PACKER NEWSLETTER

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Fungicide survey – is resistance blowing in the wind?

Peter Taverner

SARDI

The sudden appearance of decay in the marketplace is a dilemma for packers. A remedy needs to be found immediately, with the added anxiety of waiting for out-turn reports for the fruit currently in transit. Problems invariably occur during peak packing: This is a situation no packer wants to be in. We have conducted a small fungicide survey to try to establish some trends and hopefully help take some of the "knee-jerk" out dealing with decay problems.

The simple approach to solve decay problems is to increase the fungicide residues by combining fungicides, applying multiple times (e.g., drench, in-line &/or in wax) and increasing exposure time or fungicide concentration. This strategy may work in the short term but there are inherent risks. For instance, this approach can lead to fungicide MRL's being exceeded in overseas markets.

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If application has been poor or monitoring has been inadequate for some time, the damage may be done. Low fungicide deposits can lead to the selection of mould strains resistance to fungicides. The risks of resistance are greater today due to a reduction in fungicide groups, consistent use of these few groups and longer on-premises storage of fruit for marketing reasons.

There is no recognition of widespread fungicide resistance in citrus packing sheds in Australia. However, Brian Wild did collect fungicide resistance strains from fruit packed in NSW during 1994. This survey was our first attempt to discern if any fungicide resistance occurs in packing sheds.

In this study, we ran inoculated fruit through the main fungicide application inline. The study was designed to provide feedback on whether inline application methods are working effectively. The in-line application is the key fungicide and should control decay by itself. However, many packing sheds also use bulk dip/drenching and fungicide in wax as part of their decay control procedures. We were also interested in whether these additional applications provided the higher residues required for good sporulation control. As such, fruit was collected from the end of line for sporulation assessments.

For fungicide resistance, we chose very low rates of

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fungicides that control a highly sensitive mould isolate to determine if 'technical' resistance was evident. Any growth of mould spore collected in packing sheds on fungicide-amended plates indicates a trend towards fungicide resistance. However, further testing would be required to determine if any fungicide resistance is sufficient to impact on packing operations.

Fungicide residues and decay

From each packer, fruit was collected after 3 different treatments: Fruit were dipped for 30 seconds in a sample of the fungicide that was being applied inline. This is the standard test and removes the variability of individual packing line fungicide application. Fruit was run through the fungicide section of the line, including any brushes following the fungicide application, and retrieved prior to the wax application. Lastly, fruit was retrieved at the end of line after all fungicide applications, including postharvest drenching when applied. All fruit samples were sent to commercial laboratories for fungicide residue determination. The mean residues for imazalil (IMZ and thiabendazole (TBZ) are shown in Table 1.

Table 1 – Mean fungicide residues (ppm) in fruit associated with the in-line application, a 30 second dip comparison and whole of line application.

Fungicide	Application	Mean Fruit Residues (range)
IMZ	In-line	0.46 (0.3-0.6)
	Dip	0.50 (0.3-0.7)
	Whole line	0.66 (0.4-1.0)
TBZ	In-line	1.44 (0.5-3.6)
	Dip	1.66 (1.0-3.6)
	Whole line	1.69 (0.8-3.5)

Most packers surveyed applied two fungicides in-line, with average fruit residues of ~0.5ppm IMZ and ~1.5ppm TBZ for in-line application only. Generally, the in-line fungicide application resulted in good coverage and decay control.

Packers surveyed were aiming for reduced IMZ residues in packed fruit to avoid MRL issues, but not as low as recorded in this survey (0.4-1.0ppm). The TBZ residues were usually higher than IMZ, with some packers compensating for a lower IMZ level by increasing TBZ residues (>3ppm).

Samples were also taken of fungicide solutions during the surveys. The analysis of the solutions sometimes showed much lower than expected concentrations of fungicide, and consequently, some decay. Interestingly, a low concentration was usually taken just prior to a 'lunch break' top-up. A discussion on strip-out rates of fungicides and topping up regimes can be found in the article later in this edition.

Sporulation

The sporulation test involved collecting fruit from end of line (after all fungicide treatments applied). Fruit were injected with mould spores to ensure the fruit decayed. If fungicide residues in the rind are high enough fruit should develop a 'white crust' only. The development of spores in packed fruit 'foul' the carton and are a fungicide resistance risk.

The surveyed sheds all applied IMZ and TBZ to fruit. Interestingly, most target residues for sporulation control assume only one fungicide, imazalil. Generally, about 2.0 ppm IMZ in rind is considered appropriate for sporulation control (Brown & Dezman, 1990) but, as in this survey, packers rarely apply imazalil by itself.

In this survey, the IMZ range at 0.4-1.0ppm was lower than would be expected to control sporulation by itself. The TBZ range was higher at 0.8-3.5ppm. TBZ does have anti-sporulation activity but you need about >2.5ppm TBZ (4,000ppm in wax) (Gutter *et al*., 1971) or >4.4ppm TBZ (5,000ppm in water) (Eckert *et al*., 1969) for good sporulation control.

Although the sporulation results were variable across the packers; some packer did achieve good control with fungicide mixtures. For those of you statistically-minded, a simple linear regression indicated that sporulation control was highly correlated (adjusted R^2= 0.88) with the combined fungicide residues levels (see Graph 1, page 3). For those of you that just want to know the 'bottom line', total rind residues higher than 3ppm resulted in good sporulation control.

However, I wouldn't base my anti-sporulation strategy on a >3ppm total rind residue just yet. The results from this survey are from a limited number of packers; and are indicative only. The most practical finding for packers is that mixtures of TBZ and IMZ residues can provide good sporulation control, which

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should take the emphasis off a sporulation 'target' residue for each fungicide by itself.

This is an encouraging result for fungicide mixtures and I look forward to more work being conducted to validate this approach for sporulation control.

Fungicide resistance

The fungicide resistance survey results were very interesting. As expected, mould spores were common and grew well on normal agar plates placed around the packingline, but spores were less common in the cool-room. These results were a good indicator of the success of cleaning and sanitising at the beginning of the navel season.

The large amount of fungal growth on fungicide-amended plates from most packing lines surveyed was unexpected. Growth regularly occurred on 1ppm TBZ-amended plates, which is considered 'technical' resistance. Based on these results, some mould spores were tolerant of low levels of fungicide. However, this survey is only one result and repeated surveys are required to establish a pattern of resistance.

We used very low rates of IMZ and TBZ as an indicator of fungicide resistance. These low rates inhibited the growth of susceptible moulds stains held in our laboratories but we are unsure if growth at these levels is sufficient to impact on commercial out-turn. Regardless, mould spore growth does indicate a trend towards resistance and further investigation is warranted.

As such, we would like to expand this survey using higher rates to establish if fungicide resistance is a 'real' issue. We have developed a DIY resistance test kit just for this purpose. The test kit can be mailed out to packers and returned to the SARDI laboratories for analysis. Enclosed plates are exposed in three areas of your packing operations. There are also sterile swabs for testing packing line surfaces, such as wax brushes. The returned samples will be evaluated against IMZ, TBZ and the new fungicide, fludioxonil ("Scholar"). Full instructions are included in the kit.

There will be a charge of about $250 for the DIY kit and the results will be emailed to you. I hope that many packers will start to test for resistance in their packing sheds. At the moment; the situation is unknown. If packers take up the service, we will collate the results and start to develop management strategies at both the individual packer and regional levels.

The three regional citrus Boards, SA Citrus Industry Development Board, Murray Valley Citrus Board and Riverina Citrus, have all been very supportive of this work. They have the full survey report if you would like a copy and the Boards continue to be the local contact point for all of our surveys. Please contact your local Board or SARDI directly if you have an interest in testing for fungicide resistance.

Summary

Packingline procedures were usually sound, with evidence of monitoring and hygiene. In-line applications were normally sufficient to control decay.

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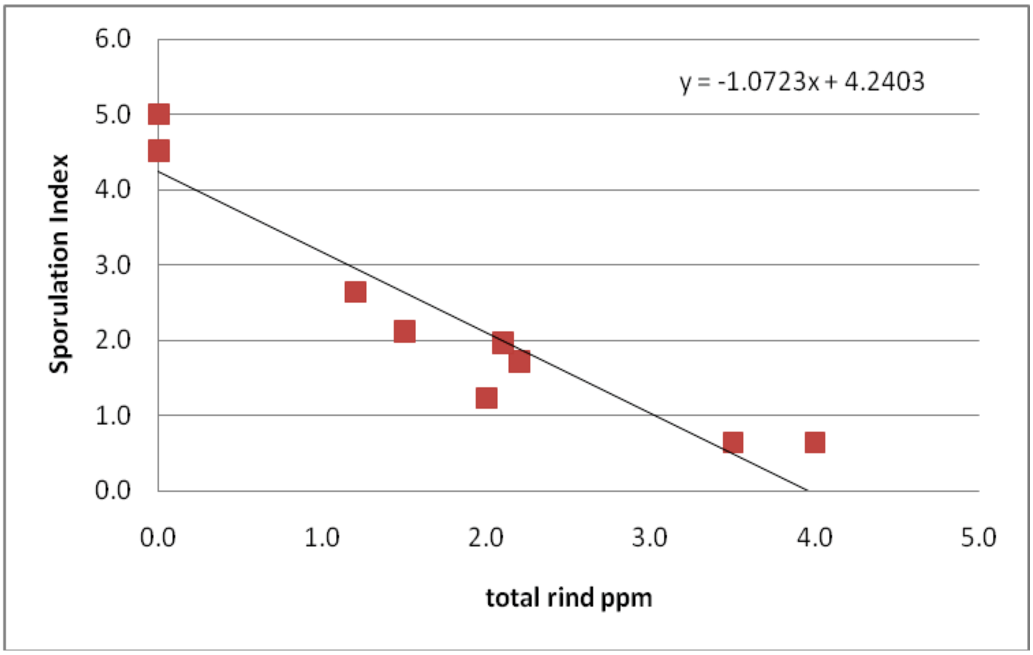

Graph 1 – The relationship of sporulation index to total fungicide deposit in the rind (TBZ + IMZ; ppm).

n.b sporulation index of 1 equivalent to an average of 10% or less rind area covered in spores

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There was a trend to increase fruit volume or flow rates over the line without altering fungicide application, which may lead to reduce decay control. Sporulation results suggest that there can be less emphasis on a high IMZ residue when the TBZ residues are high (>3ppm). There appears to be a trend toward fungicide resistance in some packingsheds. Some practices may exacerbate this trend. For instance, extended storage of fungicide-treated fruit on premises may lead to fungicide resistance problems. At the moment, we don't know if we have a problem. Let's find out by testing and then, using on the results, we can develop the appropriate strategies to deal with the situation.

References

Brown G.E. and Dezman D.J. (1990) Uptake of imazalil by citrus fruit after postharvest application and the effect of residue distribution on sporulation on Penicillium digitatum. Plant Disease. ***74****: 927-930.*

Eckert J.W, Kolbezen M.J. and Kraght (1969) A.J. Accumulation of 2-aminobutane and thiabenazole for the control of Penicillium decay in citrus. Proc. First Int. Citrus Symp. ***3****: 1301-8.*

Gutter Y., Yanko U., Davidson M. and Rahat M. (1974) relationship between mode of application of thiabendazole and its effectiveness for the control of green mould and inhibiting fungus sporulation on oranges. Phtytopathology, ***64****: 1477-8.*

Wild, B. L., (1994) Differential sensitivity of citrus green mould isolates to the fungicide imazalil. New Zealand Journal of Crop and Horticultural Science, ***22****: 167-171.*✯

Strippers – how closely are you watching?

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SARDI

It's not what you think, but now I have your attention: During the recent fungicide survey, I was amazed how quickly fungicides were stripping-out over a few hours of packing in some lines. Many packers have significantly increased the throughput of fruit over their line but the fungicide tank volume stays the same. This means that fungicides can strip-out much faster and, if you are topping up infrequently, the rates could be very low before top-up time again.

Generally, the aim has been to make sure each top-up returns you to the required rate, with little regard to the level prior to top-up. This may lead to a false sense of security. Let me give you an example;

Shed 'A' has a 500L in-line fungicide tank with 500ml product (to get 500ppm IMZ) at the start of the day. The first top-up is at 'Smoko' after 3 hours running. They have already worked out that they need to add 250 ml product to get back to 500ppm. This was based on test results after running 60 bins per hour.

They then decided to run the line 20% faster (72 bins per hour) but didn't change the fungicide top-up regime. The result is shown in the graph 1, below. As you can see, the rate sharply drops over the 3 hours, with the last 45 mins (or 54 bins) applied with less than half the label rate of fungicide. If the top-up rate hasn't been increased, the fungicide is not returned to initial rates and the concentration becomes lower as the day progresses.

This fungicide regime continues until a few consignments show decay. Fruit held on the premises in cool rooms also begin to show some decay.

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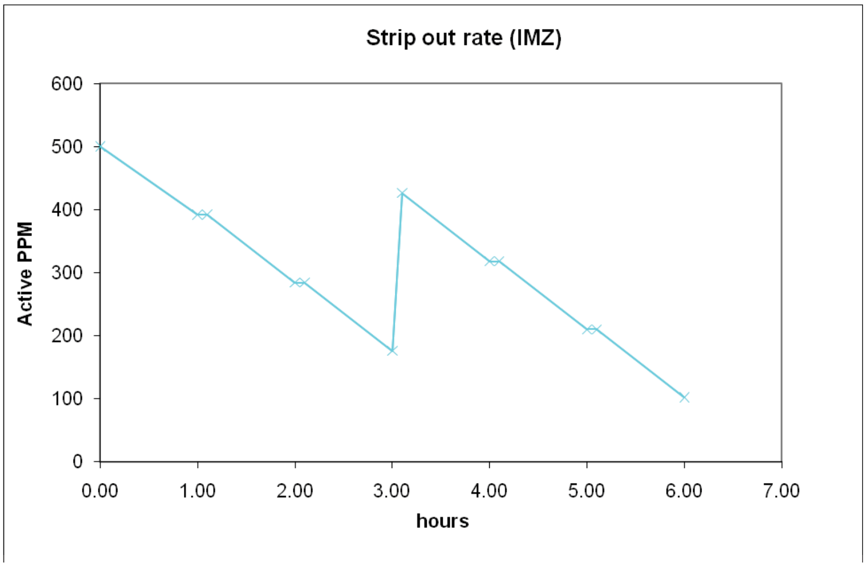

Graph 1 – Strip-out rate of imazalil from a 500L tank on a line running ~72 bins per hour.

The tank is top-up after 3 hours running and the fungicide rate drops well below 250ppm (1/2 label rate) before the top-up.

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Suddenly, a remedy is needed urgently and they decide to slightly increase the fungicide rates for initial fill and top-up. Meanwhile, they also collect fruit at different times during the day for testing, but the results are variable. However, after increasing the rates the problem seems to have disappeared (for the moment!).

The problem appears to be solved but the underlying cause has not been addressed. Yes! Increasing the fungicide rates does lift the overall fungicide residues but the trend shown in the line remains the same. Fruit is still being treated at a range of rates, with most below label rate. Applying a range of below label rates is an ideal way to select for fungicide resistance. In this case, treated fruit is being stored on the premises long enough for decay to develop spores, which sets up a cycle of resistance.

It can happen like this: The slightly resistant spores from packed fruit drift into the packing area to contaminate in-coming fruit. The higher fungicide rates may control the susceptible orchard spores on this fruit but as the rate drops over the day the slightly resistance spores reach a point where they will not be affected. If fact, over time resistance is likely to increase given this cycle of treatment, storage, sporulation, then re-contamination and re-treatment of fruit. Eventually, the increased fungicide rates will fail and the packer is faced with increasing fungicide rates even higher. We are now in a classic resistance spiral.

It can be very difficult to get out of this resistance cycle after it has been established. You can stop using the fungicide that has developed resistance and replace it with a different fungicide group; if you have another fungicide group available. You can sanitise, fumigate and clean-up (to remove resistant spores) and then arrange for all treated fruit to be immediately dispatched to an off-premises storage facility (to break the cycle).

As you can see, waiting until resistance develops can be difficult to deal with and costly. It is better to put practices in place that will delay and, hopefully, stop resistance developing; which brings me back to the strip out of fungicides and topping-up.

A factor in developing resistance is exposing spores to low levels of fungicide. You don't want the zig-zag line on the strip-out graph; you need to flatten out the line (see graph 2). This probably means setting up a low volume continuous pump or a pump on a timer. In graph 2, I have 'turned off" the pump off the last hour to reduce the amount of fungicide before dumping but be careful not to allow the concentration to fall too low while still packing.

The calculations used in this example are theoretical. I have used a figure of 125 ml product lost per 100 bins but it is a 'ball park' figure. There are many factors influencing fungicide losses, such as fungicide formulation, water quality and application method (for a more detailed discussion on maintaining fungicide concentrations, see Packer Newsletter 99; Dec 2010). As such, I have limited confidence in my estimates and have found very little data on top up rates for dips and drenches to make bold predictions. Basically, you need to work it out on your line for yourself.

I agree that this is a pretty unsatisfactory situation.

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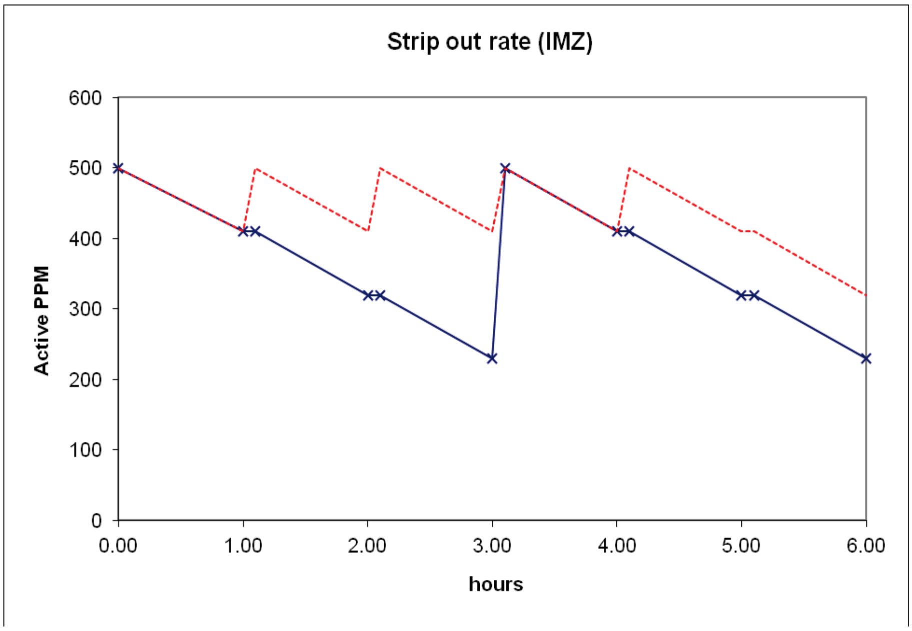

Graph 2 – Strip-out rate of imazalil from a 500L tank on a line running ~72 bins per hour.

The blue line is one 270ml top-up after 3 hours. The red line is four 90ml top-ups for the first 4 hours. The tank is dumped after 6 hours.

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The consequences of getting things wrong are serious; too low leads to decay and too high may exceed MRL's in markets. Although I can't see one approach that will fit all situations it should be possible to frame some guidelines.

In the past, we have advocated a double strength strategy, which was handed down to us from Barry Tugwell. This approach suggests replacing any lost volume with double label strength solution. In other words, if the label rate is 100ml product/100L, add 100ml product to every 50L replaced. Unfortunately, I have seen very little uptake of this strategy and the amount of solution lost during packing isn't usually recorded by packers. As such, we have had no opportunity to verify this approach under commercial conditions.

I suspect that most packers are either taking a guess based on experience, regularly monitoring solutions and fruit residues or a combination of both. In the past, this may have been good enough but MRL's are coming down and the margins between decay failure and exceeding MRL's are narrowing (especially if fungicide resistance is added to the equation).

Monitoring tank solutions and recording losses against throughput of fruit seems a reasonable approach to determine strip out rates. Using this approach in a few packing lines, I have determined a product loss for imazalil of about 80-150ml product per 100 bins for in-line high volume application. This is based on a very small sample size and I would be very grateful if anyone could provide records for their line to improve the accuracy of that estimate. You can contact me using the details on the front page of this newsletter.

A related question is: How often should I dump?' When fruit prices are so low, the temptation is to hold onto that tank for just a bit longer to save costs. However, the costs may hit you at the market end if decay develops. Again, there is very little objective information for citrus packing operations. However, the apple industry recommends a top up every 50 bins per 1,000L and max 3 times top up before replacement rule for DPA use. If you followed this rule for a 2,000L tank, you would top up fungicide after every 100 bins and dump after 400 bins. This seems to be a pretty reasonable approach for an outside drench, where fruit is dirty, but an in-line fungicide applied after a good high pressure probably requires a different approach.

These are difficult issues, which are compounded by the specter of resistance. Hey! Maybe we should just move to non-recovery systems to avoid strip-out issues. They have their own set of problems but I'll leave that discussion for another time. ✯

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