SCIENTIFIC NOTE

A SINGLE NUCLEOTIDE SUBSTITUTION IMPLICATED IN SPINOSAD RESISTANCE IN WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE)

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Summary

Western flower thrips *Frankliniella occidentalis* (Pergande) (WFT) is a serious international pest of horticulture causing severe crop losses by direct feeding or virus transmission. The insecticide spinosad is a mainstay chemical control used in Australia against *F. occidentalis* that is now undermined by resistance. Understanding the mechanism of spinosad resistance in *F. occidentalis* may improve its overall chemical management. We obtained 3 partial cDNA sequences for the acetylcholine receptor Foα6 and found a single nucleotide substitution (C→T) in spinosad resistant WFT compared to susceptible *F. occidentalis* at the molecular level.

Keywords: resistance mechanism, spinosad, Success Naturalyte

Western flower thrips (WFT), *Frankliniella occidentalis*, is a serious pest of horticulture due to feeding by adults and nymphs and transmission of tomato spotted wilt virus (Kirk and Terry 2003). WFT is now found in all six Australian states but not the Northern Territory (Medhurst and Swanson 1999). Spinosad is the most commonly used insecticide for control of *F. occidentalis* in Australia (InfoPest 2010). However, repeated and intensive use of spinosad against *F. occidentalis* has caused field control failure due to high level spinosad resistance (Bielza *et al.* 2007; Herron and James 2007).

Understanding the genetic mechanisms that confer resistance to spinosad can help in the detection of resistance alleles in the field and therefore improve integrated pest management strategies. We used immunoprecipitation to isolate the spinosad binding protein using a RaPID Assay Spinosad Test Kit (Strategic Diagnostics, Inc) in which spinosad antibody was attached to magnetic beads. Approximately 2000 reference susceptible thrips were lysed in cell lysis buffer (50 mM Tris-HCl, 150 mM NaCl and 1 % NP-40, pH 8.0). The protein lysis solution was halved, with each half made up to a total volume of 450 µL with lysis buffer. Ten microlitres of super saturated spinosad was added to one portion of the protein lysis solution and mixed using a vortex mixer. The other portion of the protein lysis solution without super saturated spinosad was used as a no-spinosad control. Five hundred microlitres of magnetic beads conjugated with spinosad antibody were added to the protein solutions and mixed by slow rotation overnight at 4 °C. The magnetic beads were washed twice at 4 °C for 2 minutes with phosphate-buffered saline (PBS) and protein was eluted in 20 µL 0.1 M (sodium) citrate acid. Eluant protein was neutralised with 1 M Tris (pH 7.5). The eluant protein was separated on a 12 % acrylamide SDS-PAGE gel. Four stained protein bands were excised from the gels (Figure 1) and analysed by liquid chromatography-mass spectrometry (LC-MS/MS). Ten candidate proteins which were found to be similar to the *F. occidentalis* excised protein bands were identified (MASCOT score >52) with the NCBI identification: gi|193624646, gi|157110721, gi|8186, gi|156544337, gi|157891, gi|45330818, gi|4191598, gi|46909203, gi|134290336, gi|54289293.

Isolation of the *nAChR* receptor *Dα6* orthologue, *Foα6* in *F. occidentalis* was carried out with RACE. Total RNA was extracted from 100 resistant and 100 susceptible WFT with TRI reagent (Ambion, Applied Biosystems). The first strand of cDNA for 3' and 5'-RACE was synthesized with gene specific primers *Fα6*_left (TTCAAGAGCACATGCAAGATAGA) and *Fα6*_right (GTCTATCTTGCATGT GCTCTTGA) based on consensus sequence 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). Three partial cDNA sequences were obtained with 249 bp, 463 bp and 539 bp. A C→T substitution at position 90 was revealed in the 249 bp partial cDNA fragment on comparison of the spinosad resistant and susceptible strains. DNA

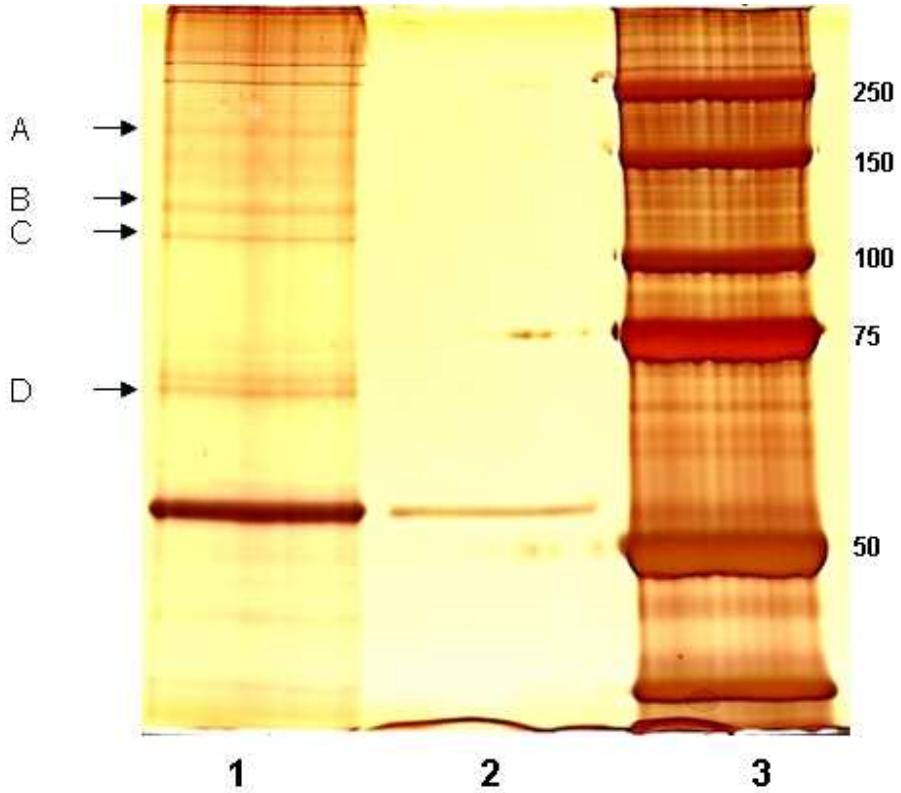

Figure 1. Separation of bound spinosad antibody on a 12 % acrylamide SDS-PAGE gel under reducing conditions and visualized using silver staining. Lane 1: Eluant by immunoprecipitation with spinosad treatment, Lane 2: Eluant by immunoprecipitation without spinosad treatment (control), Lane 3, protein marker. A, B, C, D indicate the excised band for LC-MS/MS) analysis

sequence variations were not found between the susceptible and resistant WFT strains for the 436 bp and 539 bp fragments

The identified candidate proteins may provide useful background information to aid the identification of a spinosad binding target site in future studies. Sequencing of the *nAChR* receptor *Foα6* cDNA from spinosad resistant and susceptible WFT revealed a single nucleotide substitution (C→T) that may be associated with spinosad resistance. *Dα6* and its orthologue *Pxα6* are known to be the gene responsible for high level of spinosad resistance in the ferment fly *Drosophila melanogaster* Meigen and the cabbage moth *Plutella xylostella* (Linnaeus) (Perry *et al.* 2007; Rinkevich *et al.* 2010). In both of these cases the mutation causes a prematurely truncated protein. Further sequencing the *F. occidentalis* genome may allow characterisation of the acetylcholine receptors (*nAChRs*) and aid the development of a DNA based diagnostic test for spinosad resistance in *F. occidentalis*. This study provides the first molecular based information on the spinosad resistance mechanism in *F. occidentalis*.

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