A look into the variability of prochloraz residues in the avocado industry

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ABSTRACT

The Agricultural Research Council's Institute for Tropical and Subtropical Crops was contacted by the Subtropical growers' associacion in May 2015 with a request to develop protocols for prochloraz usage on avocado and in avocado pack houses, since there seemed to be problems with stripping of prochloraz in pack houses with residue on fruit exported.

In the past season, fruit was collected from Hazyview, Nelspruit and Tzaneen areas ensuring at least four different cultivars were included. Trials were conducted comparing three concentrations of prochloraz (180, 90 and 60 ml/100 L water) for effective anthracnose control, while fruit were collected to determine residue levels of prochloraz on the fruit. Anthracnose levels were low in the initial trial, but a higher incidence was observed later in the season. Results showed that the concentration of prochloraz can be reduced to 90 ml/100 L water. Results of residue levels also indicated that residue levels of prochloraz at 180 ml/100 L water were sometimes above 2 ppm, which is the allowed MRL in four of the eight tests done.

A break down curve showed that residue levels dipped at day 7 after which it increased again, but the level was at its highest at day 0. It was also observed that cut fruit seem to have a higher residue level than uncut fruit.

An additional test was conducted where prochloraz was used in combination with HCl using the same concentrations. It was not possible to determine the efficacy of the combination on anthracnose control due to a low infection rate, but residue levels increased significantly for the three concentrations tested, which implicates that lower concentrations will have to be used if HCl is added to the fungicide bath.

The effect of storage before packing on the fruit's residue levels was also determined. Residue levels decreased when fruit was left in storage between day 1 and day 3. This implies that storage will not cause a problem with residue levels on avocado fruit.

A problem observed was the variability of residue levels on fruit between replicates and between tests, as well as residue in the solution. Although a pattern was observed between the different concentrations of prochloraz, variation within a treatment was present and this needs to be attended to in the following season.

INTRODUCTION

Anthracnose caused by *Colletotrichum gloeosporioides*, and stem-end rot caused by several species in the *Botryosphaeriaceae*, are limiting factors in the export of avocado fruit (Darvas & Kotze, 1979; Le Roux *et al*., 1985) and need to be treated with fungicides. Control consists of a combination of pre-harvest fungicides and a post-harvest treatment in the pack house.

For avocados, prochloraz (Chronos 450 g/l SC - Adama) is registered as a post-harvest treatment to control anthracnose at a concentration of 180 ml/100 L water + 0.2% HCl, while the EC formulation is registered as 1100 ml per 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). However, when visiting pack houses, it became clear that different concentrations of prochloraz for either the fungicide bath or the spray-on method that are used differ, depending on the pack house, with very little standardisation.

The allowed export default MRL for prochloraz is 2 parts per million (ppm) (DAFF, 2007), however, some importers in Europe require a lower MRL which could be problematic when the recommended concentration is used.

Also, export fruit are being analysed for residues in South Africa and/or overseas and whereas most of

the analyses are well within the allowed limit, sometimes the residue levels are for no apparent reason above the allowed MRL of 2 ppm.

The aim of the study was to investigate several factors that could possibly have an effect on residue levels of prochloraz on the fruit.

Different concentrations were tested for efficacy in the control of anthracnose and the suppression of stem-end rot, along with related residue levels found on the fruit for the different concentrations. A break down curve on the fruit was determined. In an additional trial, acid (HCl) added to prochloraz was tested for efficacy and residue levels. The effect on residue levels of fruit stored in the pack house for several days before packing was also investigated.

MATERIALS AND METHODS

Avocado fruit were collected from several pack houses from 2 July until 12 August 2016. The cultivars used included 'Fuerte', 'Hass', 'Ryan' and 'Pinkerton'. Avocado fruit were collected from Halls and Anton Hough (Nelspruit area), Koeltehof (Hazyview area), Gradly Farms (White River area), Westfalia, Univeg, Letaba Packers and ZZ2 (Tzaneen area).

Fruit were brought to the Agricultural Research Council – Institute for Tropical and Subtropical Crops (ARC-ITSC) in Nelspruit and all tests were carried out in the post-harvest laboratory. For all tests carried out, fruit were collected from the pack house and brought to the ARC-ITSC at one day while the tests were carried out the following day (except when stated otherwise).

Fruit per cultivar were always randomly divided into three groups to be used as replicates. Each replicate consisted of small and large fruit, as well as good and poorer quality fruit, while the fruit that were mostly packed in several crates were mixed properly, thus ensuring that fruit from all the crates was divided into each replicate. Most tests consisted of four treatments and three replicates for each treatment.

Tests consisted of dipping the fruit (between 20 and 30 fruit) which were placed in a container in a water solution for 3 min while shaking the container (washing). Fruit were left to drip a short while after which it was dipped for 30 sec in a separate container containing the prochloraz solution. Thereafter the fruit were left to dry. After drying, residue samples were collected and the rest of the 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 the rest of the cold chain period. Residue samples were taken to the deep freezer and stored at -20°C.

Determining the effect of different concentrations of prochloraz on residue levels and efficacy against anthracnose

The concentrations of prochloraz (Chronos® 450 SC – Prochloraz zinc complex (imidazole) 530 g/L and prochloraz equivalent 450 g/L) tested were 180 (treatment 1), 90 (treatment 2) and 60 (treatment 3) ml per 100 L water. An untreated control (treatment 4) with only water was also included. A container was filled with 25 L of water. One litre of the water was poured into another container where the correct concentration of prochloraz was added. This mixture was properly stirred and mixed after which it was added to the rest of the water where it was again thoroughly mixed. Immediately after preparation, fruit were dipped into the solution.

On each of the days where tests were performed, each concentration was prepared once and all the cultivars tested were dipped in the same solution. Each concentration was prepared separately.

Additional fruit of 'Fuerte' (2) was used to determine a break down curve of the prochloraz in the fruit during the cold chain period. Fruit were treated as above but before sampling for residues, the selected fruit (about eight fruit per treatment and replicate) was cut into three pieces and for each fruit one piece was selected at day 0, day 7 and day 28. In this way, the same fruit were used for residue analyses.

Table 1. Trials conducted in 2015 with cultivar, location and harvesting and packing date.

Cultivar	Location	Origin of fruit	Date harvested	Packing	Tests
Fuerte (1)	Nelspruit	Hall and Sons	01-Jul-15	02-Jul-15	4 concentrations
Fuerte (2)	Nelspruit	Hall and Sons	11-Aug-15	13-Aug-15	4 concentrations
Hass (1)	Kiepersol	Koeltehof	01-Jul-15	02-Jul-15	4 concentrations
Hass (2)	Tzaneen	Letaba Packers	03-Aug-15	05-Aug-15	4 concentrations
Hass (3)	Tzaneen	Westfalia	10/11-Aug-15	13-Aug-15	4 concentrations
Ryan (1)	Tzaneen	Letaba Packers	03-Aug-15	05-Aug-15	4 concentrations
Ryan (2)	White River	Gradly Farms	11-Aug-15	12-Aug-15	4 concentrations
Pinkerton	Nelspruit	Anton Hough	13-Aug-15	13-Aug-15	4 concentrations
Ryan	Tzaneen	Westfalia	10/11-Aug-15	12-14-Aug-15	180 ml only*
Ryan	Tzaneen	Univeg	10/11-Aug-15	13-Aug-15	8 treatments**
Reed	Tzaneen	ZZ2	15-Oct-15	17-Oct-15	8 treatments**

* Trial for determining effect of fruit stored before packing;
** Acid trial; 4 concentrations were 180 ml, 90 ml, 60 ml and 0 ml of prochloraz/100 L water

Determining the effect of acid on prochloraz residue level and anthracnose control

In this trial, the same procedure was followed but four additional treatments were added: each concentration of prochloraz was compared with the same concentration with the addition of acid to reduce the pH. The acid used was HCl and the pH was reducing to about 4.5.

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

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

The pH was determined for each set of solutions and can be seen in Table 2. When HCl was added for the acid trial, pH was adjusted to 4.5. Temperature of the water was between 17 and 22°C for July and August while it was 24.2°C in October.

Table 2. pH range of the solutions through the trial period.

Treatments	pH range for the different days
180 ml	6.92 - 6.64
90 ml	6.82 - 7.12
60 ml	6.91 - 7.32
only water	7.14 - 7.42

Evaluation of fruit

After 28 days, fruit were removed from the cold room and kept at room temperature for eight days after which it was evaluated for disease incidence, including anthracnose and stem-end rot. The fruit was again evaluated four days later. At this stage the fruit was ripe to slightly overripe, but this enabled the evaluation of the effect of prochloraz on anthracnose control.

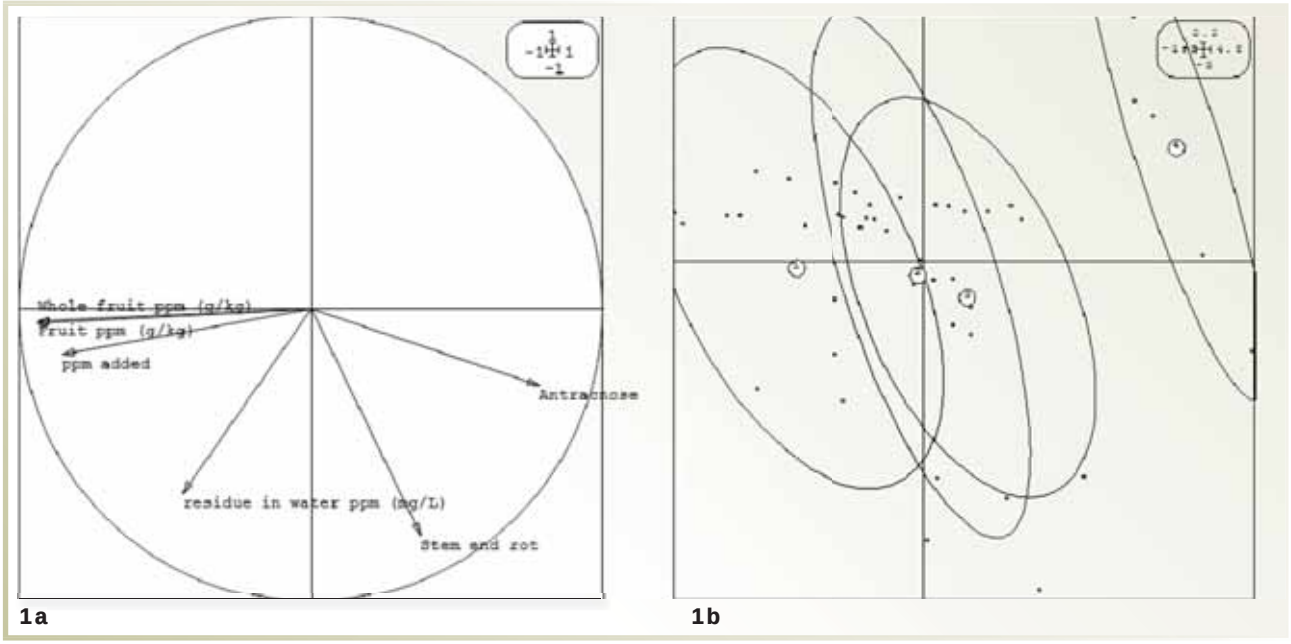
Evaluations were done by giving a value per class for disease incidence from class 0 = no disease, class 0.5 = < 5% disease symptoms, class 1 = 5 - 10% disease symptoms, class 2 = 11 - 20%, class 3 = 21 - 35%; 4 = 36 - 50%; class 5 above 50% disease incidence.

When data was analysed, the formula {(class 0 x 1) + (class 0.5 x 2) + (class 1 x 3) + (class 2 x 4) + (class 3 x 5) + (class 4 x 6)}/n (with n = number of fruits evaluated) was used to calculate incidence of anthracnose. A number of 1 indicated no disease incidence, while 3 or more indicated high disease incidence.

Residue analysis

After termination of the tests, fruit were taken to the SABS Chromatographic services (SANAS accredited) where it was analysed using the modified QuEChERS method.

The stones of the fruit of each sample were removed, weighed and discarded. The flesh of each sample was shredded in a food cutter and mixed thoroughly to render it homogeneous. Single determinations were done employing the following method: SABS In-house Method No 030/2007: 'The Determination of Relevant Pesticides Residues in Avocado Samples'. Final analysis was done with LC/MS/MS. Recovery determinations were carried out by adding known amounts of prochloraz to portions of the control samples and analysing these concurrently with the samples.



1a 1b

Figure 1a. Correlation circle with the parameters measured and **Figure 1b** factorial plan depicting the data in relation to all the parameters in the correlation circle.

RESULTS

Effect of different concentrations of prochloraz on residue levels and efficacy against post-harvest diseases, including anthracnose

Figure 1 depicts the result of a multi-variate analysis with all parameters measured. Figure 1a shows the correlation circle, indicating the position of the different parameters towards one another. When parameters are on the opposite side of the F2-axis they are negatively correlated, while a positive correlation is found when they are on the same side. It is also important to observe the position of the parameters towards the F1-axis. When the arrow is closer to F1, the parameter is more important than when the arrow is further away, as can be seen for anthracnose which is more important than stem-end rot. Also, residue in water is less important than other residue data. It is clear from Figure 1a that parts per million (ppm) on fruit and whole fruit and ppm added are negatively correlated with anthracnose. Thus, with a higher residue level, a lower anthracnose incidence was observed. Residues in the water and stem-end rot seem to be much less correlated.

In Figure 1b, the position of the different data points is shown in a factorial plan. These graphs need to be read in combination with the correlation circle. In this case, four convection circles are drawn showing the four different treatments. It is clear that, when all parameters are taken into account, the untreated control (4) is situated on the right side of the graph (with high anthracnose incidence and low prochloraz residue levels), while the prochloraz treatments are situated on the other side of the graph. Also, while the three prochloraz treatments overlap, the 180 ml/100 L water treatment (1) was the treatment with the lowest anthracnose and highest residue levels, followed by treatment 2 (90 ml/100 L of water) and 3 (60 ml/100 L water). In the analysis, data collected for the trials conducted in July were not included as these fruit had no or very low levels of anthracnose.

Figure 2 shows the correlation between anthracnose incidence and residue levels on the fruit. For this evaluation, only tests where anthracnose was observed were included. It is clear that with a higher residue level on the fruit, better anthracnose control is obtained, which confirms results seen in Figure 1.

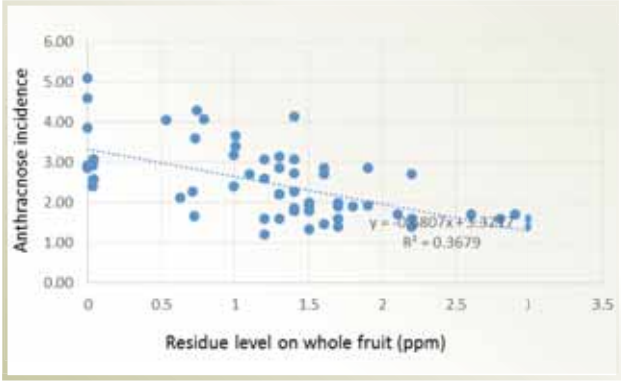


Figure 2. Correlation between residue levels on the fruit (X-axis) and anthracnose incidence (Y-axis).

Table 3 shows the incidence of anthracnose for the different concentrations of prochloraz. Fruit tests during July had very low levels of anthracnose, however, fruit tested later showed a difference in anthracnose incidence on the fruit between the different concentrations of prochloraz, with 180 ml/100 L water having the lowest levels of anthracnose followed by 90 ml, 60 ml and control the highest levels of anthracnose. Although 60 ml/100 L water seems to give some control, it is assumed that when high pressure is present, this concentration might not be sufficient.

Table 4 shows the incidence of stem-end rot (SER) and some control was seen. Prochloraz is said to suppress SER and this was observed.

Table 3. Index* for anthracnose on fruit for the different concentrations and different tests.

Anthracnose	**Fuerte (1)	Fuerte (2)	**Hass (1)	Hass (2)	Hass (3)	Ryan (1)	Ryan (2)	Pinkerton
180 ml***	1.12	1.57	1.02	2.27	1.40	2.20	3.17	1.61
90 ml	1.12	1.90	1.03	3.13	1.49	3.07	4.29	2.12
60 ml	1.10	2.59	1.05	4.14	2.29	3.78	4.04	2.27
only water	1.13	2.86	1.07	3.87	2.40	4.56	5.09	2.93

* Note that 1 = No disease; ** Test conducted in July 2015; *** Concentration is per 100 L water

Table 4. Index* for stem-end rot on fruit for the different concentrations and different tests.

Stem-end rot	**Fuerte (1)	Fuerte (2)	**Hass (1)	Hass (2)	Hass (3)	Ryan (1)	Ryan (2)	Pinkerton
180 ml***	1.10	1.42	1.03	2.30	1.58	1.00	3.62	1.45
90 ml	1.15	1.57	1.03	2.97	1.51	1.00	3.92	1.70
60 ml	1.15	1.71	1.03	3.80	2.29	1.00	3.72	1.95
only water	1.22	1.45	1.17	3.53	2.29	1.00	4.21	2.29

* Note that 1 = No disease; ** Test conducted in July 2015; *** Concentration is per 100 L water

Table 5. Mean residue levels for the different concentrations of prochloraz determined during the different tests (same colours were treated the same day) on whole fruit (mg/kg).

Whole fruit	Fuerte (1)	Hass (1)	Hass (2)	Ryan (1)	Hass (3)	Ryan (2)	Fuerte (2)	Pinkerton
180 ml*	2.2	2.2	1.4	1.0	1.4	1.8	2.9	1.2
90 ml	1.3	1.5	1.3	0.7	1.3	1.2	1.6	0.6
60 ml	0.7	1.4	1.4	0.5	1.2	0.8	1.5	0.7
Untreated	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0

* Concentration is per 100 L water.

Residue levels on fruit were determined and can be seen in Table 5 (values shown are the mean of three replicates). Treatment 1 showed the higher residue levels throughout the study, but significant variation was seen in the residue levels on the fruit. Although the tests were conducted in exactly the same way every time, residue levels on the fruit differed between the days the tests were conducted, as well as the different cultivars tested on the same day. A variation between the three replicates was also observed (data not shown). 'Fuerte' (2) had a higher residue level than the other treatments. It is believed that this might have been caused by the fact that this fruit was cut into three pieces after dipping (as part of the study for the break down curve). This procedure might have spread prochloraz from the rind of the fruit to the flesh.

From Figure 1 it is clear that residue levels measured in the water were not strongly correlated with the prochloraz added to the water. Water residue samples showed a variation between the different days for the same treatments, while it was difficult to differentiate between the different concentrations within one day (Table 6). It must be stated that the water sample was normally taken after the tests were conducted, just prior to disposal of the water. This could be part of the variation in residue levels, since on the 12th of Aug, more tests were conducted than on the other days, while one of the trials, namely the storage trial, was only done with the 180 ml/100 L water. This could have explained why the water sample of 12 Aug had a low residue level in the 180 ml/100 L compared to the other days. However, it is difficult to explain why residues for the lower concentrations varied between treatments.

Table 6. Residue (ppm) in water samples for the different dates.

	02-Jul	05-Aug	12-Aug	13-Aug
180 ml/100 L	253	259	147	255
90 ml/100 L	191	281	179	279
60 ml/100 L	100	196	178	267

Table 7 shows the results and at day 7 residue levels seem to dip after which the residue levels increase again. This is probably linked to movement of the active ingredient in the fruit and later settling down in the fruit. However, in all instances the highest residue level was found in the sample taken just after packing.

Table 7. Residue analysis (ppm) whole fruit (including pip) from 'Hass' fruit sampled at three consecutive dates.

Concentration	Sampling time after dip	Residue on whole fruit (ppm)
180 ml/100 L water	0 days	2.87
	7 days	2.00
	28 days	2.63
90 ml/100 L water	0 days	1.57
	7 days	1.57
	28 days	1.53
60 ml/100 L water	0 days	1.70
	7 days	1.23
	28 days	1.60
Only water	0 days	0.00
	7 days	0.02
	28 days	0.04

Determining the effect of acid on prochloraz residue level and efficacy against anthracnose

The acid trial revealed little on anthracnose control since disease incidence was very low in the original trial. For that reason the trial was repeated in October with cv. Reed but again anthracnose incidence was too low. However, residue levels of fruit treated with acid were significantly higher than in fruit treated with prochloraz only (Table 8). Residue levels increased to more than double the levels without acid for 60 and 90 ml/100 L water. Both 90 and 180 ml were well above the acceptable MRL of 2 ppm when acid was added. In the water sample, residue levels were also considerably higher than in the prochloraz treatments without acid (data not shown).

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

Table 9 shows the residues of fruit left in storage for several days. It would seem as if the longer the fruit is left in storage before packing, the lower the residue levels are.

Table 8. Residue levels (ppm) on fruit and whole fruit on 'Ryan' for the different treatments.

Treatments	Residue on whole fruit (ppm)
180 ml/100 L	2.1
90 ml/100 L	1.0
60 ml/100 L	0.58
Untreated	0
180 ml/100 L with acid	3.3
90 ml/100 L with acid	2.4
60 ml/100 L with acid	1.6
Untreated with acid	0.02

Table 9. Residue levels (ppm) on 'Ryan' whole fruit when fruit is left for 1, 2 and 3 days in storage before packing.

Days left before packing	Residue in whole fruit (ppm)
1 day	2.23
2 days	2.10
3 days	1.57

DISCUSSION

Inconsistent results with prochloraz residues have been observed during the past years with samples send in by producers. It was hoped that these tests would provide more insight in the reason for these inconsistencies.

During the study, it was again confirmed that prochloraz is effective in controlling anthracnose. Although 180 ml/100 L water concentration provided the best results, it often resulted in residue levels above the acceptable MRL for Europe of 2 ppm (see Table 4). Because 90 ml/100 L water also provided good control and residue levels were lower, it is preliminary advised that producers could consider using this lower concentration to prevent residue levels exceeding 2 ppm.

Although residue levels measured of the 60 and 90 ppm treatments were very similar in the samples taken, results with efficacy indicated that 60 ml/100 L water might be too low for effective control, especially when anthracnose pressure is high.

Samples taken during the cold chain showed that residue levels were highest at day 0 after treatment. It is thus best to take samples at that stage to ensure that the highest possible residue level is determined.

When fruit was left in storage for up to three days before packing, residue levels seemed to decrease, which would mean that storage would not increase the risk of excessive residue levels if the fruit cannot be packed immediately. It is, however, advised that this test is repeated again to confirm the finding.

When acid is added to the fungicide treatment, the residue level increases significantly and prochloraz at the registered dosage of 180 ml/100 L water plus acid, as recommended in the label, will cause residue levels well above the allowed MRL. This is caused by the increased solubility of prochloraz when an acid is added (Prusky *et al.*, 2006). In our trials (data not shown), it was not possible to determine efficacy since anthracnose levels were too low. But since anthracnose incidence is negatively correlated with residue levels, it is believed that all prochloraz + acid concentrations tested will provide effective anthracnose control. It is believed that a lower concentration of prochloraz can readily be used when acid is added and it might be possible to go even lower, as was seen in a study by Mavuso and Van Niekerk (2010) where 200 ppm (45 ml prochoraz/100 L water) + acid provided good control. Although adding acid can reduce the amount of prochloraz used in the fungicide bath, it will not necessarily reduce the risk of residues, since significantly higher levels of residues were observed on fruit treated with prochloraz + acid. It is also important to check pH when an acid is added, since the pH can increase quickly with high amount of fruit going through the pack line, as well as through the dilution caused by wet fruit coming into the fungicide bath. Acid may also corrode the equipment.

A problem observed was the inconsistent residue levels obtained within the water samples. Many reasons have been given to explain the reduction of prochloraz levels in the fungicide bath (Daneel, 2011), with the amount of fruit packed, impurities in water, water quality and dilution from neighbouring baths being probably the more important ones. Stripping effect caused by bacteria in the water was another reason for rapid break-down of prochloraz (Swart & Broekhuizen, 2003; Swart *et al.*, 2004; Serfontein & Serfontein, 2006 & 2007). And of course, prochloraz is added to protect the fruit during which process the concentration is also reduced. Most of the above mentioned reasons are not applicable in our trials, as solutions were not left to stand and only a limited amount of fruit was dipped. However, this last reason (protection of the fruit) is probably part of the explanation for the variation in results.

Although the samples were taken in the same way every time, water samples were not immediately taken to the freezer and it is believed that this might have caused the solution to precipitate before freezing, explaining some of the differences in residue levels as well.

It is important to note that the residue levels on the fruit were more consistent, in that residue levels on fruit showed a correlation with prochloraz added to the water.

In future, water samples should be taken as soon as the solution is prepared, while another sample can be taken afterwards to determine the amount taken out of the solution after the trial. The water sample analysis is important, as this is the means by which prochloraz levels in the fungicide water have to be checked and readjusted using the turbidity meter.

Another problem was the high variability of prochloraz residue levels in the replicates tested

and variability of similar concentrations between the different tests. When conducting the tests, fruit were randomly divided between the different treatments and replicates, ensuring that large and small fruit were present everywhere. When fruit were of weaker quality, it was divided among replicates and treatments to ensure that each replicate had fruit of similar quality. Normally, seven fruit were selected for residue analysis while the remaining fruit were used for efficacy evaluations. In the fruit selected for residue analysis, fruit of all sizes were included. However, it is maybe important to determine the different residue levels on small, medium and large fruit separately. It might be possible that the fruit size and thus surface area play a role in residue levels.

With the statistical analysis it was not possible to determine that cultivar or locations played a significant role in differences in residue levels.

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