

Development of an insecticide resistance monitoring program and identification diagnostic for silverleaf whitefly

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Introduction

The silverleaf whitefly (SLW), *Bemisia tabaci* (Gennadius) biotype B, was first recorded in Australia in 1994 (Gunning *et al.* 1995) and is a serious pest of cotton and horticultural crops, both in Australia and overseas (Oliveira *et al.* 2001). Its pest status in cotton is due to its ability to rapidly develop resistance to insecticides and secretion of honeydew resulting in sticky cotton which would seriously jeopardise marketing of Australian cotton and compromise Australian's reputation as a high quality fibre producer. It is also a vector of cotton leaf curl virus, an exotic disease that poses a potentially serious threat to the Australian cotton industry.

The Australian native *B. tabaci* (ANB) and the greenhouse whitefly (GHW) *Trialeurodes vaporariorum* (Westwood) are susceptible to all currently used insecticides whereas SLW is highly resistant to several insecticide classes. Australian native *B. tabaci* and SLW cannot be distinguished by morphological characteristics. Therefore, a diagnostic test using DNA markers is necessary to distinguish the different biotypes. Accurate identification is important if effective control is to be achieved by choosing the appropriate insecticide for the target whitefly species.

Pyriproxyfen (Admiral[®]), diafenthiuron (Pegasus[®]) and bifenthrin (Talstar[®]) are currently registered for whitefly control in cotton. Monitoring the resistance status of these insecticides in controlling SLW is critical if their usefulness is to be sustained. Resistance monitoring is also important in evaluating the relevance of the Insecticide Resistance Management Strategy (IRMS) guidelines and providing input into developing solutions for looming control issues before they impact on grower productivity. This paper outlines our initial course of action and preliminary results in developing a resistance monitoring program and whitefly identification service.

Materials and Methods

Whitefly collections

Whitefly samples were obtained from all the tropical and warm temperate cotton growing districts of Queensland, namely the Central Highlands, St. George, Dawson-Callide, Darling Downs and Burdekin where SLW is deemed to be a major pest. Samples were either collected as adults using a portable motorised aspirator and transferred directly onto a caged, potted cotton plant or as red-eyed nymphs on leaves and transferred to the Toowoomba laboratory within 24 h where they were placed in an

emergence cage with a potted cotton plant. Caged samples were maintained in a glasshouse at ambient temperature. A reference susceptible colony was obtained from CSIRO at Indooroopilly in 2007 and maintained on hibiscus, *Hibiscus rosa-sinensis*. This source colony was started in 1995 with small injections of new stock, the last being in 2004 and had only been exposed to malathion once in the previous two years to control the parasitoid, *Eretmocerus hayati* Zolnerowich and Rose.

Bioassays

The primary focus of the project in the first year has been handling and rearing whiteflies, refining bioassay techniques and initial range-finding tests. Where possible, bioassays were adapted from the Insecticide Resistance Action Committee (IRAC) susceptibility test methods series - version: 2 (<http://www.irc-online.org/>).

The bioassay protocol for pyriproxyfen involved confining 10 mated female adults in clip cages on leaves of 5-7 node cotton plants (Sicot 71) for 24 h to lay eggs. Five leaves (>20 eggs/leaf) per treatment were then dipped for 20 sec in a serial dilution emulsion. Individual leaves were maintained in 20 mL glass scintillation vials containing 1.5% agar and topped up with a weak soluble fertiliser solution. Bioassays were held in these vials for 10 days when egg hatch was assessed.

A leaf disc bioassay technique was used for both diafenthiuron and bifenthrin. This involved dipping five 38 mm leaf discs per treatment for 20 sec in a serial dilution emulsion. After drying, leaf discs were then placed on a 1.5% agar bed in a ventilated plastic Petri dish. Twenty female whiteflies (< 2 days old) were confined in each dish for 48 h (bifenthrin) and 72 h (diafenthiuron) after which time mortality was assessed. Diafenthiuron requires a longer time span as it is a pro-insecticide, which has first to be converted to its active form in the insect.

A minimum of five concentrations were used in all bioassays and were held at $27 \pm 1^\circ\text{C}$, $50 \pm 5\%$ RH and photoperiod of 16:8 (L:D) h until assessment. Mortality data from all dose response bioassays were analysed using Probit 5 for Windows (Gillespie 1995). Probit regressions were calculated and LC_{50} values estimated.

Biotype identifications and parasitism monitoring

Field collections were initially examined under a microscope to segregate and quantify the proportion of *Bemisia* biotypes and GHW in the samples as well as percentage parasitism of the nymphs. A randomly amplified polymorphic DNA polymerase chain reaction (RAPD PCR) test based on optimising techniques described by De Barro & Driver (1997) was used to determine the biotype identifications in the samples. Molecular markers were used to identify minute differences in the genetic code of the whitefly biotypes.

Results and Discussion

Resistance monitoring

Toxicity data for the SLW strains tested to date against pyriproxyfen, diafenthiuron and bifenthrin in 2007-08 are shown in Table 1.

Table 1. Dose responses of several strains of SLW to three insecticides in comparison to the susceptible laboratory strain.

| Insecticide | Strain | LC ₅₀ (ppm) | 95% Confidence Limits | Slope | Resistance Factor # |
|---------------|--------------|---------------------------|--------------------------|-------|------------------------|
| Pyriproxyfen | Susceptible | 0.023 | 0.001-0.44 | 1.47 | - |
| | St. George A | 0.043 | 0.01-0.13 | 0.091 | 1.9 |
| | St. George B | 0.78 | 0.47-1.30 | 0.84 | 33.9 |
| | Dalby | 0.012 | 0.025-0.059 | 1.37 | 0.5 |
| | Ayr | 1.52 | 1.06-2.18 | 1.19 | 66.1 |
| | Moura | 0.036 | 0.004-0.32 | 3.07 | 1.6 |
| | Emerald* | 0.039 | 0.015-0.104 | 4.51 | 1.6 |
| Diafenthiuron | Susceptible | 17.02 | 11.94-24.3 | 2.52 | - |
| | St. George | 43.9 | 29.3-65.8 | 1.89 | 2.6 |
| Bifenthrin | Susceptible | 13.01 | 9.53-17.78 | 1.99 | - |
| | Dalby | 12.03 | 3.79-38.21 | 1.07 | 0.9 |

* off late season sunflower in the Emerald Irrigation Area

LC₅₀ of tested strain/LC₅₀ of susceptible strain for the same insecticide

Preliminary data for the 2007-08 season indicate no major increases in the resistance levels in any of the populations tested when compared to the susceptible strain. However, there are early signs that resistant SLW individuals are present in some populations. The flat slopes in dose response assays are evidence of populations being made up of both susceptible and resistant individuals. This result demonstrates that there is potential to select for resistance through poor management of insecticides, particularly repeat applications of any product during the season. An area-wide approach to SLW insecticide resistance management is extremely important to ensure the long-term efficacy of available chemistry. Where cotton is grown alongside horticultural and grain crops, this will necessitate a collaborative cross-industry management strategy.

Biotype Identifications and parasitism levels

Results from the RAPD PCR diagnostics show that SLW predominates in the hotter regions (Central Qld, Burdekin and St. George). In the more temperate region of the Darling Downs, GHW, ANB and SLW were all present. Samples from the hotter areas west of Dalby were mainly SLW, and the cooler

Oliveira, M.R.V., Henneberry, T.J. & Anderson, P. (2001). History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection* 20 (9), 709-723.