RESISTANCE MONITORING IN AUSTRALIAN *FRANKLINIELLA OCCIDENTALIS*: ESTABLISHMENT OF A PHORATE BASELINE, DETECTION OF THIAMETHOXAM RESISTANCE, AND A NEW MANAGEMENT STRATEGY FOR USE IN PROCESSING TOMATOES

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Summary

Processing tomato growers in Australia recently reported difficulty controlling western flower thrips, *Frankliniella occidentalis* (Pergande), using registered insecticides. Consequently, this study was initiated to investigate the cause of those difficulties by examining the resistance status of Australian *F. occidentalis* to seven chemicals (spinosad, malathion, methidathion, acephate, abamectin, phorate and thiamethoxam) registered for use in processing tomatoes. Resistance to the above chemicals was detected using previously established discriminating doses and a discriminating dose of 3.0 g a.i. / L phorate generated. Bioassay data were collected over two consecutive seasons (2013-14/2014-15) and resistance in *F. occidentalis* to all chemicals was detected except methidathion. Spinosad resistance was detected in both seasons and often at frequencies >50% resistant individuals. Low frequency thiamethoxam resistance (<10%) was also detected in both seasons and is the first known detection of thiamethoxam resistance in Australian field-collected *F. occidentalis*. Despite the focus of this study being *F. occidentalis*, *F. schultzei* Trybom was the most abundant species within crops sampled suggesting inadequate spray coverage and/or resistance development in *F. schultzei*. The data generated was used to develop a processing tomato-specific management strategy to reduce selection pressure against those chemicals which have been compromised by resistance so facilitating sustainable control.

Keywords: neonicotinoid, spinosad, insecticide resistance, western flower thrips

INTRODUCTION

Western flower thrips, *Frankliniella occidentalis* (Pergande), was first identified in Western Australia during the early 1990s (Malipatil et al. 1993), from where it quickly spread to all States and Territories except the Northern Territory (Farrell 2003). Management of *F. occidentalis* including its chemical control and resistance status was overseen through a National Strategy that has now formally concluded (Farrell 2003).

The National Strategy ran for more than a decade, identifying chemicals compromised by resistance (some organophosphates and all pyrethroids) and eliminating their use for *F. occidentalis* control (Herron and Gullick 2001). Chemicals that remained had their efficacy demonstrated according to the mandated three-spray strategy as outlined by Broughton and Herron (2007), with residue data established to support that use pattern (see Herron et al. 2007 for detail). Newer chemicals for *F. occidentalis* control were identified including the neonicotinoid thiamethoxam that had no detectable resistance at that time (Herron and Broughton 2006). Finally, the strategic integrated pest management (IPM) compatible group 5 chemical spinosad (now replaced by chemically related spinetoram) was studied in detail to prolong its use (Herron et al. 2010).

Two years ago, Australian processing tomato growers suspected insecticide resistance in *F. occidentalis* was causing reduced efficacy. After consultation with the Australian Processing Tomato Research Council Inc. (APTRC) it was decided to test acephate, spinosad, methidathion, malathion and phorate for resistance. The APTRC further requested two more chemicals be evaluated as the study progressed; firstly thiamethoxam at the end of season one and abamectin in season two.

Following resistance testing a resistance management strategy was developed in collaboration with the APTRC and key industry agronomists. The APTRC considered any sustainable management strategy should consist of a combination of chemical groups, with at least 50% older chemistries. It was considered likely that if the resistance management strategy was heavily reliant upon newer, more expensive chemicals, Australian processing tomato growers would likely switch production to other crops. Here we present the results of that study.

MATERIALS and METHODS

Insecticides

The seven chemicals tested and their current suppliers are listed in Table 1.

Table 1. Common name, trade name, formulation, discriminating concentration (DC) and current supplier of insecticides tested.

Common name	Trade name	Formulation	DC g a.i. / L	Supplier
Acephate	Lancer 750 DF	750 g/kg GR	5.0 (Herron and James 2005)	Nufarm Australia Ltd
Abamectin	Vantal 18	18 g/L EW	0.3 (Herron and James 2005)	Cheminova Australia Pty Ltd
Malathion	Maldison 500 EC	500 g/L EC	0.3 (Herron and James 2005)	Nufarm Australia Ltd
Methidathion	Supracide 400 EC	400 g/L EC	1.0 (Herron and James 2005)	Syngenta Crop Protection Ltd
Phorate	Technical grade	91.6 %	3.0 (This study)	Barmac Industries Ltd
Spinosad	Success Naturalyte	120 g/L SC	0.01 (Herron and James 2005)	Dow AgroSciences Australia Ltd
Thimaethoxam	Actara	250 g/kg GR	0.3 (Herron and Broughton 2006)	Syngenta Crop Protection Ltd

EC, emulsifiable concentrate; EW, emulsion-in-water; GR, granular; SC, suspension concentrate

Strains and their maintenance

Frankliniella occidentalis were collected from each of the two major Australian processing tomato production districts in Victoria, Australia [Boort 'Boor' (36.1165° S, 143.7282° E) and Rochester 'Roc' (36.3619° S, 144.6994° E)]. Two strains were collected early ('E') in the season (one from each district) and two late ('L') in the season, making a total of four strains per season. Early and late strains collected at Boor were sourced from the same field while early and late strains from Rochester were often from fields opposite the initial collection site.

Frankliniella occidentalis for resistance testing were forwarded via overnight courier to the Elizabeth MacArthur Agricultural Institute (EMAI) where they were sorted under a stereo microscope. All thrips species were noted but only *F. occidentalis* were counted and cultured for use in bioassays. Briefly, *F. occidentalis* were reared in purpose built insect proof cages on potted bean plants with Cumbungi (*Typha domingensis* Pers.) pollen and sugar as a supplementary food source. Thrips were given fresh plants on a weekly basis and maintained at 25±1 °C under a 16:8 hour L: D regime (Herron and Gullick 1998).

The phorate baseline study included the reference susceptible strain NZ2 that has been isolated and maintained in an insectary at EMAI for more than a decade under insecticide free conditions. Its response against some 17 insecticides has been previously compared directly to the standard University of California Davis susceptible strain UCD90, with the data supporting its use as a reliable and appropriate reference susceptible (Herron and Gullick 1998). Other strains tested against phorate included the reference resistant strain Oasis known to be highly spinosad (>1000 fold), fipronil and pyrethroid resistant (Herron and Langfield 2011). Two other resistant strains tested, Lach Chil [Western Australia (WA)] and Lach 2 (WA), have known resistance to several chemicals via discriminating dose tests, but specific levels are not known. Finally, two field collected strains Flag Cr and Tent from Queensland, of unknown resistance status were tested. Strains Oasis and Lach Chil were maintained as above and pressured on an *ad hoc* basis with 1.0 and 0.01 g active ingredient (a.i.) / L spinosad respectively, but were not pressured at all during the phorate baseline study.

Bioassay

Methods used are as described in detail in Herron and Gullick (1998). Thrips were anaesthetised with CO_2, tipped onto French bean-leaf discs embedded in agar in small Petri dishes and then sprayed via a Potter spray tower with technical grade phorate diluted directly into reverse osmosis water (RO) (shaken vigorously until forming a suspension of small phorate droplets in water) or with formulated acephate, abamectin, malathion, methidathion and spinosad (Herron and James, 2005), and thiamethoxam (Herron and Broughton, 2006). Phorate baseline establishment required leaf discs with twenty anaesthetised adult *F. occidentalis* in place to be sprayed with serial dilutions of phorate technical insecticide with RO water (set to achieve $0 < x < 100\%$ mortality) (Herron et al. 2014). In contrast, the discriminating dose testing required the application of a single dose (g a.i. / L) using the above method to delineate % susceptible from

resistant *F. occidentalis* (Table 1). All tests were replicated at least once and included a water-only sprayed control (< 15% mortality). The Petri dish was then covered with taut plastic cling-wrap film perforated with fine holes. The dishes were stored for 48 h at 25±0.1 °C in a 18:6 L:D regime after which the numbers of alive and dead *F. occidentalis* were counted. Thrips were classified as dead if individuals did not respond when prodded with a fine paintbrush.

Data analysis

Phorate data were analysed without replicate pooling using a stand-alone probit program developed by Barchia (2001) ensuring variability between replicates was taken into account. The LC_{50} and $LC_{99.9}$ values plus their 95% fiducial-limits (FL) were calculated using the method of Finney (1971) and included control mortality correction when appropriate (Abbott 1925). A minimum effective concentration (MEC) was determined directly from the experimental bioassay data. As distinct from a calculated lethal concentration above, a MEC is the actual observed minimum insecticide concentration required to kill all insects tested across all test replicates (Herron et al. 2014). Discriminating dose data is given as percent mortality (i.e. percent susceptible) following control correction (Abbott 1925).

RESULTS

Phorate baseline study

Not all responses were a good fit to the probit model with strains NZ2, Lach Chil, Flag Cr and Tent all indicating significant heterogeneity ($P < 0.05$) (Table 2). Regression slope values were also variable, ranging from a low of 2.27 (strain Lach 2) to a high of 4.68 (strain Oasis). Calculated $LC_{99.9}$ concentrations ranged from a minimum of 0.84 g/L for strain NZ2 to a maximum of 5.9 g/L for strain Lach 2. Interestingly strain Flag Cr had the highest MEC to kill all insects tested (1.6 g/L) but not the highest calculated $LC_{99.9}$ value.

Table 2. Dose response data for reference susceptible NZ2 and reference resistant and field collected strains of *Frankliniella occidentalis* tested against phorate (91.6% technical).

Strain	Chi-square (df)	Slope (se)	LC_{50}* (95% FL)	$LC_{99.9}$* (95% FL)	MEC*
NZ2	45.11^{+} (13)	4 (0.7)	0.1 (0.078-0.13)	0.84 (0.46-3.1)	0.4
Oasis	3.21 (10)	4.68 (0.52)	0.16 (0.14-0.17)	0.97 (0.69-1.66)	0.8
Lach Chil	52.60^{+} (15)	3.13 (0.4)	0.079 (0.063-0.096)	1.22 (0.67-3.41)	0.8
Lach 2	22.95 (16)	2.27 (0.18)	0.14 (0.12-0.16)	5.9 (3.5-12.16)	0.8
Flag Cr	44.47^{+} (16)	2.99 (0.36)	0.15 (0.12-0.18)	2.6 (1.4-7.0)	1.6
Tent	94.08^{+} (16)	2.84 (0.46)	0.15 (0.11-0.20)	3 (1.3-15.85)	0.8

$^{+}$ Chi-square significant $P < 0.05$; * g a.i. / L; MEC = Minimum effective concentration to kill all test insects; FL = fiducial limit.

Resistance testing

Although not tabulated *F. occidentalis* were only a minor component of all collections (<50 *F. occidentalis* collected per strain from samples containing hundreds to thousands of thrips; including *F. schultzei* and *Thrips tabaci* Lindeman). Methidathion was the only chemical tested to which resistance was not detected (Table 3). Phorate, malathion and acephate bioassays showed a small proportion of resistant individuals in some samples (1-4%) during the 2013-14 season but only acephate and malathion produced resistant survivors in the following 2014-15 season (Table 3). Thiamethoxam resistance was detected during both seasons sampled at a maximum of 9% resistant *F. occidentalis* (strain Boor 14/15E). Abamectin resistance was detected in all strains sampled at a maximum of 9% (Table 3). Spinosad resistance was detected during both seasons with at least two strains comprising more than 50% resistant individuals (77% in strain Roc 13/14L and 71% in Boor 14/15E). Spinosad resistance levels did not always increase as the season progressed and disappeared in strains Boor 13/14L and Boor 14/15 at the end of seasons 2013-14 and 2014-15 respectively.

Table 3. Percent mortality at the discriminating dose (i.e. percent susceptible) for *Frankliniella occidentalis* collected early and late during seasons 2013-14 and 2014-15 and tested for resistance against several chemicals used for their control.

Season	Strain	Phorate	Spinosad	Methidathion	Malathion	Acephate	Thiamethoxam	Abamectin
2013-2014	Boor 13/14E	100	65	100	100	100	100	Nt
	Boor 13/14L	100	100	100	99	100	100	Nt
	Roc 13/14E	*	86	100	*	*	*	Nt
	Roc 13/14L	99	23	100	99	96	99	Nt
2014-2015	Boor 14/15E	100	29	100	100	99	91	97
	Boor 14/15L	100	100	100	100	100	100	97
	Roc 14/15E	100	86	100	96	100	100	95
	Roc 14/15L	100	99	100	100	99	100	91

* = eaten by predatory mite [*Neoseiulus cucumeris* (=*Amblyseius cucumeris*)]; Nt = not tested that season.

DISCUSSION

Phorate baseline study

Strains for the phorate baseline study were sourced widely and included a range of laboratory susceptible, pressured laboratory resistant and resistant field collected strains. Despite the many underlying resistances and consequent resistance mechanisms in the strains tested, responses to phorate consistently showed minimal variation.

The discriminating dose should be set at a rate which covers the upper limits of tolerance of a range of susceptible strains to lessen the chance of a discriminating dose false-positive result. In fact, it is an empirical compromise based on a two-stage process: firstly, define the limits of tolerance; and secondly, based on stage one, select a dose that accounts for all the susceptibles (Herron et al. 2014). The phorate baseline data produced an $LC_{99.9}$ estimate for the susceptible strain NZ2 of 0.84 g/L. In contrast, the highest $LC_{99.9}$ estimate was 5.9 g/L seen in strain Lach 2 with the actual MEC dose somewhere in between at 1.6 g/L in strain Flag Cr. It is notable then that the phorate $LC_{99.9}$ estimate of 5.9 g/L seen in strain Lach 2 is much higher than the observed MEC dose of 0.8 g/L in that strain; we consider it likely the difference can be attributed to the low probit regression slope value of 2.27. The low probit regression slope value therefore has caused an overestimate at the $LC_{99.9}$ level. For that reason we consider it appropriate to choose a dose less than the $LC_{99.9}$ level estimate of the most tolerant strain for phorate resistance monitoring, and propose a compromise discriminating dose of 3.0 g a.i. / L.

Thiamethoxam resistance

Our results provide the first evidence of thiamethoxam resistance in Australian *F. occidentalis*. *Frankliniella occidentalis* tested were from processing tomatoes where thiamethoxam is only available as a mixture of thiamethoxam and chlorantraniliprole known as Durivo® and restricted

to seedling tomatoes only (APVMA 2014). It is possible then that early season thiamethoxam resistance in strain Boor 14/15E was being selected while tomatoes were in the seedling stage; however industry agronomists indicated tomatoes grown were direct seeded and not transplanted seedlings. Additionally thiamethoxam is registered (APVMA Number: 56499) for the control of other insect pests in tomatoes, including the green peach aphid *Myzus persicae* Sulzer and some whitefly species, (APVMA 2014) so theoretically *F. occidentalis* may have been indirectly treated when controlling those pests. Confoundingly, the APTRC confirmed that thiamethoxam had not been used in any of the tomato crops from which thrips samples had been sourced (including adjacent or opposite fields).

Resistance management

Frankliniella occidentalis was only a minor component of all thrips collected with numbers better described as a minor contaminant of *F. schultzei* dominated samples. The reason for *F. schultzei* dominance in all the field collected samples is unclear because the result obtained is more consistent with fields that have not been sprayed. In an unsprayed situation *F. schultzei* quickly displaces *F. occidentalis* to dominate (GAH, unpublished observation), however the processing tomatoes were sprayed yet *F. occidentalis* did not obtain the selective advantage expected. If *F. occidentalis* were susceptible to the sprays they would die, but it is unlikely those sprays would not kill any concurrent *F. schultzei,* and they were found in large numbers. Possibly the sprays were applied in such a manner that only a very small proportion of the total thrips were exposed, allowing interspecies population dynamics to mimic an unsprayed situation. If that is true, spray application practices and methodology should be investigated by industry as a priority since resistance can't be effectively managed if spray application is not optimal. Alternatively, numerical dominance of *F. schultzei* may indicate that resistance has developed in this thrips species as well. For that reason, the authors consider that any future resistance monitoring in processing tomatoes include both thrips species.

Chemical alternation only

Methidathion remains the only chemical tested where resistance was not detected. However, as resistance management of *F. occidentalis* is based on the alternation of chemical groups after each chemical treatment cycle, the first foliar spray can't be the organophosphate methidathion, as phorate is also an organophosphate and its place in any control strategy is fixed i.e. it will always be used first. The first foliar spray needs to be from a different chemical group (e.g. spinosad or abamectin), but it is possible these will be compromised by resistance. Even newer products such as the mixture of thiamethoxam and chlorantraniliprole will likely have some individuals resistant to the *F. occidentalis* controlling thiamethoxam component, leaving only the relatively expensive spirotetramat (Movento® 240 g/L) as a first alternation product. For that reason *F. occidentalis* control in Australian processing tomatoes cannot be based exclusively around old low cost insecticides. Instead, at-planting treatments containing phorate followed by spirotetramat and methidathion in alternation will be required.

Chemical alternation and IPM

With chemicals available to control *F. occidentalis* in processing tomatoes severely limited by resistance, IPM based control is clearly desirable. It has long been known that chemicals alone will not control *F. occidentalis*, and any effective control strategy must include an IPM component of good farm hygiene (Herron et al. 2012). It is a small step then to further include biological controls; it is noteworthy then that the strain Roc 13/14E collected during 2013-2014 was destroyed in culture by the predatory mite *Neoseiulus cucumeris* Oudemans. Effective biological control agents occur in close proximity to processing tomatoes, and so have potential to provide supplementary control.

If growers want to preserve adjunct biological controls then chemical selection is critical (Maas 2014). As the organophosphate insecticide phorate is granular and applied as a side dressing and covered by soil, any acute effect on beneficials should be minimal. The first foliar spray then is the key to preserving beneficials so for that reason it is desirable to use spinosad because it has the lowest beneficial toxicity rating (Mass 2014). However, spinosad resistance detected in fields at Boort was found at high frequencies early in the season (Boor 13/14E and Boor 14/15E). Previous bioassay data indicate spinosad resistance could be at levels compromising product efficacy (Herron and Broughton 2006) so spinosad should be used with extreme caution (Bielza et al. 2008). Surprisingly, spinosad resistance disappeared in strains Boor 13/14L and Boor 14/15L when re-tested late in the season. This was unexpected as *F. occidentalis* are known to be poor dispersers (Rhainds and Ship 2004) so once resistance is selected it would be expected to stay in situ. Thus, the reasons for high level early season spinosad resistance detection subsequently disappearing in these strains may relate to reversion. Spinosad

resistance in *F. occidentalis* is thought to revert in the field without selection and from one season to the next product efficacy may be recovered (Bielza et al. 2008). If spinosad resistance is indeed reverting from one season to the next, its use then as a favourable first foliar spray due to its low beneficial toxicity rating would remain a practical control option for growers. Beyond spinosad, spirotetramat too has a low beneficial toxicity rating but is costly for growers and thus often not used. The mixture of thiamethoxam and chlorantraniliprole (Durivo®) has a moderate beneficial toxicity rating but registration does not include broad-acre use and thiamethoxam resistance may further compromise efficacy. Methidathion, acephate and malathion have low or no resistance but are all foliar applied organophosphates and have a high beneficial toxicity rating (Mass 2014) so use should be avoided.

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