5.8	88.9
90 mL + Agrigold	0.51	13.9	83.3
90 mL	0.33	11.6	86.1
60 mL + Agrigold	0.37	2.8	88.9
60 mL	0.31	19.5	66.7

The effect of alternative postharvest chemicals for the control of anthracnose and stem-end rot

Imazalil sulphate reduced the pH of the solution. However, no increased lenticel damage was observed due to this reduction in pH. The other products did not have a significant effect on pH.

The first trial was executed with 'Pinkerton' fruit but very little disease incidence was observed, even after fruit was allowed to rot. Subsequently the trial was repeated with 'Hass' fruit. The 'Hass' fruit used in the second trial was left to mature for an extended period because initial disease incidence was also low, even after a cold storage period of 28 days. Percentage anthracnose and stem-end rot incidence was calculated by scoring each fruit a 1 when disease was

observed and 0 when disease was not observed. Fruit were left to ripen far beyond the normal practice and percentage disease incidence was therefore very high, as can be seen in Table 6. In many instances both anthracnose and stem-end rot were present at the same time and percentage fruit infected with either disease thus exceeds 100%.

No significant difference was observed for anthracnose control between the different treatments. However, prochloraz 180 mL had the lowest level of anthracnose incidence, while the other products had similar anthracnose incidence than the control treatment, indicating very little efficacy (Table 6).

For stem-end rot, no significant differences were observed, but imazalil sulphate seemed to provide some control when compared to the untreated fruit (28% incidence compared to 45% incidence). Prochloraz showed the highest incidence of stem-end rot (77%) and it is difficult to explain why it is so much higher than the control fruit.

The results show that prochloraz had the highest percentage healthy fruit during evaluation, followed by fludioxonil (Table 6). The other products tested did not differ from the untreated control concerning % healthy fruit, except for propiconazole 600 ppm.

While Table 6 shows the effect of each parameter separately, Figure 1 shows the effect of the different treatments on fruit quality taking all the variables into account. In Figure 1a, which shows the correlation circle of the variables corresponding to the parameters measured during evaluation of the trial, healthy fruit is opposed to both anthracnose and stem-end rot incidence along F1 (the first axis in abscissa). On the factorial plan (Fig. 1b) of the rows (each row relates to one fruit), each circle indicates the average position of the treatment in which all variables are taken into account. It can be seen that treatment 10 (prochloraz) is situated separately from the other treatments. The position of prochloraz in Figure 1b is due to the lower anthracnose incidence and higher incidence of healthy fruit (shown by the green arrows). The other treatments were all grouped together (orange circle), showing little difference between the fruit of the control treatment and the other treatments. Figures 1a & 1b confirm that prochloraz gave the best postharvest control between all the treatments tested on avocado fruit.

Pack house visits

Pack houses visited used a variety of methods to apply the prochloraz, including dipping in the fungicide bath, spray-on system recycling the prochloraz solution and a misting system without recycling the solution. Water samples collected in the Tzaneen area were greyish, even when

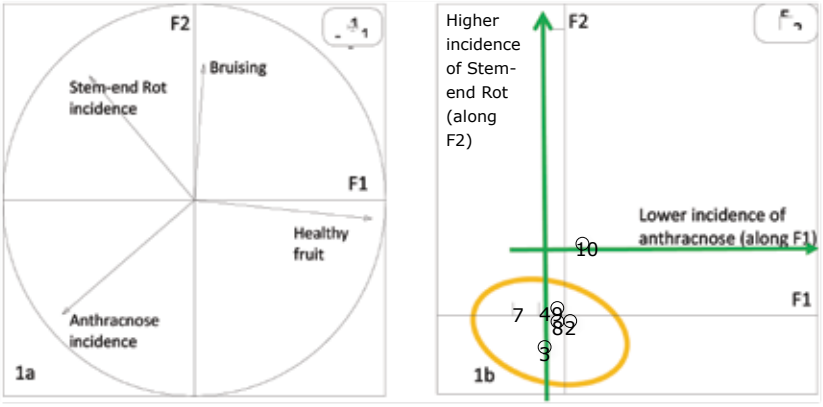

Figure 1a. Correlation circle showing the four variables evaluated and the position towards one another. **Figure 1b**. Factorial plan for the different treatments with 1 = control; 2 = imazalil sulphate 40 g; 3 = imazalil sulphate 67 g; 4 = propiconazole 145 ml; 5 = propiconazole 240 ml; 6 = pyrimethanil 150 ml; 7 = pyrimethanil 250 ml; 8 = fludioxonil 100 g; 9 = fludioxonil 170 g; 10 = prochloraz 180 ml.

Table 6. Percentage incidence of anthracnose, stem-end rot and percentage healthy avocado fruit for the different treatments.

		% Anthracnose	% Stem-end rot	% Healthy fruit
1	Untreated	62.4	45.1	14.3
2	Imazalil sulphate 40 g	59.6	29.3	11.1
3	Imazalil sulphate 67 g	86.7	26.2	10.0
4	Propiconazole 145 mL	66.7	53.3	13.3
5	Propiconazole 240 mL	56.7	57.7	18.6
6	Pyrimethanil 155 mL	66.6	45.2	11.9
7	Pyrimethanil 250 mL	67.6	75.7	3.3
8	Fludioxonil 100 g	66.7	51.3	18.7
9	Fludioxonil 170 g	61.4	64.8	19.1
10	Prochloraz 180 mL	43.3	76.7	23.3

No significant difference was observed between the different treatments; fruit was left to become overripe to enhance disease incidence.

the solution was made up fresh, in contrast to solutions in Hazyview. The cause of this grey colour has not been determined but it is probably linked to the water characteristics. However, this made the use of the turbidity meter difficult. A turbidity meter measures turbidity caused by the concentration of prochloraz in the solution. A lower concentration would mean a lower turbidity and this can be measured (Daneel and Botha, 2013). However, in this instance, two factors were influencing the turbidity of the solution, namely the prochloraz concentration as well as the grey colour. This situation resulted in the need for a very high dilution, making the estimate too vague and thus without real value.

The use of a misting system was previously proposed since it has several advantages above the other options. This system was installed in one of the pack houses visited. The water samples collected throughout the day in this pack house were similar to a freshly made up prochloraz solution throughout the day, even seven hours after preparing the fresh solution.

DISCUSSION

During the study conducted this season (2016), anthracnose levels were overall low, probably due to unfavourable climatic conditions. When fruit had to be evaluated, it was left to ripen far beyond the normal practices and this would explain the high level of anthracnose and stem-end rot of the data shown in the results. However, the focus was more on residue analysis than evaluation of the fruit, since it has been proven previously that prochloraz is an effective postharvest agent (Le Roux *et al.,* 1995; Mavuso and Van Niekerk, 2010; Daneel, 2011; Daneel *et al*., 2016).

Residue levels on fruit were much more consistent this season than last year (Daneel *et al.*, 2016), probably because fruit selected for residue analysis was similar in size, in contrast to last year when fruit were randomly selected for residue analysis, and number rather than fruit size was used. Furthermore, the prochloraz bath was filled with 60 L water compared to the 25 L water used last season, and only three containers were dipped per treatment. This ensured that the prochloraz concentration in the bath remained more or less the same when dipping the fruit.

Regarding the observation that higher residue levels were observed on smaller fruit (Table 2), an explanation could be the fact that residue analyses are done using fruit pulp and pip weight. Two kilogram small fruit consists of more fruits than 2 kg large fruits. Having more fruit means a larger surface area, resulting in a possible higher residue levels. More significant is that it confirmed the importance of selecting similar size fruit when sampling for residue analysis and preferably smaller fruit, since the highest possible residue levels need to be determined to ensure that fruit send overseas does not exceed the required MRL.

Three cultivars were dipped in similar prochloraz solutions and a considerable difference was observed in residue levels between the cultivars (Table 3). 'Hass' had the highest residue levels. The rougher skin might make it more suitable for prochloraz to stick to it. 'Ryan' seemed to have the lowest levels. This result is important since residue levels found on one cultivar can be different from residue levels on another cultivar using the same bath.

Similar to last year, fruit left in storage for up to three days before packing had similar residue levels, with fruit packed at the 1st day showing a slightly higher residue level (Table 4). As seen in the previous season, storage does not seem to increase residue levels indicating that fruit does not have to be packed immediately.

This season, Agrigold was used in combination with prochloraz since it was previously seen to enhance the efficacy of prochloraz on mango (Daneel, 2010). Similar to the addition of acid, the results showed that Agrigold also increased prochloraz residue levels on the fruit (Table 5). Agrigold might also enhance the solubility of prochloraz, as was stated to be the reason when an acid was added (Prusky *et al.,* 2006). Daneel (2015) has shown in previous studies that Agrigold also improved efficacy of imazalil sulphate when added to the fungicide bath. The higher residue levels obtained when Agrigold is added could explain why anthracnose incidence is lower when Agrigold is added. When using Agrigold in combination with prochloraz, a lower concentration of prochloraz can and maybe should be used, especially for export, since prochloraz residue level increased. Good anthracnose control was obtained even with lower prochloraz concentrations.

Imazalil sulphate provided some stem-end rot control and should be tested in combination with another postharvest product to control the important postharvest diseases on avocado. Because none of the other chemicals tested seemed to provide either anthracnose or stem-end rot control, other alternative products should be included.

The turbidity method was tested in the pack house but seemed to be inadequate in the Tzaneen area where the water turned grey almost immediately after prochloraz was added in the fungicide bath. No easy method to determine the prochloraz concentration is available. The biological method (Serfontein & Serfontein, 2006) can be used to determine the concentrations of prochloraz in the fungicide bath. However, a more accurate method is sending water samples, collected throughout the day, away for chemical analysis to a registered laboratory, but this method is expensive. For both methods, it is important when samples are collected to note time of sampling and amount of fruit packed. To reduce variation in results, samples should be replicated. However, this will have a cost implication. Results will provide information as to when prochloraz should be added to the fungicide bath throughout the day. The same applies for the spray-on method as the water is also recycled and thus concentrations will reduce throughout the day.

The misting system seems to be an improvement to the commonly used systems and could be installed in most pack houses without excessive costs.

The advantages of such a system are the lower amount of prochloraz used throughout the day, reducing the costs of the chemical. Misting uses a much smaller amount of water compared to a bath and spray-on system and 100 L solution is probably enough for the entire day. Immediately linked to this, less water and chemical have to be disposed of, resulting in a significantly lower ecological impact. The solution is never diluted since it is an open system and once the solution is sprayed on the fruit, it is not recycled. It is important to remember that the solution in the container has to stay in suspension at all times. It may be feasible to investigate the optimal concentration to be made up in the container to ensure high enough residue levels on the fruit. The starting point could be 1100 mL/100 L water which is registered for the spray-on application.

CONCLUSION

When residue samples are taken, it is important to select fruit that will provide the highest possible residue level to ensure that, when fruit is send overseas, the allowed MRL levels are not exceeded. These results will allow the pack house to make adaptations to the prochoraz bath if levels are too high.

It is therefore recommended that when samples are taken for residue analysis, the following principles be followed:

- Sample small size fruit;
- Sample immediately after packing;
- Sample 'Hass' fruit if available;
- Sample fruit that is harvested the same day or one day before.

Residue analyses should be done early in the season allowing for adaptations to the pack house procedure for the rest of the season. If possible, allow for residue analysis throughout the season.

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