AUSTRALIAN BASELINE DATA FOR WESTERN FLOWER THRIPS (*FRANKLINIELLA OCCIDENTALIS*) SUSCEPTIBILITY TO CYANTRANILIPROLE (DPX-HGW86) AND THE ESTABLISHMENT OF A DISCRIMINATING DOSE FOR RESISTANCE DETECTION

Grant A. Herron[1], Kate L. Marshall[1] and Geoff W. Cornwell[2]

[1]NSW Department of Primary Industries, PMB 4008, Narellan 2567, Australia and [2]DuPont (Australia) Pty Ltd, Locked Bag 2067, North Ryde BC, 1670, NSW, Australia
Email: grant.herron@dpi.nsw.gov.au

Summary

Laboratory susceptible, reference resistant (spinosad pressured) and field collected (multiple resistances) western flower thrips *Frankliniella occidentalis* (Pergande) were tested against formulated cyantraniliprole using a Potter spray tower to generate baseline response data. All test populations were killed at 8 g/L, with calculated $LC_{99.9}$ level responses for the six strains ranging from 7.77 to 28.48 g/L. A cyantraniliprole discriminating dose of 25 g/L for the purpose of resistance monitoring in *F. occidentalis* is proposed.

Keywords: ryanodine receptor, anthranilic diamides, Cyazypyr®

INTRODUCTION

Western flower thrips 'WFT', *Frankliniella occidentalis* (Pergande) is a serious pest of vegetables, fruit and ornamental crops in Australia (Herron *et al*. 1996) and worldwide (Kirk and Terry 2003). *F. occidentalis* causes damage directly by feeding and ovipositing on plant parts, and indirectly by transmission of several viruses including tomato spotted wilt virus (TSWV) (Ullman *et al*. 1997) that causes significant damage to susceptible crops such as lettuce or tomato (Herron *et al*. 2012). Chemical sprays have been the primary method used by growers to control *F. occidentalis* in Australian field crops, although alternate control strategies exist (Steiner and Goodwin 2005).

The use of chemical insecticides against *F. occidentalis* has led to the development of resistance to major insecticide groups, including the organophosphates, carbamates and pyrethroids (Herron and Gullick 2001), which was soon followed by resistance to fipronil and spinosad (Herron and James 2005). Resistance in Australia is managed via chemical group alternation in combination with a *F. occidentalis* specific three spray strategy (Broughton and Herron 2007).

Newer chemistries such as thiamethoxam, spirotetramat and spinetoram have been registered in Australia for *F. occidentalis* control and are now commercially available for use (APVMA 2013). These newer chemistries however are not available in Australia for use on all crops affected by *F. occidentalis* and additional products are required for an optimal resistance management plan (Herron *et al*. 2012). Cyantraniliprole, a chlorantraniliprole analogue, is a second-generation ryanodine receptor insecticide discovered by DuPont Crop Protection, and is structurally similar to chlorantraniliprole except it has a cyanosubstituent replacing a chlorine atom on the anthranilic core using a process patented from 2008 (Jeanguenat 2013). Cyantraniliprole's chemical properties differ from a classical diamide (a chemical compound containing two amide groups), by having a broader insecticidal spectrum (Jeanguenat 2013), making it likely that cyantraniliprole will be used in Australia to control *F. occidentalis*. No baseline resistance data to underpin resistance monitoring currently exists for this chemical.

Here we test *F. occidentalis* strains against formulated cyantraniliprole to generate baseline toxicity data to allow calculation of a reliable discriminating dose for resistance monitoring.

MATERIALS AND METHODS

Insecticides

Cyantraniliprole was supplied by DuPont (Australia) Pty Ltd as formulated (Cyazypyr®) (DPX-HGW86) (100 g/L oil dispersible liquid [OD]).

Insects

The reference susceptible strain of *F. occidentalis* known as 'NZ2' was sourced from lupins in New Zealand and has been isolated and maintained for more than a decade under insecticide free conditions. The lupin strain response against 17 insecticides was previously compared directly to the standard University of California, Davis susceptible strain 'UCD90' with the data supporting use of 'NZ2' as a reliable and appropriate susceptible reference strain

(Herron and Gullick 1998). WFT were reared in purpose built rearing cages on potted bean plants with Cumbungi (*Typha domingensis* Pers.) pollen and sugar as supplementary food sources. Thrips were transferred onto fresh plants in a new cage on a six weekly cycle and maintained at 25±1 °C under a 16:8 hour L:D regime. Other strains tested included the reference resistant strain 'Oasis' that is known to be highly spinosad (>1000 fold), fipronil and pyrethroid resistant (Herron and Langfield 2011). Another reference resistant strain known as 'Lach Ch' is also spinosad resistant (as confirmed via discriminating dose tests), while strain 'Lach 2' was similarly confirmed as resistant to several chemicals including abamectin, bifenthrin, dichlorvos and spinosad. Finally, two field collected strains known as 'Flag Cr' and 'Tent' of unknown resistance status were supplied by DuPont (Australia) Pty Ltd. Strains 'Oasis' and 'Lach Ch' were maintained in culture as above, and outside the experimental period they were pressured on an *ad hoc* basis with 1.0 and 0.01 g/L spinosad respectively, while all other strains were not pressured.

Bioassay

Methods used are as described by Herron and Gullick (2001) except the period between treatment and assessment was extended to 96 h, the temperature was increased to 26 °C and all diluents included the addition of Tween 60 as an emulsifier. Briefly, thrips (mean 19) were anaesthetised with CO_2 and then tipped onto French bean leaf discs embedded in agar in small petri dishes. The leaf discs with anaesthetised adult thrips in place were sprayed with aqueous insecticide (range 0.000024-0.8 g/L) plus 0.5 g/L Tween 60, or 0.5 g/L Tween 60 alone (control) using a Potter spray tower. The Petri dish was covered with taut plastic cling wrap film perforated with fine aeration holes. Tests were replicated at least once and control mortality did not exceed 20%. The dishes were stored for 96 h at 26±0.1 °C in a 16:8 L:D regime after which the numbers of alive and dead (defined as the inability to move when probed) thrips were counted.

Data analysis

Bioassay data were analysed without replicate pooling using a stand alone probit program developed by Barchia (2001) that ensures variability between replicates is taken into account during the analysis. The program applies the method of Finney (1971) including data adjustment for natural mortality (Abbott 1925). Significant heterogeneity is identified using a χ^2 test and if significant at the 5% level, the variance of the estimated parameter is scaled by the corresponding heterogeneity factor equal to the residual mean deviance (Finney 1971). Differences between dose responses were determined by pair wise comparison of their ratios at LC_{50} and $LC_{99.9}$ with significance determined when their 95% confidence interval did not overlap one (Robertson *et al*. 2007). A minimum effective concentration (MEC) required to kill all insects of a particular strain was determined directly from the experimental bioassay data. As distinct from a calculated lethal concentration, it is the lowest insecticide concentration from the serial concentration dose-response data that killed all insects tested across all replicates, and thus has no variance.

RESULTS

All probit regressions were a fit to the model except 'Lach Ch' and 'Flag Cr' that both had excessive heterogeneity and a scaled fiducial limit calculation (Table 1). Regression slope values were low ranging from 0.7 to a high of 1.1. Calculated LC_{50} concentrations ranged from 0.0018 to 0.023 g/L in strains 'Oasis' and 'Tent' respectively. Pair wise comparison of the LC_{50} level response ratios indicated some were significantly different (as indicated by the 95% confidence interval not overlapping one) including the reference resistant strain 'Oasis' being less tolerant than the reference susceptible 'NZ2' strain (Table 2). The $LC_{99.9}$ level results showed strain 'Lach Ch' to have the lowest calculated minimum value of 3.43 g/L and strain 'Flag Cr' a maximum value of 28.48 g/L. In contrast to the LC_{50} result, all $LC_{99.9}$ level pair wise comparisons were similar. Strain 'NZ2' had the highest MEC to kill all insects tested yet its calculated $LC_{99.9}$ value was second lowest.

Table 1. Dose response data for the reference susceptible 'NZ2', reference resistant and field collected *Frankliniella occidentalis* strains tested against cyantraniliprole.

Strain	Chi-square (df)	Slope (se)	LC_{50}* (95% FL)	$LC_{99.9}$* (95% FL)	MEC*#
'NZ2'	20.52	1.1	0.011	7.77	8
	(25)	(0.11)	(0.0076-0.016)	(2.86-33.40)	
'Oasis'	32.97	0.8	0.0018	18.85	4
	(24)	(0.083)	(0.00085-0.0032)	(4.88-143.67)	
'Lach Ch'	57.51^{+}	1.1	0.0053	3.43	1
	(28)	(0.15)	(0.0028-0.0086)	(0.97-30.14)	
'Lach 2'	25.47	0.9	0.0065	18.22	4
	(18)	(0.088)	(0.0040-0.0095)	(5.60-101.73)	
'Flag Cr'	44.83^{+}	0.7	0.0022	28.48	4
	(31)	(0.088)	(0.00097-0.0040)	(5.79-360.69)	
'Tent'	32.79	1.0	0.023	23.03	4
	(16)	(0.16)	(0.011-0.045)	(4.14-600.03)	

$^{+}$ Chi-square significant $p<0.05$
* g ai/L
# Minimum effective concentration to kill all test insects (g/L)

Table 2. LC_{50} and $LC_{99.9}$ dose response ratios with 95% confidence intervals (CI) for *Frankliniella occidentalis* strains tested against cyantraniliprole.

Denominator	NZ2' $LC_{99.9}$ ratio (95% CI)	'Oasis' LC_{50} ratio (95% CI)	'Lach Ch' LC_{50} ratio (95% CI)	'Lach 2' LC_{50} ratio (95% CI)	'Flag Cr' LC_{50} ratio (95% CI)	'Tent' LC_{50} ratio (95% CI)
'NZ2' $LC_{99.9}$ ratio (95% CI)	1.00	0.16 (0.075-0.34)	0.47 (0.25-0.89)	0.57 (0.32-1.00)	0.19 (0.089-0.41)	2.03 (0.98-4.21)
'Oasis' $LC_{99.9}$ ratio (95% CI)	0.41 (0.053-3.19)	1.00	2.94 (1.28-6.76)	3.58 (1.64-7.79)	1.20 (0.47-3.03)	12.76 (5.17-31.45)
'Lach Ch' $LC_{99.9}$ ratio (95% CI)	2.27 (0.31-16.29)	5.50 (0.57-53.25)	1.00	1.22 (0.62-2.38)	0.41 (0.17-0.94)	4.33 (1.92-9.77)
'Lach 2' $LC_{99.9}$ ratio (95% CI)	0.43 (0.066-2.77)	1.03 (0.12-9.18)	0.19 (0.023-1.55)	1.00	0.33 (0.15-0.74)	3.56 (1.67- 7.59)
'Flag Cr' $LC_{99.9}$ ratio (95% CI)	0.27 (0.028-2.63)	0.66 (0.053-8.30)	0.12 (0.010-1.42)	0.64 (0.059-6.96)	1.00	10.64 (4.28-26.46)
'Tent' $LC_{99.9}$ ratio (95% CI)	0.33 (0.030-3.85)	0.82 (0.056-11.95)	0.15 (0.011-2.05)	0.79 (0.062-10.11)	1.24 (0.071-21.39)	1.00

LC = Lethal concentration

DISCUSSION

The extended assay holding period (96 h as opposed to the standard 48 h used in such bioassays) significantly reduced the LC_{50} response against cyantraniliprole for the reference susceptible strain 'NZ2' by about an order of magnitude (unpublished data). Regardless of this, concentrations needed to exert a dose-response remained high. Further extending the bioassay withholding period to 120 h significantly reduced concentrations required to fully control the test strains but control mortality was unacceptably high beyond 96 h with a 28% maximum recorded at 120 h and 80% at 144 h (unpublished data).

As the bioassay data presented is a compromise between bioassay endpoint and unacceptable control mortality, it may not extrapolate reliably to true field performance. For instance, current DuPont Australia data suggest cyantraniliprole would likely be registered at a rate equivalent to 75 g ai/ha to control *F. occidentalis* in 500 L water/ha. This would produce a dose of 0.15 g/L that is well below the MEC (8 g/L) required to achieve 100% control of the *F. occidentalis* tested here. However, the comparison is not valid because if left longer than 96 h, mortality would have increased further, so the data presented here is useful only as a laboratory tool to detect resistance.

Cross-resistance explains a phenomenon where insects resistant to one insecticide are immediately resistant to another to which they had no previous exposure (Yu 2008). No strain tested had been previously exposed to cyantraniliprole. Strains 'Lach Ch' and 'Oasis' were highly spinosad resistant with the latter strain also resistant to fipronil and pyrethroids. In addition, strain 'Lach 2' was resistant to abamectin, bifenthrin and dichlorvos. As strain 'Oasis' was significantly more susceptible than reference susceptible 'NZ2' at LC_{50} and there were no significant differences between 'NZ2' and 'Lach 2' at the LC_{50} or $LC_{99.9}$ level, cross resistance to cyantraniliprole is unlikely.

A discriminating dose can be set at double the representative MEC or calculated at the $LC_{99.9}$ level of a reference susceptible strain (Busvine 1971). Essentially, it should be lethal to susceptibles in a population without affecting the resistant types. It is an empirical compromise based on a two-stage approach of defining the limits of tolerance, and then selecting a dose that accounts for all of the susceptibles. The baseline study here has defined the limits of tolerance to 8 g/L cyantraniliprole yet the most tolerant strain produced an $LC_{99.9}$ estimate of 28.48 g/L cyantraniliprole being more than 3 fold the MEC being likely due to relatively low probit regression slope values.

We propose a discriminating dose of 25g/L cyantraniliprole for the purpose of resistance monitoring in Australian field collected *F. occidentalis*. This dose is relatively high compared to the MEC data and so may be less sensitive for detection of low-level resistance. Conversely, it should be quite robust at delineating susceptible from resistant genotypes therefore minimising, the risk of producing a false positive result.

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