SPINETORAM RESISTANCE DETECTED IN AUSTRALIAN WESTERN FLOWER THRIPS *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) FROM QUEENSLAND AND VICTORIA

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Summary

A control failure to spinetoram was recently detected in a population of western flower thrips *Frankliniella occidentalis* (Pergande) from Western Australia that was verified to be resistant. Here we present data from thrips sampled at one location in Victoria and three locations in Queensland to determine if resistance is restricted to Western Australia only. Resistance was detected in both states but not in every population sampled with an LC_{50} Resistance Factor (RF) range (95% confidence interval in brackets) of 5.6 (3.8-8.4) to 55.7 (41.2-75.4) fold. Unexpectedly a response consistent with negative cross resistance was detected in some strains with one having a RF of 0.1 (0.06-0.2) fold.

Keywords: resistance management, *Frankliniella occidentalis,* negative cross resistance

INTRODUCTION

Western flower thrips, *Frankliniella occidentalis* (Pergande), was first detected in Australia in Perth, Western Australia during April 1993 (Malipatil *et al*. 1993) and by July 1996 had quickly spread to all other mainland Australian states (Miller and Moran 1996). *Frankliniella occidentalis* that occur in Australia are resistant to many insecticide classes used for their control, including spinosad (Herron *et al*. 2014). Australian growers relied heavily on spinosad for management of *F. occidentalis,* with uses in a range of crops including apples, brassicas, cucurbits, fruiting vegetables, leafy vegetables, legumes, ornamentals, potatoes, pears and stone fruit (Herron *et al*. 2014).

Spinetoram subsequently replaced spinosad in Australia for *F. occidentalis* control with label extensions including forestry and cotton (APVMA 2019) and remains a primary chemical control option due high beneficial selectivity and more than 70 registered uses (Corteva 2019). Spinetoram is a semi-synthetic active ingredient from the spinosyn chemical class, derived from fermentation of a soil organism *Saccharopolyspora spinosa,* followed by chemical modification of spinosyns J and L (Dripps *et al*. 2008). Like spinosad, spinetoram displays excellent efficacy against insect pests while still maintaining low mammalian toxicity and short environmental persistence (Dripps *et al*. 2008). Spinetoram and spinosad are therefore similar and spinosad resistance in Australian *F. occidentalis* is known (Herron and Langfield 2011) as is cross resistance between spinosad and spinetoram (Sparks *et al*. 2012).

A field failure was investigated in 2017 following use of spinetoram against *F. occidentalis* in stone fruit in Western Australia (Langfield *et al*. 2018). To determine if these reports were a consequence of resistance, a full log-dose response was produced for a field collected strain known as 'Spin WA'. When results were superimposed against the spinetoram recommended field rate (40 mL / 100 L or 0.048 g a.i. / L) data suggested control failure in combination with a 19 fold LC_{50} level resistance (Langfield *et al*. 2018).

The objective of the following study is to determine if spinetoram resistance documented by Langfield *et al*. (2018) in *F. occidentalis* was an isolated occurrence. Here we test a further six field collected *F. occidentalis* strains collected in two Australian states and a range of crops to determine their sensitivity to spinetoram.

MATERIALS AND METHODS

Chemical tested

Spinetoram 120 g / L (Success™ Neo insecticide) was supplied by Corteva Agriscience™, Agriculture Division of DowDuPont ™.

Strains and their maintenance

Thrips comprising all life stages available on leaf material were collected directly from host plants in Queensland and Victoria. Thrips were collected and dispatched according to an ISO (International Organisation for Standardisation) 9001 compliant standard operating procedure (SOP) established for that purpose. It required the collection of not less than 20 infested flowers or leaves with a minimum of 50 thrips on each while walking through the crop. Collections Cla4AB, Cla21 and ClaB19 where

collected from the same farm on the same day with other collections from different farms. Thrips on host material was then packaged according to the ISO 9001 SOP and transported via overnight courier to the Elizabeth Macarthur Agricultural Institute. Thrips were then chilled to stop movement and species identity confirmed with the aid of a stereomicroscope (Horticulture Australia 2002). All thrips were maintained on potted French bean (*Phaseolus vulgaris* L.) in an insectary maintained at 25±2°C and isolated in insect proof cages to ensure strain integrity (Herron and Gullick 1998). Strains tested included our reference susceptible NZ2 (collected off wild lupins in New Zealand (Herron and Gullick 1998) and since isolated and maintained under insecticide free conditions) and six field collected populations (Table 1). Strains were maintained for several weeks on insecticide free French bean whilst being tested and then discarded

Bioassay

A bean leaf disc procedure for generating full log-dose probit regressions, as described in Herron and Gullick (1998), was used. Briefly, 15 to 25 adult thrips were lightly anaesthetised with CO_2 and then transferred onto French bean leaf discs embedded in agar in small Petri dishes. The leaf discs containing anaesthetised thrips were then sprayed with serially diluted insecticide (4 mL aliquot) or with water (control) with the aid of a Potter spray tower (Burkard Scientific, Uxbridge, Middlesex, UK) producing an aqueous deposit of 3.2 mg / cm^2. Dose range always included 100% mortality then at least three to a maximum of seven lower doses. All tests were replicated three to four times and included a water only sprayed control that did not exceed 15% control 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 16:8 h L:D regime after which the number of alive and dead (defined as an inability to stand when prodded) thrips were counted.

Bioassay data were analysed without replicate pooling using a stand-alone probit program developed by Barchia (2001) that ensured variability between replicates was taken into account. LC_{50} values plus their 95% fiducial-limits (FL) were calculated using the method of Finney (1971) and included control mortality correction (Abbott 1925). LC_{50} values were used to calculate a resistance factor (RF) (LC_{50} field strain / LC_{50} strain NZ2) plus its associated 95% confidence interval (CI). As outlined in Robertson *et al*. (2007) the 95% CI was used to determine significance between dose responses and was considered statistically different when the CI did not overlap one.

RESULTS

Responses against spinetoram were variable producing LC_{50} estimates ranging from 0.00010 (95% fiducial limit (FL) 0.00003-0.00025) to 0.049 (95% FL 0.037-0.063) g / L (Table 1). Interestingly, the response for the reference susceptible NZ2 strain was approximately centred in the data set and was not the most susceptible although its response is statistically equivalent (as indicated by LC_{50} 95% fiducial limit overlap) to that previously produced against spinosad in this laboratory (see Herron and Langfield (2011)). Response ratios ranged from 0.1 (95% CI 0.060-0.23) in strain Cla4AB to 55.7 (95% CI 41.17-75.45) in strain ClaB19 with both from the Clare region Queensland.

DISCUSSION

The present study expands on the initial 19-fold Western Australian resistance detection (Langfield *et al*. 2018) with a further six strains of *F. occidentalis* sourced from Queensland and Victoria. Resistance was detected in 3 of 5 strains from Queensland, with the highest level of 55.7 fold, and in the single strain from Victoria at 12.8 fold. Three Queensland strains remain susceptible with one from Clare, Queensland (Cla4AB), particularly so.

In fact, the response is significantly less than that of reference susceptible NZ2 and is suggestive of an undocumented negative cross resistance (where increasing resistance to one insecticide causes increasing susceptibility to another). Limited negative cross resistance has been recently found between spinetoram and Cry1Ac in cotton bollworm *Helicoverpa armigera* from China (Wei *et al*. 2018) with Visnupriya and Muthukrishnan (2017) suggesting negative cross resistance to conventional chemistry also possible in the fruit borer *Leucinodes orbonalis* in India.

Spinosyn resistance is in *F. occidentalis* is thought recessive although an incompletely dominant finding is known (Sparks *et al.* 2012). Resistance management of *F. occidentalis* in Australia is well established (Herron and Cook 2002) and included on every product label including spinetoram (Corteva 2019). Despite favourable resistance genetics and established resistance management spinetoram resistance has continued to be detected beyond Western Australia.

This is worrisome as spinetoram has more than 70 crops specific registrations and is known IPM friendly (Corteva 2019) with uncontrolled resistance in *F. occidentalis* potentially undermining sustainable IPM based agriculture. Clearly further study is required to ensure sustainability is not undermined that should 1. Define the scope of the problem i.e. which crops and at what frequency or level and where. 2. Find the reason why resistance is increasing in the areas identified and 3. Initiate an extension campaign based on the results of one and two with the aim of preserving product sustainability.

Table 1. Dose response data for reference susceptible and field collected western flower thrips *Frankliniella occidentalis* against spinetoram.

Strain	Location	Host Plant	Chi-sq. (DF)	Slope (SE)	LC_{50} g/L (95% FL)	RF (95% CI)
NZ2	Laboratory	Laboratory	21.5 (10)	3.6 (0.56)	0.00088 (0.00070-0.0011)	reference
Cla4AB	Clare, QLD (Lat: -19.8205, Long: 147.2273)	Capsicum	33.6 (6)	2.7 (1.15)	0.00010 (0.00003-0.00025)	0.1 (0.06- 0.2)
Cla21	Clare, QLD (Lat: -19.8205, Long: 147.2273)	Capsicum	7.6 (10)	4.1 (0.45)	0.00027 (0.00024-0.00031)	0.3 (0.2-0.4)
GumSB	Gumlu, QLD (Lat: -19.8890, Long: 147.7429)	Capsicum Chilli and Eggplant	19.8 (10)	2.6 (0.39)	0.00033 (0.00024- 0.00046)	0.4 (0.3-0.5)
Aero	Applethorpe, QLD (no coordinates noted)	Apple	40.7 (22)	1.1 (0.16)	0.0049 (0.0034-0.0074)	5.6 (3.8-8.4)
Bhat	Ardmona, VIC (no coordinates noted)	Pear	9.7 (14)	1.7 (0.22)	0.01125 (0.00850-0.01425)	12.8 (9.4-17.5)
ClaB19	Clare, QLD (Lat: -19.8744, Long: 147.2273)	Eggplant	65.5 (20)	2.3 (0.29)	0.049 (0.037-0.063)	55.7 (41.2-75.4)

DF = degrees of freedom, SE = standard error, FL = fiducial limit, RF = resistance factor and CI = confidence interval, VIC = Victoria, QLD = Queensland, WA = Western Australia

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