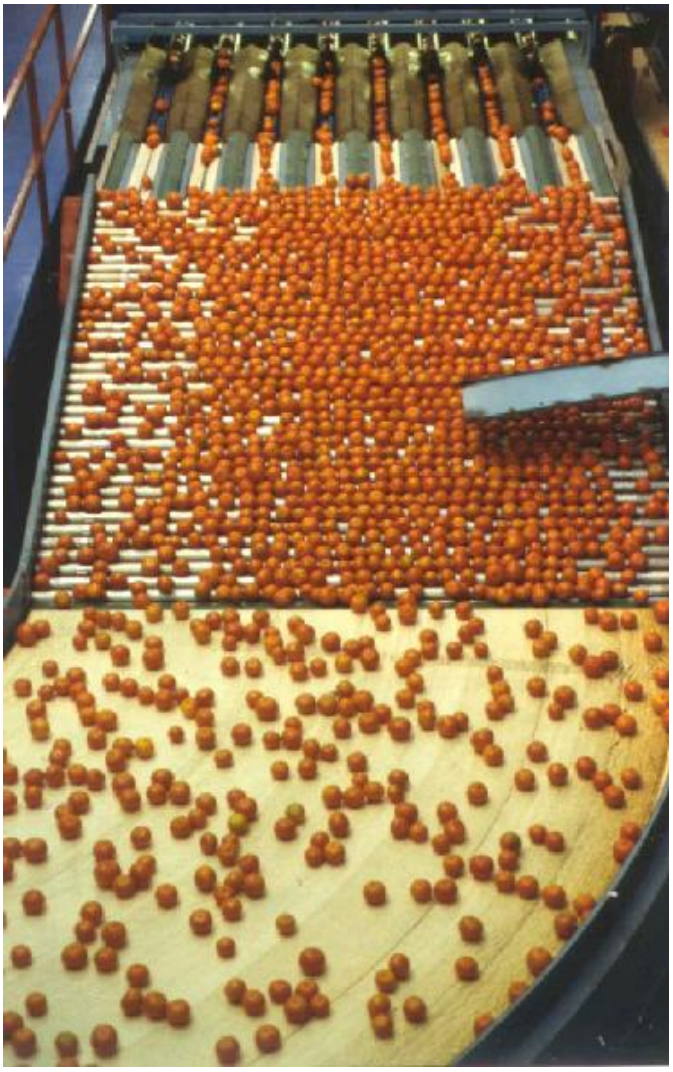
SANITATION SURVEY OF CITRUS PACKINGSHEDS



Florida Citrus Packingline,, 2000.

(incorporating fungicide resistance survey results)

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SUMMARY

This survey was conducted to determine whether packinglines are achieving good control of fungal spores on fruit. A reduction in fungal spores on fruit is desirable because the fungicides used for export do not control sour rot. We collected oranges as they past through different processing points in the packing line and measured the levels of mould spores and other microbes. In addition, we exposed media plates amended with currently registered fungicides to detect the presence of resistant spores.

To judge improvements, I have included a summary from the 2004 sanitation survey results 'side-by side' with the current 2012 summary.

2004 Survey

- Fruit sampled after the chlorinated wash showed a significant reduction in total microbe levels and to a lesser extent the fungal spore load.
- After oranges pass through the chlorinated wash they still carrying viable spores. Spores are accumulating further down the packingline.
- Spores and microbes are accumulating in fungicide tank. The best approach is to frequently change the solution. Wash out the tank and treat with a sanitiser regularly to ensure that microbes in undrained residues are not re-introduced to fresh solutions.
- Non-recovery fungicides systems can carry an accumulated spore load continuously washed from fruit over the day. Regular cleaning of the brushes will be required to maintain low microbe levels.
- Wax brushes indicate a build-up of fungal spores despite earlier processes to minimise contamination. The best option to avoid contamination at this late stage is to regularly clean wax brushes.

2012 Survey

- Fruit sampled after the HP wash showed a significant reduction in surface fungal spore load and overall microbe counts.
- Sanitation of HP washes must be monitored carefully due to the high organic matter load washed off fruit.
- The spore numbers on fruit decreased as it progressed through the packing line.
- Generally, fungicide tanks had low spore counts. Microbe counts were zero when compatible sanitisers were appropriately used with fungicides.
- Wax brushes showed few spores present; this result was supported by low spore levels on waxed fruit.
- Good cleaning and sanitation practices are evident by the comparatively clean swabs collected throughout the packingline and cool room.
- The fungicide resistance plates showed growth of mould spores on TBZ fungicide amended plates. While numbers were generally low is a concern early in the season.
- Fungicide resistance can increase over a busy season. Monitoring the fungicide resistance situation in a few months would be prudent.

A comparison of the two summaries shows an overall reduction in spore counts on fruit in 2012. High-pressure washing, sanitised fungicide tanks and wash brushes are significant improvements implemented over recent years.

SANITATION SURVEY

INTRODUCTION

This survey is the second in a series of alternating annual surveys of packinglnes to assess fungicide performance and sanitation methods. The surveys are part of the 'Showcase Sheds' initiative, which is within the National Citrus Postharvest Science Program (see acknowledgements, pg. 5). This season's survey aims to assess the effectiveness of sanitation methods and general hygiene/cleaning.

For many years, packers have been using combinations of fungicides and sanitisers to minimise decay in export consignments. The limitation of chlorine products are well understood, but still cause monitoring problems due to the products' sensitivity to pH and organic matter. In addition, many sheds regularly alter their packinglines by changing sanitation products and modifing their application methods for both chlorine and fungicides. These changes have been made with the expectation of improving their packout, including decay control. However, the interactions are complex and objective measurements are rarely made to assess the effectiveness of packingline operations.

This survey of 3 major exporters was conducted to determine whether they are achieving a reduction in fungal spores (and total microbe levels) on fruit as it progresses through packingline. To assess this, we measured the levels of spores on oranges as they passed through different processing points in the packingline.

GENERAL METHODS

At each packingshed, several fruits were samples by hand (using sterile gloves) at a number of sampling points: immediately prior to dumping, after washing and in-line fungicide application, and after waxing. Wash solutions and in-line fungicide solution were sampled and sterile swabs of rollers and brushes were also collected.

All orange samples were weighed and washed with sterile solutions to remove surface microbes. Appropriate dilutions of each fruit wash solution, collected liquids and wax swab samples were surface plated onto agar and incubated. After incubation, the numbers of colonies on agar plates were counted.

The results from each area were pooled and the graphs represent the average microbial levels. The orange surface results were converted to microbial levels per gram of orange weight (eg. spores/gm). The fungicide solution and wax brush levels were converted to microbe levels per ml of solution (eg. spores/ml).

RESULTS AND DISCUSSION

POSTHARVEST DECAY IMPLICATIONS

The fungal spore levels of oranges before dumping indicates the background level of spores entering the packingline. In this survey, the average spore levels prior to dumping was 24 viable spores/gm (see figure 1).

This equates to a background load of around 20,000,000 viable spores for every tonne of oranges and represents a significant challenge to the packingline over an extended period of packing. The figures in this survey are based of "sound" oranges and do not take into account diseased fruit, which can also enter the system adding millions more spores per fruit.

Most sheds have some form of high pressure washing early in the packing process. They are sanitised using various products, including calcium Hypochlorite, peroxyacetic acid, and chlorine dioxide. All fruit sampled after the chlorinated wash showed a large reduction in the surface spore load (~98% reduction) (see figure 1). This is in stark contrast with survey results in 2004, where average spore counts were reduced by 27% after a chlorinated wash. It is important to recognise the limitations of sanitisers in reducing spore loads on the surface of fruit. This improvement was most probably due to the change to high pressure washing, which physically removes the spores from the surface of the fruit. The spores washed off are much easier to neutralise in water and do not migrate further down the line.

After oranges pass through the chlorinated wash they may still carry some viable spores. It is important to minimise the number of spores as they are washed into the recirculating fungicide solution. The fungicides used for the export markets control mould (*Penicillium sp*.), but resistant mould spores and sour rot will accumulate in the solution. For this survey, average spore levels remained low (see figure 2). Some accumulation can occur in fungicide solutions when sanitisers are not added to the tank. Generally, spore levels could be much higher and this relatively low level reflects the overall effectiveness of the shed practices.

In 2004, directly reducing spore levels by adding sanitisers in fungicide tanks was not attempted by any packingshed. Imazalil fungicides (e.g., Fungaflor®, Magnate®), commonly used in in-line fungicide systems are incompatible with most chlorine compounds. More recently, compatibility of peroxyacetic acid (e.g., Tsunami®) with imazalil fungicides has been demonstrated but it is still not commonly used for this purpose. An option many packers have adopted to overcome the accumulation of spores in the fungicide tank is frequently changing the solution (at least daily). The tank can be cleaned with a chlorinated solution between batches to sterilise the system. Smaller packinglines can run without topping up and then dump after the concentration runs down to half strength. Initially, solutions should be analysed to determine the rate of fungicide strip out to calculate top up &/or dump times. Quality assurance requires documentation of fungicide residues on fruit and this measurement can be useful in determining top up rates. The advantages of high volume systems are that they maintain good contact of fungicides on fruit, which increases fungicide uptake.

Another option used by packers is to apply the fungicide in low volume non-recovery systems. This system has the advantage that the solution is fresh, ie. no recirculation to accumulate spores. Non-recovery systems rely heavily on the brushes to both wet fruit and distribute the fungicide evenly. An important hygiene consideration with a non-recovery system is the accumulation of spores in the brushes themselves. Although fresh solution is used, the brushes will be carrying an accumulated spore load. Low solution volumes may

actually increase the concentration of spores in the brushes compared to flooding systems. Regular cleaning of the brushes will be required to maintain low microbe levels.

Swabs of wax brushes demonstrated that spores are not accumulating throughout packing system (see figure 2). Earlier processes, such as high-pressure washes, probably minimised contamination by removing spores from the surface of fruit. Packers using fungicides in wax may gain some benefit but will not be controlling sour rot spores. The best option to avoid contamination is regular cleaning of wax brushes. There is no substitution to thorough cleaning using hot water under pressure. A proprietary chlorinated detergent may provide greater reduction of microbe levels when cleaning brushes and packingline surfaces.

Overall, the packing lines surveyed achieved high fungal control. The survey shows that spore levels decline as fruit was carried through the system. The accumulation of spores in fungicide solutions and wax brushes have been addressed since earlier surveys but vigilance must be maintained. It is hoped that the packers surveyed are representative of the industry.

PUBLIC SAFETY IMPLICATIONS

The results of the survey show total microbe numbers and are not classified according to health risk. The majority of microbes on fresh produce are harmless soil-dwelling bacteria. As a consequence, high microbe numbers do not necessarily constitute a public health risk but are indicative of a potential area of risk. Use this survey to identify areas where potential problems can occur.

High populations of microbes were found on pre-dump fruit at packingsheds (see figure 3). The high pressure wash significantly reduced levels of all microbes, however, fruit collected after the fungicide solution had increased total microbe numbers. The fungicide solution had high levels of bacteria that were presumably washed off the oranges and accumulating in the system (see figure 4). As the fungicide did not control the bacteria they could proliferate. This accumulation also carried over the wax brushes where high numbers were found, probably deep in the brush-beds (see figure 4). It is important to note that the levels of fruit remained relatively low. The reasons for this were not determined in this study, but it seems likely that regular cleaning of these areas would reduce overall microbe counts on fruit. Fungicide tanks and wax brushes were identified in pervious surveys as potential risk areas and, perhaps, mitigation measures are being applied more frequently in these areas.

Acknowledgements

The National Citrus Postharvest Science Program, based at SARDI, will be facilitated by HAL in partnership with Citrus Australia for the period of June 2011–Mar 2015. It has been funded by citrus grower levies and voluntary contributions pledged from the three regional citrus marketing boards (at the time). The Australian Government provides matched funding for all HAL's R&D activities. Several citrus packers and service providers have also provided support for the specific activities recorded in this report.

Mean mould levels on fruit surfaces and packingline solutions

Below: Schematic diagram of packingline. Arrows show where oranges were collected

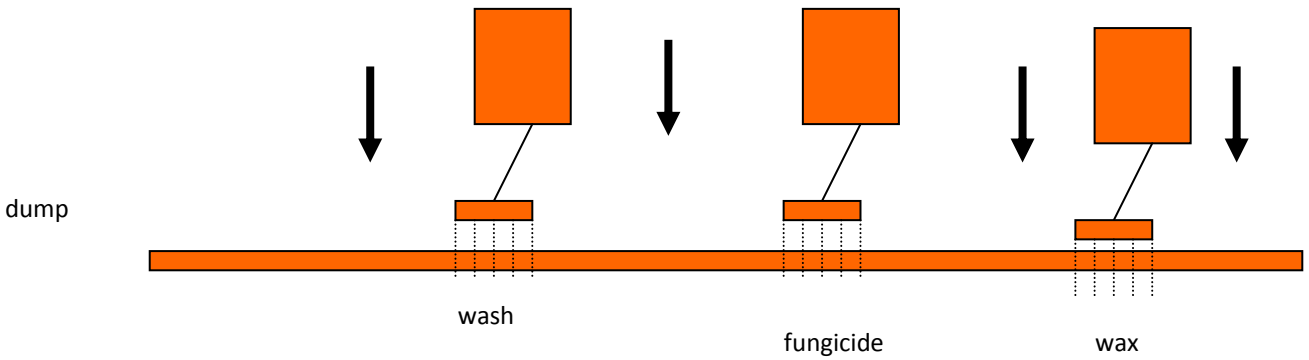
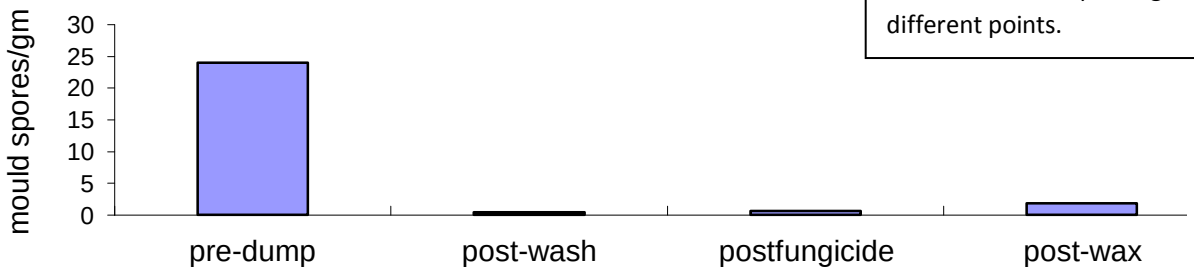


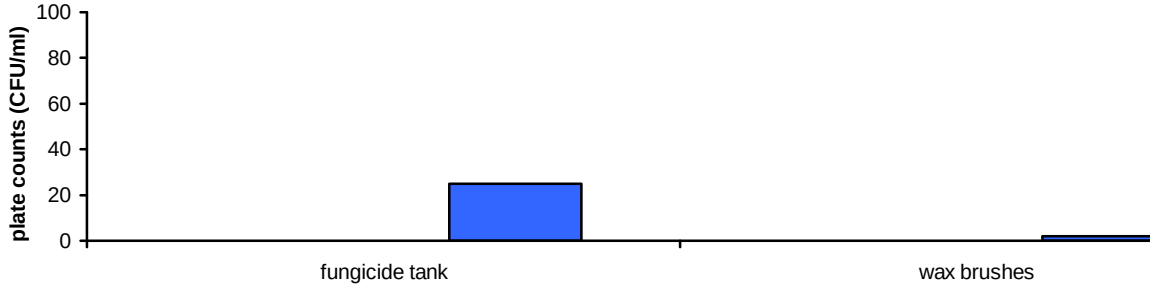
Figure 1

Below: Mould levels on fruit surfaces are shown. Oranges were retrieved from packinglines at 3 different points.



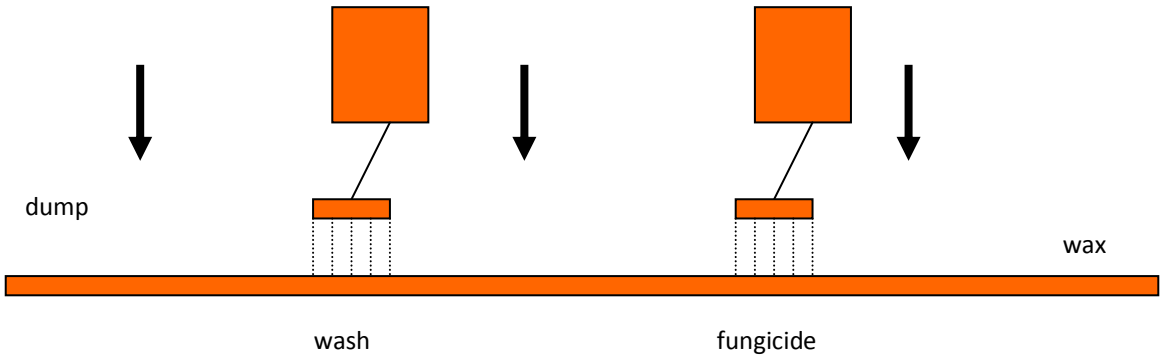
Below: Fungi levels in fungicide tanks and wax brushes are indicative of fungal spores not controlled by the fungicide used, eg., sour rot

Figure 2



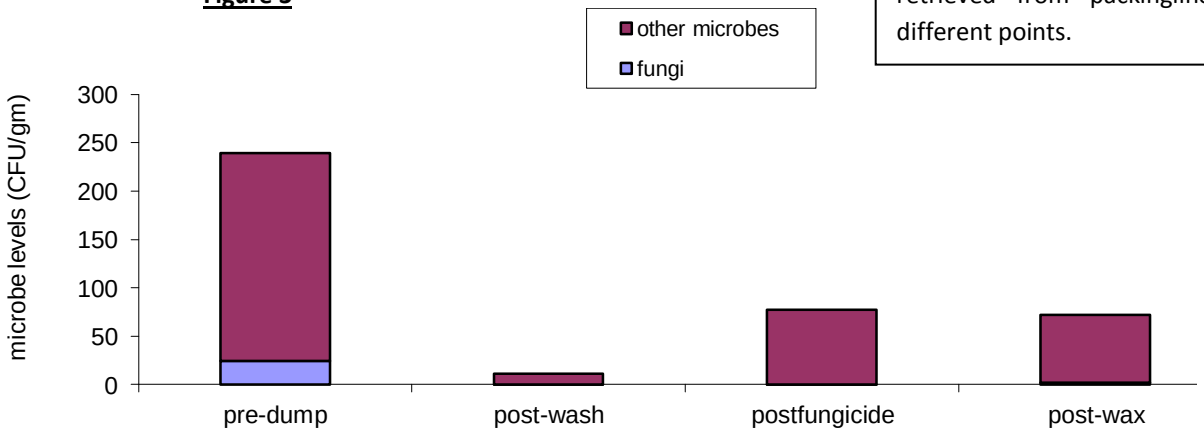
Mean total microbe levels on fruit surfaces and packingline solutions

Below: Schematic diagram of packingline. Arrows show where oranges were collected



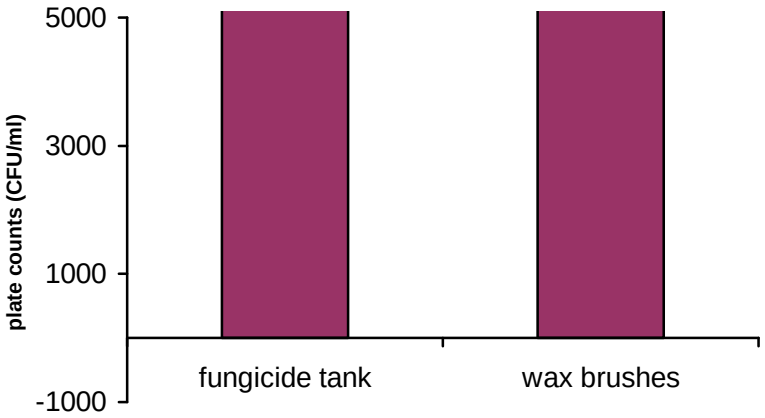
Below: Microbe levels on fruit surfaces are shown. Oranges were retrieved from packinglines at 3 different points.

Figure 3



Right: Microbe levels in fungicide tanks and wax brushes are indicative of microbes not controlled by the fungicide used. Eg. bacteria.

Figure 4



APPENDIX – FUNGICIDE RESISTANCE TESTS

INTRODUCTION

In 2011, SARDI introduced fungicide resistance testing; it involved exposing media plates amended with low concentrations of fungicide in various parts of the packing operations. Initially, we chose very low rates of fungicides that controlled a highly sensitive mould isolate to determine if 'technical' resistance was evident. We found high mould spore growth on plates amended with thiabendazole (TBZ), and some growth of imazalil (IMZ) plates. A trend was established but we were unsure if fungicide resistance on these plates was sufficient to impact on packing operations.

After review of the literature[1], we increased the concentrations of TBZ to 5ppm and 15ppm, and IMZ to 0.5ppm and 1.5ppm for monitoring resistance in packing sheds. In addition, we included fludioxonil (FLU) at 1.0ppm and 2.5ppm to provide a baseline prior to commercial use in Australian citrus packing. This June, fungicide resistance surveys were conducted in 4 packingsheds.

The results presented are representative of the sheds evaluated. However, there were significant variations between each shed. These results do not necessarily represent the situation in other citrus packing operations.

RESULTS AND DISCUSSION

The images on the following page are from plates collected during the survey. They show many different fungi, yeast and bacteria. Our interest is with the mould spores only, which are dark circles.

The unamended (control) plates contained no fungicide and indicate overall level of microbes. The plates exposed in the cool room shows no mould (dark circles) and fewer colonies indicating a cleaner environment. A comparison of the control and 15ppm TBZ plates indicate similar mould growth on both sets of plates suggesting a high portion of mould spores are resistant to TBZ. In contrast, a comparison of the controls with the FLU plates reveals no mould growth on any FLU plates. Ideally, all fungicide-amended plates should have no mould growth (i.e., susceptible mould spores). However, the consistent use of TBZ and IMZ appears to be leading to increased resistance.

Mould spores have been isolated from fungicide-amended plates collected during this survey. Although rarer, some IMZ resistant spores were collected during the survey. Further work is planned to verify resistance by inoculating and treating fruit with label rates of fungicides. Isolates will be exposed to different fungicides, which should allow evaluation of possible multiple resistance.

Evidence of fungicide resistance early in the season (after summer break and cleaning) is a concern because resistance is likely to increase during the season. Periodic monitoring of packinglines is recommended.

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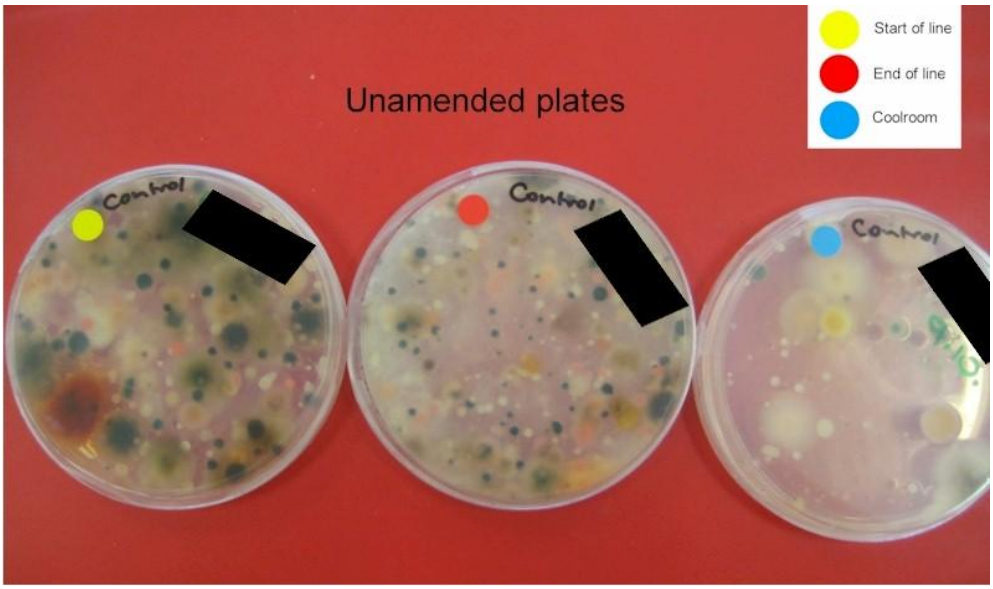
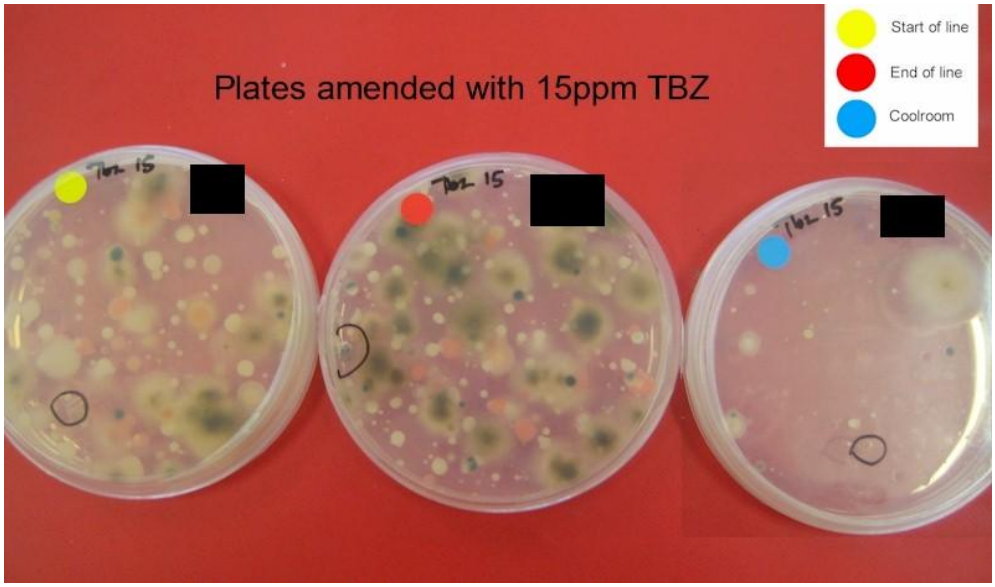
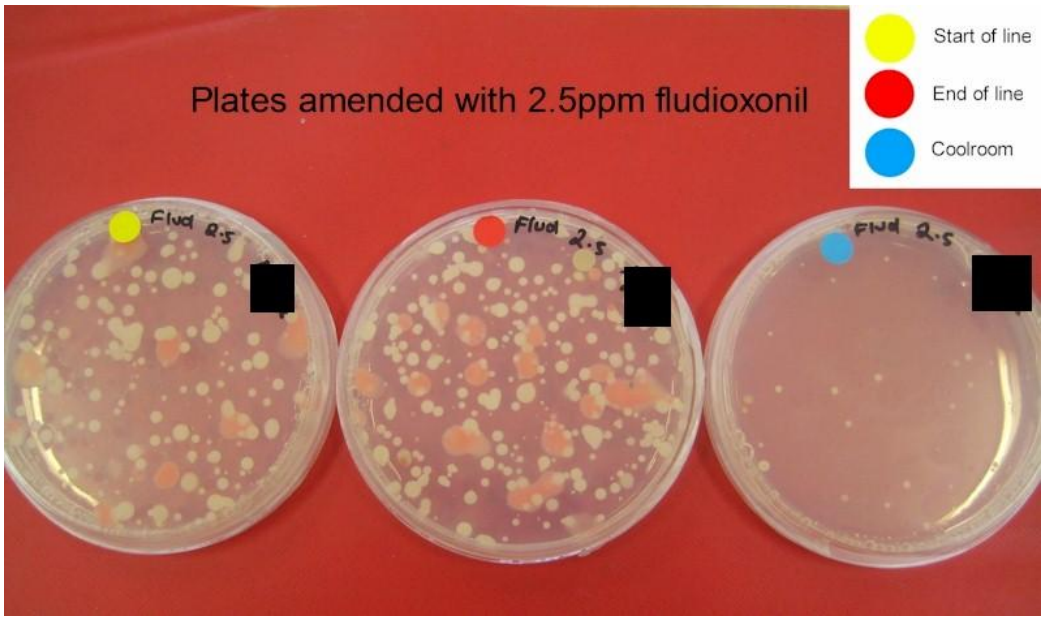
[1] Thanks to Andrea Pastore for kindly providing a useful reference on monitoring IMZ resistance in citrus packinghouses in Uruguay. [E. Perez et al., Postharvest Biology and Technology 60 (2011) 258-262].


Unamended plates
Start of line
End of line
Coolroom
Control
Control
Control


Plates amended with 15ppm TBZ
Start of line
End of line
Coolroom
Tbz 15
Tbz 15
Tbz 15


Plates amended with 2.5ppm fludioxonil
Start of line
End of line
Coolroom
Flud 2.5
Flud 2.5
Flud 2.5