SCIENTIFIC NOTE

# A NEW DISCRIMINATING DOSE TO MONITOR DIAFENTHIURON (CGA-140408) RESISTANCE IN COTTON APHID (*APHIS GOSSYPII* GLOVER) (HEMIPTERA: APHIDIDAE)

**Grant A. Herron[1], Brendan J. Langfield[1] and Lewis J. Wilson[2]**

[1]NSW DPI, Elizabeth Macarthur Agricultural Institute, Private Bag 4008, Narellan, 2567 Australia [2]CSIRO, Australian Cotton Research Institute, Locked Bag 59, Narrabri 2390, Australia
Email: grant.herron@dpi.nsw.gov.au

## Summary

During the nine year interval spanning the 1999 to 2008 cotton growing seasons monitoring of *Aphis gossypii* Glover populations for resistance failed to identify survivors of the discriminating dose of diafenthiuron (CGA-140408). In 2009-2010, although a significant 4.9 fold (95% CI 2.0-12.0) increase in response relative to a reference susceptible strain (potential resistance) was detected in one population, it was not associated with field control failure. Moreover, the dose required to kill all aphids in laboratory bioassays did not change with selection, nor did the proportion of aphids capable of surviving the discriminating dose increase, and the level of field resistance did not increase over the next two seasons. Ultimately, the detection of field survivors brings into question the validity of the discriminating dose rather than heralding the development of resistance. In 2011-2012 aphid populations were monitored using a diagnostic dose of 0.03 g $L^{-1}$ (double the previous dose) and no survivors were detected.

**Keywords:** Pegasus®, discriminating dose, false positive, bioassay

Diafenthiuron is an insecticidal/acaricidal inhibitor of mitochondrial ATP synthase belonging to the group 12A mode of action group (InfoPest 2011). Under field conditions diafenthiuron (Pegusus®) is transformed into the active carbodiimide derivative of diafenthiuron, CGA-140408, by exposure to ultra-violet light. It was first registered in Australia in April 1996 (APVMA 2012) to control two-spotted mite (*Tetranychus urtice* Koch) and cotton aphid (*Aphis gossypii* Glover) in cotton (APVMA 2012). In 2002 an additional claim for the control of silver leaf whitefly, *Bemisia tabaci* (Gennadius) was registered (InfoPest 2002). Given the multiple pest targets, it is likely that applications targeting one pest also affect the others, even if they are present at sub-economic control levels.

Managing the risk of resistance development and implementation of resistance management strategies requires on-going monitoring of the target pests for resistance. Baseline data for resistance monitoring against *A. gossypii* was developed from 1996 using two reference susceptible strains (A and B) that were completely controlled at the same 0.005 g $L^{-1}$ dose with a discriminating dose for resistance monitoring set during 1996 at 0.015 g $L^{-1}$ (Herron *et al*. 2000). We have monitored for diafenthiuron resistance in *A. gossypii,* using a discriminating dose approach (Roush and Miller 1986), since the 1999-2000 cotton season. Three populations containing survivors of the discriminating dose were detected during the 2008-2009 season (Figure 1). Here we present discriminating dose mortality data for *A. gossypii* against diafenthiuron (CGA-140408).

Aphids were collected from commercial cotton fields or from cotton plants in the vicinity of commercial crops in New South Wales and Queensland. Samples were located by contacting local crop consultants to determine the prevalence of aphids. Aphids were sent to the Elizabeth McArthur Agricultural Institute at Menangle where each isolate was cultured as a separate strain on pesticide-free cotton plants maintained at 25 ± 4°C under natural day light. Aphids were tested by placing them on an excised cotton leaf disc fixed onto agar in a 35 mm petri dish (Herron *et al*. 2001). Testing entailed the Potter spray tower treatment of three batches of approximately twenty-five adult female aphids per leaf disc with insecticide at the discriminating dose (0.015 g $L^{-1}$) (Herron *et al*. 2000). Because ultra-violet light activation was prevented under the laboratory conditions, aphids were treated with CGA-140408 instead of the parent compound diafenthiuron. After spraying, petri dishes were maintained at 25 ± 0.1°C under 16:8 (light:dark) illumination for 24 h after which mortality was assessed. Two replicates were performed giving a total of six insecticide treatment and two control (water only) discs. Control mortality did not exceed 10%.

Strains found to contain survivors at the discriminating dose were studied further in tests with a serial range of CGA-140408 concentrations (selected with the aim of achieving 0-100% mortality) using the methods outlined above. After correction for control mortality (Abbott 1925) these data were analysed in log-dose probit regressions (Finney 1971) to calculate LC50s and to determine resistance factors (RF) relative to a reference susceptible strain. Confidence intervals (CI) for each RF (Robertson *et al*. 2007) were calculated using a Genstat computer software routine (Barchia 2001). Each full log-dose probit regression was replicated up to four times and each replicate included a control as above.

Figure 1. Percentage of *A. gossypii* populations with survivors of CGA-140408 discriminating dose treatment (number of strains tested in brackets) per season.

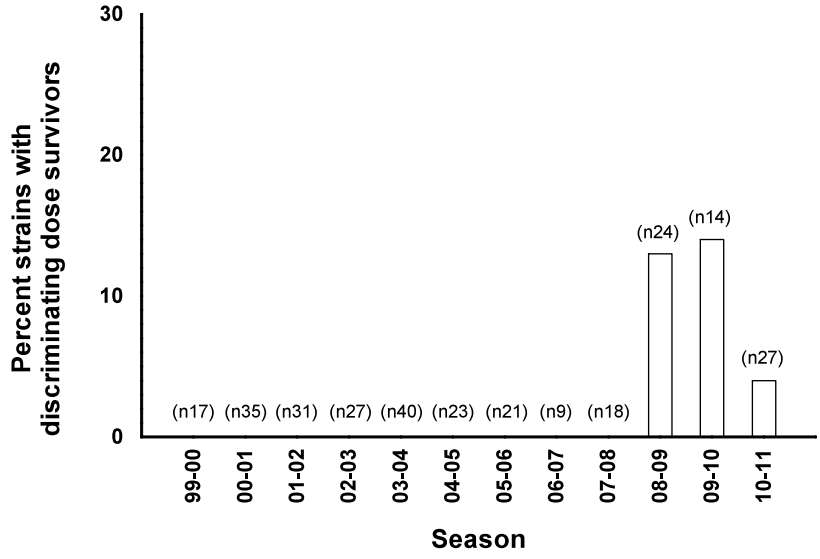



**Table 1. Mortality at the discriminating dose (DD) (0.015 $gL^{-1}$) and probit analysis resistance factor (RF) for susceptible and field *A. gossypii* strains with CGA-140408 discriminating dose survivors.**

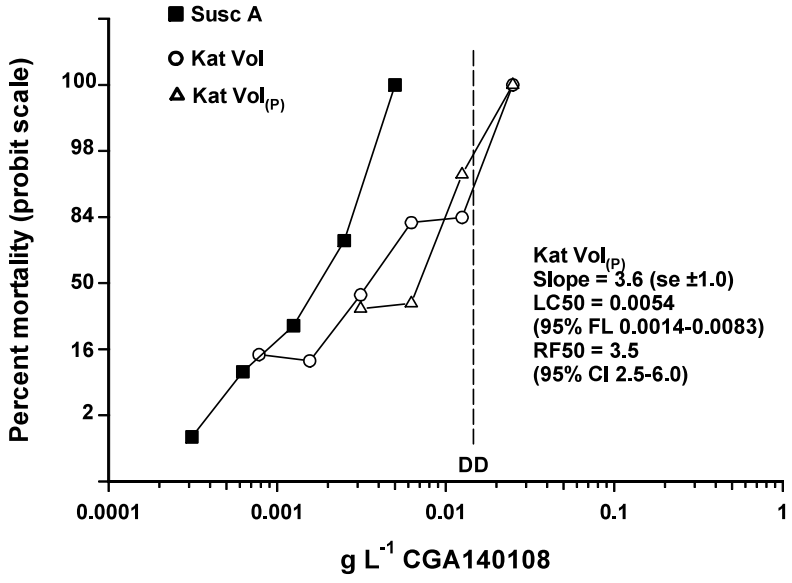
| Season | Strain | Mortality at DD (%) | Chi-square (DF) | Slope (SE) | $LC_{50}$ g $L^{-1}$ (95% FL) | RF (95% CI) |
|---|---|---|---|---|---|---|
| | Susc. A | 100 | 67 (18) | 3.3 (0.6) | 0.0015 (0.0011-0.0022) | - |
| 08/09 | Kat Vol | 95 | 40 (10) | 2.1 (0.4) | 0.0040 (0.0024-0.0064) | 2.6 (1.6-4.2) |
| | Nar B9 | 95 | 51 (10) | 2.1 (0.5) | 0.0055 (0.0032-0.011) | 3.6 (2.1-6.2) |
| | E Wer | 99 | 40 (10) | 2.5 (0.5) | 0.0041 (0.0026-0.0063) | 2.7 (1.7-4.2) |
| 09/10 | Arm | 89 | 134 (5) | 3.4 (2.2) | 0.0075 (0.0020-0.037) | 4.9 (2.0-12.0) |
| | West F8 | 98 | not tested | | | |
| 10/11 | S Gin Block | 99 | not tested | | | |

During the nine seasons between 1999-2000 and 2007-2008 no survivors of the discriminating dose of diafenthiuron (CGA-140408) were detected (Figure 1). During the 2008-2009 season a small proportion of survivors were detected in three strains (12% of strains tested). This suggested the presence of resistant individuals. Results of subsequent full probit analysis indicated a significant 3.6 (95% CI 2.1-6.2) fold difference in response in one strain ('NarB9') (Table1).

Two strains with survivors of the discriminating dose were further pressured with CGA-140408. The first, 'Kat Vol' from season 2008-09 was sub-cultured for three consecutive generations and subsequently sprayed with the discriminating dose (0.015 g $L^{-1}$) of CGA-140408. This new 'pressured' strain ('Kat $Vol_{(P)}$') was then subjected to full log-dose probit analysis. Strain 'Arm' from season 2009-10 was sub-cultured and sprayed over following generations with the discriminating dose and subsequently with 0.02 g $L^{-1}$ CGA-140408. Doses above this treatment caused 100% mortality so this strain was not tested further.

The LC50 of the strain pressured with CGA-140408 'Kat $Vol_{(P)}$' was not significantly different from that of the parental strain (as indicated by overlapping 95% CI). Moreover, the minimum effective dose required to control the new pressured strain remained the same (0.025 g $L^{-1}$). However, the response of this strain was deemed to be more homogenous based on an increase in slope value to 3.6 (Figure 2). Again in 2009-2010 low level CGA-140408 resistance was detected in two strains with the maximum level of 4.9 fold in strain 'Arm' (Table 1). When strain 'Arm' was subsequently pressured with CGA-140408 all insects died when the dose exceeded 0.02 g $L^{-1}$. In 2010-2011 there was again a single survivor of treatment with the CGA-140408 discriminating dose but there were no field control failures reported associated with this, or any of the other suspect resistant strains (Table 1) (unpublished data).

Figure 2. Dose response for a susceptible (Susc A) and suspect resistant strain of *A. gossypii* before (Kat Vol) and after (Kat $Vol_{(P)}$) laboratory selection with the discriminating dose of CGA-140408



Resistance is often monitored via a single discriminating or diagnostic dose that separates resistant from susceptible individuals (Roush and Miller 1986). When detected, suspect resistant strains can be further studied using full probit analysis (Finney 1971) to generate response ratios. Robertson *et al*. (2007) cautioned that resistance ratios alone cannot identify resistant populations. Ffrench-Constant and Roush (1990) stated that resistance level (intensity, or RF) should relate to field control. The finding in 2008-2009 of three strains containing discriminating dose survivors might be interpreted as the beginning of a resistance problem that might be expected to worsen quickly in both level and abundance. This scenario has occurred twice in the recent past for. Australian *A. gossypii*, firstly against pirimicarb (Herron *et al*. 2001), and again several years later against the neonicotinoids (Herron and Wilson 2011). In contrast, subsequent testing of putative resistance to CGA-140408 obtained during the 2009-2010 season did not show an increase in discriminating dose survivors. Likewise, response levels remained statistically static, peaking at 4.9 fold and importantly strain Kat Vol did not respond to CGA-140408 selection. Although there is still a possibility of true resistance developing such a result is indicative of high level vigour tolerance (the natural ability of a population to withstand the toxic effect) (Yu 2008) rather than resistance and serves as a warning not to prematurely consider resistance without confirmatory studies.

Baseline data for resistance monitoring against *A. gossypii* was developed in 1996 using two naïve reference susceptible strains collected from unsprayed backyard gardens (A and B) (Herron *et al*. 2000) that were completely controlled at the same dose of CGA-140408. From these data the discriminating dose (0.015 g $L^{-1}$) was established. Herron *et al.* (2003) reported that four field collected *A. gossypii* strains with resistances to various insecticides (carbamate, organophosphate, pyrethroid and cyclodiene) succumbed to treatment with 0.02 g $L^{-1}$ CGA-140408; this too, supported the 1996 discriminating dose. However, based on the work reported here we now believe the dose used to monitor aphid populations was too low. For season 2011-2012 the dose used to monitor for CGA-140408 was doubled to 0.03 g $L^{-1}$ and no survivors were detected supporting use of the higher dose to discriminate resistance.

## ACKNOWLEDGMENTS

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