Industry-led Research Sub-Program

INDUSTRY REPORT

Developing a fungicide resistance service for citrus packers

[South Australian River Murray Sustainability Program (IRSP)]

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Summary

This project aimed to develop a fungicide resistance service and provide resistance management advice/training for citrus packers. It involved conducting resistance monitoring on five major citrus packers from the Riverland and surrounds, on four occasions from October 2015 to October 2016. Craig Wooldridge, E.E Muir & Sons postharvest technical consultant at Renmark, jointly participated in surveys, resistance management training and provided a local resource for packers.

The results of each survey was collated and remain confidential to the individual packer. The overall survey results provided a revealing depiction of the fungicide resistance pattern during 2016. Each packer had different experiences but there were a few consistent trends. They were;

- The 'summer break' did not always lead to a total loss of fungicide resistance acquired the previous season.
- The use of the combination of thiabendazole (TBZ) and imazalil (IMZ) controlled decay but did not halt single fungicide resistance developing within one season.
- Resistance to the fungicide TBZ is acquired before resistance to IMZ.
- Sampling in different areas of the packing line resulted in different levels of spores and the proportion resistant to a fungicide. The results in packing lines and cool rooms of the same packer can be very different.

The program also included a component of awareness and training for packers. Packers gained understanding as a result of participating in the fungicide resistance surveys. In particular;

- The fungicide-amend plates used in the surveys provide a visual guide of mould growth, which highlighted their risk.
- The information and training provided options for maintaining or improving their situation.
- The surveys identified potential problem areas, leading to a changes in cleaning and sanitation
- Survey plate tests can be used to verify the treatment efficiency.
- The surveys provide an early warning system that resistance is developing and can be used to guide an alternating fungicide program.

It seems likely that packers would value an on-going fungicide resistance service. At this stage, packers still prefer expert advice to confirm suspect results. As such, any service should include some interpretation of the results and advice on possible treatment options. Partnership with a local service provider has been very valuable in providing detailed follow-up and product advice.

Background

There have been postharvest fungicide residue breaches (exceeding MRLs) on citrus fruit exported to Japan, the Riverland citrus industry's most valuable market. More fungicide to control decay is not sustainable and jeopardises our markets. Previous surveys indicated pockets of resistance to current fungicides and the potential for this to escalate. Packers require 'real-time' information on their fungicide resistance levels to make informed decisions to reverse the current trend.

This project aimed to develop a fungicide resistance service and provide resistance management advice/training for packers. It involved conducting resistance monitoring on five major citrus packers from the Riverland and surrounds on four occasions from October 2015 to October 2016. SARDI and E.E Muir & Sons, a major distributor of chemicals and other farm supplies to the Australian agriculture industry, jointly conducted the surveys. We interpreted the resistance results and provided advice on sustainable strategies to avoid the over-use of fungicides. Craig Wooldridge, E.E Muir & Sons postharvest technical consultant at Renmark, jointly participated in surveys, resistance management training and provided a local resource for packers.

We also sought the packers' chemical residue records to estimate average fungicide residues. The residue levels were compared with fungicide residues required for decay and spore control of moulds.

Survey Methods

SARDI

For fungicide resistance surveys, potato dextrose agar (PDA) was amended with fungicide, resulting in nil fungicide or 15 mg/L thiabendazole (TBZ) (Tecto®, Syngenta Australia, Macquarie Park, NSW), or 1 mg/L fludioxonil (FLU) (Scholar®, Syngenta Australia, Macquarie Park, NSW).

Petri dishes (9cm diameter) containing amended agar for each fungicide concentration were exposed in 3 areas of commercial packing operations for ~60 minutes. Typically, plates were exposed near where fruit was dumped onto the line, near the waxing or packing area, and inside the cool rooms.

The exposed plates were incubated for 3 days at 25^0C before being assessed for mould spore coverage. The results of each establishment is collated in a survey sheet is sent to the individual packer.

EE Muir & Sons

Concurrently, citrus fruit were scratched and exposed in the same areas and for the same time period as plates (see above). This wounded fruit was incubated at ~20^0C until fruit produced spores. Spores were collected on sterile swabs and sent to Janssen PMP (Beerse, Belgium) for fungicide resistance evaluation. The fungicide actives evaluated were imazalil, thiabendazole, pyrimethanil and pyrimethanil + imazalil. Janssen's detailed methods are proprietary but involve comparing the growth of mould colonies on fungicide-amended plates relative to unamended

control standards to determine a resistance factor. The higher the factor value, the greater the resistance to the fungicide evaluated.

Survey Results

Late Season Fungicide resistance surveys (October 2015)

The late season surveys were conducted when fungicide resistance is likely to occur; after a busy season exporting navel oranges. This survey is a benchmark for current practice. Five packers were involved in fungicide resistance surveys. They will be referred to as Packer A through to Packer E in this report.

Packer A

The following are the results for Packer A. The area of the control plate covered by mould indicates relative hygiene of the packing line. The fungicide amended plates indicate if resistant spores are present. The control plates can also be compared to the fungicide amended plates to assess the proportion of resistance. The proportion of resistance can be used to better assess

The plates were exposed in different areas as indicated by the coloured dots. They are at the start of line (green), end of line (red) and cool room (yellow)

Control plates (no fungicide)
Brief description: These plates show the level of mould spores and other fungi in the air. The numbers/area covered can indicate relative hygiene and sanitation.

Mould coverage on plates *(circle appropriate level)*
Start of line: 0%, <25%, <50% or >50%
End of line: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Additional notes: Visibility of Control –Start of Line plate was low due to Aspergillus sp. Growth on 100% of the plate. Low levels of yeast and/or bacterial growth observed on cold room plate.

Thiabendazole (TBZ plates) (rate 15ppm)
Brief description: Mould growth on these plate indicate technical resistance. Comparing the number of mould spores on control and TBZ plates can indicate the proportion of resistant mould spores. [TBZ products; e.g., Tecto and Vorlon]

Proportion of TBZ resistance spores (15ppm)
Start of line: 0%, <25%, <50% or >50%
End of line: 0%, <25%, <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Additional notes: Apergillus sp. Present on "Start of Line" plate. Low levels of yeast and/or bacterial growth observed on end of line and cold room plates.

Fludioxonil (FLU) plates (rate 1ppm)
Brief description: Mould growth on these plate indicate technical resistance. Comparing the number of mould spores on control and TBZ plates can indicate the proportion of resistant mould spores. [FLU products; e.g., Scholar]

Proportion of FLU resistance spores (1ppm)
Start of line: 0%, <25% <50% or >50%
End of line: 0%, <25% <50% or >50%
Cool room: 0%, <25%, <50% or >50%

Additional notes: Low levels of yeast and/or bacterial growth observed on all FLU 1ppm plates.

risk. Higher proportions of resistant spores constitute higher risk, especially when there are numerous spores in the control plates (unhygienic conditions).

The plates at the start of the line had a moderate level of mould spores and a high proportion grew on TBZ amended agar, suggesting most airborne spores in this area were resistant to TBZ. The end of the line had higher levels of spores but a lower proportion was resistant to TBZ. The levels of spores in cool rooms was low but some spores were still resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer A are summarised in Table 1.

Table 1. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer A.

	Start line	*End line*	*Cool room*
Control plate coverage	<50%	>50%	<25%
TBZ resistance	high	low	medium
FLU resistance	low	low	very low

Interpretation: Moderate to high levels of spore around the line indicate a build up over the season. Sanitation and hygiene may be less diligent due to the pressures of a busy packing season. TBZ has probably been used all season for resistance to be high. High spore number and a high level of resistance constitutes a high risk of problems developing. Cool rooms spore levels and TBZ resistance levels are lower indicating they are still relatively clean. The low spore growth on FLU indicates that this fungicide would control the TBZ resistant spores in this environment.

Packer B

The plates at the start of the line had high levels of mould spores but a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were still susceptible to TBZ. The end of the line had lower levels of spores but a higher proportion was resistant to TBZ. The levels of spores in cool rooms was low but a high proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer B are summarised in Table 2.

Table 2. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer B.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	<50%	<25%
TBZ resistance	low	high	high
FLU resistance	low	low	very low

Interpretation: The spore levels around the line are similar to Packer A. Again, sanitation and hygiene may be less diligent due to the pressures of a busy packing season. TBZ has probably been used all season leading to high resistance. High spore numbers and a high levels of resistance constitutes the highest risk of problems developing. Cool rooms spore levels are low but TBZ resistance levels are high suggesting that small amounts of TBZ-treated fruit may have decayed and produced spores during storage. The low spore growth on FLU indicates that this fungicide could be introduced to successfully control the TBZ resistant spores.

Packer C

The plates at the start of the line had very high levels of mould spores and a high proportion grew on TBZ amended agar, suggesting most airborne spores in this area were resistant to TBZ. The end of the line had high levels of spores but a lower proportion was resistant to TBZ. The levels of spores in cool rooms was moderate but a low proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer C are summarised in Table 3.

Table 3. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer C.

	Start line	*End line*	*Cool room*
Control plate coverage	>95%	<95%	<50%
TBZ resistance	high	low	low
FLU resistance	low	low	low

Interpretation: Very high levels of spores around the line indicate a build up over the season. Sanitation and hygiene was lacking due to the pressures of a busy packing season. TBZ has probably been used all season for resistance to be high. High spore number and a high level of resistance constitutes a high risk of problems developing. Cool rooms spore levels are moderate

but TBZ resistance levels are still relatively low suggesting some migration of spores from outside rather than storage problems. The low spore growth on FLU indicates that this fungicide could be introduced to successfully control the TBZ resistant spores.

Packer D

The plates at the start of the line had very high levels of mould spores and a high proportion grew on TBZ amended agar, suggesting most airborne spores in this area were resistant to TBZ. The end of the line had high levels of spores but a lower proportion was resistant to TBZ. The levels of spores in cool rooms was moderate but a low proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer D are summarised in Table 4.

Table 4. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer D.

	Start line	*End line*	*Cool room*
Control plate coverage	>95%	<95%	<50%
TBZ resistance	high	medium	low
FLU resistance	low	low	very low

Interpretation: Similar to Packer C. Very high levels of spores around the line indicate a build up over the season. Sanitation and hygiene was lacking due to the pressures of a busy packing season. TBZ has probably been used all season for resistance to be high. High spore number and a high level of resistance constitutes a high risk of problems developing. Cool rooms spore levels are moderate but TBZ resistance levels are still relatively low suggesting some migration of spores from outside rather than storage problems. The low spore growth on FLU indicates that this fungicide could be introduced to successfully control the TBZ resistant spores.

Packer E

The plates at the start of the line had low levels of mould spores and a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were susceptible to TBZ. The end of the line had moderate levels of spores but a lower proportion was resistant to TBZ. The levels of spores in cool rooms was very high and a high proportion were resistant to TBZ. Spores collected in all areas around the line were susceptible to FLU, but spores in the cool room were less susceptible.

The results for Packer E are summarised in Table 5.

Table 5. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer E.

	Start line	*End line*	*Cool room*
Control plate coverage	<25%	<50%	>95%
TBZ resistance	low	low	very high
FLU resistance	low	low	high

Interpretation: Packer E is significantly different to the other packers. There are low to moderate levels of spores around the line, which is a good result. Sanitation and hygiene may have been exceptional but the cool room results suggest otherwise. Cool rooms spore levels were very high and relatively unclean. TBZ resistance levels in the cool room was very high and FLU resistance was also high. It is likely that both FLU and TBZ have been used during the season, which with hygiene measures, has resulted in maintaining susceptibility to both fungicides in the packing area. A good result. In contrast, a lack of hygiene and long-term storage of treated fruit has probably contributed to fruit decaying to produce resistant spores in the cool room. At this stage, the fungicide-resistant spores are isolated in the cool room. However, this is a risk for future fruit stored in these premises.

EE Muir & Sons samples

The majority of samples taken for analysis by Janssen did not result in any pathogen being isolated. From the samples evaluated, the isolates were sensitive to the fungicides imazalil and the pyrimethanil + imazalil mixture, but isolates were generally highly resistant to TBZ. Isolates showed increase resistance values to pyrimethanil at 2 packing facilities. Janssen considered these resistance values 'not uncommon' late in the season. They expect everything to return to 'normal' by start of next season.

Early season surveys (June 2016)

Packer A

The following are the results for Packer A. The area of the control plate covered by mould indicates relative hygiene of the packing line. The fungicide amended plates indicate if resistant spores are present. The control plates can also be compared to the fungicide amended plates to assess the proportion of resistance. The proportion of resistance can be used to better assess risk. Higher proportions of resistant spores constitute higher risk, especially when there are numerous spore in the control plates (unhygienic conditions).

The plates at the start of the line had a high level of mould spores and a high proportion grew on TBZ amended agar, suggesting most airborne spores in this area were resistant to TBZ. The end of the line had higher levels of spores but a lower proportion was resistant to TBZ. The levels of spores in cool rooms was low and susceptible to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer A are summarised in Table 6.

Table 6. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer A.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	>50%	<25%
TBZ resistance	high	low	low
FLU resistance	low	low	very low

The average fungicide residues on fruit for Packer A in June were estimated at 1.3 mg/Kg for IMZ and 2.3 mg/Kg for TBZ. The average total fungicide residue due to postharvest application was 3.6 mg/Kg.

Interpretation: Moderate to high levels of spore around the line indicates either a rapid build-up or a remnant population from last season. It seems more likely to be a carryover from last year for TBZ resistance to be high this early in the season. High spore number and a high level of resistance constitutes a high risk of problems developing. Cool rooms spore levels and TBZ resistance levels are low indicating effective cleaning. The low spore growth on FLU indicates that this fungicide would control the TBZ resistant spores in this environment.

The residues for each fungicide alone (TBZ or IMZ) were sufficient to control decay of susceptible spores but when decay occurs may be insufficient to arrest spore formation, leading to resistant isolates. Although each fungicide residue is insufficient on its own to control spore formation, the combination of the two fungicides (total 3.6 mg/Kg) may have an additive effect reducing overall spore formation.

Packer B

The plates at the start of the line had high levels of mould spores but a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were still susceptible to TBZ. The end of the line was similar. The levels of spores in cool rooms was too low to detect. Spores collected in all areas were susceptible to FLU.

The results for Packer B are summarised in Table 7.

Table 7. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer B.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	>50%	0%
TBZ resistance	low	low	very low
FLU resistance	Very low	low	very low

The average fungicide residues on fruit for Packer B in June were estimated at 2.1 mg/Kg for IMZ and 2.2 mg/Kg for TBZ. The average total fungicide residue due to postharvest application was 4.3 mg/Kg.

Interpretation: The spore levels around the line are high but the break (with sanitation and hygiene) has reduced last season's TBZ resistance to low levels. Cool rooms spore levels are very low suggesting good pre-season cleaning. As fungicide resistance is low there is no imperative, but FLU could be introduced in rotation with TBZ to avoid resistant mould spore developing.

The residues for each fungicide alone (TBZ or IMZ) were sufficient to control decay of susceptible spores and IMZ residue (>2.0 mg/Kg) may be enough to arrest spore formation alone. The combination of the two fungicides (total 4.3 mg/Kg) may have an additive effect reducing overall spore formation.

Packer C

The plates at the start of the line had high levels of mould spores but a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were still susceptible to TBZ. The end of the line had moderate levels of spores and a low proportion was resistant to TBZ. The levels of spores in cool rooms was low and a low proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer C are summarised in Table 8.

Table 8. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer C.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	<50%	<25%
TBZ resistance	low	low	low
FLU resistance	low	low	low

The average fungicide residues on fruit for Packer C in June were estimated at 2.0 mg/Kg for IMZ and 2.0 mg/Kg for TBZ. The average total fungicide residue due to postharvest application was 4.0 mg/Kg.

Interpretation: The spore levels around the line are high but the break (with sanitation and hygiene) has reduced last season's high TBZ resistance to low levels. Cool rooms spore levels are low and TBZ resistance levels are low, which is a good result for the start of the season. The low spore growth on FLU indicates that this fungicide could be introduced successfully in rotation control the TBZ resistant spores.

The residues for each fungicide alone (TBZ or IMZ) were sufficient to control decay of susceptible spores and 2.0 mg/Kg IMZ may be enough to arrest spore formation. The combination of the two fungicides (total 4.0 mg/Kg) may have an additive effect reducing overall spore formation.

Packer D

The plates at the start of the line had moderate levels of mould spores and a moderate proportion grew on TBZ amended agar, suggesting some airborne spores in this area were resistant to TBZ. The end of the line had lower levels of spores and a lower proportion was resistant to TBZ. The levels of spores in cool rooms was low and a low proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer D are summarised in Table 9.

Table 9. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer D.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	<50%	<25%
TBZ resistance	medium	low	very low
FLU resistance	low	low	low

Packer D did not supply fungicide residue data for the month of June.

Interpretation: Sanitation and hygiene has reduced spore levels from the end of last season. Some spores are still resistance to TBZ and monitoring is required to assess any build up in fungicide resistance. Cool rooms spore levels are low and resistance is also low, suggesting good sanitation in these areas.

Packer E

The plates at the start of the line had moderate levels of mould spores and a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were susceptible to TBZ. The end of the line had moderate levels of spores and a slightly higher proportion was resistant to TBZ. The levels of spores in cool rooms was high and a moderate proportion were resistant to TBZ. Spores collected in all areas around the line were susceptible to FLU, including in the cool room.

The results for Packer E are summarised in Table 10.

Table 10. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer E.

	Start line	*End line*	*Cool room 2*
Control plate coverage	<50%	<50%	>50%
TBZ resistance	low	medium	medium
FLU resistance	Very low	Very low	Very low

The fungicide residues on fruit for Packer E in June were ranged from 3.5 to 1.8 mg/Kg for IMZ and averaged 0.44 mg/Kg for TBZ. FLU was recorded in one sample at 1.8 mg/Kg. The total fungicide residue due to postharvest application was 3.6 mg/Kg.

Interpretation: Packer E has moderate levels of spores around the line, with some TBZ resistance evident. Cool rooms spore levels were high and relatively unclean. TBZ resistance levels in the cool room was moderate, which is a concern when coupled with high overall spore levels. FLU resistance is low in all areas, which allows the use of FLU to mitigate any TBZ resistance.

The residues for each fungicide alone (FLU or IMZ) were sufficient to control decay of susceptible spores but TBZ would be insufficient to control decay. Samples with >2 mg/kg IMZ should be sufficient to control sporulation alone. The combination of the two or three fungicides (total >3.0 mg/Kg) may have an additive effect reducing overall spore formation.

Late season surveys (Sep 2016)

Packer A

The following are the results for Packer A. The area of the control plate covered by mould indicates relative hygiene of the packing line. The fungicide amended plates indicate if resistant spores are present. The control plates can also be compared to the fungicide amended plates to assess the proportion of resistance. The proportion of resistance can be used to better assess risk. Higher proportions of resistant spores constitute higher risk, especially when there are numerous spore in the control plates (unhygienic conditions).

The plates at the start of the line had a high level of mould spores and a high proportion grew on TBZ amended agar, suggesting most airborne spores in this area were resistant to TBZ. The end of the line had lower levels of spores and a lower proportion was resistant to TBZ. The levels of spores in cool rooms was low and susceptible to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer A are summarised in Table 11.

Table 11. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer A.

	Start line	*End line*	*Cool room*
Control plate coverage	>90%	>25%	<25%
TBZ resistance	high	low	low
FLU resistance	low	low	very low

The average fungicide residues on fruit for Packer A in August ranged from 1.0 to 2.0 mg/Kg for IMZ and 1.3 to 2.7 mg/Kg for TBZ. Some lines of fruit were also treated with FLU, with an average of 1.5 mg/Kg. The average total fungicide residue due to postharvest application was 3.7 mg/Kg for IMZ and TBZ, and 4.4 mg/Kg for IMZ, TBZ and FLU.

Interpretation: Moderate to high levels of spore around the line was found during the early season surveys. High spore numbers and a high level of resistance has persisted at the start of

the line. Cool rooms spore levels and TBZ resistance levels remain low indicating effective spore control during storage. The low spore growth on FLU suggests that this fungicide would control the TBZ resistant spores in this environment. The cool room is the main area of improvement compared to surveys late last season.

The residues for each fungicide alone (TBZ, IMZ or FLU) were sufficient to control decay of susceptible spores but when decay occurs may be insufficient to arrest spore formation. Although each fungicide residue is insufficient on its own to control spore formation, the combination of the two or three fungicides may have an additive effect reducing overall spore formation.

Packer B

The plates at the start of the line had high levels of mould spores but a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were still susceptible to TBZ. The end of the line also had low number of spores with TBZ resistance. The levels of spores in cool rooms was unusually high and resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer B are summarised in Table 12.

Table 12. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer B.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	<50%	>95%
TBZ resistance	low	low	high
FLU resistance	low	very low	very low

The average fungicide residues on fruit for Packer B in Aug were estimated at 1.3 mg/Kg for IMZ and 1.7 mg/Kg for TBZ. The average total fungicide residue due to postharvest application was 3.0 mg/Kg.

Interpretation: The spore levels around the line are medium to high but TBZ resistance remains at low levels. Overall, the packing practices were successful in maintaining low fungicide resistance levels around the line. Cool rooms spore levels were high and showed TBZ resistance, which suggests fruit is being stored for too long in this cool room. The main change from late last season is the general increase in spore numbers and TBZ resistance in the cool room.

The residues for each fungicide alone (TBZ or IMZ) were sufficient to control decay of susceptible spores but when decay occurs may be insufficient to arrest spore formation, leading to resistant isolates. Although each fungicide residue is insufficient on its own to control spore formation, the combination of the two fungicides (total 3.0 mg/Kg) may have an additive effect reducing overall spore formation.

Packer C

The plates at the start and end of the line had high levels of mould spores and a moderate proportion grew on TBZ amended agar, suggesting airborne spores in these area were becoming resistant to TBZ. The levels of spores in cool rooms was low and a very low proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer C are summarised in Table 13.

Table 13. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer C.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	>50%	<25%
TBZ resistance	medium	medium	low
FLU resistance	very low	very low	very low

The average fungicide residues on fruit for Packer C in Aug were estimated at 1.8 mg/Kg for IMZ and 2.7 mg/Kg for TBZ. The average total fungicide residue due to postharvest application was 4.5 mg/Kg.

Interpretation: The spore levels around the line are high and TBZ resistance levels were slowly building. Cool rooms spore levels are low and FLU and TBZ resistance levels are low, which is a good result for the end of the season. The low spore growth on FLU indicates that this fungicide could be introduced successfully in rotation control the TBZ resistant spores.

The residues for each fungicide alone (TBZ or IMZ) were sufficient to control decay of susceptible spores but may not be enough to arrest spore formation on their own. However, the combination of the two fungicides is high (total 4.5 mg/Kg) and may have an additive effect reducing overall spore formation.

Packer D

The plates at the start of the line had moderate levels of mould spores and a moderate proportion grew on TBZ amended agar, suggesting some airborne spores in this area were resistant to TBZ. The end of the line had lower levels of spores and a lower proportion was resistant to TBZ. The levels of spores in cool rooms was low and a low proportion were resistant to TBZ. Spores collected in all areas were susceptible to FLU.

The results for Packer D are summarised in Table 14.

Table 14. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer D.

	Start line	*End line*	*Cool room*
Control plate coverage	>50%	<50%	<25%
TBZ resistance	medium	low	low
FLU resistance	low	low	very low

Packer D did not supply fungicide residue data for the month of September.

Interpretation: Some spores are still resistance to TBZ but the levels have not significantly changed since the start of the season. Cool rooms spore levels are low and resistance is also low, suggesting fruit has not decayed and produced spores in these areas.

Packer E

The plates at the start of the line had high levels of mould spores and a low proportion grew on TBZ amended agar, suggesting most airborne spores in this area were still susceptible to TBZ. The end of the line had low levels of spores with low resistant to TBZ. The levels of spores in cool rooms was high and a high proportion were resistant to TBZ. Spores collected in all areas around the line were susceptible to FLU, including in the cool room.

The results for Packer E are summarised in Table 15.

Table 15. The mould coverage and relative proportion of mould growth on fungicide amended plates compared to control plates, for Packer E.

	Start line	*End line*	*Cool room 2*
Control plate coverage	<90%	<25%	>50%
TBZ resistance	low	low	high
FLU resistance	Very low	Very low	Very low

The fungicide residues on fruit for Packer E in September averaged 1.6 mg/Kg for IMZ, 0.4 mg/Kg for TBZ, and 1.3 mg/Kg for FLU. The total fungicide residue due to postharvest application was 3.3 mg/Kg.

Interpretation: Packer E has variable levels of spores around the line, with low TBZ resistance evident. Cool rooms spore levels were high and relatively unclean. TBZ resistance levels in the cool room was high, suggesting treated fruit has decayed and produced spores in this cool room. This is a concern when coupled with high overall spore levels. FLU resistance is low in all areas, which allows the use of FLU to mitigate any TBZ resistance.

The residues for each fungicide alone (FLU or IMZ) were sufficient to control decay of susceptible spores but TBZ would be insufficient to control decay. Samples with >2 mg/kg IMZ should be sufficient to control sporulation alone. The combination of the two or three fungicides (total >3.0 mg/Kg) may have an additive effect reducing overall spore formation.

EE Muir & Sons samples

The majority of samples taken for analysis by Janssen were lost in transit. Samples were evaluated from two packers. These isolates were sensitive to the fungicides imazalil and pyrimethanil, but two isolates (both from the same packer) were highly resistant to TBZ and showed increased resistance to the Imazalil + pyrimethanil mixture. The isolates from the second packer was sensitive to all fungicides evaluated.

Postharvest fungicide residues 2016

The fungicide residues on fruit provided by packers indicated a wide range of residues for each of the three fungicide used (see Graph 1). Fruit treated with TBZ had the majority of residues between 0.5 to 4.0 mg/kg, whereas fruit treated with IMZ ranged from 1.0 to 3.5 mg/Kg. Fruit were infrequently treated with FLU but the residues were most consistent, ranging from 1.0 to 2.0 mg/Kg.

In previous years, some fruit residues for IMZ have exceeded the CODEX maximum residue limit (MRL) for IMZ at 5.0 mg/Kg. This MRL was not exceeded in fruit from the results provided this season.

Packer surveyed applied at least two different fungicides to their fruit. These fungicides are applied to provide extra protection from decay and to mitigate against fungicide resistance. The total fungicide residue on fruit was reasonably consistent, with most residues between 2.0 and 5.0 mg/Kg (see Graph 2).

There were no significant linear relationships between fruit residues (total or single fungicide) and levels of resistance (results not presented).

Graph 1. Imazalil (IMZ) fludioxonil (FLU) and thiabendazole (TBZ) fungicide residues (mg/Kg) on citrus fruit treated at surveyed packing lines during June and early September 2016, and their relationship to the CODEX maximum residue limit (MRL) for IMZ.

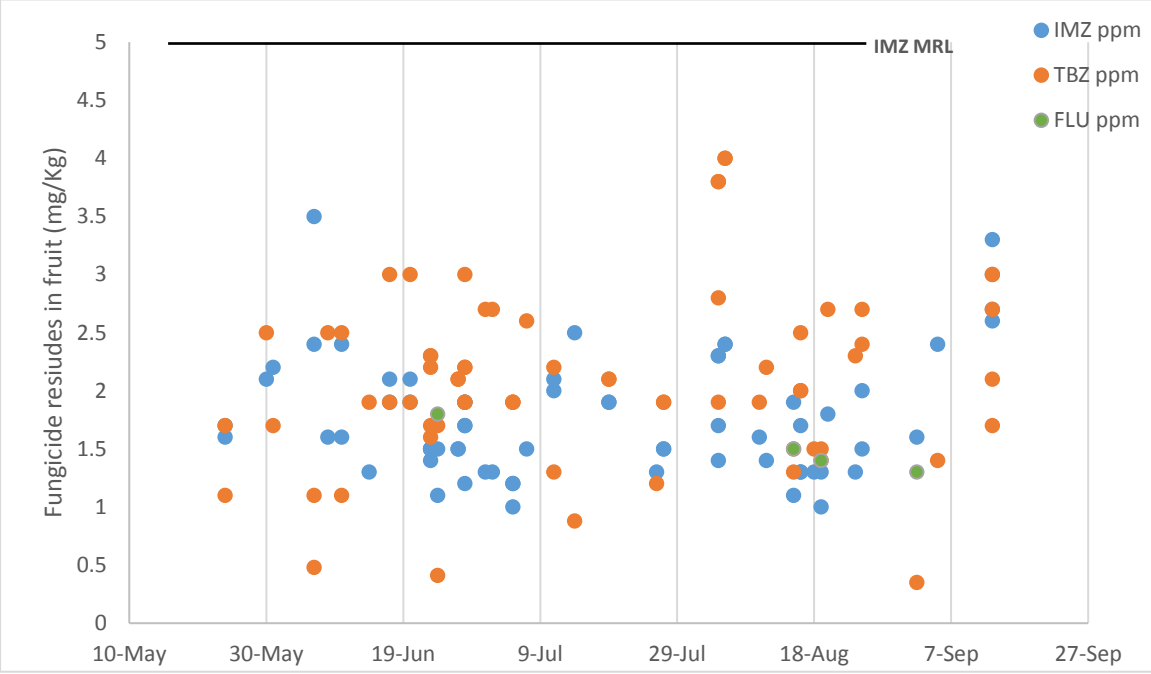
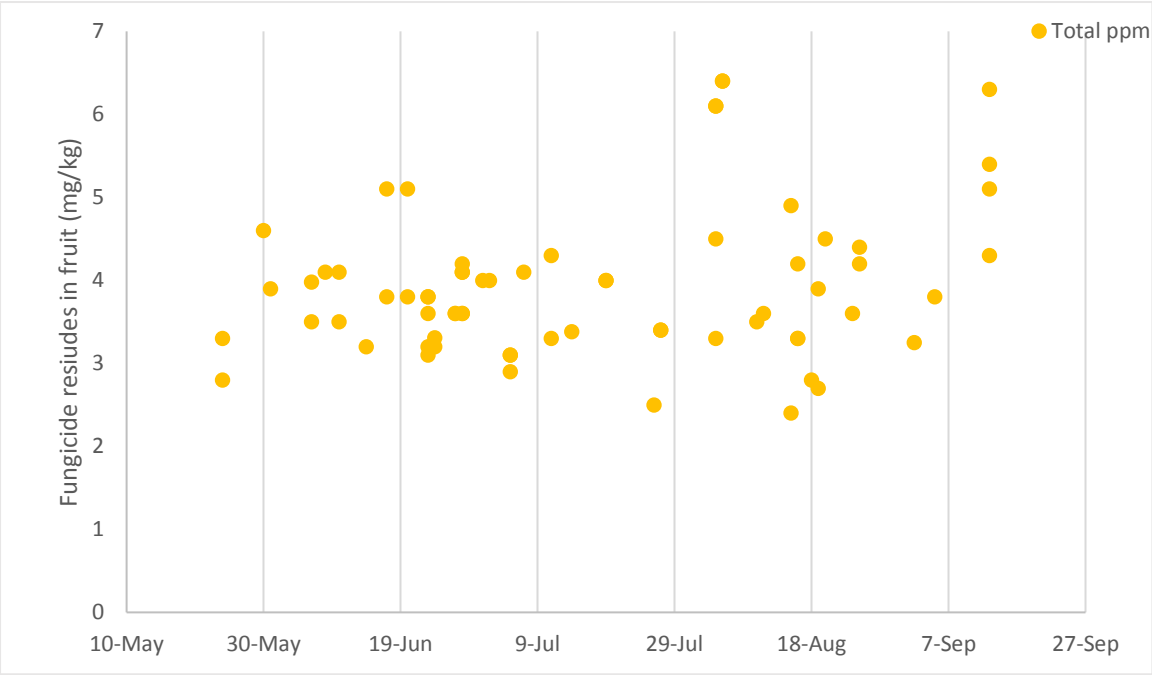


Table 2. Total postharvest fungicide residues (mg/Kg) on citrus fruit treated at surveyed packing lines during June and early September 2016.



Discussion

The survey results from the five different packers provided a revealing depiction of the fungicide resistance pattern during 2016. Each packer had different experiences but there were a few consistent trends. They were;

- The 'summer break' did not always lead to a total loss of fungicide resistance acquired the previous season.
- The use of the combination of thiabendazole (TBZ) and imazalil (IMZ) does not halt fungicide resistance developing within one season.
- Resistance to the fungicide TBZ is acquired before resistance to IMZ, when both fungicide are used concurrently.
- Decay was not rampant after TBZ resistance was acquired suggesting that treatment continues to control decay if sufficient IMZ &/or fludioxonil (FLU) residues are present. At least, until cross-resistance develops.
- FLU was used by only two packers and residue data was limited, which makes conclusions conditional. Surveys show that resistance to FLU can occur; it occurred in the cool rooms of one regular user in 2015 but did not reappear after instruction and monitoring for resistance in 2016.
- Sampling in different areas of the packing line resulted in different levels of spores and the proportion resistant to a fungicide. The results in packing lines and cool rooms of the same packer can be very different.

The program also included a component of awareness and training for packers. Packers gained confidence, perception and understanding as a result of participating in the fungicide resistance surveys. The build-up of fungicide resistant spores is insidious and packer had no cues to resistance developing before these surveys. The fungicide-amend plates used in the surveys provide a visual guide of mould growth, which highlighted their risk. The information and training provided options for maintaining or improving their situation. In particular, the surveys identified cool rooms as potential problem areas. Packers with fungicide resistance in 2015 responded by seeking methods to improve cleaning. The local service provider (E.E. Muir & Sons) was very valuable in providing products and advice on treatment application. After treatment, the packers requested extra survey plate tests to verify the treatment efficiency. This indicates that packers see practical value in the surveys and have confidence in the results.

However, one season's training has not been sufficient for packers to independently assess the risks and make final judgements. They have continued to seek expert advice to confirm suspect results and then ask for guidance. Packers are usually time poor, dealing with many competing issues during the season. Good independent advice is keenly sought when potential issues arise.

It seems likely that packers would support an on-going fungicide resistance service. At this stage, the service should include some interpretation of the results and advice on possible treatment

options to mitigate any potential resistance issues. Partnership with a local service provider has been very valuable in providing detailed follow-up and product advice.

Fungicide resistance management

The first principle for fungicide use is to apply sufficient residues on fruit to control decay. Fungicide application in commercial packing lines is notoriously variable, with single applications often resulting in low residues (Erasmus et al. 2011). To mitigate, packers have used a combination of TBZ and IMZ to provide sufficient residues (Ismail & Zhang, 2004). In this study, most single fungicide applications were sufficient to control decay but insufficient to control sporulation. IMZ requires <2.0 mg/Kg to arrest spore formation of green mould (Erasmus et al. 2015). Some fungicide residues for a single fungicide was lower than required but the total residues on fruit was consistently between 2.0 and 5.0 mg/Kg. Previous fungicide surveys results showed that total residues of IMZ and TBZ of >3 mg/Kg resulted in negligible spore formation (packer newsletter). We were unable to assess sporulation as packers indicated that there was little or no decay during the survey period.

The use of fungicides in mixtures is also used to overcome fungicide resistance. Mould spores resistance to one fungicide can be controlled by a second different fungicide (Eckert et al. 1994). However, cross resistance can occur when the combination is used year round without a pause to conduct through sanitation procedures (Holmes and Eckert 1999). In this study, TBZ resistance increased within the season and some resistance spores persisted after the summer break. Some packers process and store fruit year round and are at greater risk.

Recently, two new fungicide, pyrimethanil (PYR) and FLU, were introduced into the Australian citrus industry, primarily to use in alternating strategies with existing fungicides (Smilanick et al. 2006). When resistance is detected, an alternate fungicide with a different mode of action should be used (Kinay P. et al. 2007). The surveys conducted in this study provide an early warning system that resistance is developing and could be used to guide an alternating fungicide program.

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