The sustainable use of pesticides (especially spinosad) against WFT in vegetables

Dr Grant Herron
NSW Department of Industry and Investment

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FINAL REPORT

"The sustainable use of pesticides (especially spinosad) against WFT in vegetables"

VG06010

01 July 2006 to 31 May 2010

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Purpose of the report: Outlines resistance monitoring data, cross resistance data, resistance mechanism data, molecular genetics data, glasshouse field trial data designed to better understand spinosad resistance and improve WFT chemical control and management.

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Media Summary

Western flower thrips (WFT) *Frankliniella occidentalis* (Pergande), is a serious pest of vegetable crops. Feeding by adults and larvae causes severe physical crop damage, and WFT also spreads viruses such as tomato spotted wilt virus (TSWV) that can wipe out entire crops. Insecticides are the most common method of controlling WFT, but since WFT develops insecticide resistance, a national resistance management strategy was developed. The strategy requires that growers alternate an insecticide from one chemical class, followed by the use of another insecticide from a different chemical class with a different mode of action. Recently, insecticide resistance monitoring has detected WFT populations resistant to spinosad. Spinosad is a unique insecticide in that it is highly efficacious against WFT, but can be used in an integrated pest management program (IPM). Spinosad is now considered to be at risk due to resistance.

Resistance monitoring for WFT against several key registered products including spinosad in this project (VG06010) demonstrated that spinosad resistance is increasing in both level and abundance. Resistance was found to be still increasing and now exceeds 200 fold in one strain, with 100 fold resistance in four of the 14 strains tested. Monitoring showed that 43% of the strains collected contained 20% or more resistant WFT, and 28% of the strains contained 30% or more resistant WFT. Of the more resistant strains, 75% were associated with control failure in the field.

To improve resistance management, a more detailed knowledge of how WFT develops resistance to spinosad is required. We examined the mechanism of spinosad resistance using three methods, bioassays, molecular methods and by determining the cross resistance profile for spinosad. Cross resistance occurs when resistance to one insecticide (such as spinosad) confers resistance to another insecticide. Cross-resistance testing against existing insecticides identified chlorfenapyr (Secure®) and methidathion (Nitofol®) as suitable alternation candidates for spinosad to help manage resistance, but the chloronicotinyl chemicals (eg imidacloprid, Confidor®) were not considered suitable for alternation with spinosad. This information is very important because growers may be using inappropriate chemicals as an alternation partner with spinosad, which exacerbates resistance. Growers may also be including an inappropriate chemical in a mixture with spinosad to control multiple pests.

To identify resistance pathways, bioassays were used in combination with chemicals that interfere with specific resistance pathways. Using this process we eliminated detoxification (enzymes produced by WFT that can break down chemicals) as the likely cause of spinosad resistance. As detoxification was unlikely, it allowed the subsequent molecular genetic studies to concentrate on target site resistance (the biochemical site where the insecticide works) as the primary cause of spinosad resistance. Susceptible and resistant WFT strains were compared using molecular genetic techniques which identified a specific genetic difference that seems to be associated with spinosad resistance in spinosad resistant WFT. The sequencing of the nAChR receptor Dα6 cDNA sequence from spinosad resistance and susceptible WFT revealed a singe nucleotide substitution (C>T), but more research is needed to confirm exactly what it does. However, since resistant thrips are genetically distinct, the mutation should be further studied to develop a molecular based method to detect spinosad resistance. We are confident that it will be possible to detect resistance ‘in real time’, with the information used to support spray decisions.

To relate spinosad resistance to field control in WFT, we trialled specific frequencies of susceptible and resistant thrips under glasshouse conditions. From this study we concluded that spinosad resistance affected control, and the data indicated that spinosad resistance would

adversely affect control when a population was comprised of 10-40% resistant individuals. This is encouraging information because we additionally consider that spinosad resistance could probably be managed with IPM. Interestingly, the study also found that WFT was never fully controlled even if the population is completely susceptible. However, more research is required to more precisely define where resistance becomes an issue in the 10-40% range.

In conclusion, spinosad resistance doesn't necessarily mean that growers will experience total control failure. However, resistance monitoring indicates that if WFT management continues at present, then resistance levels to key chemicals such as spinosad will continue to rise. A radical new way in which growers manage WFT is required to change this! Resistance management needs to better integrate with IPM as a matter of urgency. Funding is required to develop IPM programs in the field and glasshouse for WFT. New resistance monitoring methodology (both bioassay and molecular) needs to be developed for new strategic chemicals that affect WFT growth, or are slower acting (e.g. Movento®). Old methodology is currently based on insecticides that kill WFT within a short period of time.

Finally, several new chemicals that could possibly be used for WFT control have been studied. The new active ingredients pyridalyl, acrinathrin, clothianidin and DPX-HGW86 were tested, with the latter product showing the most promise for WFT control.

Article II. Technical Summary

(a) Project outputs and outcomes: success and failures

Outputs

- *Continued resistance monitoring for WFT against strategic chemicals (including spinosad)*. Nineteen chemicals were screened for resistance. Resistance to spinosad was found to be increasing and exceeded 200 fold in one strain, with 100 fold resistances in four of the 14 strains tested. Resistance monitoring showed 43% of strains collected contained 20% or more resistant WFT, and 28% contained 30% or more resistant WFT. Of the more resistant strains, 75% were associated with spinosad field control failure when collected.
- *A cross-resistance profile for spinosad against existing chemicals used for its control will be established*. Abamectin, acetamiprid, chlorfenapyr, fipronil, methamidophos, methomyl, thiamethoxam, and spinosad were evaluated against susceptible and spinosad resistant WFT to determine cross-resistance. Cross-resistance testing indicated likely problems with the neonicotinoid chemicals acetamiprid and thiamethoxam; their alternation with spinosad should be avoided. Similarly, fipronil cross-resistance is considered highly likely and it should not be used in rotation or in a tank mix with spinosad. Abamectin, methomyl, chlorfenapyr, and methamidophos did not show cross–resistance to spinosad.
- *The mechanism of spinosad resistance will be identified using bioassay, biochemical and molecular methods*. Bioassays in combination with biochemical synergists (piperonyl butoxide (PBO) used to detect cytochrome P450 monooxygenases, diethyl maleate (DEM) used to detect glutathione-S-transferases and triphenyl phosphate (TPP) used to detect esterases) were used to eliminate metabolic detoxification as a cause of spinosad resistance. Subsequent molecular genetic assays then concentrated as target site mutations as a likely cause of resistance and sequencing of nAChR receptor Dα6 cDNA sequence from spinosad resistance and susceptible WFT revealed a singe nucleotide substitution (C>T).
- *The field impact of resistance (ie at what level will spinosad cause control failure will be related to specific resistance frequencies using molecular methods developed in the project*. Specific frequencies of susceptible and resistant WFT were released onto caged whole plant plots and left to breed for two weeks. Thrips were then sprayed with spinosad at the registered rate, three days apart according to be published chemical use strategy. Results suggested that resistance causes spinosad failure between 10 and 40% resistant WFT.

Outcomes

- *A revised resistance management strategy for spinosad that allows sustainable product use (especially spinosad)*. Resistance monitoring over the life of the study indicated that if WFT management continues as it has till now, resistance levels to key chemicals such as spinosad will continue to rise. Growers will need to change their current management practices and we consider that resistance management should be integrated with IPM as a matter of urgency.
- *If spinosad resistance is related to mutations within the nAChR or GABA receptors it will be possible to create a molecular test for resistance using PCR and restriction enzymes*. The sequencing of nAChR receptor Dα6 cDNA sequence from spinosad resistance and susceptible WFT revealed a singe nucleotide substitution (C>T). However, more research is needed to confirm exactly what it does and so create a molecular test for resistance using PCR and restriction enzymes.

- *A revised WFT resistance management strategy that includes both cross-resistance and mechanism knowledge*. Cross-resistance testing suggests that there are likely to be problems if the neonicotinoid chemicals acetamiprid and thiamethoxam are used in alternation with spinosad. Similarly, fipronil cross-resistance is a possibility and its use should also be avoided with spinosad. This should be included into a revised strategy that integrates strongly with IPM.

(b) WFT and insecticide resistance

Insecticide resistance was monitored in field collected populations of WFT from 2006-2009. WFT were screened against nineteen chemicals, but not all strains were screened against all products. Organophosphate resistance was sporadic and generally low level except for dichlorvos and methamidophos resistance in strain Ornamental F 05/2007, which showed 68 and 78 percent susceptible respectively. Fipronil resistance when detected was at a low frequency, with a maximum of 11% resistant individuals in strain Capsicum Penfield 10/2007. Spinosad resistance was detected in eight of the fourteen strains tested (60% of strains), with four strains having resistance levels above 100-fold, with a maximum of 201-fold in strain cucumber Rossmore 03/2007. Spinosad resistance continues to increase in both level and abundance putting sustainable spinosad use against WFT at risk.

(c) WFT and cross-resistance studies

Cross-resistance refers to the situation where resistance to one insecticide confers resistance to other insecticide to which the insect has not been exposed. Here we studied cross-resistance in western flower thrips (WFT) that were spinosad resistant by exposing susceptible and resistant strains to existing and potential chemicals used for their control and compared their response. Strains were tested against abamectin, acetamiprid, chlorfenapyr, fipronil, methamidophos, methomyl, spinosad and thiamethoxam. Field strains tested were highly spinosad resistant and results suggest that cross-resistance to the chloronicotinyl (neonicotinoid) insecticides acetamiprid and thiamethoxam is likely from spinosad use. Their alternation with spinosad should be avoided. Similarly, fipronil cross-resistance is also considered a possibility and its use should also be avoided with spinosad. Abamectin and methomyl did not show cross–resistance, but their alternation with spinosad is compromised for other reasons. Chlorfenapyr and methamidophos are the only two products likely to be useful for alternation with spinosad. Even so, methamidophos use in Australia is restricted to ornamentals (registered) and head lettuce only (permit PER10416). Chlorfenapyr is currently restricted for use in spring onion and shallots (permit PER11508) for WFT control in Australia.

(d) WFT resistance mechanisms - bioassay with synergists

Resistance is caused by a number of mechanisms with most being either target site or detoxification. The presence of specific detoxification enzymes can be deduced with synergists that can neutralise specific enzyme detoxification pathways. As compared to the response of a spinosad pressured and resistant strain LRp, the addition of synergists did not significantly influence (by overlapping 95% CI) the RF_{50} (resistance Factor at the LC_{50} level) ratio for abamectin, spinosad or thiamethoxam. Interestingly, the RF_{50} ratio was significant for acetamiprid with the monooxygenase synergist PBO (piperonyl butoxide), but not for the other synergists tested. Calculated S (synergist) ratios were similar in that there was a significant difference with acetamiprid and PBO. Unlike the RF_{50} ratios, there was an additional significant difference with the esterase synergist TPP (triphenyl phosphate) and abamectin. As there were no significant differences with any synergist detected with spinosad,

we suspect resistance may be target site rather than metabolic. As the synergist study eliminated metabolic detoxification as a cause of spinosad resistance, the molecular component of the project VG06010 can pursue the target site resistance model of *Drosophila melanogaster* Meigen with a high degree of confidence.

(e) WFT resistance mechanisms - molecular genetics and spinosad resistance

Improved management of spinosad resistance requires a good understanding of the spinosad resistance mechanism so products causing cross-resistance can be eliminated from the chemical control strategy. As part of this process we aimed to find the molecular basis for spinosad resistance to improve resistance management by developing a molecular diagnostic method to monitor spinosad resistance in WFT. By immunoprecipitation we identified 10 candidate spinosad binding proteins in WFT. We also obtained 4 partial cDNA sequences for acetylcholine receptor Dα6. A singe nucleotide substitution (C>T) from spinosad resistance and susceptible WFT stains has been identified showing a clear difference between spinosad susceptible and resistant WFT at the molecular level. These findings provided the first useful information for understanding the molecular mechanisms of spinosad resistance in WFT as a first step to developing a molecular based diagnostic assay.

(f) The relationship between spinosad resistance and field control

Susceptible and resistant WFT were prepared at EMAI by placing them in vials at specific initial resistance frequencies. Each vial contained 50 WFT adults that were ready for use immediately in WA. An initial trial done in 2008 included ratios of 100% susceptible (sprayed), 10% resistant (sprayed), 20% resistant (sprayed), 40% resistant, (sprayed) 60% resistant (sprayed) and 80% resistant (sprayed). Thrips were released onto caged whole plant plots and left to breed for two weeks. Thrips were then sprayed with spinosad at the registered rate, three days apart according to be published resistance strategy. The initial trial was replicated twice but plant quality declined during trial due to problems with fungi. At the end of the trial whole plants were destructively sampled and total number of adults and larvae counted. Interestingly, not all susceptible WFT were killed with 4 adults and 22 larvae counted. Based on that initial data we consider chemical control with spinosad seemed to fail somewhere between 20 and 30% resistant WFT. A second trial was under taken in 2009 using the same methodology but with 100% Susceptible (water control), 100% Susceptible (sprayed), 10% Resistant (sprayed), 20% Resistant (sprayed), 40% Resistant (sprayed), 60% Resistant (sprayed), 80% Resistant (sprayed) and 100% Resistant (sprayed). Results were obtained for two replicates but final replication had to be abandoned because reference strains at EMAI were destroyed by the predatory mite *Neoseiulus barkeri* (Hughes). For this reason statistical significance could not be determined, but we suggest that resistance causes spinosad failure between 10 and 40% resistant WFT. Spinosad resistance is thus likely to be manageable within an integrated pest management system. However, the experiment needs to be repeated to determine exact statistical significance.

(g) New or experimental chemicals for WFT control

The chemicals pyridalyl, acrinathrin, clothianidin and DPX-HGW86 were evaluated for efficacy against susceptible and resistant WFT. Pyridalyl was thought to show some low level cross-resistance, possibly limiting chemical use for WFT control. Again cross- resistance to acrinathrin was also likely, but a mixture of acrinathrin and malathion appeared to reduce acrinathrin resistance. High level cross-resistance to clothianidin was evident, indicating that this chemical should not be used for WFT control. Finally, the evaluation of DPX-HGW86

did not find any evidence of cross-resistance. It may be useful for WFT control if practical field rates prove lower than the relatively high rates suggested from the bioassay.

Article III. General Introduction

Western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is regarded to be one of the most important economic pests of agricultural and horticultural crops. It causes direct damage by feeding on leaves and other plant parts, and indirect damage via transmission of plant viruses. *Frankliniella occidentalis* is regarded to be a pesticide induced problem, though pesticides are usually the main strategy used for its control. Glasshouse and field populations resistant to insecticides in several major chemical classes have been recorded in different parts of the world, including Australia. Effective control of WFT can only be achieved with an integrated approach that includes cultural methods to reduce thrips numbers and the removal of virus infected plant material (Herron *et al*. 2007). In addition, crop monitoring is considered essential because it allows insecticides to be used only when necessary. That in turn reduces the insecticide impact on beneficials and the likelihood of resistance development. Currently, integrated pest management (IPM) is being trialed through HAL-funded projects (eg the projects VG05086, VG05056) with heavy reliance on biologically-based control. The only IPM compatible chemical currently available for use with WFT is spinosad.

IPM is an important progression for WFT control because of increasing and ubiquitous resistance to some insecticides (Herron and James 2005). Resistance puts the whole Australian chemical control strategy at risk. Resistance monitoring between 2000 and 2003 (the project HG00015) found insecticide resistance in many populations of WFT, with resistance detected against acephate, dimethoate, endosulfan, fipronil, methamidophos, methidathion, and spinosad. Subsequent laboratory selection of fipronil and spinosad found that resistance could quickly increase to these insecticides. Current resistance monitoring data generated in 2005 (the project HG03003) detected further increases in the frequency of spinosad resistance. Alarmingly, spinosad resistance has now been detected at a frequency of 97% on a single Sydney flower grower practising IPM (Herron and Broughton 2006). Spinosad must now be considered at risk, yet spinosad remains the only chemical compatible with IPM and biological control. If improved resistance management is not funded. Spinosad will surely be lost to industry and the development of IPM will be jeopardised. If effective WFT IPM is to progress then improved resistance management of strategic chemicals including spinosad is paramount.

WFT management is based on chemical alternation. However, alternation with chemicals that inadvertently confer cross resistance will undermine spinosad efficacy. For this reason it is very important to research spinosad cross resistance. Bioassays can be used to define which chemical(s) are likely to be cross resistant to spinosad. However, the technique is limited to phenotypic response, the actual mechanism remains unknown. Cross resistance bioassays (with synergists) can identify possible causes of cross resistance, though the diagnosis is not definitive (Raffa and Priester 1985). Even so, the bioassay technique is useful because it can be used to refine molecular methods to more quickly find the actual mechanism causing spinosad cross resistance. Identifying the mechanism at a molecular level would have a huge benefit to practical spinosad resistance management. Knowledge of the molecular mechanism of spinosad resistance will provide the basis for a spinosad resistance “test kit”. A "test kit" can then be used by growers to better manage spinosad resistance. Additionally, a "test kit" could also be used to study the practical field implications of spinosad resistance. That is, at what frequency would the grower expect control problems and is that problem the same in conventional and IPM situations.

A test kit for spinosad resistance is not an unrealistic goal. Although the exact mode of spinosad action is unknown, it is thought to be based on binding at the nicotinic-aetylcholine (nAChE) receptor (Yu 2008). That implies a similar mode of action as imadacloprid (Confidor®), a chemical that is effective against thrips generally, but ineffective against WFT. Studies have also implicated gamma amino butyric acid (GABA) receptors in spinosad efficacy that are known to cause endosulfan (Thiodan) and fipronil (Regent®) resistance. For this reason spinosad could affect the “cys-loop” superfamily of ionotropic neurotransmitter receptors that include nAChE receptors, GABA receptors and glutamate gated chloride channels. Finally, research at the University of Melbourne (Perry *et al*. 2007) suggested that spinosad resistance may be due to a polymorphism in a single receptor subunit (the receptor type was not identified). This infers that a PCR diagnostic test could be designed to identify resistant individuals.

Here we outline our findings as a series of self contained referenced reports in a scientific journal style that give:

- project resistance monitoring data,
- cross resistance data,
- resistance mechanism data,
- molecular genetics data, and
- glass-house trial data designed to better understand spinosad resistance and improve WFT chemical control and management.

Individual section findings are then integrated into a General Discussion with overall project highlights and conclusions.

Resistance monitoring of insecticides currently used in Australia to control western flower thrips, *Frankliniella occidentalis* (Pergande)(Thysanoptera, Thripidae): Data for years 2006 – 2009. Grant A. Herron, Tanya M. James and Graeme C. Gullick

I&I NSW, Elizabeth Macarthur Agricultural Institute, PMB 4008, Narellan NSW 2567.

(a) Abstract

Insecticide resistance was monitored in field collected populations of western flower thrips (WFT) *Frankliniella occidentalis* (Pergande) from 2006-2009. WFT were screened against some nineteen chemicals, but not all strains were screened against all products. Organophosphate resistance was sporadic and generally low level except dichlorvos and methamidophos resistance in strain Ornamental F 05/2007 that showed 68 and 78 percent susceptible respectively. Fipronil resistance when detected was at a low frequency with maximum 11% resistant individuals in strain Capsicum Penfield 10/2007. Spinosad resistance was detected in eight of the fourteen strains tested with four strains having resistance levels above 100-fold, maximum 201-fold in strain cucumber Rossmore 03/2007. Spinosad resistance continues to increase in both level and abundance putting sustainable spinosad use against WFT at risk.

(b) Introduction

Resistance, a heritable characteristic that results directly from insecticide use and its evolutionary selection for resistance (Plapp and Wang 1983), has been a problem of agriculture since the 1940s (Georguiou and Mellon 1983) despite first being noted nearly a hundred years ago by Melandier (1914). Since the introduction of synthetic organic insecticides, such as DDT, insect resistance to insecticides has necessitated more frequent insecticide applications, higher doses, and required the substitution of inexpensive, older compounds with newer and more expensive products (Georguiou and Mellon 1983). Globally, resistance to one or more pesticides has been recorded in some 550 arthropod species (Whalon *et al*. 2009). The loss of efficacy of pesticides due to resistance puts a huge demand on remaining products and presents an enormous threat to sustainable agriculture. Resistance is frequently quantified by measuring the “fold” resistance, e.g., the increase in the amount of insecticide that needs to be applied to a population considered resistant over that of a population that has not experienced selection pressure for resistance.

Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is one of the most serious pests of horticulture and feeding by adults and nymphs can cause severe crop losses (Kirk 2002). Damage is caused directly through feeding and oviposition (Childers and Achor 1995), and indirectly through transmission of tomato spotted wilt virus (Kirk, 2002). It has a wide host range, and because of its ability to invade and establish in new habitats WFT is considered the most damaging thrips species in California (Kirk 2002). Since first being detected in Australia in Perth during 1993 (Malipatil *et al*. 1993), WFT has spread throughout Australia and is now found in all six states but not the Northern Territory (Medhurst and Swanson 1999). In Australia, WFT is largely controlled with the insecticide spinosad (Herron *et a*l. 2007). Spinosad is a relatively new insecticide belonging to the group 5A acetylcholine receptor modulators (InfoPest 2008). It is known to have efficacy against a range of pest species, including thrips (e–Pesticide Manual 2002). Spinosad was first registered for use in Australia in September 1999 (APVMA 2008) and is currently available

to control WFT on some 67 host crops (InfoPest, 2008). Unlike the organophosphate or carbamate alternatives, spinosad is considered compatible with IPM (Jones *et al.*, 2004), despite concerns about non–target impacts (as with many insecticides), and is now the most widely registered product in Australia for WFT control (InfoPest 2008).

Despite resistance management including IPM strategies, a low 1.8–fold spinosad resistance was detected in a single population of WFT collected from lettuce during the 2001/2002 growing season (Herron and Jame, 2005). Until 2003/2004, resistance to spinosad in field–collected populations of WFT remained relatively static at 2.6–fold. Unfortunately, resistance in field–collected populations started to dramatically increase during the 2004/2005 season, to a maximum of 40–fold resistance. That increased again to 87–fold by season 2005/2006 (Herron and James 2007).

Aims: (1) to monitor crop-specific insecticide resistance in field-collected populations of WFT with specific emphasis on spinosad and;
(2) to use the data to adapt the WFT resistance management strategy as appropriate.

(c) Materials and methods

(i) Insecticides tested

Table 1. Common name, trade name, formulation and supplier of insecticides tested.

Common name	Trade name	Formulation	Supplier
Abamectin	Vertimec	18 g / L EC	Syngenta Crop Protection Pty Ltd
Acetamiprid	Mospilan	225 g / L SL	Du Pont (Australia) Ltd
Acephate	Orthene Extra	750 g / kg GR	Arvesta Corportation
Bifenthrin	Talstar 100 EC	100 g / L EC	FMC Australasia Pty Ltd
Chlorfenapyr	Secure 360 EC	360 g / L EC	Crop Care Australasia Pty Ltd
Chlorpyrifos	Lorsban 500 EC	500 g / L EC	Dow AgroSciences Australia Ltd
Dichlorvos	Dichlorvos 500	500 g / L EC	Barmac Industries Pty Ltd
Dimethoate	Dimethoate	400 g / L EC	Nu Farm Australia Ltd
Endosulfan	Thiodan EC	350 g / L EC	Bayer CropScience Pty Ltd
Fipronil	Regent 200 SC	200 g / L SC	Nu Farm Australia Ltd
Malathion	Hy-Mal Insecticide	1150 g / L EC	Crop Care Australasia Pty Ltd
Methamidophos	Nitofol Insecticide	580 g / L EC	Bayer Crop Science Pty Ltd
Methidathion	Supracide 400 EC	400 g / L EC	Syngenta Crop Protection Pty Ltd
Methiocarb	Mesurol 750	750 g / kg WP	Bayer CropScience Pty Ltd
Methomyl	Marlin Insecticide	225 g / L AC	Du Pont (Australia) Ltd
Pyrazophos	Afugan	295 g / L EC	AgrEvo Pty Ltd
Thiacloprid	Calypso 480 SC	480 g / L SC	Bayer CropScience Pty Ltd
Thiamethoxam	Actara Insecticide	250 g / kg WG	Syngenta Crop Protection Pty Ltd

Spinosad	Success2 Naturalyte	240 g / L SC	Dow AgroSciences Australia Ltd

AC = aqueous concentrate
EC = emulsifiable concentrate
GR = granule
SC = suspension concentrate
WP = wettable powder

(ii) Bioassay method

Thrips were placed in ventilated, thrips-proof containers and forwarded by overnight courier from their point of collection to the laboratory in NSW. The thrips were confirmed as WFT under a stereo microscope prior to culture establishment using the diagnostic guide of Palmer *et al*. (1992).

Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, the WFT were reared in purpose built rearing cages on potted bean plants with Cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. Thrips were transferred onto fresh plants in a new cage on a six-weekly-cycle and maintained at 25±1 ^{o}C under an 18:6 hour L: D regime.

The bioassay procedure is given in Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean-leaf discs embedded in agar in small Petri dishes. The leaf discs with anaesthetised thrips in place were then sprayed with aqueous insecticide (Table 1)(4 mL aliquot) or with water (control) with the aid of a Potter spray tower (deposit of 3.2 mg cm^{-2}). The Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine holes. The dishes were stored for 48 h at 25 ± 0.1 ^{o}C in a 18:6 hour L: D regime after which the numbers of alive and dead thrips were counted. Most tests were done to achieve full probit regressions but in some instances discriminating dose tests only were done to reduce testing time. If a discriminating dose test indicated that a strain was susceptible the resistance factor was assumed to be approximately 1.0-fold. Each test was replicated at least once (unless otherwise stated) and control mortality did not exceed 15%.

(iii) Bioassay assessment

Thrips were confirmed alive or dead with the aid of a stereo-microscope.

(iv) Analysis of bioassay data

Data were analysed using GENSTAT 5 statistical software (Barchia, 2001). The LC50 plus its 95% fiducial-limits, were calculated using the probit method outlined in Finney (1971) and included control mortality correction (Abbott 1925). The LC_{50}s were used to calculate resistance factor (RF) values at the LC_{50} level (ie RF_{50}) plus their associated 95% confidence intervals (CI) as outlined in Robertson and Preisler (1992)

(d) Results

Over the three years study some sixteen strains of WFT were collected and cultured for bioassay (Tables 2, 3 and 4). Of these, fourteen were successfully tested with two strains lost to predatory mites before testing could commence (Table 4). Worryingly, all strains tested showed resistance to at least one chemical tested with some strains having a very high proportion of spinosad resistant individuals and consequent resistant factors that peaked at 201-fold in strain Cucumber Rossmore 03/2007. Encouragingly, spinosad resistance was shown to decline in the absence of selection in strain Ornamental F that showed a small but measurable decline in resistance from 5.5 fold in July 2006 (Table 2) to 2.5 fold in May 2007 (Table 3). Additionally, fipronil resistance when detected was at a low frequency and level (Tables 2 and 3). Resistance to organophosphate chemicals was sporadic and generally low level except dichlorvos and methamidophos resistance in strain Ornamental F 05/2007 (Table 3) that showed 68 and 78 percent susceptible respectively.

Table 2. WFT resistance testing 2006 for the projectVG006010: dose-response summary giving LC50 level resistance factors for fipronil and spinosad with confidence interval (CI); and percent mortality at the discriminating dose (% mort) for remaining chemicals (0% survivors at the discriminating dose are = susceptible, 0-10% survivors at the discriminating dose are = suspect resistant, >10% surviving the discriminating dose = resistant).

		Ornamental F 2006 NSW 07/2006	**Chilli Bundaberg Qld 09/2006**	**Capsicum Wallace Qld 10/2006**	**Chilli Patane Qld 10/2006**	**Honeydew Gumlu Qld 10/2006**	**Research Station Qld 12/2006**
Spray history		No spinosad for 10 months	Nothing mentioned	Sprayed with thiodicarb methomyl and spinosad	Nothing mentioned	Nothing mentioned	Sprayed with methomyl bifenthrin spirotetram at methamidophos pyridalyl and fipronil
abamectin	% Mort					97	
acetamiprid	% Mort						
acephate	% Mort		100	100	100		100
bifenthrin	% Mort						55
chlorfenapyr	% Mort						
dichlorvos	% Mort		99	99	100	96	100
dimethoate	% Mort		100				
endosulfan	% Mort		100	100	100	100	100
fipronil	RF	•	0.2	•	•		•

	CI	•	0.07-0.7	•	•		•
	% Mort	100*	100	99	100	96	100
malathion	% Mort						
methamidophos	% Mort						100
methidathion	% Mort		99	100	100		100
methiocarb	% Mort						
methomyl	% Mort		88	98	97	93	100
pyrazophos	% Mort					96	
thiacloprid	% Mort						
thiamethoxam	% Mort						
spinosad	RF	5.5	1.2	•	•	•	•
	CI	2.7-11.1	0.9-1.6	•	•	•	•
	% Mort	80	100	100	100	100	100

NB Grey fill not tested
• Discriminating Dose (DD) test only.
⁎ unreplicated
Collected from:
Ornamental F 2006 NSW: 10 July 2006, ex roses Flora International Leppington, houses 5,7 and 8 (not spinosad sprayed for 10 months). Collector, John Prinslou
Chilli Bundaberg Qld: 12 September 2006, ex chilli S. De Pooli, Douglas Rd. Collector Iain Kay, QDPI & Fisheries
Capsicum Wallace Qld: 23 October 2006, ex capsicum J. Manera, Wallace Rd. (sprayed with methomyl and 2-3 spinosad) Collector Iain Kay, QDPI & Fisheries
Chilli Patane Qld: 23 October 2006, ex chilli Pantane, Gumlu. Collector Melissa Fellows
Honey dew Gumlu Qld: 23 October 2006, ex honeydew R. Chapman. Collector Melissa Fellows
Research Station Qld: 4 December 2006, ex capsicum Research Station Bundaberg. (Sprayed methomyl 7x, DC072, methamidophos, pyridalyl, fipronil) Collector Iain Kay, QDPI & Fisheries

Table 3. WFT resistance testing 2007 for the project VG006010: dose-response summary giving LC50 level resistance factors for fipronil and spinosad with confidence interval (CI); and percent mortality at the discriminating dose (% mort) for the remaining chemicals (0% survivors at the discriminating dose are = susceptible, 0-10% survivors at the discriminating dose are = suspect resistant, >10% surviving the discriminating dose = resistant).

		Capsicum WA 02/2007	**Cucumber Rossmore NSW 03/2007**	**Lettuce G 2007 NSW 03/2007**	**Ornamental F 2007 NSW 05/2007**	**Tomato Horvath Qld 08/2007**	**Capsicum Penfield SA 10/2007**

Spray history		Having control problems with spinosad	IPM practiced with Eco-Oil Natrasoap and abamectin (for TSM)	Sprayed with methomyl spinosad pyrethrum dimethoate α-cypermethrin imidacloprid and pirimicarb	Sprayed with methomyl abamectin Eco oil and dichlorvos	Noted reduced efficacy with spinosad. Used with predatory mites	Noted control failure with methamidophos. Sprayed methamidophos spinosad bifenthrin and abamectin
abamectin	% Mort				99	100	
acetamiprid	% Mort						
acephate	% Mort	99				100	96
bifenthrin	% Mort						
chlorfenapyr	% Mort						
dichlorvos	% Mort	87	82	90	68	94	87
dimethoate	% Mort						
endosulfan	% Mort	100				100	98
fipronil	RF	2.3				•	•
	CI	1.2-4.5				•	•
	% Mort	91*				93	89
malathion	% Mort						
methamidophos	% Mort	100			78		95
methidathion	% Mort	100				100	99
methiocarb	% Mort						
methomyl	% Mort	98	98	94	96	100	81
pyrazophos	% Mort						
thiacloprid	% Mort						
thiamethoxam	% Mort						
spinosad	RF	110	201	180	2.5	•	21
	CI	75-160	109-369	118-275	1.7-3.6	•	(14-32)
	% Mort	43*	36*	12*	89*	100	76

NB Grey fill not tested

• Discriminating Dose (DD) test only.
⁎ unreplicated
Collected from:
Capsicum WA: 2 February 2007. ex capsicum Grower north Perth. (problems with spinosad) Collector David Cousins
Cucumber Rossmore NSW: 22 March 2007, Twin Cam Farms, Kelly St, Rossmore, Moustafa Osman. Collecter Stacey Azzopardi and bub.
Lettuce G 2007 NSW: Glenorie Hydroponics, Old Northern Rd, Glenorie, Joe D'Anastasi. Collector Stacey Azzopardi and bub.
Ornamental F 2007 NSW: 08 May 2007, Stuart Lowrie, Stuart @ Flora International
Tomato Horvath Qld: Horvath Hydroponics, Bundaberg. Try to be soft and use *montdorensis* and some spinosad use. Collector Iain Kay DPI&F
Capsicum Penfield SA: Capsicums from Vandy Yon property, Penfield Rd Virginia. Sprayed Nitofol, Success and probably Talstar and Vertimec. Collector Tony Burfield, SARDI.

Table 4. WFT resistance testing 2008 for the project VG006010: dose-response summary giving LC50 level resistance factors for spinosad only with confidence interval (CI); and percent mortality at the discriminating dose (% mort) for remaining chemicals (0% survivors at the discriminating dose are = susceptible, 0-10% survivors at the discriminating dose are = suspect resistant, >10% surviving the discriminating dose = resistant).

		Lettuce Glenorie 02/2008	**Ornamental Manjimup WA 02/2008**	**White Acres 12/2008**	**Lettuce Glenorie 11/2008**
Spray history		Nothing mentioned Predatory mites in sample	Nothing mentioned	Nothing mentioned	Sprayed with spirotetramat and spinosad
abamectin	% Mort	P#	P#		
acetamiprid	% Mort	R	R		
acephate	% Mort	E	E		
bifenthrin	% Mort	D	D		
chlorfenapyr	% Mort	A	A		
dichlorvos	% Mort	T	T		
dimethoate	% Mort	O	O	100	
endosulfan	% Mort	R	R	100	
fipronil	% Mort	Y	Y	100	
malathion	% Mort				
methamidophos	% Mort	M	M		100

methidathion	% Mort	I	I		
methiocarb	% Mort	T	T		
methomyl	% Mort	E	E		100
pyrazophos	% Mort	S	S		
thiacloprid	% Mort				
thiamethoxam	% Mort	A	A	100	
spinosad	RF	T	T	3.3	156
	CI	E	E	(1.8-5.7)	(92-263)
	% Mort	!	!	95	21

NB Grey fill not tested
• Discriminating Dose (DD) test only.
Mite identified as *Neoseiulus barkeri* (Hughes 1948) synonym for *Amblyseius masiaka* (Blommer & Chazeau 1974)
Collected from:
Lettuce Glenorie (G) 02/2008: Lettuce from Mr Joe D'Antastasi, Glenorie Hydroponics. Collector Sylvia Jelinek
Ornamental Manjimup: Collected off lupin. Collector Sonya Broughton, WA Dept of Agriculture.
White Acres Qld: Collected off White Acres (cotton), Bongeen, Dalby, by CSD consultant J Marshall
Lettuce Glenorie 11/2008: Lettuce from Mr Joe D'Antastasi, Glenorie Hydroponics. Currently under trial with Spirotetramat that has been used a few times. Also had a few spinosad applications recently. Collector Sylvia Jelinek

(e) Discussion

Despite a resistance management strategy for WFT insecticide resistance being implemented, resistance has increased above the 87-fold detected in a previous study of Herron and Broughton (2006). Spinosad resistance peaked at 201–fold in the 2006/2007 season with a further three instances of resistance above 100 fold. A major factor in the selection for resistance is the lack of effective alternatives to spinosad, and field levels of resistance to spinosad continue to increase. Take the Ord River Irrigation Area (ORIA) as an example that had high reliance on a single insecticide. Poor fiber quality and pyrethroid insecticide resistance in the cotton boll worm, *H. armigera*, caused cotton production to be abandoned in the ORIA in 1975 (Yeates *et al.* 2006). If available, new chemical(s) would have been used and the cycle repeated; however, new chemicals were not available in the ORIA and cotton production ceased. The Australian situation with WFT is not much different from the cotton example, with all strains tested showing resistance to at least one insecticide. Even when growers practice integrated pest management (IPM) such as in strain Cucumber Rossmore 03/2007, very high 201-fold spinosad resistance was detected.

Unfortunately, lack of alternative chemistry is not the only reason spinosad resistance continues to increase. The published strategy requires a treatment cycle of three consecutive sprays in close succession (Broughton and Herron, 2007), because eggs are hidden in the leaf

tissue and pupae in the soil, largely preventing contact with sprayed chemicals (Herron *et al.* 2007). A detailed chemical spray history supplied with strain Lettuce Glenorie 11/2008 (unpublished data) showed that chemicals including spinosad were not applied according to the published resistance management strategy. Chemicals were being sprayed weekly rather than as recommended close together and in quick succession dependant on temperature (Broughton and Herron 2007). Additionally, chemicals were often not appropriately alternated with sequential use with an insecticide from another chemical class. This would have resulted in single chemical exposure to multiple WFT generations and so increase the probability of resistance selection. Interestingly then, spinosad resistance was associated with Lettuce Glenorie on two separate occasions. It is clear more is required to educate growers on correct chemical use for WFT control.

Promisingly, laboratory data indicates spinosad resistance will revert without selection, and has been shown to drop in the absence of spinosad use. Strain Ornamental F 07/2006 had been tested by discriminating dose some 10 months earlier and found 97% spinosad resistant (G. Herron, unpublished data). When retested in this study the resistance frequency had dropped to 20% in strain Ornamental F 07/2006 and further reverted to 11% in the subsequent collection Ornamental F 05/2007.

Additional research is required to relate specific spinosad resistance frequencies seen in the laboratory to field management plans, and how those specific resistance frequencies influence subsequent associated field control. To allow chemical control to be sustained and IPM to endure with diagnosed insecticide resistance the pesticide must function effectively in conjunction with IPM, and if resistance is quantifiable then growers must be assured of product efficacy to prevent chemical overuse. We consider resistance management integral to sustainable IPM, and, resistance management will become increasingly more important as the suite of IPM compatible chemicals is reduced for any reason, including the incidence of resistance or legislative pressure.

(f) Acknowledgments

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(h) Appendices

(i) Full log-dose probit data

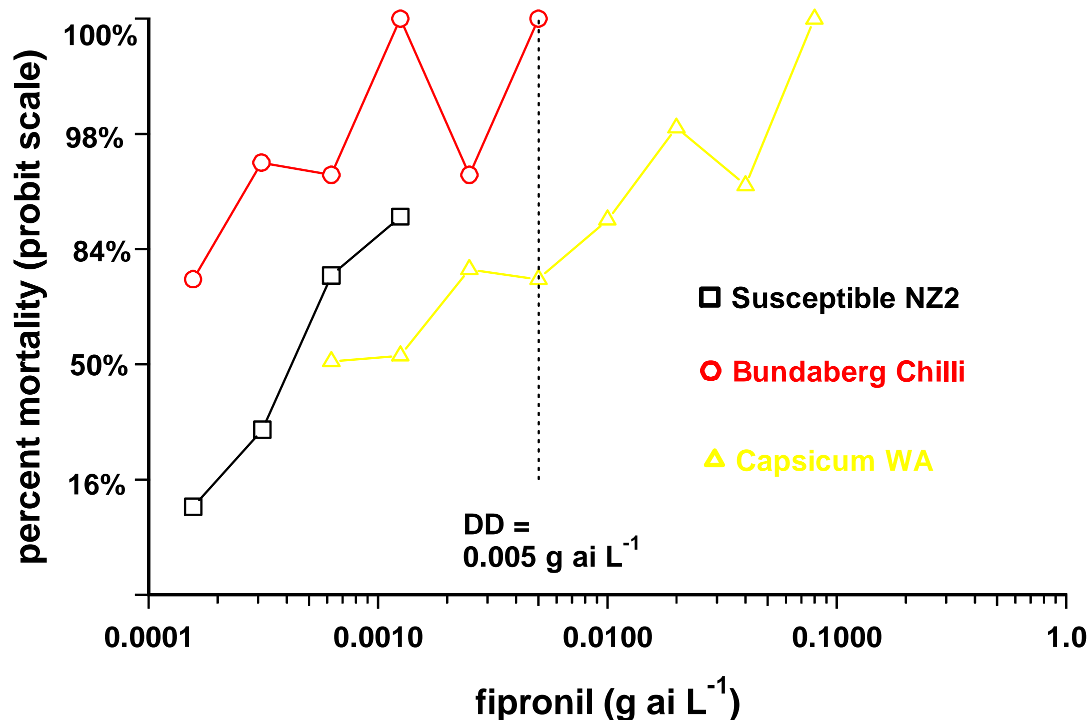

Figure 1. Control mortality corrected dose-response data against fipronil for field collected and reference susceptible NZ2 strain. DD = discriminating dose.

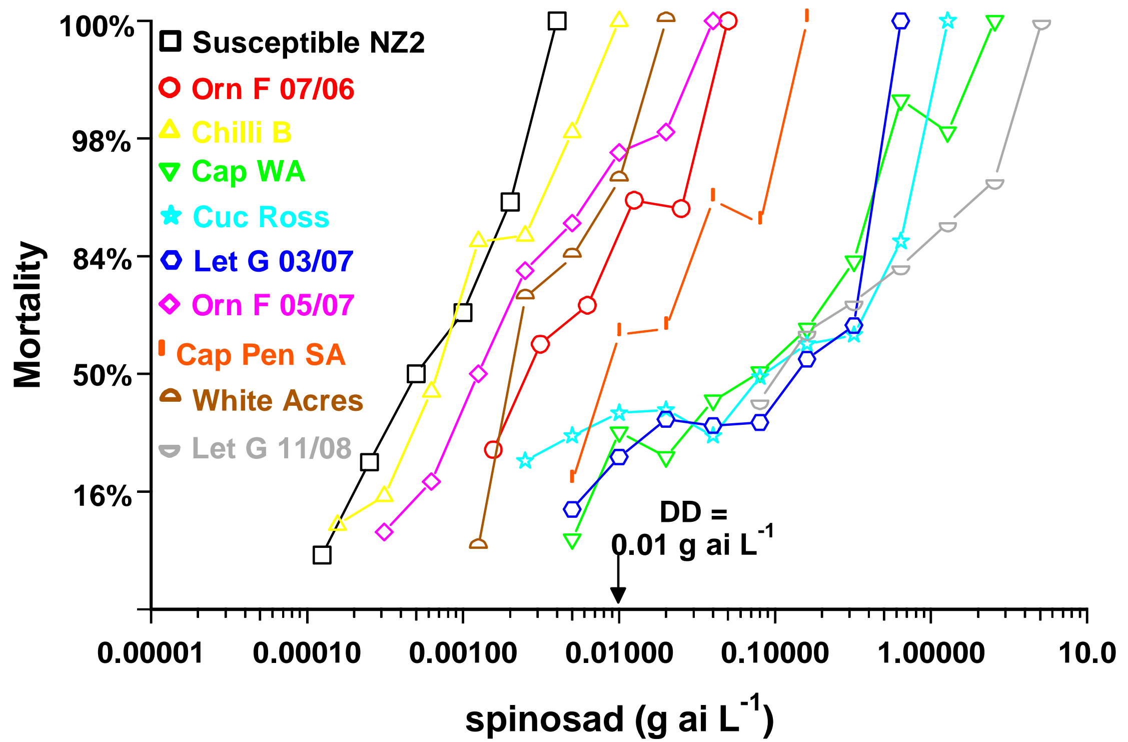

Figure 2. Control mortality corrected dose-response data against spinosad for field collected and reference susceptible NZ2 strain. DD = discriminating dose.

Cross - resistance bioassay of some existing and potential insecticides to control western flower thrips (WFT), *Frankliniella occidentalis* (Pergande)(Thysanoptera, Thripidae).
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(i) Abstract

Cross-resistance refers to the situation where resistance to one insecticide automatically causes resistance to other insecticide to which an insect has not been previously exposed. Here we studied cross-resistance in western flower thrips (WFT) that were spinosad resistant by exposing susceptible and resistant strains to existing and potential chemicals used for their control, and compared their response. Strains were tested against abamectin, acetamiprid, chlorfenapyr, fipronil, methamidophos, methomyl, spinosad and thiamethoxam. Field strains tested were highly spinosad resistant and results suggest that cross-resistance to the chloronicotinyl (neonicotinoid) insecticides acetamiprid and thiamethoxam is likely from spinosad use, and their alternation with spinosad should be avoided. Similarly, fipronil cross-resistance is also considered a possibility and its use should also be avoided with spinosad. Abamectin and methomyl did not show cross–resistance, but their alternation with spinosad is compromised for other reasons. Chlorfenapyr and methamidophos are currently the only two products likely to be useful for alternation with spinosad. Even so, methamidophos use in Australia is restricted to ornamentals (registered) and head lettuce only (permit PER10416). Chlorfenapyr is restricted to use in spring onion and shallots (permit PER11508) for WFT control in Australia.

(j) Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most serious pests of horticulture where feeding by adults and nymphs can cause severe crop losses (Kirk 2002). Damage is caused directly through feeding and oviposition (Childers and Achor 1995), and indirectly through transmission of tomato spotted wilt virus (Kirk, 2002). It has a wide host range, and because of its ability to invade and establish in new habitats, WFT is considered the most damaging thrips species (Kirk 2002). Since first being detected in Australia in Perth during 1993 (Malipatil *et al*. 1993), WFT has spread throughout Australia and is now found in all six states, but not the Northern Territory (Medhurst and Swanson 1999). This distribution has not changed in the subsequent decade.

Resistance is a heritable characteristic that directly results from insecticide use and its evolutionary selection for resistance (Plapp and Wang 1983). Insecticide resistance has been a problem of agriculture since the 1940s (Georguiou and Mellon 1983), despite first being noted nearly a hundred years ago by Melandier (1914). Since the introduction of synthetic organic insecticides such as DDT, insect resistance to insecticides has necessitated more frequent insecticide applications, higher doses, and required the substitution of inexpensive compounds with newer and more expensive products (Georguiou and Mellon 1983). Globally, resistance to one or more pesticides has been recorded in some 550 arthropod

species (Whalon *et al*. 2009). The loss of efficacy of pesticides due to resistance puts a huge demand on remaining products and presents an enormous threat to sustainable agriculture.

Resistance to newer groups of insecticide has now been reported in Australian strains of WFT (Herron and James 2005) and appear to be increasing in both frequency and abundance (Herron and James 2007). This includes the group 2C product fipronil (Regent®) and the group 5A product spinosad (Success™)(InfoPest 2008). The potential loss of spinosad is of particular concern, as it is the most widely used chemical in Australia to control *F. occidentalis.* Spinosad is registered in more than 60 individual uses in a range of crops including apples, brassicas, cucurbits, fruiting vegetables, leafy vegetables, legumes, ornamentals, potatoes, pears and stone fruit (Infopest 2008). Spinosad is also the only insecticide regarded to be safe to many beneficial insects that is available to growers using integrated pest management (IPM) programs (Elzen 2001).

Cross-resistance refers to a situation where an insect resistant to one insecticide automatically develops resistance to other insecticides to which it has not been exposed (Yu 2008). This happened in Australia with the chemical control of two-spotted spider mite (TSM) *Tetranychus urticae* Koch when resistance to the ovicide clofentezine (Apollo®) caused cross-resistance to the chemically unrelated compound hexthiazox (cailibre®) (Herron *et al*. 1993). Loss of hexythiazox to cross-resistance from clofentezine use caused severe problems with TSM control and made some crops uneconomic (Herron *et al*. 1993). In Australia, WFT is largely controlled with the single insecticide spinosad (Herron *et a*l. 2007). It is critical then that spinosad does not cause cross-resistance with other chemicals used against WFT. If that was to occur it would exacerbate spinosad resistance development and further reduce potential alternative control options.

AIM: To screen a reference susceptible (NZ2) and (OrnY) and spinosad resistant (LR_P) and (WA_P) strains of WFT against a range of chemicals used for their control to determine likely cross-resistance

(k) Materials and Methods

(i) Insecticides tested

Table 1. Common name, trade name, formulation and supplier of insecticides tested.

Common name	Trade name	Formulation	Supplier
Abamectin	Vertimec	18 g / L EC	Syngenta Crop Protection Pty Ltd
Acetamiprid	Mospilan	225 g / L SL	Du Pont (Australia) Ltd
Chlorfenapyr	Secure 360 EC	360 g / L EC	Crop Care Australasia Pty Ltd
Fipronil	Regent 200 SC	200 g / L SC	Nu Farm Australia Ltd
Methamidophos	Nitofol Insecticide	580 g / L EC	Bayer Crop Science Pty Ltd
Methomyl	Marlin Insecticide	225 g / L AC	Du Pont (Australia) Ltd
Thiamethoxam	Actara Insecticide	250 g / kg WG	Syngenta Crop Protection Pty Ltd
Spinosad	Success2 Naturalyte	240 g / L SC	Dow AgroSciences Australia

AC = aqueous concentrate
EC = emulsifiable concentrate
SL = soluble concentrate
SC = suspension concentrate
WG = water dispersible granule

(ii) Culturing and bioassay method

The thrips were confirmed as WFT under a stereo microscope prior to culture establishment using the diagnostic guide of Palmer *et al*. (1992). Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, the WFT were reared in purpose built rearing cages on potted bean plants with Cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. Thrips were transferred onto fresh plants in a new cage on a six-weekly-cycle and maintained at 25±1 °C under an 18:6 hour L: D regime. Strain integrity was assured by isolating the reference susceptible (NZ2 and Orn Y) from the resistant strains (LR_P and WA_P). It should be noted that NZ2 did contain small proportion of fipronil resistant WFT and so was not used for that chemical.

Resistant strain LR_P was collected from commercial roses in Leppington NSW on the 09/08/2005 and was associated with spinosad control failure. Resistant strain WA_P was collected from capsicum north Perth WA on the 02/02/2007 and also associated with spinosad control failure. Being field collected both resistant strains could have had previous exposure to a range of chemicals in addition to spinosad. Although spinosad was used significantly on both resistant strains causing control failure other chemicals permitted for WFT control on ornamentals at the time included abamectin, acephate, dichlorvos, fipronil, malathion, methamidophos, methidathion, methomyl and spinosad (Herron 2005). Chemical use for WFT control on capsicum at the time included acephate, dichlorvos, endosulfan, fipronil, methamidophos, methidathion, methomyl and spinosad at the time of collection (Herron 2005a). Consequently, strains should not have had any exposure to acetamiprid, chlorfenapyr or thiamethoxam. The resistant strains (LR_P and WA_P) were pressured with a discriminating concentration of spinosad on an ad hoc basis to maintain resistance post collection and had no other chemical exposure.

The bioassay procedure is given in Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean-leaf discs embedded in agar in small Petri dishes. The leaf discs with anaesthetised thrips in place were then sprayed with aqueous insecticide (Table 1)(4 mL aliquot) or with water (control) with the aid of a Potter spray tower (deposit of 3.2 mg cm^{-2}). The Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine holes. The dishes were stored for 48 h at 25 ± 0.1 °C in a 18:6 hour L: D regime after which the numbers of alive and dead thrips were counted. Tests were done to achieve full probit regressions. Each test was replicated at least once and control mortality did not exceed 15%.

(iii) Bioassay assessment

Thrips were confirmed alive or dead with the aid of a stereo-microscope.

(iv) Analysis of bioassay data

Data were analysed using GENSTAT 5 statistical software (Barchia, 2001). The LC_{50} or LC_{99} plus its 95% fiducial-limit, were calculated using the probit method outlined in Finney (1971) and included control mortality correction (Abbott 1925). The LC_{50}s and LC_{99}s were used to calculate cross- resistance factor (CRF) values at the LC_{50} (ie RF_{50}) or LC_{99} (ie RF_{99}) level plus their associated 95% confidence intervals (CI) as outlined in Robertson and Preisler (1992).

(l) Results

Table 2. Probit regression summaries for reference susceptible strains NZ2 or OrnY and reference resistant strains LR_P and WA_P giving cross-resistance estimates for each chemical tested.

Chemical	Strain	Slope (SE)	LC_{50}* (95% FL)	CRF (95% CI)	LC_{99}* (95% FL)	CRF (95% CI)
abamectin	NZ2	3.6 (0.45)	0.0054 (0.0044-0.0064)	-	0.024 (0.020-0.031)	-
	LR_P	1.6 (0.32)	0.0095 (0.0051-0.015)	1.7 (1.0-2.9)	0.24 (0.13-0.67)	10.1 (4.8-21.2)
	WA_P	2.1 (0.23)	0.0075 (0.0051-0.0098)	1.4 (0.9-2.0)	0.094 (0.063-0.18)	3.8 (2.2-6.6)
acetamiprid	NZ2	3.4 (0.46)	0.028 (0.020-0.035)	-	0.13 (0.099-0.20)	-
	LR_P	2.3 (0.28)	0.055 (0.042-0.070)	1.9 (1.4-2.7)	0.56 (0.35-1.18)	4.3 (2.3-8.0)
	WA_P	2.5 (0.39)	0.046 (0.035-0.056)	1.6 (1.2-2.3)	0.38 (0.27-0.67)	3.0 (1.7-5.1)
chlorfenapyr	NZ2	2.5 (0.65)	0.0032 (0.0020-0.0066)	-	0.027 (0.011-0.66)	-
	LR_P	3.2 (0.38)	0.0053 (0.0042-0.0064)	1.6 (1.0-2.6)	0.028 (0.020-0.050)	1.0 (0.2-4.1)
	WA_P	2.4 (0.48)	0.0026 (0.0014-0.0038)	0.8 (0.4-1.4)	0.048 (0.021-0.36)	0.8 (0.2-3.9)
Fipronil	OrnY	4.3 (1.3)	0.00035	-	0.0012	-

			(0.00017-0.00054)		(0.00063-0.0094)	
	LR_P	1.7 (0.35)	0.00037 (0.000089-0.00073)	1.0 (0.4-2.7)	0.0079 (0.0046-0.023)	6.4 (2.4-16.8)
	WA_P	1.0 (0.17)	0.00084 (0.00024-0.0017)	2.4 (1.0-6.0)	0.20 (0.062-2.48)	165 (32-856)
Methamidophos	NZ2	4.3 (1.0)	0.029 (0.014-0.040)	-	0.10 (0.071-0.23)	-
	LR_P	3.0 (0.49)	0.10 (0.078-0.14)	3.5 (2.3-5.5)	0.61 (0.36-1.73)	6.0 (2.8-13.0)
	WA_P	1.9 (0.25)	0.024 (0.016-0.032)	0.8 (0.5-1.4)	0.38 (0.25-0.74)	3.8 (1.9-7.5)
Methomyl	NZ2	5.3 (0.94)	0.051 (0.038-0.061)	-	0.14 (0.11-0.23)	-
	LR_P	3.2 (0.41)	0.059 (0.044-0.073)	1.1 (0.9-1.5)	0.31 (0.23-0.51)	2.2 (1.4-3.4)
	WA_P	2.7 (0.30)	0.037 (0.028-0.047)	0.7 (0.5-1.0)	0.28 (0.20-0.48)	2.0 (1.2-3.3)
Spinosad	NZ2	3.3 (0.49)	0.00046 (0.00034-0.00057)	-	0.0023 (0.0016-0.0046)	-
	LR_P	0.7 (0.28)	0.18 (0.053-1.83)	398 (120-1322)	411.20 (0.60-2.39×10^9)	178536 (171-1.85×10^8)
	WA_P	2.4 (0.36)	0.044 (0.022-0.069)	97 (54-174)	0.41 (0.31-0.60)	181 (106-308)
thiamethoxam	NZ2	3.2 (0.42)	0.020 (0.015-0.024)	-	0.11 (0.076-0.19)	-
	LR_P	2.2 (0.27)	0.083 (0.057-0.11)	4.1 (2.9-6.0)	0.94 (0.62-1.81)	8.6 (4.6-16.2)
	WA_P	1.0 (0.35)	0.011 (0.00028-0.041	0.5 (0.1-2.0)	1.92 (0.17-2202.50)	18 (1.4-222)

* g ai / L
LC = lethal concentration
CRF = cross-resistance factor
FL = fiducial limit
CI = confidence interval

(m) Discussion

Cross-resistance refers to a situation in which a strain that has become resistant to one insecticide automatically develops resistance to other insecticides to which it has not been exposed (Yu 2008) with management based on mode of action groups (InfoPest 2008). Spinosad belongs to the spinosyn group 5A chemicals with a primary target site of acetyl choline receptor modulators (InfoPest 2008). Ideally, the strain(s) used in a cross-resistance study would only have been exposed to a single chemical, in this case spinosad, without any other chemical selection. However, this was not possible, and both resistant strains tested LR_P and WA_P would have been previously exposed to a range of chemicals used for WFT control. For this reason two spinosad resistant strains were tested for the cross-resistance. They were sourced from two distinct geographic areas and host crops to reduce the likelihood of two strains having significant previous exposure to the same chemicals.

Previous testing of the WA_P strain immediately post collection and prior to pressuring had shown it to have some methomyl (2%), acephate (1%), fipronil (9%), dichlorvos (13%) and spinosad (57%) resistant individuals (see resistance monitoring data). The initial LR_P collection was found spinosad resistant (97%) (no other testing was done at the time) but a subsequent collection from the same site some 10 months later (where spinosad had not been used since) found the WFT fipronil susceptible but containing some abamectin (1%), dichlorvos (32%), methamidophos (22%), methomyl (4%) and spinosad resistant (11%) WFT (see resistance monitoring data). It should be noted then that there was a small proportion of fipronil resistant individuals detected in strain LR_P indicated by the 6.4 fold difference in response at the LC_{99} level. It is interesting then that fipronil (GABA), as spinosad (nACh receptor), both belong to the super family of ligand-gated ion channels known as *Cys-loop* receptors (Lester *et al*. 2004) and so cross-resistance from spinosad to fipronil is a possibility. It is unlikely that either population had dichlorvos exposure because it is used to disinfest empty structures only (Herron *et al*. 2007). As both strains showed some dichlorvos resistance it may have resulted from an undetermined cross-resistance with chlorpyrifos as previously suspected by Herron and James (2005). The abamectin resistance detected in strain LR_P may relate to previous product use as that strain undoubtedly would have had previous abamectin exposure.

Encouragingly, there was no cross-resistance methomyl or chlorfenapyr with resistance detected to methamidophos explained by previous chemical use. Therefore these chemicals appear to be suitable candidates for alternation with spinosad because they will not compromise spinosad efficacy due to cross resistance. Encouragingly, Broughton and Herron (2009) concluded that chlorfenapyr did have potential for WFT control but there are currently no registrations for use (Herron *et al*. 2007) and only a single permit PER11508. However, the usefulness of methomyl and methamidophos is limited for this purpose due to efficacy and use limitations respectively. Methamidophos is classified as an S7 product in Australia using the 'Standard for the Uniform Scheduling of Drugs and Poisons' and following the NRA (2002) review use was restricted for WFT control and now includes only a single registration (ornamentals) and permit (lettuce) (Herron *et al*. 2007). Although methomyl resistance is not always detected and then only at low level (Herron and James 2007) field trials in Qld rated methomyl efficacy to be poor and often not better than control plots (Kay and Herron 2010). Similarly, abamectin efficacy at the permit rate of 0.018 g ai / L is questionable (Broughton and Herron 2007) and again alternation with spinosad should be avoided.

Although cross-resistance should only occur within the same mode of action group it is noteworthy that both acetamiprid and thiamethoxam that belong to to chloronicotinyl group 4A chemicals with a primary target site of acetyl chloline receptor agonists/antagonists also

showed a proportion of resistant individuals when tested against a spinosad resistant strain. Yu (2008) notes that spinosad and chloronicotinyls such as thiamethoxam and acetamiprid all mimic acetylcholine by acting as agonists to activate the nicotinic acetylcholine receptor with all chemical therefore in the *Cys-loop* super family group. Consequently cross-resistance is plausible and probably likely in the two populations tested. Consequently, chloronicotinyl (neonicotinoid) alternations with spinosad should be avoided.

(n) Acknowledgments

We thank Dr Idris Barchia, NSW DPI, for analysing the bioassay data and the researchers and growers who forwarded strains for resistance testing.

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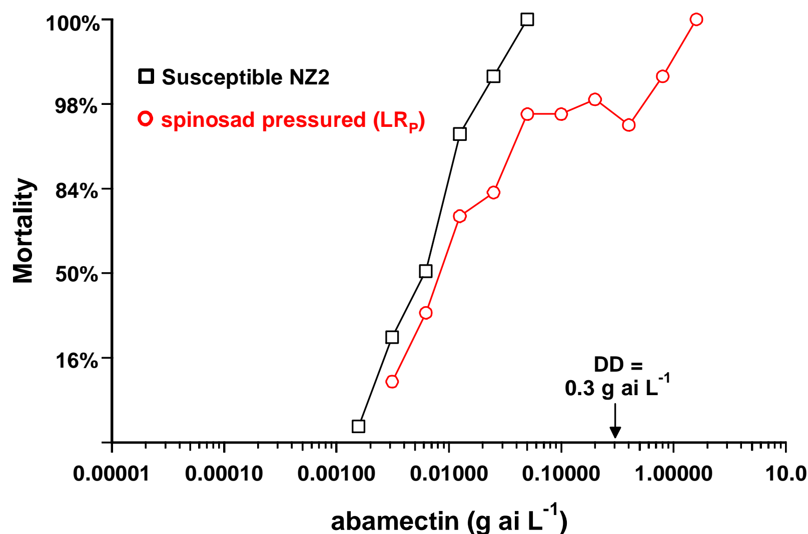
(p) Appendices

(i) Full log-dose probit data



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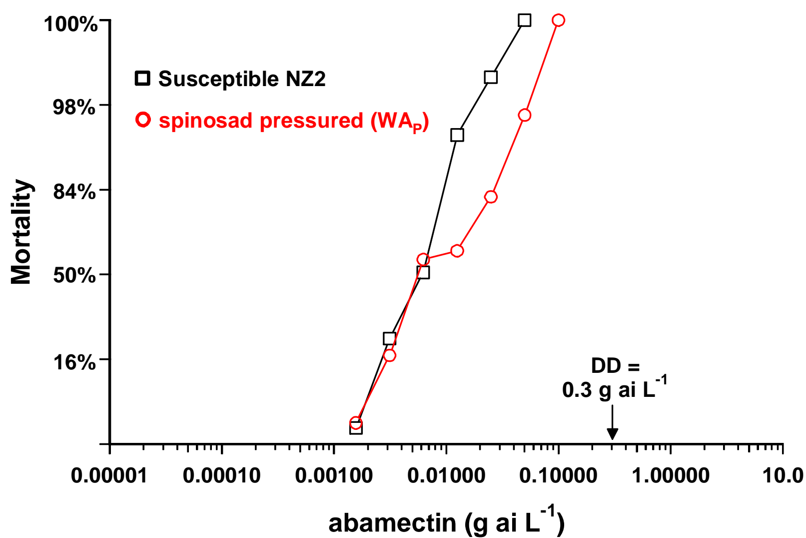
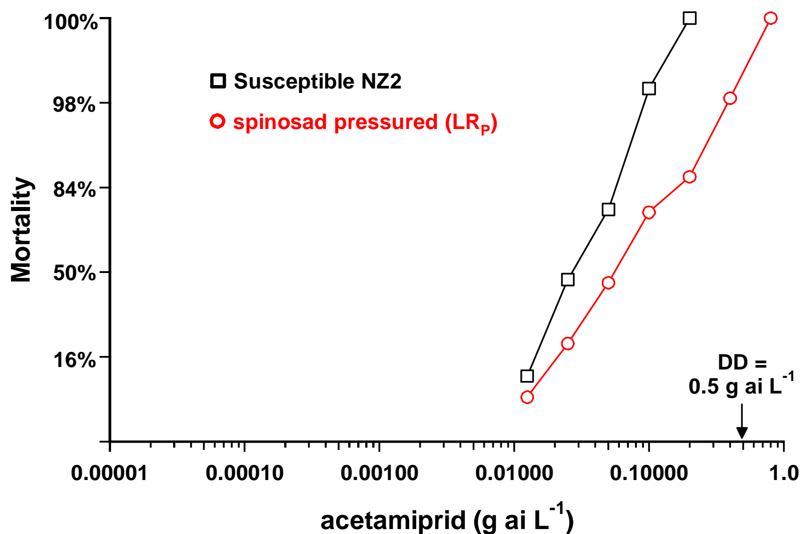


B

Figure 1. Control mortality corrected dose-response data against abamectin for reference susceptible strain NZ2 and reference spinosad resistant strains (LR_P) (Fig 1 A) and (WA_P) (Fig 1 B)(DD = Discriminating Dose)



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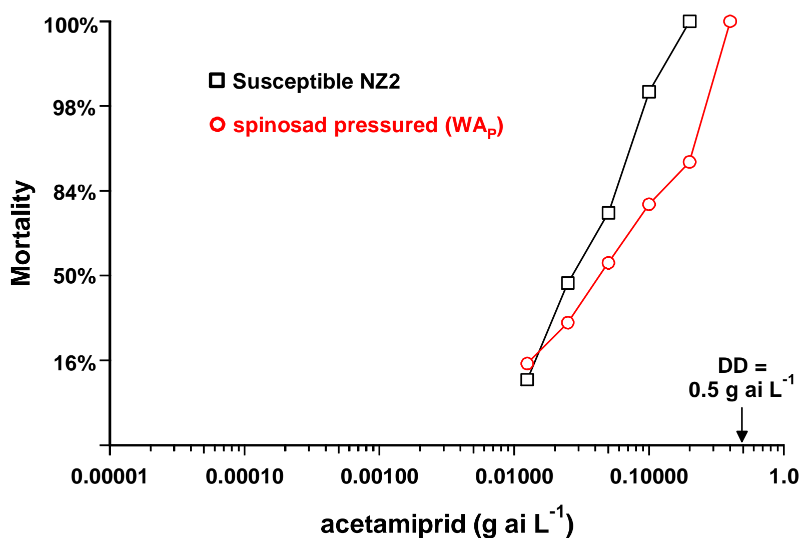
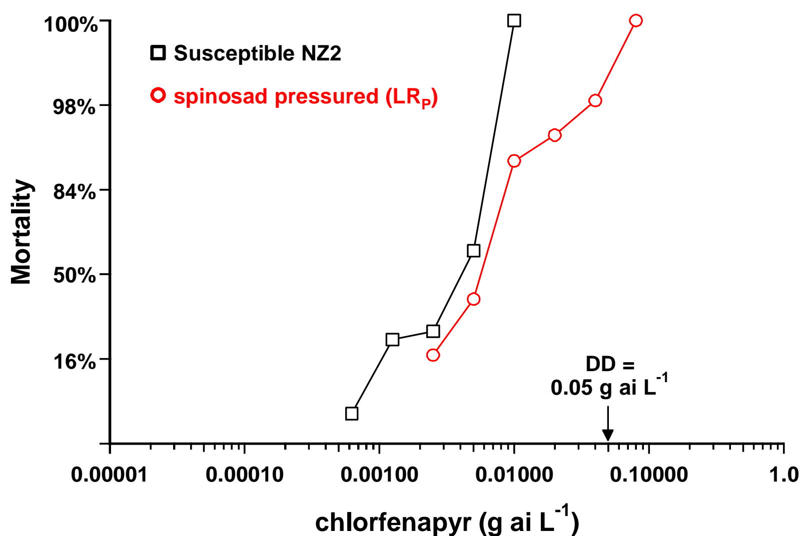


B

Figure 2. Control mortality corrected dose-response data against acetamiprid for reference susceptible strain NZ2 and reference spinosad resistant strains (LR$_P$) (Fig 2 A) and (WA$_P$) (Fig 2 B) (DD = Discriminating Dose)



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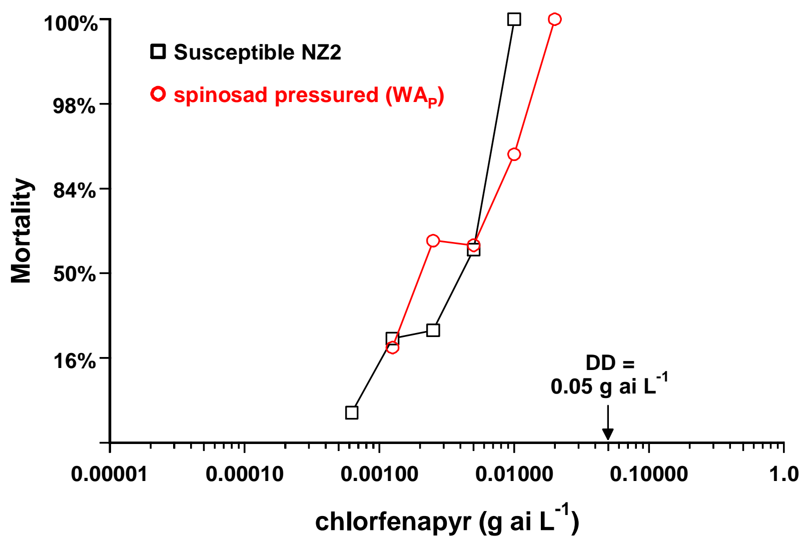
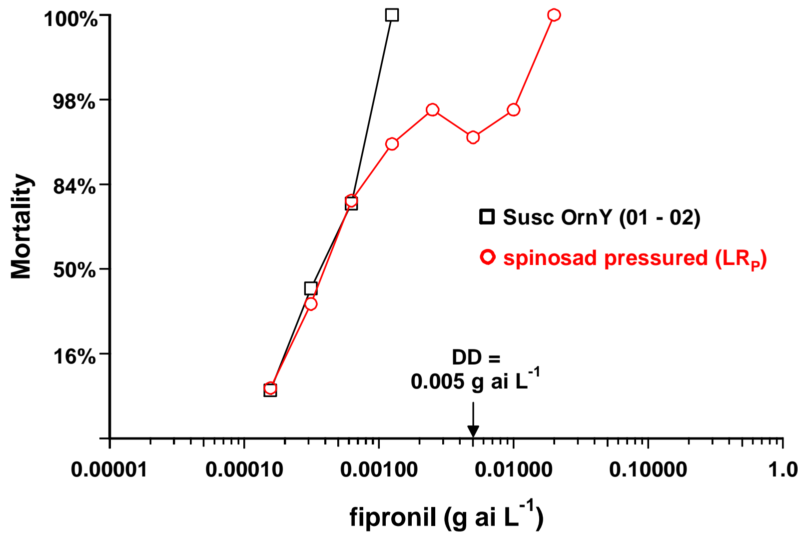


B

Figure 3. Control mortality corrected dose-response data against chlorfenapyr for reference susceptible strain NZ2 and reference spinosad resistant strains (LR$_P$) (Fig 3 A) and (WA$_P$) (Fig 3 B) (DD = Discriminating Dose)



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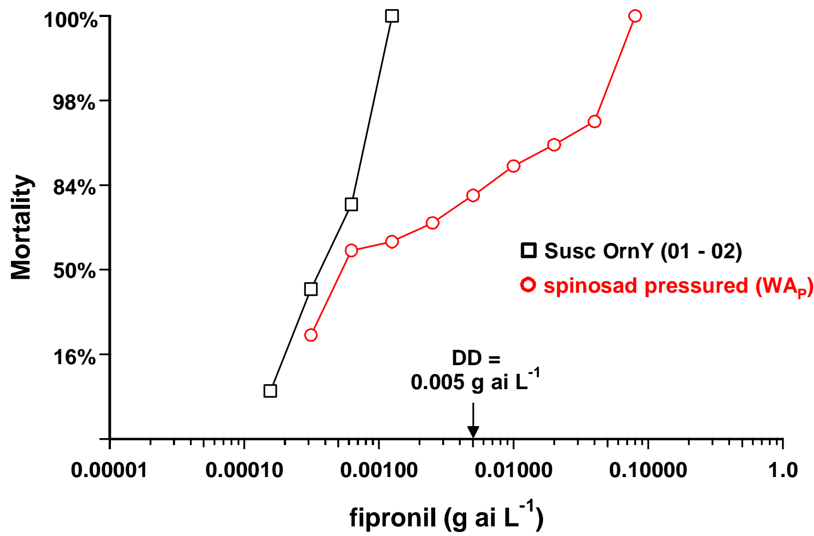
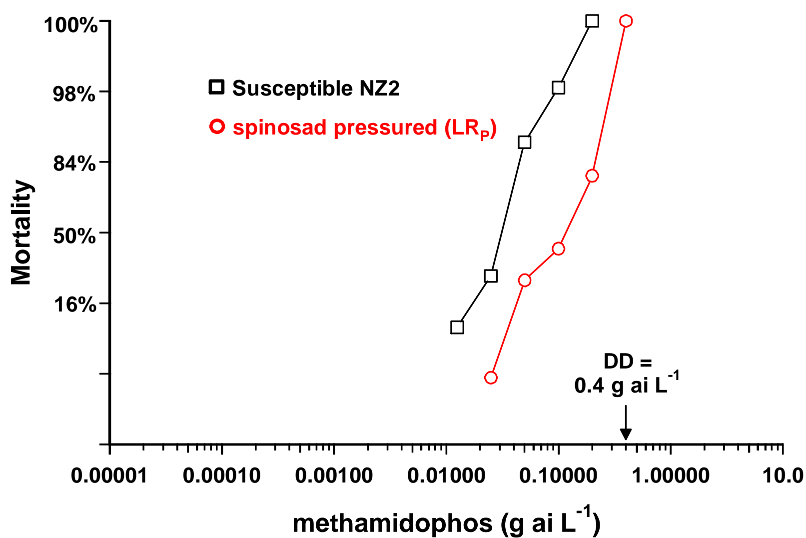


B

Figure4. Control mortality corrected dose-response data against fipronil for reference susceptible strain OrnY (01-02) and reference spinosad resistant strains (LR_P) (Fig 4 A) and (WA_P) (Fig 4 B) (DD = Discriminating Dose)



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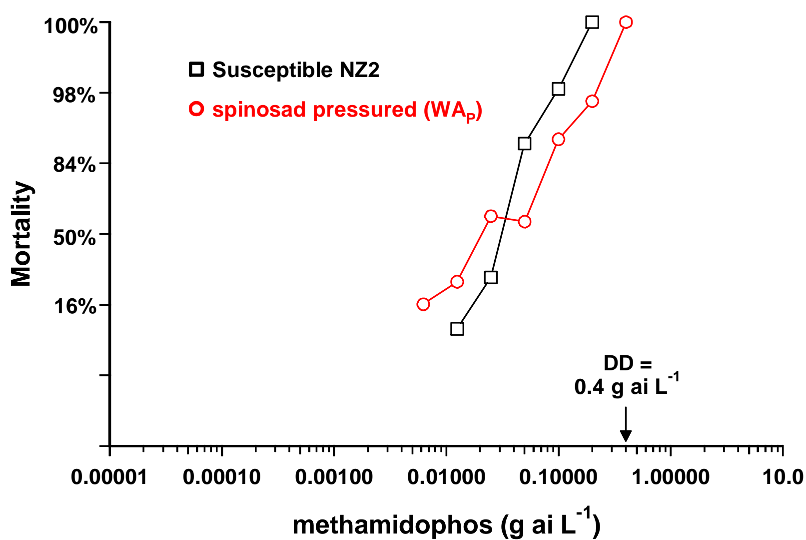
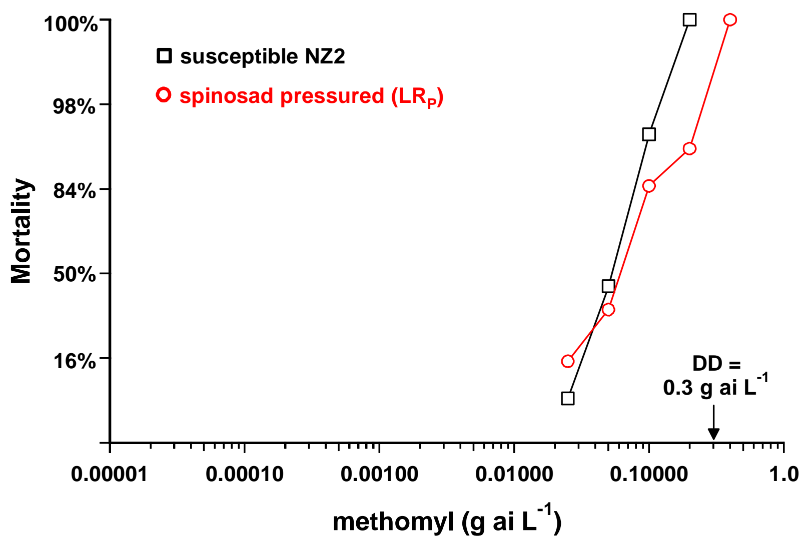


B

Figure 5. Control mortality corrected dose-response data against methamidophos for reference susceptible strain NZ2 and reference spinosad resistant strains (LR_P) (Fig 5 A) and (WA_P) (Fig 5 B) (DD = Discriminating Dose)



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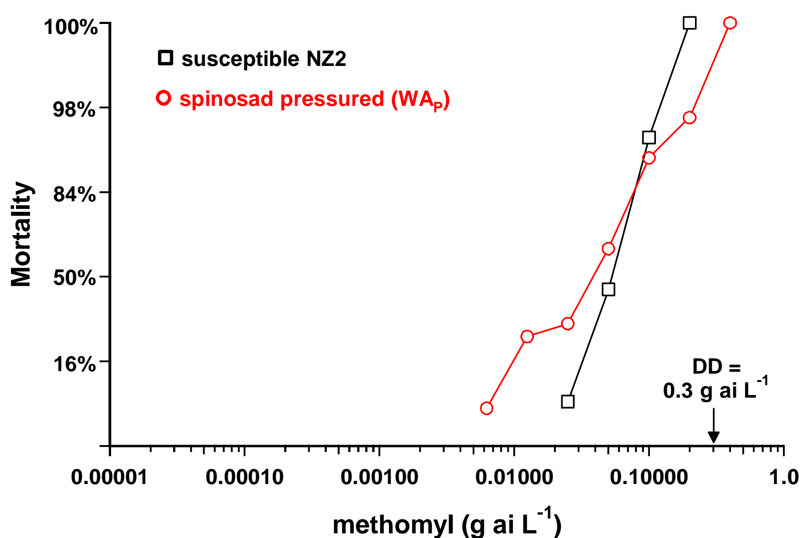
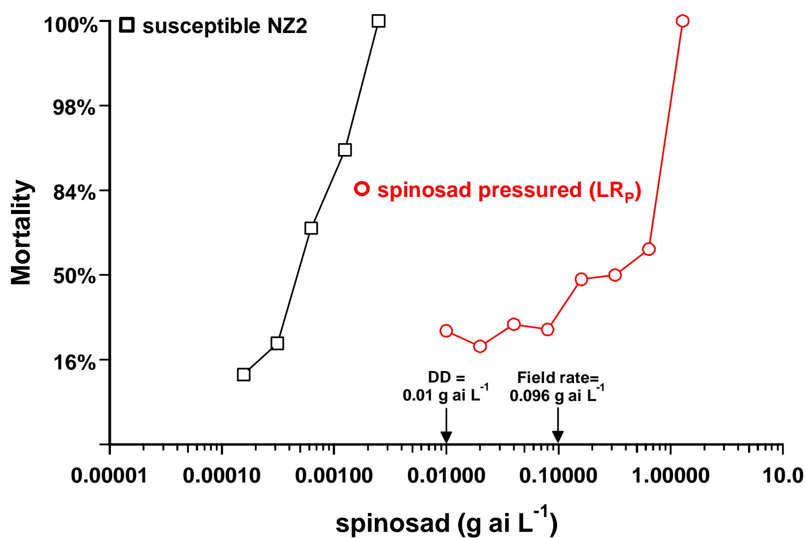


B

Figure 6. Control mortality corrected dose-response data against methomyl for reference susceptible strain NZ2 and reference spinosad resistant strains (LR$_P$) (Fig 6 A) and (WA$_P$) (Fig 6 B) (DD = Discriminating Dose)



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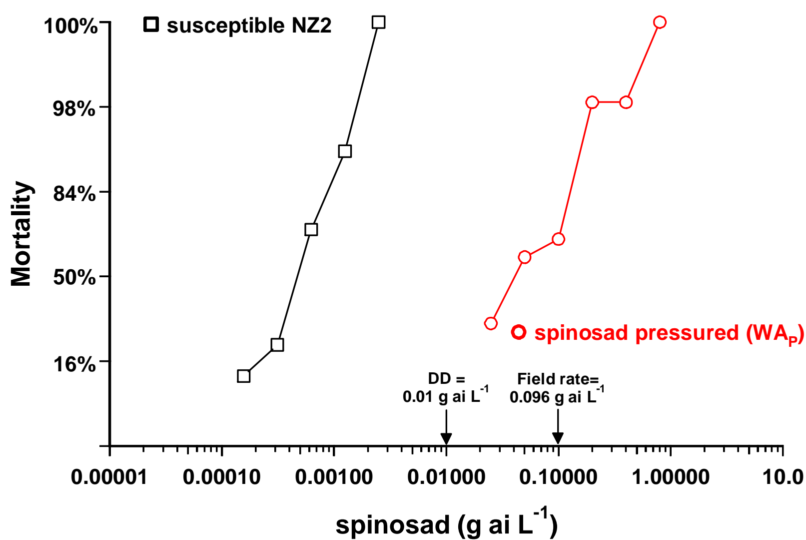
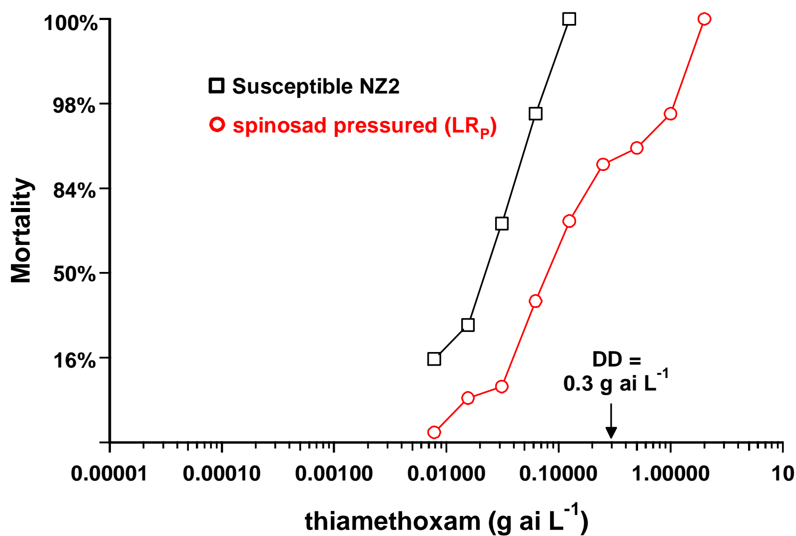


B

Figure 7. Control mortality corrected dose-response data against spinosad for reference susceptible strain NZ2 and reference spinosad resistant strains (LR$_P$) (Fig 7 A) and (WA$_P$) (Fig 7 B) (DD = Discriminating Dose)



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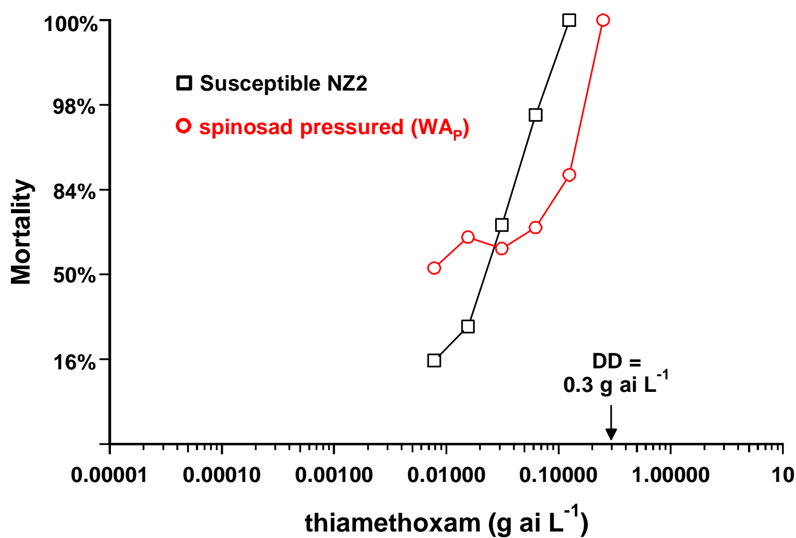

B

Figure 8. Control mortality corrected dose-response data against thiamethoxam for reference susceptible strain NZ2 and reference spinosad resistant strains (LR$_P$) (Fig 8 A) and (WA$_P$) (Fig 8 B) (DD = Discriminating Dose)

Bioassay of susceptible and resistant western flower thrips (WFT), *Frankliniella occidentalis* (Pergande)(Thysanoptera, Thripidae) with synergists as a first step to determine possible resistance mechanisms. Grant A. Herron and Tanya M. James

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(q) Abstract

Resistance is caused by a number of mechanisms with most being either target site or detoxification. The presence of specific detoxification enzymes can be deduced with synergists that can neutralise specific enzyme detoxification pathways. As compared to the response of a spinosad pressured and resistant strain LRp the addition of synergists did not significantly influences (by overlapping 95% CI) the RF_{50} (resistance Factor at the LC_{50} level) ratio for abamectin, spinosad or thiamethoxam. Interestingly, the RF_{50} ratio was significant for acetamiprid with the monooxygenase synergist PBO (piperonyl butoxide) but not for the other synergists tested. Calculated S (synergist) ratios were similar in that there was a significant difference with acetamiprid and PBO but unlike the RF_{50} ratios there was an additional significant difference seen with the esterase synergist TPP (triphenyl phosphate) and abamectin. As there were no significant differences with any synergist detected with spinosad and so we suspect resistance may be target site rather than metabolic. As the synergist study eliminated metabolic detoxification as a cause of spinosad resistance the molecular component of the project VG06010 can pursue the target site resistance model of *Drosophila melanogaster* Meigen with a high degree of confidence.

(r) Introduction

Resistance is a heritable characteristic that results directly from insecticide use and its evolutionary selection for resistance (Plapp and Wang 1983). It has been a problem of agriculture since the 1940s (Georguiou and Mellon 1983) despite first being noted nearly a hundred years ago by Melandier (1914). Since the introduction of synthetic organic insecticides, such as DDT, insect resistance to insecticides has necessitated more frequent insecticide applications, higher doses, and required the substitution of inexpensive compounds with newer and more expensive products (Georguiou and Mellon 1983). Globally, resistance to one or more pesticides has been recorded in some 550 arthropod species (Whalon *et al.* 2009). The loss of efficacy of pesticides due to resistance puts a huge demand on remaining products and presents an enormous threat to sustainable agriculture.

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most serious pests of horticulture where feeding by adults and nymphs can cause severe crop losses (Kirk 2002). Damage is caused directly through feeding and oviposition (Childers and Achor 1995), and indirectly through transmission of tomato spotted wilt virus (Kirk 2002). It has a wide host range, and because of its ability to invade and establish in new habitats WFT is considered the most damaging thrips species in California (Kirk 2002). Since first being detected in Australia in Perth during 1993 (Malipatil *et al.* 1993), WFT has spread throughout Australia and is now found in all six states but not the Northern Territory (Medhurst and Swanson 1999).

Australian WFT is now resistant to varying degrees to all chemicals used for their control (Herron and James 2005 and 2007). Resistance is caused by a number of mechanisms with most being either target site or detoxification (Georgiou and Saito 1983). Increased detoxification is caused by a number of broad spectrum enzymes known as cytochrome P450 monooxygenases, hydrolases or glutathione-S-transferases (Yu 2008). The presence of these specific detoxification enzymes can be deduced with synergists that can neutralise specific enzyme detoxification pathways (Raffa and Priester 1985) and provide strong evidence that resistance is caused by increased metabolic activity (Yu 2008). Specifically piperonyl butoxide (PBO) is used to detect cytochrome P450 monooxygenases, diethyl maleate (DEM) to detect glutathione-S-transferases and triphenyl phosphate (TPP) to detect esterases (a specific hydrolase often associated with resistance). The synergists are used to determine a synergist ratio that is defined as the LC50 value of an insecticide applied alone divided by the LC50 value of the observed when the insecticide is applied with a synergist and applied similarly (Yu 2008).

AIM: To screen a reference susceptible (NZ2) and spinosad resistant (LRp) strain of WFT against a four chemicals used for their control with and without the synergists PBO, TPP and DEM as a first step to determine if resistance is likely metabolic or target site.

(s) Materials and methods

(i) Insecticides tested

Table 1. Common name, abbreviation and supplier of synergists tested

Common name	Abbreviation	Supplier
diethyl maleate	DEM	Sigma-Aldrich
triphenyl phosphate	TPP	Sigma-Aldrich
piperonyl butoxide	PBO	Endura Fine Chemicals

Table 2. Common name, trade name, formulation and supplier of insecticides tested.

Common name	Trade name	Formulation	Supplier
Abamectin	Vertimec	18 g / L EC	Syngenta Crop Protection Pty Ltd
Acetamiprid	Mospilan	225 g / L SL	Du Pont (Australia) Ltd
Thiamethoxam	Actara Insecticide	250 g / kg WG	Syngenta Crop Protection Pty Ltd
Spinosad	Success2 Naturalyte	240 g / L SC	Dow AgroSciences Australia Ltd

EC = emulsifiable concentrate
SL = soluble concentrate
SC = suspension concentrate
WG = water dispersible granule

(ii) Culturing and bioassay method

The thrips were confirmed as WFT under a stereo microscope prior to culture establishment using the diagnostic guide of Palmer *et al*. (1992). Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, the WFT were reared in purpose built rearing cages on potted bean plants with Cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. Thrips were transferred onto fresh plants in a new cage on a six-weekly-cycle and maintained at 25±1 ^{o}C under an 18:6 hour L: D regime. Strain integrity was assured by isolating the reference susceptible (NZ2) from the resistant strain. Resistant strain LR_P was collected from commercial roses in Leppington NSW on the 09/08/2005 and was associated with spinosad control failure. Being field collected the resistant strain had previous exposure to a range of chemicals in addition to spinosad (see *Resistance Monitoring* section). The resistant strain (LR_P) was pressured with a discriminating concentration of spinosad on an ad hoc basis to maintain resistance.

The bioassay procedure is given in Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean-leaf discs embedded in agar in small Petri dishes. Thrips were pre-treated with synergist (Table 1) using intervals and methods as outlined in Thalavaisundaram *et al*. (2008). The pre treated leaf discs with anaesthetised thrips in place were then sprayed with aqueous insecticide (Table 2)(4 mL aliquot) or with water (control) with the aid of a Potter spray tower (deposit of 3.2 mg cm^{-2}).The Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine holes. The dishes were stored for 48 h at 25 ± 0.1 ^{o}C in a 18:6 hour L: D regime after which the numbers of alive and dead thrips were counted. Tests were done to achieve full log-dose probit regressions. Each test was replicated at least once and control mortality did not exceed 15% (unless otherwise stated).

(iii) Bioassay assessment

Thrips were confirmed alive or dead with the aid of a stereo-microscope.

(iv) Analysis of bioassay data

Data were analysed using GENSTAT 5 statistical software (Barchia, 2001). The LC50 plus its 95% fiducial-limits, were calculated using the probit method outlined in Finney (1971) and included control mortality correction (Abbott 1925). The LC_{50}s were used to calculate resistance factors at the LC_{50} level (ie RF_{50}) with or without synergist plus associated 95% confidence intervals (CI) as outlined in Robertson and Preisler (1992). As defined by Yu (2008) the LC_{50} was used to calculate an S ratio plus associated 95% confidence intervals (CI) by dividing the LC_{50} value for the insecticide applied alone by the LC_{50} when the insecticide is applied with synergist.

(t) Results

As compared to the response of strain LRp the addition of synergists did not significantly influences (by overlapping 95% CI) the RF_{50} ratio for abamectin, spinosad or thiamethoxam (Table 3). Interestingly, the RF_{50} ratio was significant for acetamiprid with PBO but not for the other synergists tested. Calculated S ratios were similar in that there was a significant

difference with acetamiprid and PBO but unlike the RF_{50} ratios there was an additional significant difference seen with abamectin with TPP.

Table 3. Probit regression summaries for against abamectin acetamiprid, spinosad and thiamethoxam for reference susceptible strain NZ2 and reference resistant strain LR_P with and without synergists DEM, TPP and PBO and calculated RF_{50} and S ratio.

Chemical & synergist	Strain	Slope (SE)	LC_{50}* (95% FL)	RF_{50} ratio (95% CI)	S ratio (95% CI)
abamectin	NZ2	3.6 (0.45)	0.0055 (0.0044-0.0064)	-	-
	LRp	1.6 (0.23)	0.0096 (0.0051-0.015)	1.7 (1.0-2.9)	-
+10.0 g/L DEM	LRp	0.7 (0.19)	0.012 (0.00067-0.033)	2.0 (0.5-9.0)	1.2 (0.3-5.0)
+ 2.0 g/L TPP	LRp	1.4 (0.28)	0.029 (0.011-0.056)	5.3 (2.7-10.7)	3.0 (1.3-7.0)
+0.625g/L PBO	LRp	0.9 (0.13)	0.0046 (0.0017-0.0087)	0.8 (0.4-1.9)	0.5 (0.2-1.2)
acetamiprid	NZ2	3.5 (0.46)	0.028 (0.020-0.035)	-	-
	LRp	2.3 (0.28)	0.055 (0.042-0.070)	2.0 (1.4-2.7)	-
+10.0 g/L DEM	LRp	1.9 (0.25)	0.069 (0.048-0.095)	2.5 (1.7-3.6)	1.2 (0.8-1.8)
+2.0 g/L TPP	LRp	1.7 (0.37)	0.038 (0.016-0.075)	1.4 (0.7-2.6)	0.7 (0.4-1.3)
+0.625 g/L PBO	LRp	2.0 (0.31)	0.011 (0.0073-0.014)	0.4 (0.3-0.6)	0.2 (0.1-0.3)
Spinosad	NZ2	3.3 (0.49)	0.00046 (0.00034-0.00057)	-	-
	LRp	0.7 (0.28)	0.18 (0.053-1.83)	398 (120-1322)	-
+10.0 g/L DEM	LRp	2.3 (0.70)	0.28 (0.092-0.56)	613 (325-1158)	1.5 (0.4-5.8)
+ 2.0 g/L TPP	LRp	1.6 (0.39)	0.078 (0.026-0.14)	170 (87-331)	0.4 (0.1-1.6)
+0.625g/L PBO	LRp	1.6 (0.41)	0.33 (0.15-0.72)	717 (394-1306)	1.8 (0.5-6.7)
thiamethoxam	NZ2	3.2 (0.42)	0.020 (0.015-0.024)	-	-
	LRp	2.2 (0.27)	0.083 (0.057-0.11)	4.1 (2.9-6.0)	-
+10.0 g/L DEM	LRp	1.3 (0.37)	0.038 (0.0075-0.093)	1.9 (0.8-4.7)	0.4 (0.2-1.1)
+ 2.0 g/L TPP	LRp	2.1 (0.27)	0.065 (0.045-	3.3	0.8

			0.084)	(2.3-4.7)	(0.5-1.2)
+0.625g/L PBO	LRp	1.6 (0.28)	0.062 (0.038-0.099)	3.1 (2.0-4.9)	0.7 (0.5-1.1)

* g ai / L
LC = lethal concentration
S ratio = synergist ratio
FL = fiducial limit
CI = confidence interval

(u) Discussion

Insecticide resistance can be either behavioural or physiological with the latter further subdivided into reduced penetration, altered target site or increased detoxification (Yu 2008). Chemical detoxification causing insecticide resistance can be can be suppressed by the addition of synergists (Raffa and Priester 1985) with this current study results implying monooxygenases may be involved in acetamiprid resistance and esterases with abamectin resistance in Australian populations of WFT.

Similar to our results Liu *et al*. 2003 found that PBO synergised acetamiprid caused as a cross resistance from imidacloprid in the brown plant hopper *Nilaparvata lugens* Stil and that esterases or glutathione-S-transferases were not involved. However, another study by Ninsin and Tanaka (2005) found acetamiprid synergism with both PBO and DEF (S,S,S-tributyl phosphorotrithioate, an esterase inhibitor) thus implying both monooxygenases and esterases are involved in acetamiprid resistance in the diamondback moth, *Plutella xylostella* (L). Consequently acetamiprid resistance may be variable between species. Results for abamectin are similar to that of Siqueira *et al*. (2001) who found abamectin resistance in the tomato leafminer *Tuta absoluta* (Meyrick) linked to esterases in 4 of the 6 resistant populations tested.

Interestingly, there were no significant differences with any syngergist detected with spinosad suggesting resistance may be target site rather than metabolic. This is consistent with the findings of Scott (2008) and Zhang (2008) who found spinosad resistance could not be overcome by synergists and Perry *et al*. (2007) that found spinosad resistance in *Drosophila melanogaster* Meigen linked to the nicotinic acetylcholine receptor (nAChR) (ie target site) subunit Dab, conferring 1181-fold spinosad resistance. For that reason the molecular component of the project VG06010 can pursue the *D. melanogaster* resistance model with confidence.

(v) Acknowledgments

We thank Dr Idris Barchia, NSW DPI, for analysing the bioassay data and the researchers and growers who forwarded strains for resistance testing.

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(x) Appendices

(i) Full log-dose probit data

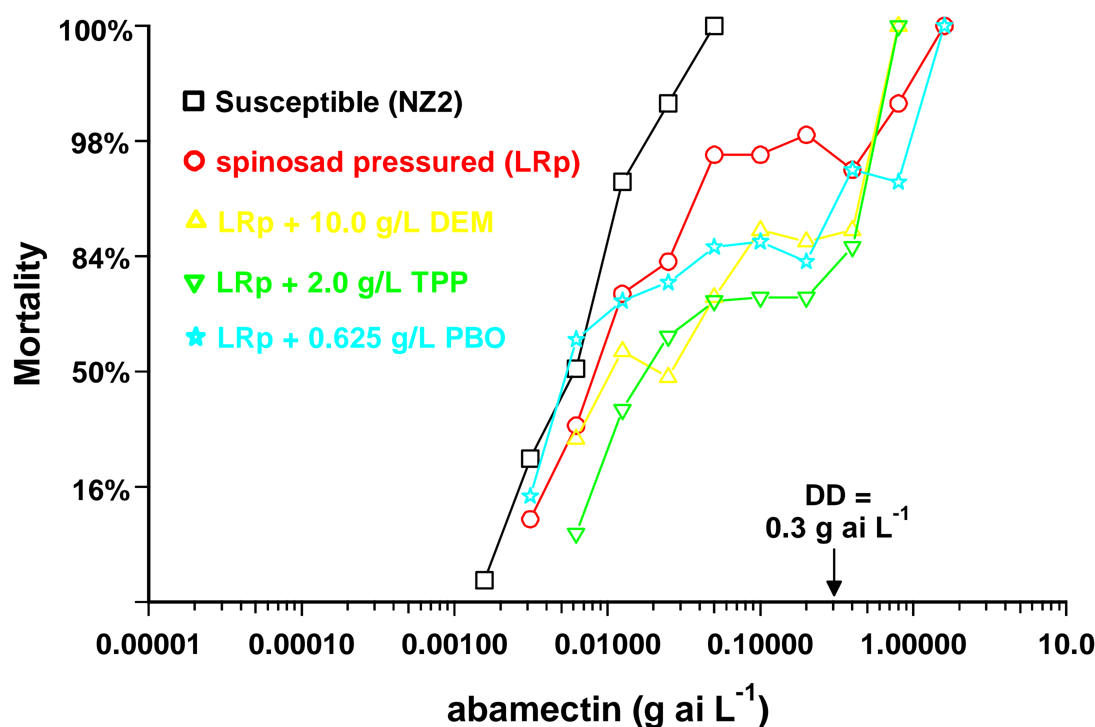

Figure 1. Control mortality corrected dose-response data against abamectin for reference susceptible strain NZ2 and reference spinosad resistant strain LRp with and without pre-treatment with 10.0 g/L di-methyl maleate (DEM), 2.0 g/L triphenyl phosphate (TPP) and 0.625 g/L piperonyl butoxide (PBO)(DD = Discriminating Dose)

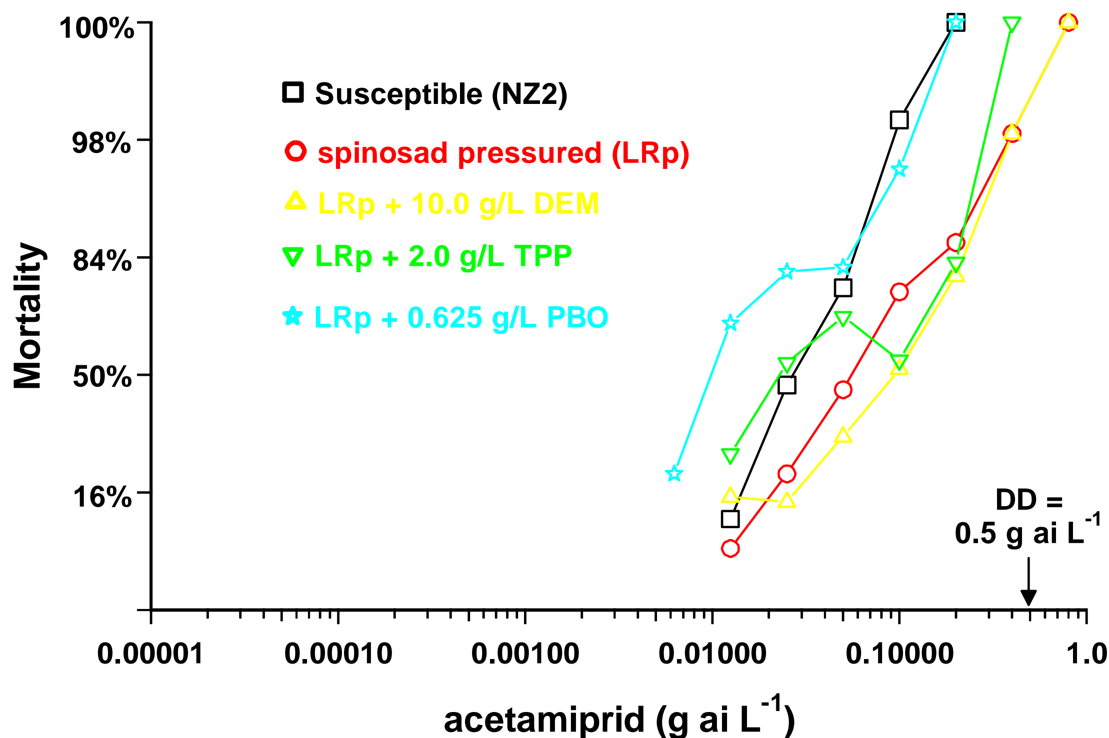

Figure 2. Control mortality corrected dose-response data against acetamiprid for reference susceptible strain NZ2 and reference spinosad resistant strain LRp with and without pre-treatment with 10.0 g/L di-methyl maleate (DEM), 2.0 g/L triphenyl phosphate (TPP) and 0.625 g/L piperonyl butoxide (PBO)(DD = Discriminating Dose)

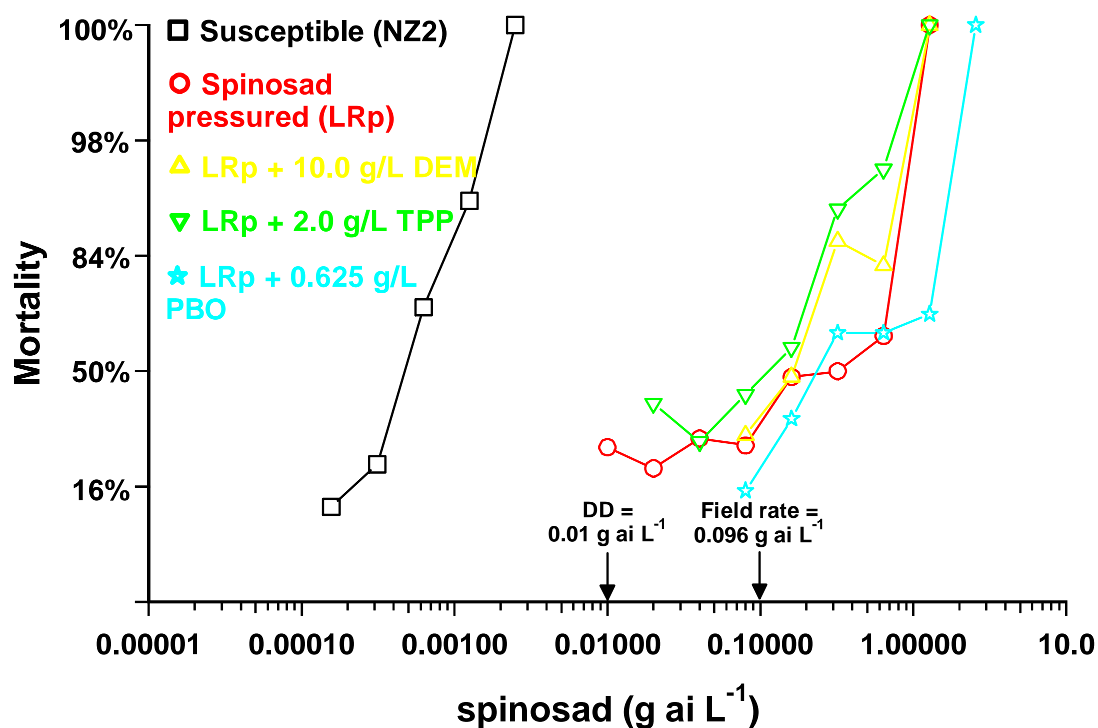

Figure 3. Control mortality corrected dose-response data against spinosad for reference susceptible strain NZ2 and reference spinosad resistant strain LRp with and without pre-treatment with 10.0 g/L di-methyl maleate (DEM), 2.0 g/L triphenyl phosphate (TPP) and 0.625 g/L piperonyl butoxide (PBO)(DD = Discriminating Dose)

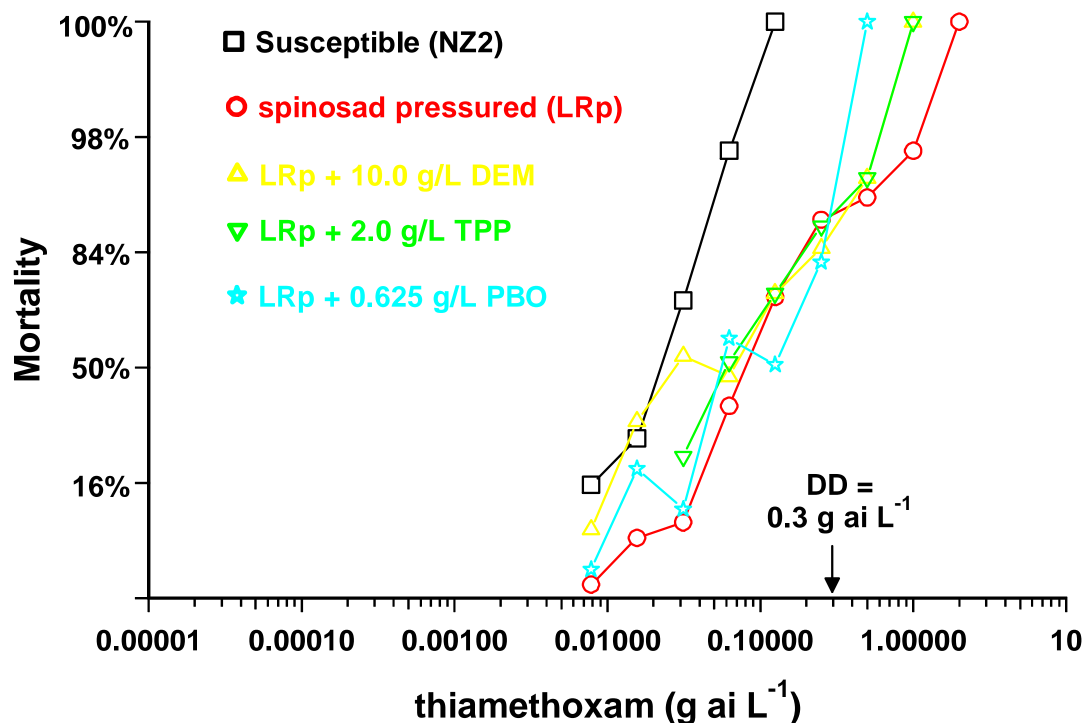

Figure 4. Control mortality corrected dose-response data against thiamethoxam for reference susceptible strain NZ2 and reference spinosad resistant strain LRp with and without pre-treatment with 10.0 g/L di-methyl maleate (DEM), 2.0 g/L triphenyl phosphate (TPP) and 0.625 g/L piperonyl butoxide (PBO)(DD = Discriminating Dose)

The molecular mechanism of spinosad resistance in western flower thrips (WFT), *Frankliniella occidentalis* (Pergandae). Yizhou Chen, Daniel Bogema, Brendan Langfield, Martin O McLoon, and Grant A. Herron

Industry and Investment NSW, Elizabeth Macarthur Agricultural Institute, PMB 2008, Narellan NSW 2567.

(y) Abstract

Western flower thrips (WFT), *Frankliniella occidentalis*, is one of the most serious pests of horticulture and cause severe crop losses. In Australia, the most common insecticide used for its control is spinosad (a mixture of spinosyn A and spinosyn D). Unfortunately spinosad resistance causing control failure has been detected, putting the sustainability of the spinosyn class of chemicals in doubt.. Improved management of spinosad resistance requires a good understanding of the spinosad resistance mechanism, so that insecticides causing cross-resistance can be eliminated from the chemical control strategy. As a part of this process we aimed to find the molecular basis for spinosad resistance to improve resistance management by developing a molecular diagnostic method to monitor spinosad resistance in WFT. Using immunoprecipitation we identified 10 candidate spinosad binding proteins in WFT. We also obtained 4 partial cDNA sequences for acetylcholine receptor Dα6. A singe nucleotide substitution (C>T) from spinosad resistance and susceptible WFT stains has been identified showing a clear difference between spinosad susceptible and resistant WFT at the molecular level. These findings provided the first useful information for understanding the molecular mechanisms of spinosad resistance in WFT as a first step to developing a molecular based diagnostic assay.

(z) Introduction

Insecticide resistance is a heritable characteristic that results directly from insecticide use and its evolutionary selection for resistance (Plapp and Wang 1983). It has been a problem of agriculture since the 1940s (Georguiou and Mellon 1983) despite first being noted nearly a hundred years ago by Melandier (1914). Since the introduction of synthetic organic insecticides, such as DDT, insect resistance to insecticides has necessitated more frequent insecticide applications, higher doses, and required the substitution of inexpensive compounds with newer and more expensive products (Georguiou and Mellon 1983). Globally, resistance to one or more pesticides has been recorded in some 550 arthropod species (Whalon *et al*. 2009). The loss of efficacy of pesticides due to resistance puts a huge demand on remaining products and presents an enormous threat to sustainable agriculture.

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), is one of the most serious pests of horticulture where feeding by adults and nymphs can cause severe crop losses (Kirk 2002). Damage is caused directly through feeding and oviposition (Childers and Achor 1995), and indirectly through transmission of tomato spotted wilt virus (Kirk 2002). It has a wide host range, and because of its ability to invade and establish in new habitats WFT is considered the most damaging thrips species in California (Kirk 2002). Since first being detected in Australia in Perth during 1993 (Malipatil *et al*. 1993), WFT has spread throughout

Australia and is now found in all six states but not the Northern Territory (Medhurst and Swanson 1999).

Spinosad is widely used in Australia for WFT control and is the most commonly used product for that purpose (InfoPest 2009). Unfortunately, spinosad resistance causing control failure has been found in Australia putting the whole chemical control strategy under threat (Herron and James 2005 and 2007). Insecticide resistance is caused by a number of mechanisms with most being either target site or detoxification (Georghiou and Saito 1983). The mechanism of action of spinosad is not clear although nicotinic acetylcholine receptor (nAChR) was implied as primary attacking site and g-aminobutyric acid (GABA) receptors as secondary attacking site (Scott 2008) and metabolic mediated detoxification was ruled out to be responsible for spinosad resistance in WFT (Bielza *et al.* 2007). Little information on spinosad resistance is known at a molecular level but studies showing the deletion of the Dα6 nAChR subunit in strains of *Drosophila melanogaster* (Fabricius) resulted in insects that were highly resistant to spinosad (Perry *et al.* 2007).

AIM: 1. To identify spinosad bind proteins in spinosad resistance WFT strain (LRp) via comparision to a reference susceptible (NZ2) as the first step to elucidate the molecular mechanism of spinosad resistance in WFT.

2. To use the Dα6 nAChR *D. melanogaster* model for possible spinosad resistance in WFT to identify sequence variations between spinosad resistant and susceptible WFT strains.

(aa) Materials and Methods

(i) WFT strains

Reference susceptible (NZ2) and spinosad resistant (LRp) WFT strains were maintained in insect proof cages in separate rearing rooms in a purpose built insectary at the Elizabeth Macarthur Agricultural Institute (EMAI)

(ii) Immunoprecipitation of the spinosad binding protein

Approximately 2000 reference susceptible (NZ2) and 1000 spinosad resistant (LRp) WFT were collected in 1.5 mL Eppendorf tubes and homogenised in 200µl NP-40 cell lysis buffer (50 mM Tris-HCl, 150 mM NaCl and 1% NP-40, pH 8.0). The protein lysis solution was divided equally and cell lysis buffer added to total volume of 450µL. Ten µL super saturated spinosad was added to one portion of the protein lysis solution and mixed using a vortex mixer. The mixed solution was kept at room temperature for 15 minutes with further 2 hours at 4°C. The other portion of the protein lysis solution without super saturated spinosad was used for a no-spinosad control.

Immunoprecipitation was used to isolation of spinosad binding protein with RaPID Assay Spinosad Test Kitn (Strategic Diagnostics, Inc) in which anti-spinosad antibody was attached to magnetic beads. Five hundred µL of magnetic beads were conjugated with an anti-spinosad antibody (RaPID Assay Spinosad Test Kit, Strategic Diagnostics, Inc) and added to the protein solutions and mixed by slow rotation overnight at 4°C. Magnetic beads were washed twice at 4°C for 2 minutes with PBS washing buffer (10 mM sodium phosphate, 150 mM NaCl, 0.1% Tween-20, pH 7.4.) Suspended beads were collected after each wash with a

magnetic particle collector (Dynal MPC-E; Dynal, Lake Success, NY). Protein was eluted in 20µl 0.1 M citrate (pH 3.0) by rotation for 5 minutes at room temperature. Eluant protein was neutralized with 1M Tris (pH 7.5).

The eluant protein was separated on a 12% acrylamide SDS-PAGE gel under reducing conditions and visualized using silver staining (Figure 1). Stained protein bands were excised from the gels and liquid chromatography-mass spectrometry (LC-MS/MS) analyses were carried out at the Bioanalytical Mass Spectrometry Facility, the University of New South Wales, Sydney. Peaks data was generated by the MASCOT searching program (Matrix Science) and protein identification was achieved as described (Coumans *et al.* 2009), by combining spectrum quality scoring obtained from a conventional database search program Mascot (Version 2.1 or 2.2, Matrix Science, London, England), with automated *de novo* sequencing on unassigned high quality spectra using the PEAKS studio 4.5 (Bioinformatics Solution Inc., Waterloo, Ontario, Canada).

(iii) Isolation nAChR receptor Dα6 in WFT

Total RNA was extracted from 100 WFT with TRI reagent (Ambion, Applied Biosystems) according to the manufacturer's instructions. Gene specific primer Fa6_left (TTCAAGAGCACATGCAAGATAGA) and Fa6_right (GTCTATCTTGCATGT GCTCTTGA) was designed based consensus sequence with alignment of Dα6 mRNA sequences of the ferment fly *Drosophila* sp., the house fly *Musca* sp., the flour beetle *Tribolium* sp. and the brown plant hopper *Nilaparvata lugens* (Stål) by CLUSTAL W (1.81) (see Appendix 1).

The first strand of cDNA for 3' and 5'-RACE was synthesized with gene specific primers and adaptor primers provided by the SMARTer RACE cDNA Amplification Kit (Clontech) according to the manufacturer's instructions. Two-step PCR protocol was employed to amplify the 5' end cDNA sequence. PCR products were purified with a Jet Quick Gel Extraction Spin kit (Genomed, Löhne, Germany) and cloned into a TOPO vector (TOPO TA Cloning® Kit for Sequencing, Invitrogen). Sequencing was carried out by the Australian Genome Research Facility (AGRF) with the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems). Sequence data was analysed with the Sequencher software v. 4.10 (Gene Codes Corporation).

(bb)Results

(i) Candidate spinosad binding protein

The affinity of the antibody to spinosad was confirmed as the control (lane 2: Eluant by immunoprecipitation without spinosad treatment) with no spinosad had a markedly reduced spinosad band (Figure 1) with only the faintest visual band from the no-spinosad control. Binding capacity of the spinosad binding proteins were separated in SDS-PAGE gel excised for analysis by LC-MS/MS. Candidate proteins were listed in Table 1 with MASCOT score >52 (the absolute probability that the observed match is a random event $p<0.05$).

(ii) cDNA sequencing of nAChR receptor Dα6 in WFT

Four gene specific fragments were visualized in agarose gel with 5' Race (Figure 2). These fragments were cloned into TOPO vector and sequenced with ABI sequencer. The candidate cDNA sequences were obtained with 278bp, 442bp, 496bp and 539bp. Comparison of the spinosad resistance and susceptible strain of 278bp cDNA fragment sequences revealed a C>T substitution at position 119 (Figure 3).

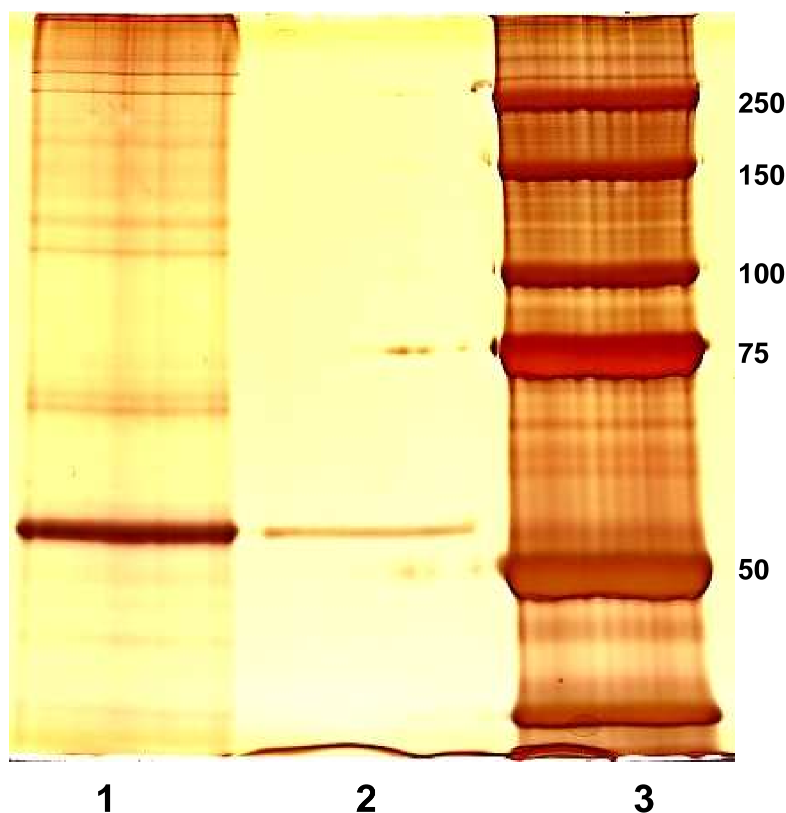

Figure 1. Separation of bound spinosad antibody on a 12% acrylamide SDS-PAGE gel under reducing conditions and visualized using silver staining. Lane 1: Eluant by immunoprecipitation with spinosad treatment, lane 2: Eluant by immunoprecipitation without spinosad treatment (control), lane 3, protein marker (kDa).

Table 1. Insect proteins identified as similar to the bound WFT spinosad resistance molecule by chromatography-mass spectrometry and MASCOT Search

Protein	NCBI GI	Mass	Score
myosin heavy chain, nonmuscle or smooth muscle [*Aedes aegypti*]	gi|157110721	221327	203
similar to CG17927-PF [*Nasonia vitripennis*]	gi|156544337	224385	190
fructose-bisphosphate aldolase [*Enallagma aspersum*]	gi|46909203	21890	92
vitellogenin [*Pteromalus puparum*]	gi|134290336	202819	72
vitellogenin C1 [*Culex pipiens quinquefasciatus*]	gi|54289293	241413	56
PREDICTED: similar to muscle myosin heavy chain isoform 1 [*Acyrthosiphon pisum*]	gi|193624646	224643	204

unnamed protein product [*Drosophila melanogaster*]	gi|8186	104050	195
fructose 1,6-bisphosphate aldolase [*Antheraea yamamai*]	gi|45330818	39684	108
myosin heavy chain [*Drosophila melanogaster*]	gi|157891	224288	113
sarco(endo)plasmic reticulum-type calcium ATPase [*Heliothis virescens*]	gi|4191598	109509	104

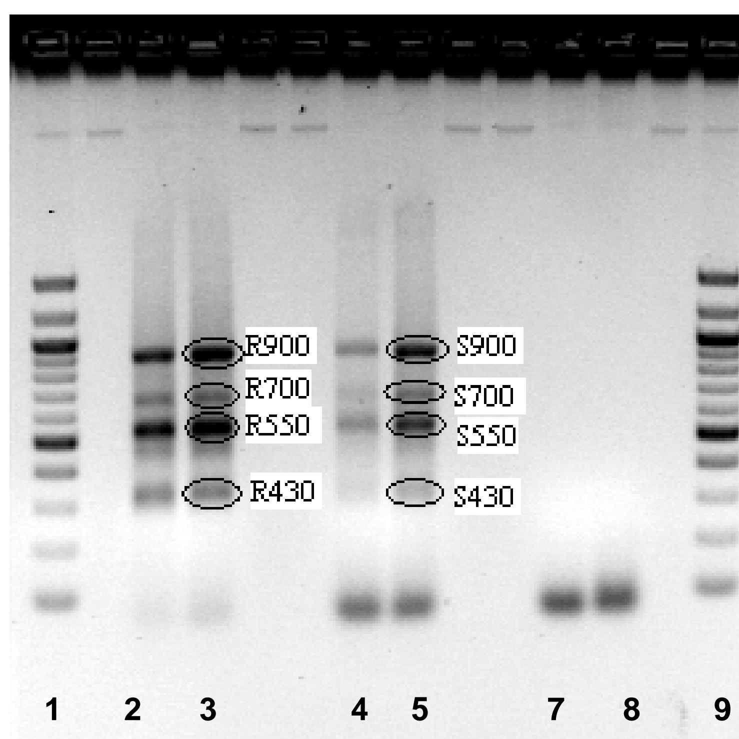

Figure 2. 5’and 3’ RACE PCR from WFT with consensus primers. Lane 1 and 9: DNA Marker (NEB 100bp), Lane 2-3: 5’RACE cDNA from spinosad resistant WFT, Lane 4-5 5’RACE cDNA from spinosad susceptible WFT.

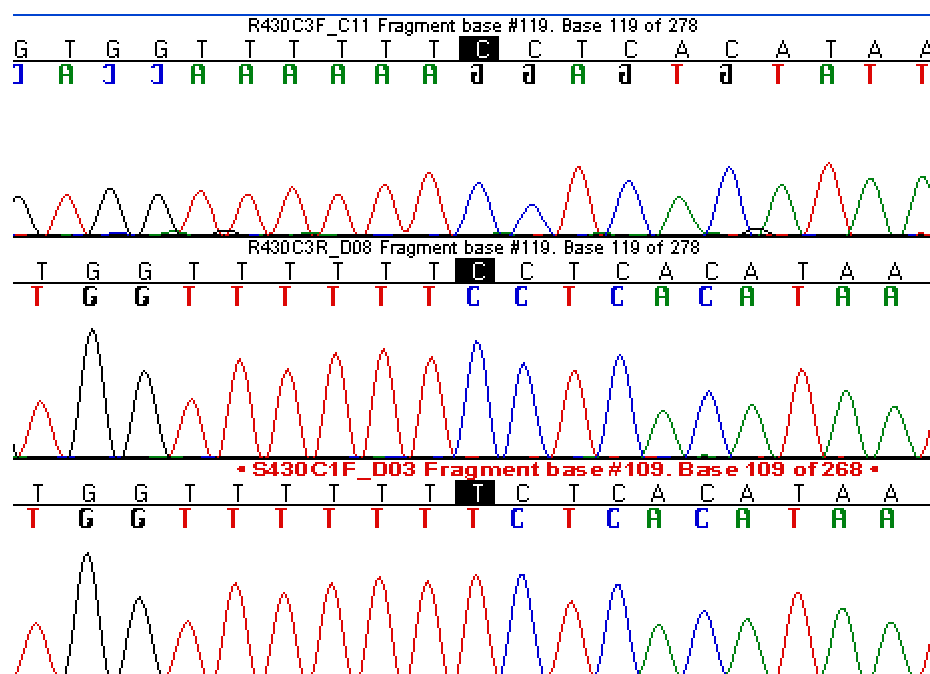

Figure3. Histogram of alignment of the cDNA sequences of resistance strain (clone R4303F_C11) and susceptible strain (clone S430F-D03). A C>T substitution is evident at position 119.

(cc) Discussion

Spinosad is a new and highly promising insecticide derived from the bacteria *Saccharopolyspora spinosa*, with efficacy against a wide range of insects including WFT, *F. occidentalis*. The mechanism of action of spinosad is thought to activate nAChRs and the deletion of the Dα6 nAChR in strains of *Drosophila melanogaste*r caused them to be highly resistant to spinosad (Perry *et al.* 2007).

Our immunoprecipitation testing identified 10 insect proteins with similarities to the spinosad binding protein in WFT. However, because there is a lack of WFT protein sequences in the protein database no clear conclusions could be drawn except that the spinosad binding protein was homologous to other insect proteins in the species listed. None the less, the identified candidate proteins may provide useful information for identifying the spinosad target site in the future. Encouragingly, the sequencing of nAChR receptor Dα6 cDNA sequence from spinosad resistance and susceptible WFT did reveal a singe nucleotide substitution (C>T) that may well be associated with spinosad resistance. Although we can not definitively confirm this as the causative mutation for the resistance, we are one big step closer to characterising the spinosad resistance gene.

To this end both proteomic and transcriptome approach should be used to further identify the molecular mechanism(s) of spinosad resistance in WFT. This will help circumvent the challenge encountered by the lack WFT specific sequence information in the Genebank. We suggest sequencing all acetylcholine receptors (nAChRs) and g-aminobutyric acid (GABA) receptors in our spinosad resistant and susceptible WFT strains as a first priority to identify the causative mutations and aid the rapid development of a DNA based diagnostic test for spinosad resistance detection in WFT.

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(ee) Appendices

(i) Alignment of mRNA sequences from the ferment fly *Drosophila* sp., the house fly *Musca* sp., the flour beetle *Tribolium* sp. and the brown plant hopper *N. lugens*

```
CLUSTAL W (1.81) Multiple Sequence Alignments


Sequence type explicitly set to DNA
Sequence format is Pearson
Sequence 1: Drosophila              1588 bp
Sequence 2: Nilaparvata_lugens      1881 bp
Sequence 3: house_fly               1601 bp
Sequence 4: Tribolium               1410 bp
Start of Pairwise alignments
Aligning...
Sequences (3:4) Aligned. Score:  69
Sequences (2:3) Aligned. Score:  58
Sequences (1:2) Aligned. Score:  62
Sequences (2:4) Aligned. Score:  66
Sequences (1:3) Aligned. Score:  76
Sequences (1:4) Aligned. Score:  70
Guide tree        file created:   [clustalw.dnd]
Start of Multiple Alignment
There are 3 groups
Aligning...
```

```
Group 1: Sequences:    2      Score:23599
Group 2: Sequences:    3      Score:19769
Group 3: Sequences:    4      Score:19487
Alignment Score 39554
CLUSTAL-Alignment file created  [clustalw.aln]
```

---

clustalw.aln

```
CLUSTAL W (1.81) multiple sequence alignment


Drosophila              ------------------------------------------------------------
house_fly               CGCACCAACCAACCAACTCAGCCAGCCACCCCCCGGCTTTAAGGAACACACACCAAAAAA
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      ----------------------------------------AGATCCGGGTCAACATGCAC


Drosophila              -------------GGACTCCCCGCTGCCAG-----CGT-CGCTGTCGCTGTTTGTCCTGT
house_fly               AGGATATCATCACAGACTGACCATAATGGATT---CGT-CAACATCGCTGTATTTGGTTT
Tribolium               ---------------ATGGTCCGGAGCGAGCAA-GCGC-TGGCGCTGCTGGCCACAGCCT
Nilaparvata_lugens      CCGGCAAGCTTATGAGCCAGCCGTCATGGAGGCAGTGTACAGCCTCATTGTGTTAGTCGC
                                            **              *           **

Drosophila              TGATCTTTCTGGCGATAATTAAAGAAAGCTGTCAAGGACCTCATGAAAAGCGCCTGCTGA
house_fly               TGCTTATATTTGTGATAATAAAAGAAAGCTGCCAAGGACCACACGAAAAACGTTTACTAA
Tribolium               TCCTGCTCTTCATCATGCCTCCAGGTTCGCAACAGGGGCCGCACGAAAAGCGGCTACTAA
Nilaparvata_lugens      TTGCATAGCTTCTC-TACCACACGAATCTCTGCAAGGCCCCCATGAAAAACGTCTTTTAC
                        *        *     *       *        ** ** ** ** ***** **  *  *

Drosophila              ACCACCTGCTGTCCACCTACAACACGCTGGAGCGACCCGTGGCCAACGAATCGGAGCCCC
house_fly               ACCACCTCTTATCCACCTATAATACTTTAGAAAGACCTGTAGCAAATGAATCCGATCCCC
Tribolium               ACAACTTGTTGGGGCCCTATAACGTCTTGGAGAGACCCGTAGCCAACGAGTCAGAACCTC
Nilaparvata_lugens      ACAACCTTCTGGATCATTACAACGTTCTGGAACGTCCGGTTGCCAACGAATCCGATCCTC
                        ** ** *  *       ** **     * **  * ** ** ** ** ** ** ** ** *

Drosophila              TGGAGGTCAAGTTCGGACTGACGCTGCAGCAGATCATCGACGTGGATGAAAAGAATCAGA
house_fly               TGGAAGTGAAATTTGGACTGACCCTACAACAGATCATCGATGTGGATGAAAAGAATCAGA
Tribolium               TTGAAGTCAAGTTCGGCCTAACTTTGCAGCAAATCATTGATGTGGACGAAAAGAATCAGA
Nilaparvata_lugens      TCCAGCTAAGCTTCGGATTAACGCTCATGCAGATCATCGATGTGGACGAGAAGAATCAGC
                        *  *  * *  ** **  * **  *    ** ***** ** ***** ** *********

Drosophila              TTCTGACCACAAATGCGTGGTTAAATTTGGACGAGAAGAATCAGCTTCTCATAACGAATC
house_fly               TTCTGACCACAAATGCGTGGTTAAATTTGGA-----------------------------
Tribolium               TTCTCACGACAAACGCGTGGTTGAATTTGGA-----------------------------
Nilaparvata_lugens      TACTAATTACAAACATTTGGCTAAAACTGGA-----------------------------
                        * ** *  *****    *** * **  ****

Drosophila              TTTGGCTTTCGTTGGAGTGGAACGACTACAATCTCCGCTGGAATGAAACGGAATACGGCG
house_fly               ----------------GTGGAATGACTACAATCTCAGATGGAATGATTCCGAGTATGGCG
Tribolium               ----------------ATGGAACGACTATAATCTCAAATGGAACGAATCGGAATATGGCG
Nilaparvata_lugens      ----------------ATGGAACGACGTCAACTTGAGATGGAATTCTACAGAGTACGGCG
                                         ***** ***   **  *    *****     * ** ** ****

Drosophila              GGGTCAAGGATCTGCGAATCACGCCCAACAAGCTGTGGAAGCCCGACGTGCTCATGTACA
house_fly               GTGTCAAAGACTTGAGAATAACGCCAAATAAACTGTGGAAACCCGATGTGCTCATGTACA
Tribolium               GGGTCAAAGACTTGCGGATTACTCCAAACAAGCTGTGGAAGCCTGATGTTCTTATGTATA
Nilaparvata_lugens      GAGTGAAAGATCTCAGAATTCCACCTCACCGGATATGGAAGCCCGATGTCCTCATGTACA
                        * ** ** **  *  * **  * **  *     * ***** ** ** ** ***** *

Drosophila              ACAGCGCGGATGAGGGATTCGATGGCACGTATCACACCAACATTGTGGTCAAACATAACG
house_fly               ACAGTGCTGATGAGGGATTCGATGGCACGTATCACACCAACATTGTGGTCAAACATAACG
Tribolium               ACAGTGCTGATGAGGGTTTCGACGGGACTTTCCAAACAAACGTTGTGGTCAAACATAACG
Nilaparvata_lugens      ACAGTGCTGATGAAGGGTTCGACGGGACTTACCCGACCAACGTTGTGGTCAGGAATGGCG
                        **** ** ***** ** ***** ** ** *  *  ** *** *********   **  **

Drosophila              GCAGTTGTCTGTACGTGCCCCCTGGTATCTTCAAGAGCACATGCAAGATAGACATCACGT
house_fly               GCAATTGTCTGTACGTGCCCCCTGGCATCTTCAAGAGCACATGCAAGATAGACATCACGT
Tribolium               GCAGCTGCTTGTACGTCCCTCCGGGTATCTTCAAGAGCACATGCAAGATAGACATTACGT
Nilaparvata_lugens      GCAGCTGCCTGTATGTGCCGCCGGGTATCTTCAAGAGCACATGCAAGATAGACATAACGT
                        ***  **  **** ** ** ** ** ***************************** ****

Drosophila              GGTTCCCATTTGATGACCAACATTGCGAAATGAAATTCGGTAGTTGGACTTACGATGGAA
house_fly               GGTTCCCATTCGATGACCAACACTGCGAAATGAAATTCGGTAGTTGGACTTATGATGGAA
Tribolium               GGTTCCCCTTTGATGACCAACACTGTGACATGAAGTTTGGTAGCTGGACCTATGACGGCA
Nilaparvata_lugens      GGTTCCCCTTTGATGACCAGCGCTGTGAAATGAAGTTCGGTAGCTGGACCTATGACGGCT
                        ******* ** ******** *  ** ** ***** ** ***** ***** ** ** **

Drosophila              ATCAGTTGGATTTGGTTTTGAATTCCGAAGATGGAGGGGATCTTTCCGATTTCATAACAA
house_fly               ATCAGTTGGATTTGGTTTTGAATTCCGAAGATGGAGGGGATCTATCCGATTTTATAACAA
Tribolium               ACCAGCTCGACCTGGTGCTCAATTCCGAATCGGGTGGTGATTTATCAGACTTCATTACAA
```

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Nilaparvata_lugens      TCCAGCTCGATCTCCAACTCCAAGACGAAGGCGGTGGAGATATTAGTAGTTTCATAACGA
                          *** * **  *     *  *   ****   ** ** *** *       ** ** ** *

Drosophila              ATGGCGAGTGGTACTTGCTTGCCATGCCGGGAAAGAAGAATACGATAGTCTACGCCTGCT
house_fly               ACGGCGAATGGTATTTAATCGCCATGCCGGGCAAAAAGAATACCATCGTATATGCCTGCT
Tribolium               ACGGAGAATGGTACTTGATAGGGATGCCCGGCAAGAAGAACACCATCGTCTACGCATGTT
Nilaparvata_lugens      ATGGAGAGTGGGATCTTTTAGGTGTTCCAGGGAAACGGAACGAAATTTACTACAATTGCT
                        * ** ** *** *  *  * *   * ** ** **   ***    **    **    ** *

Drosophila              GCCCAGAACCATATGTCGATATCACCTTTACTATACAAATTCGTCGCCGTACATTATATT
house_fly               GTCCGGAACCGTATGTGGATGTGACGTTCACAATACAAATACGAAGACGGACATTATATT
Tribolium               GCCCCGAACCGTATGTGGATGTGACGTTCACGATTAAGATCCGGAGGCGAACGCTGTACT
Nilaparvata_lugens      GCCCCGAACCTTATATAGATATCACGTTTATCATAATCATACGGAGGCGGACACTCTACT
                        * ** ***** *** * *** * ** ** *  **    ** **  * ** **  * ** *

Drosophila              ATTTTTTCAATTTAATCGTGCCATGTGTGCTAATCTCATCGATGGCCCTACTGGGCTTCA
house_fly               ATTTTTTTAATTTAATTGTGCCGTGTGTGCTGATATCCTCAATGGCTTTATTAGGATTTA
Tribolium               ACTTCTTCAACCTTATAGTTCCTTGCGTGCTCATCTCCTCCATGGCGCTACTGGGCTTCA
Nilaparvata_lugens      ACTTTTTCAACCTTATCGTACCCTGCGTGCTGATCGCCTCCATGGCGGTGCTAGGATTTA
                        * ** ** **   * ** ** ** ** ***** **  * ** *****  *  * ** ** *

Drosophila              CATTGCCGCCGGATTCGGGCGAGAAACTGACGCTGGGCGTAACTATACTACTATCATTAA
house_fly               CATTACCACCCGATTCGGGTGAAAAACTAACCTTAGGTGTAACCATACTTTTGTCACAAA
Tribolium               CGTTGCCACCGGATTCTGGCGAGAAGCTCACT----------------------------
Nilaparvata_lugens      CTCTACCACCTGACTCGGGCGAAAAACTCTCCTTAGGTGTGACGATTCTGCTGTCGCTCA
                        *  * ** ** ** ** ** ** ** **  *

Drosophila              CAGTATTTCTAAACCTTGTCGCCGAGTCCATGCCGACAACGTCGGATGCTGTTCCTCTTA
house_fly               CTGTATTTTCA----CTATTG----------GTTGGTAATGTC-----------------
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      CCGTTTTCCTAAACATGGTGGCTGAGACGATGCCTGCCACCTC-----------------


Drosophila              TAGTGTTTCTCAACCTTGTAGCTGATACATTGCCCCAAGTATCTGATGCAATCCCCTTGT
house_fly               ----------------------------ATTACCAAAACCAG-TGAAGCTGTACCGCTGT
Tribolium               -----------------------------------------------------------T
Nilaparvata_lugens      -------------------------------------------TGACGCTGTCCCTCTTC


Drosophila              TAGGCACCTACTTCAATTGCATCATGTTCATGGTCGCATCGTCGGTGGTGCTGACAGTAG
house_fly               TAGGTACCTATTTCAATTGCATTATGTTTATGGTTGCCTCCTCGGTGGTCCTGACGGTTG
Tribolium               TAGGGACCTATTTCAACTGCATCATGTTCATGGTAGCGTCGTCAGTGGTCTTGACTGTGG
Nilaparvata_lugens      TAGGCACATACTTCAATTGCATAATGTTCATGGTGGCTTCATCGGTCGTGTCAACTATAT
                        **** ** ** ***** ***** ***** ***** ** ** ** ** **    **  *

Drosophila              TGGTGCTCAACTACCACCATCGCACAGCGGACATTCACGAGATGCCACCGTGGATCAAGT
house_fly               TGGTGCTGAACTATCATCATCGCACGGCGGACATACATGAAATGCCACCATGGATACGTT
Tribolium               TGGTGTTAAATTACCATCACCGCACGGCTGATATCCACGAAATGCCTCAATGGATCAAGA
Nilaparvata_lugens      TGATTCTCAACTATCATCATAGAAACGCTGACACTCATGAAATGTCACCTTGGATCAAAT
                        ** *  * ** ** ** **  * *  ** ** *  ** ** *** * *  *****

Drosophila              CCGTTTTCCTACAATGGCTGCCCTGGATCTTGCGAATGGGTCGACC------CGGCCGCA
house_fly               CCGTATTTCTACAATGGCTGCCCTGGATTTTACGCATGAGCCGCCC------CGGCCGTA
Tribolium               CGGTATTCTTACAATGGCTGCCGTGGATGTTGGGGATGAGTCGACC------GGGCAAGA
Nilaparvata_lugens      CGGTGTTCCTGCACTGGATGCCGTGCCTGCTGCGCATGTCGCGGCCGGCGGGCGGAGGCG
                        * ** **  * ** *** **** **  *  *  * ***   ** **        **

Drosophila              AGATCACACGCAAAACAATACTATTAAGCAATCGCA------------TGAAGGAGCTGG
house_fly               AAATCACAAGAAAACACATACTCTTAACGAATCGCA------------TGAAGGAATTGG
Tribolium               AGATAACCCGGAAGACGATTCTGATGAACAGCCGAA------------TGAAAGAGTTGG
Nilaparvata_lugens      GGAACGGGGGCGGCGAGGCGACATCGGACAGTCGCAAGTCGCTGCAGATGCGGGAGCTGG
                          *      *                   *  ** *            **   **  ***

Drosophila              A------GCTAAAGGAGCGCTCCTCCAAATCCCTGCTGGCCAATGTCCTCGACATCGACG
house_fly               A------ACTGAAAGAGCGTTCTTCCAAATCGCTGCTGGCCAATGTGCTGGACATCGATG
Tribolium               A------GTTGAAAGAACGATCTTCGAAAAGTCTTCTTGCCAACGTTTTGGACATCGACG
Nilaparvata_lugens      ACGCCTCGCTCAAGGACCGCTCCAGCAAGTCACTCCTGGCCAACGTGCTAGACATCGACG
                        *         * ** ** ** **     **     ** ** ***** **  * ******** *

Drosophila              ACGACTTCCGGCACACAATATCTGGCT---------CCCAGACCGCCATTGGCTC-GTCG
house_fly               ACGATTTCCGGCATACAGTGTCGGGGT---------CACAGACGGCAATTGGGTC-GTCA
Tribolium               ACGATTTCCGGAATGTGTCAACCGGCGGGAACAACGCTTCGATGACGACTAGTTTAGGCG
Nilaparvata_lugens      ACGACTTCCGACACGGAGGCGGCGGGG-----------GCGGCACTCTCCAGTCCAGCAG
                        **** *****  *          **                *          *    *

Drosophila              GCCAGCTTCGG--TCGGCCCACAACGGTGGAGGAGCACCACACGGCCATCGGCTGCAATC
house_fly               GCGAGTTTCGG--TCGGCCCACAACGGTGGAGGAACATCACAATGCCATCGGTTGTAATC
Tribolium               GTACGTTTATGCGTCACCCTACGACGATCGAGGAGGCAGCCGTCCCCAGCTCGGGCACGC
Nilaparvata_lugens      TCACAGCTTCCTGCGAGGCCACGAGGACGGATCAGTCCTGCACTCCTGTCTCGGGC-CGC
                               *          * ** * *   **  *       *    *   *    *    *
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Drosophila              ACAAAGATCTTCATCTAATTCTCAAAGAATTGCAATTTATTACGGCGCGGATGCGCAAAG
house_fly               ACAAGGATCTACATTTAATTCTCAAAGAATTGCAATTTATAATGGCGCGCATGCGCAAGG
Tribolium               AACGCGACCTCCAGAATATCCTACGTGAGCTTCAATTCATCACCAACCGCATGAAGAAAG
Nilaparvata_lugens      ATCGAGAACTCACGCTCATCCTCAAGGAGCTGCGGGTCATCACCGACAAACTGCGCAAGG
                        *    ** **       ** **    **  * *   * ** *         **   ** *

Drosophila              CTGACGACGAAGCGGAATTGATCGGCGATTGGAAGTTCGCGGCAATGGTTGTGGATAGAT
house_fly               CCGATGATGAGGCTGAGCTAATAAGCGATTGGAAGTTCGCGGCTATGGTTGCGGATCGAT
Tribolium               CCGACGAGGAGGCCGAAGTGATCAGCGATTGGAAGTTCGCCGCGATGGTGGTGGACCGGT
Nilaparvata_lugens      AAGACGAGGCCTCCGAGGTCACCAACGATTGGAAGTACGTCGCCATGGTAGTTGATAGAT
                          ** ** *   * **  * *    *********** **  ** ***** *  **  * *

Drosophila              TTTGTTTAATTGTTTTCACGCTCTTCACGATTATTGCAACGGTTACGGTGCTGCTCTCCG
house_fly               TTTGTTTAATTGTCTTTACACTCTTCACAATAATAGCCACAGTGACAGTTCTGCTGTCAG
Tribolium               TTTGTCTGATAATCTTCACGATGTTCACGATCATCGCCACGGTGGCCGTGCTGCTTTCGG
Nilaparvata_lugens      TTTGCTTGTTTGTCTTCACCCTGTTCACGATCATCGCGACGGTAGCAGTCCTGTTATCCG
                        ****  *  *  * ** **  * ***** ** ** ** ** **  * ** *** * ** *

Drosophila              CTCCGCACATAATCGTGCAATAA-------------------------------------
house_fly               CACCCCACATAATAGTTCAATAAAATTAGGATATAAATGACACAACAACAACAATGCAAG
Tribolium               CGCCGCACATAATCGTGCAGTAG-------------------------------------
Nilaparvata_lugens      CTCCGCATATTATCGTGCAATGAGTT--GGTCTTGATCTTGCTTCTGGCAACTCTTCAGA
                        * ** ** ** ** ** ** *

Drosophila              ------------------------------------------------------------
house_fly               AAACCT------------------------------------------------------
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      GCACTGAAGAGGGCTATGATTATGGTCTTGATTATGGTGATGCAGCATGATATGGTCCAG

Drosophila              ------------------------------------------------------------
house_fly               ------------------------------------------------------------
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      AAGTTGATGGATGAGTAATGAATCCTGCTGCTGGATCTGGAAAGAATTTTGCAGGATTTG

Drosophila              ------------------------------------------------------------
house_fly               ------------------------------------------------------------
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      GTCTATATGCTTTTGGAGGATACTATCCATGAATTGAGGCTTATGGATCTGGATAGGGAC

Drosophila              ------------------------------------------------------------
house_fly               ------------------------------------------------------------
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      CTGCTGCACGCCGATCTTATGGTAGACCTCGATACTATCCCTATGCTGAGGTTGAATATT

Drosophila              ------------------------------------------------------------
house_fly               ------------------------------------------------------------
Tribolium               ------------------------------------------------------------
Nilaparvata_lugens      CTATGTTTCCTGGGAATAAATTGTTTGAGTATCACAATTCCAAAAAAAAAAAAAAAAAAA

Drosophila              -
house_fly               -
Tribolium               -
Nilaparvata_lugens      A
```

(ii) Sequences of cDNA clones

>R430C3
TACATGGGGTTGCGCGTTGTGGTTGCGGCTGTGTGCAGTCAGCTGAGTTC
CCTTGTCCATTTCAGCAGTGGTTCGACATTTCGGCTTTGTATTTTGGTTG
ATAATTGTGGTTTTTTCCTCACATAATATGGAGGATAGGTTGCGGAGATA
CGAAAAAATTGAATTCCTGGGAGAGGGACAGTTTGCAACTGTTTATAAAG
CAAGAGATACACACTCAGACAATATAGTTGCAGTTAAAAAGATCAAAATT
GGATCAAGAGCACATGCAAGATAGAC

>S430C1
TACATGGGGGCGGCTGTGTGCTGTGTGCAGTCAGCTGAGTTCCCTTGTCC
ATTTCAGCAGTGGTTCGACATTTCGGCTTTGTATTTTGGTTGATAATTGT

GGTTTTTTTCTCACATAATATGGAGGATAGGTTGCGGAGATACGAAAAAA
TTGAATTCCTGGGAGAGGGACAGTTTGCAACTGTTTATAAAGCAAGAGAT
ACACACTCAGACAATATAGTTGCAGTTAAAAAGATCAAAATTGGWTCAAG
AGCACATGCAAGATAGAC

>R550C1
TACATGGGGGACATTCAGCGGGCGGAAAGACATCGTCTGGTTGTGCTTGT
ACACTGTACACGTAGGTTACCGGCCAGCTTCACAATGAAAACAGCTGGGT
TTTGTGTCCTACTCTTAGGCGTTTTTTCATTGTGTGATGCGCAGCAACGG
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The relationship between spinosad insecticide resistance and field control in *Frankliniella occidentalis* (Pergandae) under glasshouse conditions. Sonya Broughton and Grant A. Herron*

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(ff) Abstract

Susceptible and resistant western flower thrips *Frankliniella occidentalis* were prepared at EMAI by placing them in vials at specific initial resistance frequencies. Each vial contained 50 *F. occidentalis* adults that were ready for use immediately in WA. An initial trial done in 2008 included ratios of 100% susceptible (sprayed), 10% resistant (sprayed), 20% resistant (sprayed), 40% resistant, (sprayed) 60% resistant (sprayed) and 80% resistant (sprayed). Thrips were released onto caged whole plant plots and left to breed for two weeks. Thrips were then sprayed with spinosad at the registered rate, three days apart according to be published resistance strategy. The initial trial was replicated twice but plant quality declined during trial due to problems with fungi. At the end of the trial whole plants were destructively sampled and total number of adults and larvae counted. Interestingly, not all susceptible *F. occidentalis* were killed with 4 adults and 22 larvae counted. Based on that initial data we consider chemical control with spinosad seemed to fail somewhere between 20 and 30% resistant WFT. A second trial was under taken in 2009 using the same methodology but with 100% Susceptible (water control), 100% Susceptible (sprayed), 10% Resistant (sprayed), 20% Resistant (sprayed), 40% Resistant (sprayed), 60% Resistant (sprayed), 80% Resistant (sprayed) and 100% Resistant (sprayed). Results were obtained for two replicates but final replication had to be abandoned because reference strains at EMAI were destroyed by the predatory mite *Neoseiulus barkeri* (Hughes). For this reason statistical significance could not be determined, but we suggest that resistance causes spinosad failure between 10 and 40% resistant *F. occidentalis.* Spinosad resistance is thus likely to be manageable within an integrated pest management system. However, the experiment needs to be repeated to determine exact statistical significance.

(gg) Introduction

Until recently, chemical control of *F. occidentalis* in Australia was considered the principal control strategy. However, effectively controlling *F. occidentalis* with pesticides is extremely difficult because of their cryptic feeding behaviour, high mobility, soil-dwelling life stages, and short generation time combined with high fertility (reviewed by Jensen 2000). Consequently, for insecticides to be effective they must have good coverage, be correctly timed, and will only be effective if the insecticide comes into contact with the adult or larval stages. Finally, *F. occidentalis* develops insecticide resistance and most glasshouse and field populations resistant to older chemistry insecticides (eg organochlorines, organophosphates, carbamates and pyrethroids) have been recorded throughout the world (Brødsgaard 1994, Broadbent and Pree 1997, Jensen 1998, Jensen 2000), including Australia (Herron and James 2007). Australian growers currently have access to a limited range of chemicals for *F. occidentalis* control including avermectins, chlorfenapyr, carbamates, organophosphates, imidacloprid, fipronil, spinosyn and spirotetramat (APVMA 2010). Populations are known to be resistant to some newer chemicals including spinosad (Herron and James 2005, Loughner *et al*. 2005, Bielza *et al*. 2008) and fipronil (Herron and James 2005).

Resistance to spinosad is of particular concern as it is a unique insecticide that it is highly efficacious against *F. occidentalis* (Funderburk *et al.* 2000; Broughton and Herron 2009a,b), but is also classified as a reduced-risk bioinsecticide (Sparks *et al.* 1998). Spinosad is regarded to have low to moderate toxicity to beneficial insects, though the toxicity varies from species to species (Pietrantonio and Benedict 1999, Williams et al. 2003, Jones *et al.* 2005). Despite its detrimental effect on some species, spinosad can be integrated with biological control for *F. occidentalis* management (Funderburk *et al*. 2000, Ludwig and Oetting 2001), if a period of time between spray application and release of beneficial insects is maintained (Jones *et al*. 2005, Khan and Morse 2006, Kongchuensin and Takafuji 2006).

In Australia, spinosad resistant populations have been linked to chemical control failure. This includes greenhouse populations in the Sydney Basin, NSW, greenhouse capsicum in Perth, WA, but interestingly not in Qld (Herron and James 2005). Overseas, spinosad resistant greenhouse populations have been recorded in the USA (Loughner *et al*. 2005), leading to the temporary withdrawal of spinosad to manage development of spinosad resistance in WFT in some counties in Florida (P. Downard, Dow Agrosciences pers. comm. 2010). Spinosad resistance has also been recorded from greenhouse capsicum populations in southern Spain (Bielza *et al*. 2008). In both countries, spinosad resistant populations were associated with overuse of spinosad, with up to 8 applications per crop in the USA (Loughner *et al*. 2005), and 10 applications per crop in Spain (Bielza *et al*. 2008).

The resistance mechanism that confers spinosad resistance in WFT populations was investigated in Spain by Bielza *et al*. (2009). They found that resistance appears to be an almost completely recessive trait. Resistance management is easier when resistance genes are recessive, because heterozygotes (individuals with two different alleles of the same gene ie S:R = susceptible and resistant) should be easier to kill under field conditions because the heterozygote S:R has the same or similar phenotype as the susceptible S:S. Spinosad resistance has been shown to decline in the absence of spinosad applications and in the presence of susceptible WFT populations (Bielza *et a*l. 2008), and so may be amenable to manipulation within an integrated management system.

The aim of this study was to determine how spinosad resistance in Australian populations relates to effective field control. We aimed to relate specific frequencies of resistant *F. occidentalis* against quantifiable degrees of control to establish a point where resistant populations could be manipulated within an integrated control system.

Aims: (1) to artificially make populations of *F. occidentalis* with known frequencies of susceptible and spinosad resistant individuals;

(2) to release those populations into experimental plots sprayed with spinosad at the registered rate using a 3 spray strategy; and

(3) to relate specific resistance frequencies to field control of *F. occidentalis*.

(hh)Materials and Methods

(i) Trial dates

Two trials were conducted in an unheated glasshouse at DAFWA, South Perth in September 2008 and October 2009..

(ii) Insects tested

The susceptible strain used has been isolated under insecticide free conditions for several years and it's response to a range of chemicals has been verified as typically susceptible (Herron and Gullick 1998). The spinosad resistant population was collected from greenhouse capsicum in Serpentine, WA, on the 2nd February 2007 and its response to spinosad was confirmed at 110 fold resistant (see the resistance monitoring report). All thrips were cultured on potted dwarf French bean, *Phaeseolus vulgaris* L., using methods given in Herron and Gullick (2001). Briefly, *F. occidentalis* were reared in purpose-built rearing cages on potted bean plants with Cumbungi, *Typha domingensis* Persoon, pollen, and honey as a supplemental food source. Thrips were transferred onto fresh plants in a new cage on a six weekly cycle and maintained at 25 ± 1°C under a photoperiod of 16:8 (L:D) h. Susceptible and resistant WFT were prepared at EMAI by placing them in vials at specific initial resistance frequencies. Each vial contained 50 WFT adults that were ready for use immediately in WA. Treatments for each trial are outlined in Table 1.

Table 1. Frequencies of susceptible and resistant *Frankliniella occidentalis* sprayed with three consecutive field rate applications of spinosad

Trial 1 September 2008	Trial 2 October 2009
	0% Resistant ie 50S (water control)
0% Resistant ie 50S (sprayed)	0% Resistant ie 50S (sprayed)
10% Resistant ie 45S,5R (sprayed)	10% Resistant ie 45S,5R (sprayed)
20% Resistant ie 40S,10R (sprayed)	20% Resistant ie 40S,10R (sprayed)
40% Resistant ie 30S,20R (sprayed)	40% Resistant ie 30S,20R (sprayed)
60% Resistant ie 20S,30R (sprayed)	60% Resistant ie 20S,30R (sprayed)
80% Resistant ie 10S,40R (sprayed)	80% Resistant ie 10S,40R (sprayed)
	100 % Resistant ie 50 R (sprayed)

S=Susceptible
R=Resistant

In 2009 an extra 100% resistant treatment was included plus a 0% Resistant water only sprayed control. On receipt from EMAI, thrips were checked to determine that they were alive and then released onto lettuce. Thrips were allowed to breed for two weeks prior to treatment. Trials were repeated once each year (ie two replicates/trial), although a second replicate in 2008 was attempted but abandoned and the first replicate for the 2008 trial was affected by deteriorating lettuce (see appendices).

(iii) Plants

Iceberg lettuce seedlings (commercial cultivar Levistro) were transplanted into 20-cm pots containing Baileys potting mix (Baileys Fertilisers, Rockingham, WA, Australia). Pots were enclosed in a thrips-proof bag (105 µm mesh net) (Figure 1). The bag was placed over a steel frame and the bottom end of the cage was secured around the pot with an elastic band. The top of the cage was similarly closed with a band. All plants were hand watered daily through the side of the cage to ensure that thrips did not escape. Plants were used in experiments at 3 weeks of age.

Figure 1. Individual *F. occidentalis* trial plot showing thrips proof mesh over potted lettuce

(iv) Insecticides

Solutions of spinosad (Success[2], 240g spinosad /L, Dow AgroSciences) were freshly prepared and sprayed onto lettuce at the recommended rate of 40mL/100L. Insecticides were applied to the foliage with a hand-held atomizer (Hills Sprayers, BH220063) once every 3 days, giving a total of three successive applications (as per the three spray strategy). Control plants were sprayed with water only.

(v) Sample Collection

Three days after the last spray application, the plant was cut at soil level with a sharp knife and placed into a plastic zip-loc bag for transportation to the laboratory. A plastic plate coated with tanglefoot on one side was placed sticky side down on the top of the pot to trap any emerging thrips. The plate was secured in place with metal clips and removed 1 week later and examined under a binocular microscope for thrips.

In the laboratory, lettuce was washed through a series of three increasingly finer sieves (100 µm screening on the bottom one) to remove debris and extract thrips. Paper towelling was placed under the final screen to remove excess moisture. Sieves were examined under a binocular microscope and thrips were identified to their developmental stage.

(vi) Statistical analysis

A generalized linear mixed model (Schall, 1991) was used to analyse larval and adult counts of WFT with fixed treatment effects and random replicate effects. The experimental errors were assumed to follow a Poisson distribution and a logarithmic link function was used to relate the data to the treatment effect. Data was analysed with Genstat 12.1.

(ii) Results

The trial results are presented in Figures 2 and 3 and Table 2. More adults and immature stages (larvae, pupae, prepua) survived in the 80% (Figure 2) and 100% treatments (Figure 3) as expected. Interestingly, not all susceptible WFT were killed in either the 2008 or 2009 trials.

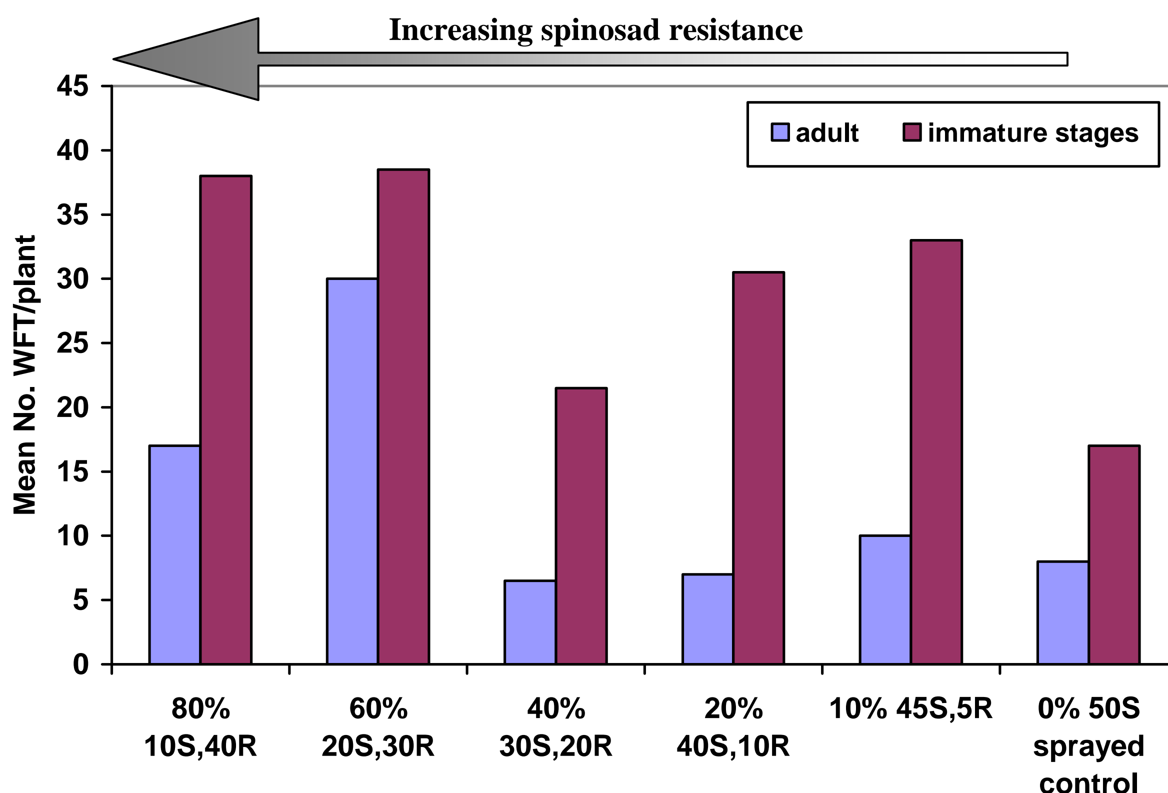

Figure 2: Mean WFT adult (blue) and larvae (red) numbers in two replicates in the 2008 greenhouse trial

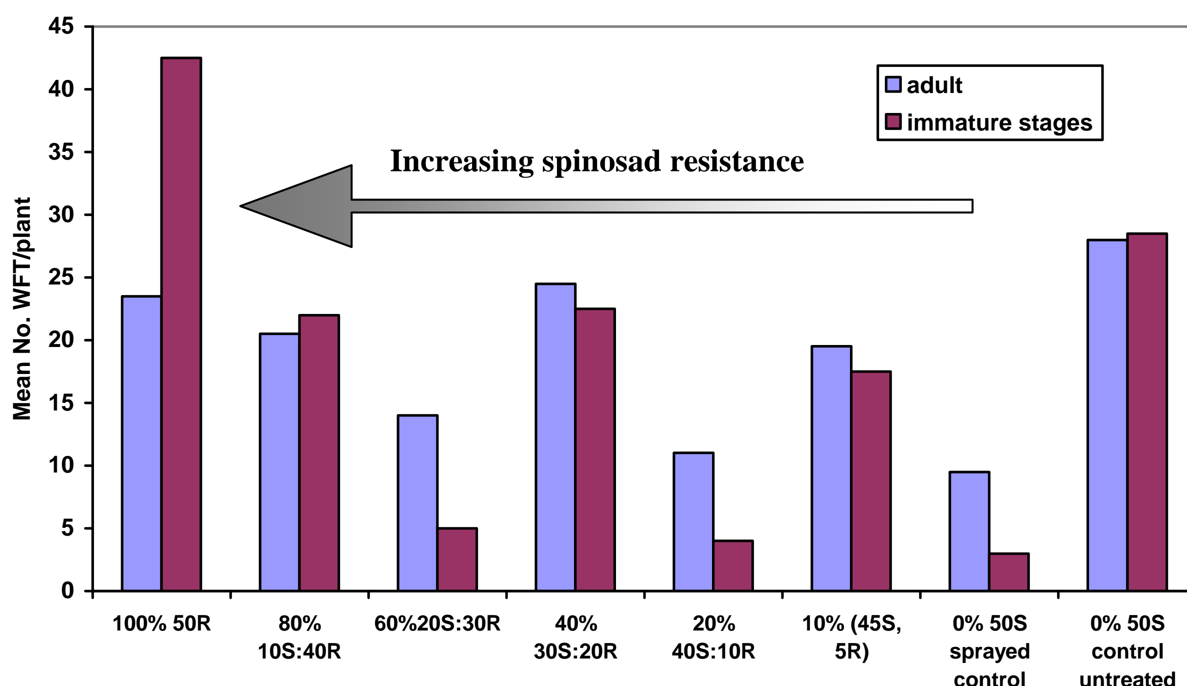

Figure 3. Mean WFT adult (blue) and larvae (red) numbers in two replicates in the 2009 greenhouse trial

Table 2: Wald statistics for the two replicates of data available from the 2009 trial

	Larvae		Adult	
TREATMENT	Log	Antilog	Log	Antilog
100% Resistant ie 50R	3.489	32.77	2.942	18.96
80% Resistant ie 10S, 40R	2.769	15.94	2.769	15.94
60% Resistant ie 20S, 30R	1.265	3.54	2.416	11.2
40% Resistant ie 30S, 20R	2.849	17.27	2.942	18.96
20% Resistant ie 40S, 10R	0.977	2.66	2.048	7.76
10% Resistant ie 45S, 5R	1.958	7.08	2.655	14.22
0% Resistant ie 50S water control (untreated)	2.923	18.6	3.109	22.41
0% Resistant ie 50S sprayed control	0.284	1.33	1.643	5.17
SED	1.617		0.630	
LSD5%	3.824		1.491	
Prob(Wald)	0.449		0.522	

Statistical analysis (Wald statistic) showed that neither the larval count nor adults were affected significantly by any of the treatments (P=0.449 and 0.522 respectively). When the positive control treatment was excluded from statistical analysis (0% 50S sprayed), the results remained unchanged. The lack of significance of any of the treatments is likely due to the low number of replicates per trial. Although attempts were made to repeat trials in 2009 and 2010, there were insufficient thrips available from EMAI for further testing (see discussion).

(jj) Discussion

Reference strains held at EMAI have been attacked by predatory mites in 2008 preventing thrips being dispatched to WA for further trials making the 2008 trial unreplicated. The predatory mites identified as *Neoseiulus barkeri* (Hughes) synonym for *Amblyseius masiaka* (Blommer & Chazeau) came with a *F. occidentalis* sample collected during February 2008. The property was Glenorie Hydroponics (all protected cropping) that has previously been associated with high level spinosad resistance causing control failure. Normally, predatory mites can be controlled with a low dose of bifenthrin that would not kill *F. occidentalis,* but the Glenorie mites were tolerant and they progressively contaminated reference strains. That slowly brought the field trial and resistance testing to a halt. Live mites were dispatched to Dr Leigh Pilkington at Gosford as a potential resistant biocontrol agent for *F. occidentalis* control.

Staff at EMAI Entomology, Insecticide Resistance Unit tried for a year to clean up the reference strains but all safe insecticidal and non insecticidal controls failed. As last resort EMAI staff was instructed to spray out all the *F. occidentalis* strains with deltamethrin (the most toxic pyrethroid available). Strains did appear predator free for a time, allowing a second trial to commence in 2009, but predators re established destroying all but the susceptible strain. The second trial then had to be abandoned after two replicates. Unfortunately, there was a great deal of variability between those replicates and a significant result could not be determined

Based on the available data, we concluded that chemical control with spinosad seems to fail somewhere between 10 and 40% resistant WFT. This suggests that resistance to spinosad is Australian *F. occidentalis* may be managed with an integrated system, but more work is required to more closely define the critical frequency of resistance. For growers with spinosad resistant populations, alternate control tactics will be required for a few generations of thrips if resistance frequencies are high. For example, by releasing natural enemies such as *Orius armatus* and beneficial mites. After a few generations resistance should decline with spinosad again becoming efficacious.

(kk)Acknowledgments

We thank Graeme Gullick and Brendan Langfield for preparing the thrips at EMAI for dispatch to WA. David Cousins is thanked for conducting the WA glasshouse trials and Idris Barchia for the statistical analysis. Danuta Knihinicki is thanked for the predatory mite identification.

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(mm) Appendices

Table 1. Raw trial results relating initial WFT resistance frequency with spinosad performance applied at the field rate for the 2008 trial.

Replicate	Treatment	Larvae	Adults	Notes
1	10S,40R	60	31	
	20S,30R	31	33	
	30S,20R	11	4	
	40S,10R	34	9	
	45S,5R	10	5	
	50S	22	4	
2	10S,40R	1	3	Plant quality declined
	20S,30R	12	27	
	20S,30R	21	9	Plant quality declined
	40S,10R	17	5	Plant quality declined
	45S,5R	33	15	
	50S	1	12	Plant quality declined
3	10S,40R	-	-	Abandoned
	20S,30R	-	-	Abandoned
	20S,30R	-	-	Abandoned
	40S,10R	-	-	Abandoned
	45S,5R	-	-	Abandoned
	50S	-	-	Abandoned

S= Susceptible
R = Resistant

Table 3. Raw trial results relating initial WFT resistance frequency with spinosad performance applied at the field rate for the 2009 trial.

	TREATMENT	Lar	Ad	Prepup/ pup	♀	♂	1st In	2nd In	Prepup	pup
Rep 1	100% 50R	72	34	11	28	6	27	45	5	6
02/11	80% 10S:40R	35	31	7	26	5	20	15	3	4
	60% 20S:30R	0	9	0	9					
	40% 30S:20R	36	35	5	33	2	12	24	3	2
	20% 40S:10R	5	16	0	15	1	2	3		
	10% 45S, 5R	15	31	11	27	4	12	3	3	8
	0% 50S H_2O control	12	43	9	37	6	4	8	4	5
	0% 50S spinosad control	1	6	0	4	2	1			
Rep 2	100% 50R	2	10		10		2			
23/11	80% 10S:40R	1	6		5	1	1		1	
	60% 20S:30R	8	17		14	3	8			
	40% 30S:20R	3	9		7	2	3		1	
	20% 40S:10R	1	2		2		1		1	
	10% (45S, 5R)	1	2		2		1			4
	0% 50S H_2O control	30	9		7	2	30		1	2
	0% 50S spinosad control	2	6		5	1	2		2	

S= Susceptible
R = Resistant
Lar =Larvae
Ad = Adult
Prepup = Prepupae
Pup = Pupae
1st In = 1st Instar
2nd In = 2nd Instar

New or Experimental Chemical Evaluations

(nn)Report for pyridalyl - Grant A. Herron

Aim: to test a single formulation of Pyridalyl against reference susceptible and resistant strains of western flower thrips (WFT).

Chemical tested: Pyridalyl 500 g/L SC (Symphony).

Thrips: Two laboratory reference strains of WFT were evaluated. A reference susceptible strain known as NZ2 and a field collected multiple resistant strain that is pressured with spinosad and known as WA (P).

Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, WFT were reared in purpose-built rearing cages on potted bean plants with cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. 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 regimen.

Bioassay method: The bioassay procedure was that of Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean leaf discs embedded in agar in 35 mm diameter Petri dishes. The leaf discs with anaesthetised thrips in place were sprayed with of insecticide (4 mL aliquot) or with water (control) via a Potter spray tower producing a deposit of 3.2 ± 0.08 mg cm^{-2}. Once sprayed each Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine ventilation holes. The dishes were stored for 48 h at 25 ± 0.1 °C under a 16:8 hour L: D regimen. The numbers of live and dead thrips were counted with the aid of a stereo-microscope. Each test was replicated at least once. Control mortality did not exceed 15%. Data were analysed using a computer program based on the Probit method of Finney (Barchia 2001)

Results: There was a significant difference in response between the susceptible and resistant strains when using Pyridalyl (as indicated by the RF value (3.53) being > 1.00 (ie 95% CI 1.78-6.99)) (Figure 1).

Discussion: The difference in response between susceptible and resistant thrips (Figure 1) is typical of insecticide resistance or cross-resistance but still within the limits of vigour tolerance. However, more testing is required to absolutely eliminate vigour tolerance as a cause. If the WA (P) strain is pyridalyl resistant any pyridalyl resistance detected is due to cross resistance from previous insecticide use. That would limit the usefulness of pyridalyl for WFT control.

Acknowledgement: Graeme Gullick, Swami Thalavaisundaram and Tanya Tomlinson provided technical assistance.

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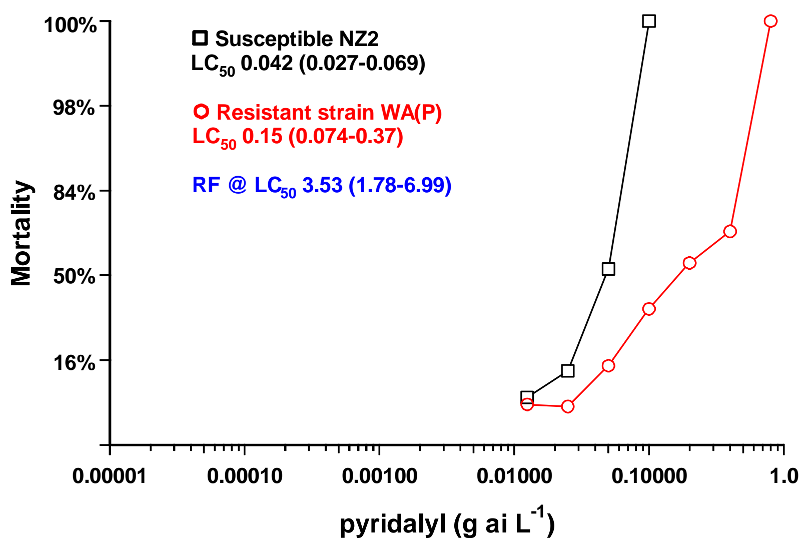

Figure 1. Replicated dose responses for pyridalyl sprayed against susceptible (NZ2) and resistant WA(P) strains of western flower thrips.

(oo) Report for acrinathrin – Grant A. Herron

Aim: to test various formulations of acrinathrin against reference susceptible and resistant strains of western flower thrips (WFT).

Chemicals tested: acrinathrin 75 g/L, acrinathrin 22.5 g/L + abamectin 12.6 g/L, and acrinathrin 15 g/L + malathion 440 g/L.

Thrips: Two laboratory reference strains of WFT were evaluated. A reference susceptible strain known as NZ2 and a field collected multiple resistant strain that is pressured with spinosad and known as WA(P).

Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, WFT were reared in purpose-built rearing cages on potted bean plants with cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. 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 regimen.

Bioassay method: The bioassay procedure was that of Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean leaf discs embedded in agar in 35 mm diameter Petri dishes. The leaf discs with anaesthetised thrips in place were sprayed with of insecticide (4 mL aliquot) or with water (control) via a Potter spray tower producing a deposit of 3.2 ± 0.08 mg cm^{-2}. Once sprayed each Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine ventilation holes. The dishes were stored for 48 h at 25 ± 0.1 °C under a 16:8 hour L: D regimen. The numbers of live and dead thrips were counted with the aid of a stereo-microscope. Each test was replicated at least once. Control mortality did not exceed 15%. Data were analysed using a computer program based on the Probit method of Finney (Barchia 2001)

Results: There was a significant difference in response between the susceptible and resistant strains when using 75 g/L acrinathrin (as indicated by the RF value (7.00) being > 1.00 (ie 95% CI 4.37-11.20)) (Figure 1). Interestingly, there was no significant difference in response between the two reference strains when evaluated against the acrinathrin mixture of acrinathrin 15 g/L + malathion 440 g/L (Figure 2) or acrinathrin 22.5 g/L + abamectin 12.6 g/L (Figure 3). However, the dose response data for the mixtures indicate they are less potent than acrinathrin alone with acrinathrin 15 g/L + malathion 440 g/L the least potent of the two mixtures.

Discussion: The difference in response between susceptible and resistant thrips (Figure 1) is typical of insecticide resistance or cross-resistance. Consequently, the WA(P) strain is likely acrinathrin resistant although it has not had previous acrinathrin exposure. It would be reasonable to conclude that the acrinathrin resistance detected is due to cross resistance from previous insecticide use including pyrethroids. Importantly, such significant differences in response were not evident in the two mixtures of acrinathrin + malathion or acrinathrin + abamectin suggesting that they both neutralise the cross resistance detected in Figure 1. As mixture efficacy (ie increase/decrease in absolute LC_{50}) seems to relate to the reduced acrinathrin rate only in the mixture it would seem acrinathrin 22.5 g/L + abamectin 12.6 g/L to be the superior formulation for further development against WFT.

Acknowledgement: Graeme Gullick and Tanya Tomlinson provided technical assistance.

References: Barchia I. 2001. Probit analysis and fiducial limits in Genstat. In: *Genstat 2001 Program and Abstracts* (eds V Doogan, D Mayer & T Swain), p, 3. Mecure Resort, Surfers Pardise, Gold Coast, 31 January - 2 February 2001, Australia.

Herron, G.A., Rophail, J. and Gullick, G.C. (1996). Laboratory-based, insecticide efficacy studies on field-collected *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and implications for management. *Australian Journal of Entomology* **35**: 161-164.

Herron, G.A. and Gullick, G.C. (2001). Insecticide resistance in Australian populations of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) causes the abandonment of pyrethroid chemicals for its control. *General and Applied Entomology* **30**: 21-26.

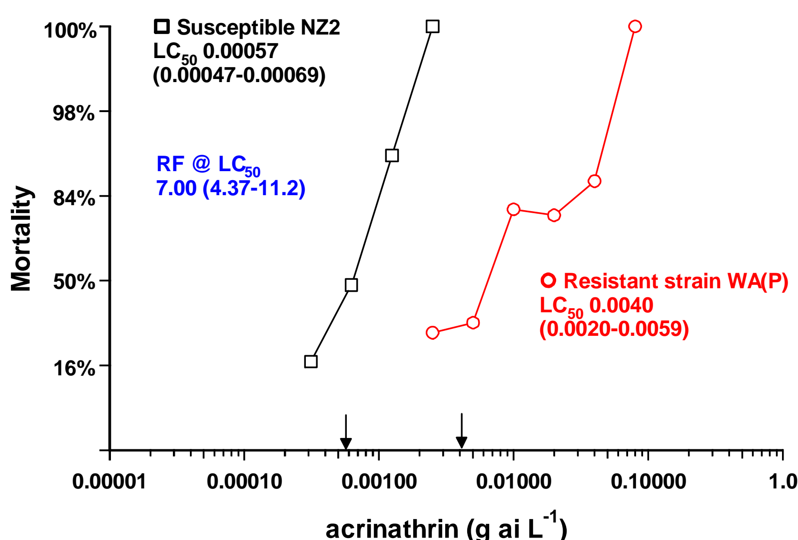

Figure 1. Replicated dose responses for 75 g/L acrinathrin sprayed against susceptible (NZ2) and resistant WA(P) strains of western flower thrips. The arrows show the approximate LC_{50} values.

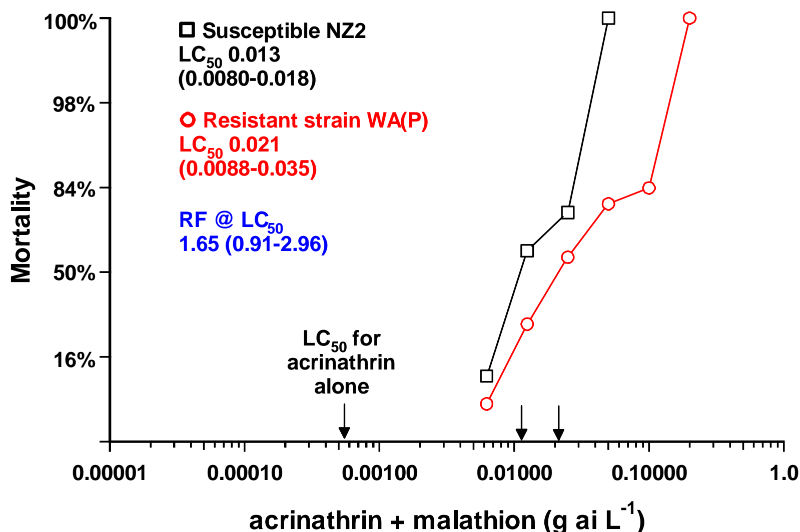

Figure 2. Replicated dose response for acrinathrin 15 g/L + malathion 440 g/L sprayed against susceptible (NZ2) and resistant (WA(P)) strains of western flower thrips. The arrows show the approximate LC_{50} values compared to acrinathrin 75 g/L

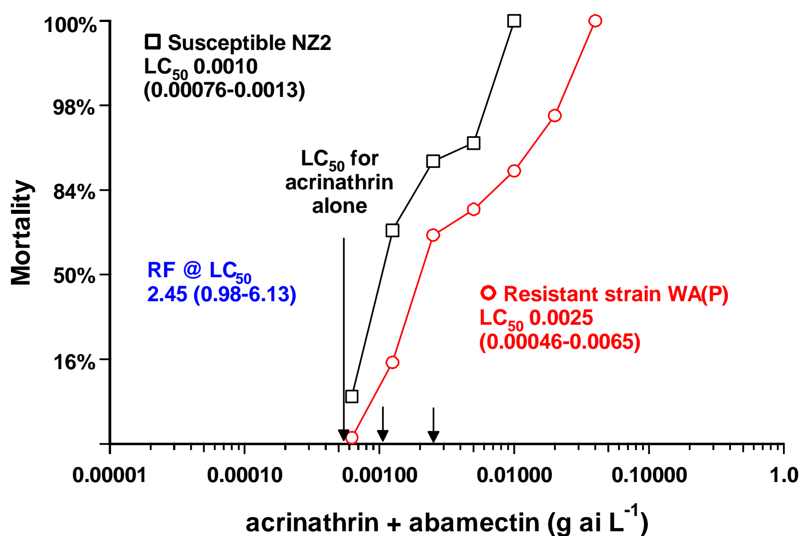

Figure 3. Replicated dose responses for acrinathrin 22.5 g/L + abamectin 12.6 g/L sprayed against susceptible (NZ2) and resistant (WA(P)) strains of western flower thrips. The arrows show the approximate LC_{50} values compared to acrinathrin 75 g/L

(pp)Report for clothianidin – Grant A. Herron

Aim: to test a single formulation of clothianidin against reference susceptible and resistant strains of western flower thrips (WFT).

Chemical tested: OZ-T1 435 200SC (Clothianidin 200 g/L SC).

Thrips: Two laboratory reference strains of WFT were evaluated. A reference susceptible strain known as NZ2 and a field collected multiple resistant strain that is pressured with spinosad and known as WA (P).

Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, WFT were reared in purpose-built rearing cages on potted bean plants with cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. 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 regimen.

Bioassay method: The bioassay procedure was that of Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean leaf discs embedded in agar in 35 mm diameter Petri dishes. The leaf discs with anaesthetised thrips in place were sprayed with of insecticide (4 mL aliquot) or with water (control) via a Potter spray tower producing a deposit of 3.2 ± 0.08 mg cm^{-2}. Once sprayed each Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine ventilation holes. The dishes were stored for 48 h at 25 ± 0.1 °C under a 16:8 hour L: D regimen. The numbers of live and dead thrips were counted with the aid of a stereo-microscope. Each test was replicated at least once. Control mortality did not exceed 15%. Data were analysed using a computer program based on the Probit method of Finney (Barchia 2001)

Results: There was a significant difference in response between the susceptible and resistant strains when using clothianidin (as indicated by the RF value (7.14) being > 1.00 (ie 95% CI 3.03-16.84)) (Figure 1).

Discussion: The difference in response between susceptible and resistant thrips (Figure 1) is typical of insecticide resistance or cross-resistance. As the response is above 5 fold it is likely beyond the realms of vigour tolerance. As the WA (P) strain is clothianidin resistant any clothianidin resistance detected is due to cross resistance from previous insecticide use. That would limit the usefulness of clothianidin for WFT control.

Acknowledgement: Graeme Gullick, Swami Thalavaisundaram and Tanya Tomlinson provided technical assistance.

References: Barchia I. 2001. Probit analysis and fiducial limits in Genstat. In: *Genstat 2001 Program and Abstracts* (eds V Doogan, D Mayer & T Swain), p, 3. Mecure Resort, Surfers Pardise, Gold Coast, 31 January - 2 February 2001, Australia.

Herron, G.A., Rophail, J. and Gullick, G.C. (1996). Laboratory-based, insecticide efficacy studies on field-collected *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and implications for management. *Australian Journal of Entomology* **35**: 161-164.

Herron, G.A. and Gullick, G.C. (2001). Insecticide resistance in Australian populations of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) causes the abandonment of pyrethroid chemicals for its control. *General and Applied Entomology* **30**: 21-26.

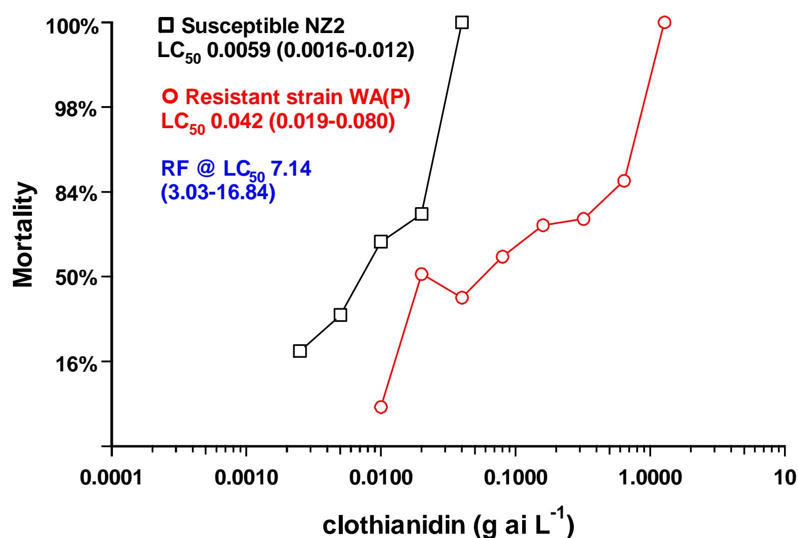

Figure 1. Replicated dose responses for clothianidin sprayed against susceptible (NZ2) and resistant WA(P) strains of western flower thrips.

(qq)Report for DPX-HGW86 – Grant A. Herron

Aim: to test a single formulation of DPX-HGW86 against reference susceptible and resistant strains of western flower thrips (WFT).

Chemical tested: DPX-HGW86 (oil dispersable).

Thrips: Two laboratory reference strains of WFT were evaluated. A reference susceptible strain known as NZ2 and a field collected multiple resistant strain that is pressured with spinosad and known as WA (P).

Thrips were cultured on potted dwarf French bean (*Phaeseolus vulgaris* L.) using methods given in Herron and Gullick (2001). Briefly, WFT were reared in purpose-built rearing cages on potted bean plants with cumbungi (*Typha domingensis* Pers.) pollen and honey as a supplementary food source. 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 regimen.

Bioassay method: The bioassay procedure was that of Herron *et al*. (1996). Briefly, WFT were lightly anaesthetised with CO_2 and then tipped onto French bean leaf discs embedded in agar in 35 mm diameter Petri dishes. The leaf discs with anaesthetised thrips in place were sprayed with of insecticide (4 mL aliquot) or with water (control) via a Potter spray tower producing a deposit of 3.2 ± 0.08 mg cm^{-2}. Once sprayed each Petri dish was covered with taut plastic cling-wrap film perforated with 40-50 fine ventilation holes. The dishes were stored for 48 h at 25 ± 0.1 °C under a 16:8 hour L: D regimen. The numbers of live and dead thrips were counted with the aid of a stereo-microscope. Each test was replicated at least once. Control mortality did not exceed 15%. Data were analysed using a computer program based on the Probit method of Finney (Barchia 2001)

Results: There was no significant difference in response between the susceptible and resistant strains when using DPX-HGW86 (as indicated by the RF value (0.99) being = 1.00 (ie 95% CI 0.47-2.09)) (Figure 1).

Discussion: The difference in response between susceptible and resistant thrips (Figure 1) is typical of insecticide susceptible populations. Encouragingly then, the dose response data do not suggest any resistance or cross-resistance in the resistant WFT tested. However, the bioassay with its 48 h withholding period, suggests a likely field rate in excess of 10 g ai / L. If the bioassay does accurately reflect field efficacy then such high rates may not be economic. Then again, as initial anecdotal discussions with DuPont indicated DPX-HGW86 to be efficacious against WFT it is possible the bioassay result is not accurately reflecting field efficacy. If that is true then further bioassay methods development is required so that the bioassay does accurately reflect field performance.

Acknowledgement: Graeme Gullick, Swami Thalavaisundaram and Tanya Tomlinson provided technical assistance.

References: Barchia I. 2001. Probit analysis and fiducial limits in Genstat. In: *Genstat 2001 Program and Abstracts* (eds V Doogan, D Mayer & T Swain), p, 3. Mecure Resort, Surfers Pardise, Gold Coast, 31 January - 2 February 2001, Australia.

Herron, G.A., Rophail, J. and Gullick, G.C. (1996). Laboratory-based, insecticide efficacy studies on field-collected *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and implications for management. *Australian Journal of Entomology* **35**: 161-164.

Herron, G.A. and Gullick, G.C. (2001). Insecticide resistance in Australian populations of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) causes the abandonment of pyrethroid chemicals for its control. *General and Applied Entomology* **30**: 21-26.

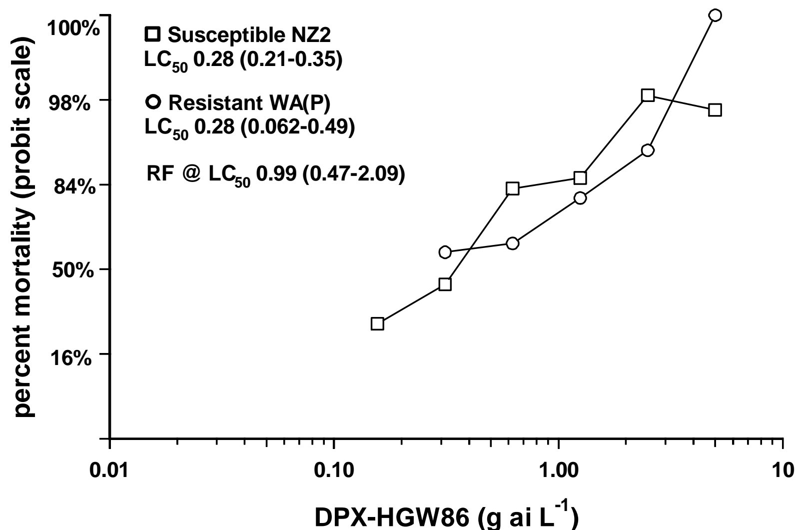

Figure 1. Replicated dose responses for DPX-HGW86 sprayed against susceptible (NZ2) and resistant WA(P) strains of western flower thrips.

Article IV. General Discussion

Despite a resistance management strategy being implemented for the horticultural Australian industry, spinosad resistance has increased above the 87-fold detected in the previous study HG03003 of Herron and Broughton (2006). Spinosad resistance in the current study (project) VG06010 peaked at 201–fold in the 2006/2007 season, with a further three instances of resistance above 100 fold. A major factor in the selection for resistance is the lack of effective alternatives to spinosad, and field levels of resistance to spinosad continue to increase. Unfortunately, lack of alternative chemistry is not the only reason spinosad resistance continues to increase. The published strategy requires a treatment cycle of three consecutive sprays in close succession (Broughton and Herron, 2007), because WFT eggs are hidden in the leaf tissue and pupae in the soil, largely preventing contact with sprayed chemicals (Herron *et al*. 2007). A detailed chemical spray history supplied with strain Lettuce Glenorie 11/2008 for example, showed that chemicals including spinosad were not applied according to the published resistance management strategy. Chemicals were being sprayed weekly rather every 3-5 days, dependant on temperature (Broughton and Herron 2007). Additionally, chemicals were often not appropriately alternated with sequential use of the same product common. Although information about the resistance management strategy has been made available to growers in this (see Appendix – project communications) and previous projects, correct spray procedures continue to be ignored.

Cross-resistance refers to a situation in which a strain that has become resistant to one insecticide automatically develops resistance to other insecticides to which it has not been previously exposed (Yu 2008). As the insecticide resistance management strategy is based on chemical alternation, cross-resistance will undermine the strategy and could cause efficacious chemicals to prematurely fail if alternated with a cross-resistant compound. The cross-resistance study suggested that dichlorvos resistance may have resulted from an undetermined cross-resistance with chlorpyrifos, as previously suspected by Herron and James (2005). Cross-resistance testing also found a possible cross-resistance between spinosad and the chloronicotinyl (neonicotinoid) insecticides acetamiprid and thiamethoxam. Consequently, alternations with any chemicals from the chloronicotinyl group with spinosad should be avoided. This conclusion is unfortunate because the chlornicotinyl chemical group is the fastest growing class of insecticides in modern crop protection (Jeschke and Nauen 2007). Encouragingly, there was no cross-resistance to methomyl, chlorfenapyr or methamidophos, so these chemicals appear to be suitable candidates for alternation with spinosad.

Resistance is caused by a number of mechanisms with most being either target site or detoxification. The presence of these specific detoxification enzymes can be deduced with synergists that can neutralise specific enzyme detoxification pathways (Raffa and Priester 1985). The bioassay mechanism study suggests that acetamiprid and abamectin resistance was associated with an esterase mediated detoxification pathway, but importantly detoxification was not associated spinosad resistance; a result consistent with Bielza *et al*. (2007). Consequently the following molecular genetics study on spinosad resistance was then able to pursue a target side cause for resistance with a high degree of confidence. The molecular genetics research identified several candidate proteins that may prove useful for identifying the spinosad target site in the future. Additionally, the sequencing of nAChR receptor Dα6 cDNA sequence as outlined in Perry *et al.* (2007) using spinosad resistant and susceptible WFT revealed a singe nucleotide substitution (C>T), that may be associated with spinosad resistance. Although this cannot be confirmed definitively as the causative mutation for spinosad resistance, we are one big step closer to characterising the spinosad resistance gene and so establishing a real time capability for resistance detection.

In our attempt to relate spinosad resistance to field control, we concluded that chemical control with spinosad seems to fail somewhere between 10 and 40% resistant WFT. This suggests that resistance to spinosad is Australian *F. occidentalis* may be managed with an integrated pest management system, but more work is required to more closely define the critical frequency of resistance. For growers with spinosad resistant populations, alternate control tactics will be required for several generations when resistance frequencies are high. If spinosad can be removed by the grower for a period of time (i.e. for a few WFT generations), resistance to spinosad should decline and spinosad should once again become effective against WFT.

(a) Acknowledgements

This project was facilitated by HAL in partnership with AUSVEG. It was funded using the vegetable industry levy and voluntary contributions from Dow AgroSciences Australia Ltd, with matched funds from the Federal Government. This project is part of a collaborative effort with significant in-kind contributions from I&I NSW and DAFWA.

(b) References

Broughton S. and Herron G.A. (2007). *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) chemical control: insecticide efficacy associated with the three consecutive spray strategy. *Australian Journal of Entomology* **46,** 140–145.

Herron GA. and Broughton S. (2006) Final Report HG03003: Evaluation of insecticides for western flower thrips resistance. NSW DPI, Orange.

Herron GA. and James TM. (2005) Monitoring insecticide resistance in Australian *Frankliniella occidentalis* (Pergandae) detects fipronil and spinosad resistance. *Australian journal of Entomology* **44**, 299-303.

Herron G., Steiner M., Gollnow B. and Goodwin S. 2007. Western Flower thrips (WFT) insecticide resistance management plan. http://www.dpi.nsw.gov.au/agriculture/horticulture/pests-diseases-hort/multiple/thrips/wft-resistance. Accessed 15 May 2010

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Perry T., MaKenzie JA. and Bratterham P. 2007. A D alpha 6 knockout strain of *Drosophila melahogaster* confers a high level of resistance to spinosad. *Insect Biochemistry and Molecular Biology* **37**, 184-188.

Raffa KF and Priester TM. 1985. Synergists as research tools and control agents in agriculture. *Journal of Agricultural Entomology* **2**, 27-45.

Yu S.J. 2008. *The toxicology and biochemistry of insecticides*. CRC Press, Boca raton.

Article V. Conclusions

This project (VG06010) has shown that spinosad resistance doesn't necessarily mean that growers will experience total control failure. However, resistance monitoring indicates that if growers continue to manage WFT the same way as they have in the past, then resistance levels to key chemicals such as spinosad will continue to rise. To conserve existing insecticides and to stop resistance increasing to key IPM chemicals, a major change in the national WFT resistance management strategy is required. As a matter of urgency, chemical control needs to be an integrated with IPM. In addition, additional research to fully characterise spinosad resistance by molecular genetic techniques is required. New resistance monitoring methodology (both bioassay and molecular) also needs to be developed for new strategic chemicals as they become available to the vegetable industry.

Article VI. Recommendations

- WFT chemical control and the national WFT resistance management strategy should be integrated with evolving IPM practices such as biological control as a matter of urgency.
- Additional research is required to fully characterise spinosad resistance by using molecular genetic techniques to provide improved resistance monitoring capability.
- Resistance monitoring should continue, but needs to be narrowly focussed on a few key conventional and IPM compatible chemicals.
- New IPM compatible chemicals will necessitate the development of new resistance monitoring methodology (both bioassay and molecular), as new strategic chemicals become available eg Movento®. New techniques need to be developed because the newer insecticides cannot be tested or monitored using standard bioassay techniques.
- New insecticides should be studied as they are made available to industry to determine baseline susceptibility levels that will facilitate resistance detection.
- Compatibility of new insecticides with biological control agents used to control WFT and other pests needs to be determined.
- Any new insecticide proposed for WFT control should be screened for possible cross resistance to existing chemicals (especially spinosad).

Article VII. Appendix

(a) Project communications

1. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Capsicum. http://www.dpi.nsw.gov.au/reader/thrips/wft-capsicum-table.htm

2. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- cucumber. http://www. dpi.nsw.gov.au/reader/thrips/wft-cucumber-table.htm

3. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Culinary Herbs. http://www. dpi.nsw.gov.au/reader/thrips/wft-culinaryherbs-table.htm

4. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Egg Plant. http://www.dpi.nsw.gov.au/reader/thrips/wft-eggplant-table.htm

5. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- lettuce. http://www. dpi.nsw.gov.au/reader/thrips/wft-lettuce-table.htm

6. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Tomatoes. http://www.dpi.nsw.gov.au/reader/thrips/wft-tomatoes-table.htm

7. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Strawberries. http://www.dpi.nsw.gov.au/reader/thrips/wft-strawberries-table.htm

8. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Stone Fruit. http://www.dpi.nsw.gov.au/reader/thrips/wft-stonefruit-table.htm

9. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Spring onions and shallots. http://www.dpi.nsw.gov.au/reader/thrips/wft-springonions-shallot-table.htm

10. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Silverbeet. http://www.dpi.nsw.gov.au/reader/thrips/wft-silverbeet-table.htm

11. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Parsley and coriander. http://www.dpi.nsw.gov.au/reader/thrips/wft-parsley-coriander-table.htm

12. Herron, G. (2007) Western flower thrips (WFT) insecticide management plan- Ornamentals. http://www.dpi.nsw.gov.au/reader/thrips/wft-ornamentals-table.htm

13. Herron, G., Steiner, M., Gollnow, B. and Goodwin S. (2007) Western flower thrips (WFT) insecticide management plan- Spring onions and shallots. http://www.dpi.nsw.gov.au/reader/thrips/wft-insecticide-mgt-plan.htm

14. *Broughton, S and Herron, G.A. (2007) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) chemical control: insecticide efficacy associated with the three consecutive spray strategy *Australian Journal of Entomology*, **46**: 140-145

15. *****Herron, G., Broughton, S. and Clift, A. (2007) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) chemical control: residues associated with the three consecutive spray strategy *Australian Journal of Entomology*, **46**: 146-151.

16. *****Herron, G.A**.** and James, T.M. (2007) Insecticide Resistance in Australian Populations of western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) *General and Applied Entomology*, **36**: 1-5.

17. *Thalavaisundaram, S., Herron, G.A.**,** Clift, A.D. and Rose H. (2008) Pyrethroid resistance in *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae) and implications for its management in Australia. *Australian Journal of Entomology* **47**: 64-69.

18. Herron, GA. (2008) Spinosad resistance in *Frankliniella occidentalis* Pergandae (Western Flower Thrips) is a serious threat to biocontrol. p. 88. *In*: Australia & New Zealand Biocontrol Conference, Sydney Australia 10-14 February 2008.

19. *****Broughton S and Herron GA (2009) Potential new insecticides for the control of western flower thrips (Thysanoptera, Thripidae) on sweet pepper, tomato and lettuce. *Journal of Economic Entomology*, **102**: 646-651

20. *Broughton S and Herron GA (2009) Management of western flower thrips, *Frankliniella occidentalis* (Pergandae)(Thysanoptera, Thripidae) on strawberries. *General and Applied Entomology*, **38**: 37-42

21. *Kay, I.R. and Herron G.A. (2010) Evaluation of existing and new insecticides including spirotetramat and pyridalyl to control *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae) on peppers in Queensland. *Australian Journal of Entomology*, **49**: 175-181.

* = refereed scientific journal article