

Integrating bioassays and transcriptomics to detect neonicotinoid resistance in Australian *Bemisia tabaci* MEAM1

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Abstract

BACKGROUND: *Bemisia tabaci* MEAM1 is a highly invasive and polyphagous pest species of agricultural and horticultural crops worldwide. It has developed resistance to numerous insecticides, including neonicotinoids. Over-expression of the cytochrome P450 *CYP6CM1* gene has been associated with neonicotinoid resistance. In this study, we used transcriptional analysis (RNAseq and RT-qPCR) to assess the expression of *CYP6CM1* and bioassays to quantify levels of neonicotinoid resistance in the *B. tabaci* resistant GR15-1R strain and 10 field populations collected from Australian cotton in 2023.

RESULTS: Foliar bioassays showed that the GR15-1R strain has partial cross-resistance to neonicotinoids with responses ranked as very high to imidacloprid, clothianidin and thiacloprid, high to thiamethoxam, moderate to acetamiprid, but susceptible to dinotefuran. The transcriptional analysis revealed constitutive over-expression of multiple cytochrome P450 genes, with *CYP6CM1* being the most highly expressed. Bioassays of field populations and *CYP6CM1* expression levels indicated moderate to high levels of resistance to clothianidin, while the response to imidacloprid was more variable, ranging from low to very high resistance; no resistance to dinotefuran or acetamiprid was observed.

CONCLUSIONS: The over-expression of *CYP6CM1* is associated with resistance to imidacloprid, clothianidin, thiacloprid and thiamethoxam. Combining molecular techniques with bioassays is useful for identifying biologically relevant levels of resistance.

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1 INTRODUCTION

The silverleaf whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a cryptic species complex that contains several highly invasive species.^{1–3} *Bemisia tabaci* Middle East Asia Minor 1 (MEAM1) and Mediterranean (MED) are widespread pests of agricultural and horticultural crops,⁴ causing serious damage through direct feeding and as a vector of plant viruses.^{5,6} Furthermore, *B. tabaci* excretes a sugary liquid, 'honeydew', that creates major issues for cotton producers. The nutrient-rich honeydew promotes the growth of sooty mold that discolours and reduces the quality of cotton, and its high carbohydrate content results in stickiness,⁷ causing problems in cotton gins and textile mills.⁸

Managing *B. tabaci* is challenging due to the rapid evolution of insecticide resistance.⁹ In Australian cropping systems, resistance to organophosphates, carbamates, pyrethroids, pyriproxyfen and spirotetramat has been detected in *B. tabaci* MEAM1 through phenotyping bioassays and/or sequencing (to identify target site mutations and determine resistance allele frequencies).^{10–13} Although neonicotinoid resistance has also been reported,¹⁴ its

distribution, severity and the underlying mechanism(s) remain uncharacterized.

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Neonicotinoids target nicotinic acetylcholine receptors (nAChRs). These receptors are ligand-gated ion channels that mediate rapid excitatory neurotransmission in the insect central nervous system.¹⁵ Compounds such as imidacloprid bind to nAChRs, mimicking the action of acetylcholine.¹⁶ However, unlike acetylcholine, neonicotinoids are resistant to enzymatic degradation, which causes prolonged receptor activation that disrupts normal synaptic transmission and leads to paralysis and death.¹⁷ Since their introduction in 1991, neonicotinoids have become among the most widely used insecticides for controlling agricultural pests such as aphids, whiteflies, planthoppers and mealybugs.^{18,19} Neonicotinoids are popular due to application versatility that includes drip or drench systems, seed treatments and foliar applications.²⁰ However, widespread intensive use has resulted in the development of resistance.¹⁸

Two mutations (A58T and R79E) that cause target-site resistance to neonicotinoids²¹ have been previously identified in the nAChR subunit gene family of *B. tabaci* (*BTβ1*). Moreover, resistance to neonicotinoids is frequently linked to the overexpression of detoxification enzymes, particularly cytochrome P450 monooxygenases.^{22–26} For example, over-expression of P450 genes has been described to confer resistance against neonicotinoids in *Nilaparvata lugens* (*CYP6ER1*),²⁷ *Myzus persicae* (*CYP6CY3*)²⁸ and also in *B. tabaci* (*CYP6CM1*).^{24,25,29} A recent study reported a single nucleotide polymorphism (SNP) A387G in *CYP6CM1* of *B. tabaci* MED that is associated with enhanced neonicotinoid resistance.²⁶ Furthermore, MAPK-mediated CREB activation has been linked to an overexpression of *CYP6CM1* and neonicotinoid resistance.³⁰

In this study, we reviewed bioassay data collected over the previous decade to determine when resistance to neonicotinoids appeared in Australian *B. tabaci* MEAM1 populations. We then analyzed the transcriptome to explore likely mechanisms involved in neonicotinoid resistance. Finally, we examined the current distribution and levels of neonicotinoid resistance in field populations of *B. tabaci* MEAM1 from cotton-growing regions in Queensland and New South Wales (NSW), combining bioassay results with RT-qPCR analysis to study neonicotinoid resistance.

2 MATERIALS AND METHODS

2.1 Insect collections and plants

Whitefly populations were collected yearly from field crops, predominantly cotton in Queensland and NSW between 2012 and 2023, as previously described¹⁰ (Supplementary data, Table S1). Furthermore, our study includes two *B. tabaci* MEAM1 laboratory strains: the insecticide-susceptible strain SU07-1, maintained in the laboratory on cotton plants since 1995 without exposure to insecticides, and the insecticide-resistant strain GR15-1R that was established from neonicotinoid-resistant whiteflies, collected

in 2015 near Griffith (NSW). The GR15-1R strain has since been maintained in a glasshouse on cotton plants under selection with clothianidin; adults of each generation were exposed to a cotton plant sprayed to run off with 1000 mg L⁻¹ of clothianidin for 4 days and then transferred to an untreated plant for oviposition. Whitefly populations and cotton plants were reared and maintained as previously described.¹⁰

2.2 Insecticides

For all serial dilution bioassays, the following insecticides and concentrations were used: clothianidin at 200 g L⁻¹ (Shield[®], Sumitomo Chemical, Epping, NSW, Australia), imidacloprid at 200 g L⁻¹ (Confidor[®]; Bayer CropScience, Hawthorn East, VIC, Australia), thiacloprid at 480 g L⁻¹ (Claypso[®]; Bayer CropScience), thiamethoxam at 250 g L⁻¹ (Actara[®]; Syngenta Australia, Macquarie Park, NSW, Australia), acetamiprid at 200 g L⁻¹ (Primal[®]; Adama Australia, St Leonards, NSW, Australia) and dinotefuran at 200 g kg⁻¹ (Starkle[®]; AgNova Technologies, Box Hill North, VIC, Australia). From 2012 to 2022, testing of neonicotinoids varied by year, active ingredient and scale in response to changing cotton industry priorities, including new registrations (dinotefuran and acetamiprid). To ensure broad geographic coverage of resistance monitoring of lower priority insecticides, a minimum of one population per region was included in the annual testing regimen. In 2023, to complement the molecular studies, clothianidin, imidacloprid, acetamiprid and dinotefuran were tested on the same *B. tabaci* populations.

2.3 Dose response bioassays of field populations

The phenotypic response of *B. tabaci* MEAM1 to neonicotinoids was determined using foliar leaf-dip and systemic uptake bioassays.^{31–34} The bioassays were conducted with 4–7 insecticide concentrations increasing in half log increments, designed to achieve mortality ranging from 10 to 100% (Table 1). Each treatment concentration was replicated five times, and each bioassay included a water control, also replicated five times. Foliar bioassays completed in 2023 used the following concentrations: clothianidin (1, 3.2, 10, 32, 100, 320, and 1000 mg L⁻¹), imidacloprid (1, 3.2, 10, 32, 100, 320, and 1000 mg L⁻¹), acetamiprid (1, 3.2, 10, 32, 100, and 320 mg L⁻¹) and dinotefuran (1, 10, 32, 100, and 320 mg L⁻¹). For each complete replication of the concentration range, 100–140 insects were tested.

In 2023, where bioassays indicated resistance, the relevant populations were subjected to selection pressure to validate the result. To accomplish this, a potted cotton plant was sprayed with 600 mg L⁻¹ of insecticide to the point of run off. After drying for approximately 1 h, the plant was placed inside a cage, and adult whiteflies (*n* ≈ 1000) were introduced and exposed to the insecticide for 4 days, after which any survivors were transferred to an untreated plant to oviposit. At approximately 30 days, the cage

Table 1. Timeline of neonicotinoid insecticide testing, bioassay methodology and concentrations used

Insecticide	Year	Bioassay methodology	Concentration (mg L ⁻¹)
Clothianidin	2012–2016, 2023	Foliar	1, 3, 10, 32, 100, 320, 1000, 3200, 10 000 [†]
Dinotefuran	2017–2023	Foliar	0.32, 1, 3.2, 10, 32, 100, 320
Acetamiprid	2017–2023	Foliar	1, 3.2, 10, 32, 100, 320
Imidacloprid	2020	Systemic	0.1, 0.32, 1, 3.2, 10, 32, 100
	2023	Foliar	1, 3.2, 10, 32, 100, 320

[†] From 2012 to 2014, only four concentrations up to 320 mg L⁻¹ used. From 2015 onwards, five or more concentrations were used.

was inspected, and the presence of viable progeny was assessed. Populations were considered resistant if live whiteflies were present.

2.3.1 Foliar leaf-dip bioassay

Cotton leaves were collected from glasshouse plants and, using a sterile scalpel, leaf petioles were reduced to a uniform length with a clean 30° angle cut. If necessary, the leaf surface area was reduced to a size suitable for leaf dipping into 300-mL crystallizing dishes. Leaves were dipped in treatment concentrations for 20 s, air dried on test tube racks for 30 min and transferred to 40-mL glass vials containing potable water to maintain leaf freshness throughout the assay.³⁴ Clip cages were placed onto the treated leaves and whiteflies were aspirated from colony cages and transferred onto the abaxial leaf surface inside the clip cage. Adults were tested shortly after emergence (max. age < 3 days) and experiments were kept in controlled environment rooms (27 °C, 60% room humidity, 12 h light:12 h dark photoperiod).³⁴ Mortality was assessed after 72 h of exposure by counting live and dead insects under a stereo microscope.³⁴

2.3.2 Systemic uptake bioassay

Leaf preparation followed the foliar leaf-dip bioassay methodology, but instead of dipping the leaves, the petioles were placed into 18-mL plastic tubes filled with insecticide solution to facilitate uptake by the petiole vascular bundle and translocation of the insecticide to the leaf. Leaves were left in the insecticide-filled tubes for 24 h and then transferred to 40-mL glass vials with water.^{31–34} The use of clip cages, handling of the insects, environmental conditions, duration and mortality assessment were the same as described for the foliar leaf-dip bioassay.

2.4 Cross-resistance in neonicotinoid-resistant strain GR15-1R

The cross-resistance of strain GR15-1R to clothianidin, imidacloprid, thiacloprid, thiamethoxam, acetamiprid and dinotefuran was investigated using both the foliar leaf-dip and systemic uptake bioassay methodologies described above. Adult whiteflies were exposed to seven treatment concentrations, with half log increments between 0.1 and 100 mg L⁻¹ for the systemic uptake bioassay and half log increments between 1 and 1000 mg L⁻¹ for the foliar leaf-dip bioassay.

2.5 Data analysis of bioassays

Mortality data from bioassays were corrected for control mortality (0–5.4%, $\bar{x}$ = 1.6%)³⁵ and analyzed as non-pooled data using probit regression in Genstat 22.³⁶ From this analysis, the dose-dependent mortality response including the slope, median lethal concentration (LC₅₀) estimate and associated 95% fiducial limits were determined. During the analysis, heterogeneity was assessed using a χ^2 test and, if significant at the 5% level, the variance of the estimated parameter was scaled by the corresponding heterogeneity factor equal to the residual mean deviance.³⁷ The minimum effective concentration (MEC), defined as the lowest concentration required to kill 100% of insects in all replicates³⁸ was recorded. For each field population, a resistance ratio (RR) and 95% confidence interval (CI) was calculated as outlined by Robertson *et al.*³⁹ Field populations collected in 2023 were defined as resistant if they survived both a diagnostic dose of 320 mg L⁻¹ and a subsequent selection test at 600 mg L⁻¹. These concentrations were adapted from a previous diagnostic test protocol.³⁴ The level of resistance was then interpreted by using the

following RR categories: susceptible (RR = 1), tolerance to low resistance (RR = 2–10), moderate resistance (RR = 11–30), high resistance (RR = 31–100) and very high resistance (RR > 100).⁴⁰

2.6 DNA extraction and species identification

Adult whiteflies from the susceptible strain SU07-1, the resistant strain GR15-1R and the 2023 field populations were used for DNA extraction (Fig. 1). Subsamples of whiteflies ($n \approx 100$) were preserved in 90% ethanol and sent from the Toowoomba laboratory of the Queensland Department of Primary Industries and Fisheries to the CSIRO Black Mountain Laboratory in Canberra (ACT). Upon arrival, DNA was extracted using QIAGEN DNeasy Blood & Tissue kit following the manufacturer's instructions. The mtCOI barcode primers (wfly-PCR-F1/R1 and F2/R2) and PCR conditions were previously described.^{10,41} High-throughput sequencing libraries were prepared as per the Illumina protocol (#15044223 Rev. B) with modification.⁴¹ The sequencing was performed using an Illumina MiSeq at the CSIRO Black Mountain Laboratory using a 300-bp PE kit. The mtCOI contigs were mapped to a cleaned mtCOI database⁴² using Geneious Prime version 2025.1.3 (www.geneious.com) for species identification.

2.7 RNA extraction and transcriptomic analysis

Newly emerged adult whiteflies ($n = 100$) from SU07-1, GR15-1R and the 2023 field populations with no direct exposure to clothianidin were aspirated from their cages into 1.5-mL Eppendorf tubes and stored at –80 °C. Additionally, adult whiteflies ($n = 43–91$, $\bar{x}$ = 71) from GR15-1R and the field populations that survived 72 h of exposure to 1000 mg L⁻¹ of clothianidin were retained and preserved at –80 °C. Samples were transported on dry ice to the CSIRO Black Mountain Laboratory for transcriptomic analysis.

Three replicates of total RNA were extracted from each whitefly population using the Quick-RNA™ Tissue/Insect Microprep Kit (Cat. #R2030, Zymo Research, Irvine, CA, USA). RNA from unexposed (susceptible SU07-1, resistant GR15-1R and field populations) and exposed insects (clothianidin surviving individuals from GR15-1R and field populations) was transferred to RNA stabilization tubes (Azenta Life Sciences, Burlington, MA, USA) and submitted for sequencing. Transcriptome sequencing was conducted by Azenta Life Sciences (<https://www.azenta.com/>) using paired-end (PE), stranded 2 × 150 bp short-read technology.

Differential expression analyses were performed between untreated resistant (GR15-1R) and untreated susceptible (SU07-1) strains to identify genes associated with neonicotinoid resistance. In addition, transcriptomic comparisons between clothianidin-treated and untreated GR15-1R samples were performed to identify genes responsive to insecticide exposure.

2.8 RNAseq

The RNAseq analysis pipeline was based on two published protocols.^{43,44} The pair-end reads were trimmed and quality checked using Fastp/0.22.0⁴⁵ to ensure a Q-score > 30. After trimming, paired reads were mapped to the *B. tabaci* MEAM1 reference genome (RefSeq: GCF_001854935.1)⁴⁶ using HISAT/2.2.1.⁴⁷ A raw count table was generated using the 'featureCounts' function of the Subread/2.0.3 package⁴⁸ and DESeq2⁴⁹ was used to analyze gene expression changes. To identify neonicotinoid induced changes, expression profiles were compared between unexposed insects from the resistant strain GR15-1R, unexposed insects from the susceptible SU07-1, and between clothianidin exposed and unexposed insects from the GR15-1R population. Differential

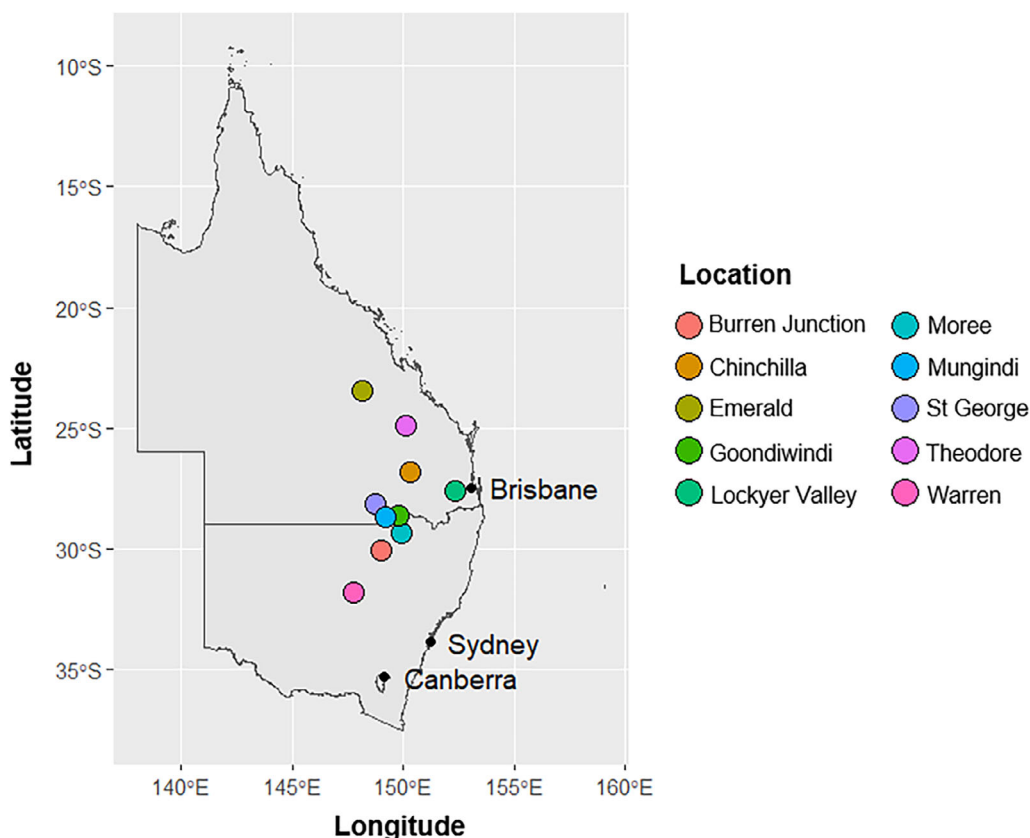


Figure 1. Location of the 2023 collection sites of *B. tabaci* MEAM1 from cotton fields in Queensland and New South Wales.

expression was assessed using the negative binomial generalized linear model implemented in DESeq2, with significance tested using the Wald test. *P*-values were adjusted for multiple testing using the Benjamini–Hochberg procedure. Genes with $|\log_2\text{FoldChange}| > 1$ and an adjusted *P*-value (*padj*) < 0.05 were considered significantly differentially expressed.

Gene ontology (GO) analysis was based on the differentially expressed genes from the output of DESeq2 using TopGO version 2.46.0.⁵⁰ Based on differentially expressed genes, significant GO terms (*P* < 0.05) were identified under specific ontologies of biological process, molecular function and cellular component. The GO terms were then clustered based on semantic similarity measures.

2.9 RT-qPCR

The gene expression was analyzed in the resistant GR15-1R strain, the 2023 field populations and the survivors of these populations after 72 h of exposure to 1000 mg L⁻¹ of clothianidin. Three biological replicates were analyzed for each whitefly population and treatment group. Each biological replicate was measured in technical triplicates, and mean Ct values were used for subsequent statistical analyses. The RT-qPCR experimental design and analysis follow the published minimum information for publication of quantitative real-time PCR experiments (MIQE) guideline.⁵¹ RT-qPCR was used to assess *CYP6CM1* gene expression with Verso 1-step RT-qPCR SYBR Green ROX kit (Cat. #AB4105A/C, ThermoFisher Scientific, Waltham, MA, USA). A 25-μL PCR reaction contained 0.25 μL of the enzyme mix, 12.5 μL of 1-step SYBR ROX mix, 1 μL of RT-Enhancer, 1.75 μL of 10 μM forward primer, 1.75 μL of the reverse primer, 5 μL of total RNA template (1 ng/μL) and 2.75 μL of nuclease-free water.

Reactions were incubated at 50 °C for 15 min (cDNA synthesis), 95 °C for 15 min (Thermo-Start activation) and in 35 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s, followed by a standard melt-curve condition of temperature increase from 65 to 95 °C with 0.5 °C increments per cycle.

A 128-bp fragment of *CYP6CM1* was amplified using primers *CYP6CM1*-f (5'-GCCATCGGTGATAAAGGAGA) and *CYP6CM1*-r (5'-AACTCGGTTTCCTCATCGTG).⁵² The amplicons were Sanger sequenced at the Biomolecular Resource Facility at the Australian National University. For normalization, the housekeeping reference elongation factor 1- α (*EF1 α*) was amplified with primers *EF1 α* -f (5'-GATGGCAGGAGATAACATG) and *EF1 α* -r (5'-TTGTCTGTGGGCTGCTGGG).⁵² Primer sequences were modified to match the coding region of our reference population SU07-1. The gene expression level was quantified using the $2^{-\Delta\Delta C_t}$ method.⁵³ A two-tailed *t*-test was used on each field population to evaluate whether there was any significant change in *CYP6CM1* expression before and after neonicotinoid treatment. A one-way analysis of variance (ANOVA) was performed on ΔC_t values to assess differences in *CYP6CM1* expression among the untreated field populations, followed by Fisher's least significant difference procedure to identify significant differences.

3 RESULTS

3.1 Phenotypic response of *B. tabaci* MEAM1 to neonicotinoids

3.1.1 Historical evidence of field resistance to neonicotinoids (2012–2022)

Over the past decade, resistance to neonicotinoids has been periodically observed in *B. tabaci* MEAM1. Initially, the detection of

resistance was infrequent, but recent results demonstrate a rapid increase in both the distribution and severity of resistance (Supplementary data, Tables S2 and S3). When reviewing historical bioassay data and based solely on resistance ratios, we found that moderate resistance ($RRs > 11$) to clothianidin was present in whiteflies collected from tomatoes in the Lockyer Valley in 2012, cotton at Emerald in 2013, melons near Griffith in 2015 (the source of GR15-1R), and cotton at Wee Waa in 2015 (Supplementary data, Table S2). In 2019, testing of whiteflies from cucurbits from Bowen and Gumlu indicated that imidacloprid resistance was present; testing of populations collected in the following year from cotton regions of Queensland and NSW revealed widespread resistance to imidacloprid with 11 out of 16 populations being resistant with a $RR > 11$ (Supplementary data, Table S3). Testing between 2017 and 2022 has revealed no clear evidence of resistance to either dinotefuran (Supplementary data, Table S4) or acetamiprid (Supplementary data, Table S5).

3.1.2 Field resistance to neonicotinoids (2023)

All populations tested in 2023 were resistant to clothianidin with survival observed at the 320 mg L^{-1} diagnostic dose and following selection at 600 mg L^{-1} , $RRs \geq 12.6$ (Table 2). Moderate resistance was observed in the Chinchilla and Theodore populations, while the remaining populations were highly resistant. For many of the populations, accurate LC_{50} data could not be determined as there was a limited change in mortality with increasing doses. This is best exemplified by the Lockyer Valley population, which recorded 2.5% mortality at the 320 mg L^{-1} diagnostic dose.

Increased sensitivity to imidacloprid was observed compared to clothianidin, with most assays recording a mortality of $> 50\%$ at 320 mg L^{-1} (Table 3). More accurate LC_{50} estimates could therefore be determined and used to calculate RRs . All populations were resistant to imidacloprid with survival observed at the 320 mg L^{-1} diagnostic dose and following selection at 600 mg L^{-1} . Resistance ranged from low at Theodore to very high at St George and Goondiwindi.

Whitefly populations were sensitive to acetamiprid, with $RRs < 5$ (Table 4). The St George population was initially indicative of low resistance when assayed with a RR of 4.3, and a 1.2% survival rate at 320 mg L^{-1} but did not survive the selection pressure test at 600 mg L^{-1} and is therefore considered not resistant.

Based on uniform LC_{50} estimates and 95% fiducial limits, there was limited phenotypic variation within field populations when tested with dinotefuran (Table 5). In all populations, the LC_{50} 95% fiducial limits of the field populations overlapped those of the susceptible laboratory population (SU07-1). There was no conclusive evidence of resistance to dinotefuran in the field populations, as all populations had $RRs < 2$, and only the population from Mungindi had surviving insects, 1.3% at the diagnostic dose (320 mg L^{-1}), but in subsequent glasshouse selection testing, all individuals failed to survive exposure to dinotefuran at 600 mg L^{-1} .

3.1.3 Cross-resistance profile of strain GR15-1R

Cross-resistance within the neonicotinoid insecticide group was investigated using the resistant laboratory strain GR15-1R. We found partial cross-resistance to six insecticides (Table 6). In the foliar assay, the GR15-1R strain exhibited very high resistance ($LC_{50} > 1000 \text{ mg L}^{-1}$) to imidacloprid, clothianidin and thiacloprid. Due to a limited change in mortality in response to increasing concentration, accurate LC_{50} estimates could not be calculated, and only minimum resistance ratios could be determined.

Population (G^+)	Bioassay date	n	χ^2 (df)	Slope (SE)	LC_{50} (mg L^{-1}) (95% FL)	$RR^{\dagger}$ (95% CI)	Mortality (%) at 320 mg L^{-1}
SU07-1	10 June 2023	528	51.7 (33)	1.89 (0.19)	15.2 (11.6–19.8)		100
Emerald (G_9)	22 September 2023	661	81.9 (33)	0.42 (0.09)	996.1 (296–12 603)	65.6 (13.5–319.4)	34
Theodore (G_7)	19 August 2023	563	96.8 (33)	0.47 (0.10)	460.0 (152.1–4209)	30.4 (7.5–123.9)	41
Chinchilla (G_6)	22 September 2023	637	87.0 (33)	0.35 (0.09)	283.3 (86.9–3219)	18.6 (4.3–81.3)	44
Lockyer Valley (G_6)	18 September 2023	598	60.1 (33)	0.06 (0.14)	> 1000	> 66	3
St George (G_6)	18 September 2023	617	57.1 (33)	0.47 (0.11)	> 1000	> 66	14
Goondiwindi (G_3)	9 January 2023	650	76.6 (33)	0.45 (0.09)	> 1000	> 66	33
Mungindi (G_6)	9 January 2023	597	87.4 (33)	0.28 (0.10)	> 1000	> 66	35
Moree (G_6)	10 June 2023	663	57.2 (33)	0.30 (0.08)	> 1000	> 66	25
Burren Junction (G_6)	10 June 2023	672	51.9 (33)	0.26 (0.07)	> 1000	> 66	27
Warren (G_6)	10 June 2023	796	61.6 (33)	0.36 (0.08)	> 1000	> 66	20

Abbreviations: n , number of insects tested; df, degrees of freedom; SE, standard error; FL, fiducial limit; CI, confidence interval.

† Generation tested.

$^{\#}$ The resistance ratio (RR) is calculated by dividing the LC_{50} of the field populations by LC_{50} of the susceptible population (SU07-1).

Table 3. Toxicity of imidacloprid to *B. tabaci* MEAM1 field populations in a foliar leaf-dip bioassay

Population (G [†])	Bioassay date	<i>n</i>	χ^2 (df)	Slope (SE)	LC ₅₀ (mg L ⁻¹) (95% FL)	RR [‡] (95% CI)	Mortality (%) at 320 mg L ⁻¹
SU07-1	10 October 2023	330	40.8 (17)	2.30 (0.44)	1.4 (0.8–1.9)		100
Emerald (G9)	25 September 2023	452	36.5 [§] (28)	0.84 (0.08)	14.8 (10.3–21.0)	10.7 (6.4–17.7)	91
Theodore (G7)	19 September 2023	546	44.9 (28)	0.99 (0.10)	12.2 (8.3–17.7)	8.8 (5.3–14.8)	92
Chinchilla (G6)	25 September 2023	490	36.5 [§] (28)	0.94 (0.08)	21.3 (15.7–29.2)	15.4 (9.6–24.8)	89
Lockyer Valley (G6)	19 September 2023	486	46.2 (28)	1.65 (0.18)	76.8 (56.5–105.7)	54.9 (32.4–87.6)	91
St George (G6)	19 September 2023	642	67.7 (28)	0.87 (0.13)	213.2 (115.6–558.2)	154.3 (69.3–343.4)	71
Goondiwindi (G6)	29 September 2023	560	35.4 [§] (28)	0.82 (0.09)	281.8 (176.2–538.7)	203.6 (105.5–393.0)	50
Mungindi (G6)	29 September 2023	581	48.2 (28)	0.66 (0.09)	127.3 (69.5–312.2)	91.9 (42.3–199.9)	66
Moree (G6)	10 October 2023	593	56.1 (28)	0.81 (0.10)	70.6 (43.4–131.4)	51.0 (27.1–95.9)	76
Burren Junction (G6)	10 October 2023	561	42.7 (28)	0.96 (0.10)	69.6 (47.3–110.9)	50.4 (29.3–86.6)	73
Warren (G6)	10 October 2023	694	57.6 (28)	0.99 (0.10)	51.1 (35.0–79.0)	36.9 (21.7–62.6)	76

Abbreviations: *n*, number of insects tested; df, degrees of freedom; SE, standard error; FL, fiducial limit; CI, confidence interval.

[†] Generation tested.

[‡] The resistance ratio (RR) is calculated by dividing the LC₅₀ of the field populations by LC₅₀ of the susceptible population (SU07-1).

[§] χ^2 test significant at *P* < 0.05, for heterogeneity scaling. Chi-square values report how well bioassay data fit the probit model.

Table 4. Toxicity of acetamiprid to *B. tabaci* MEAM1 field populations in a foliar leaf-dip bioassay

Population (G [†])	Bioassay date	<i>n</i>	χ^2 (df)	Slope (SE)	LC ₅₀ (mg L ⁻¹) (95% FL)	RR [‡] (95% CI)	Mortality (%) at 320 mg L ⁻¹
SU07-1	26 February 2018	514	55.1 (23)	2.42 (0.28)	4.0 (3.1–5.2)		100
Emerald (G3)	3 October 2023	451	44.6 (28)	1.11 (0.12)	4.5 (2.9–6.6)	1.1 (0.7–1.8)	100
Theodore (G4)	30 May 2023	555	40.7 [§] (28)	1.63 (0.12)	9.0 (7.3–10.9)	2.2 (1.6–3.1)	100
Chinchilla (G4)	17 July 2023	482	34.4 [§] (28)	1.19 (0.10)	6.3 (4.7–8.2)	1.6 (1.1–2.3)	100
Lockyer Valley (G3)	30 May 2023	545	31.8 [§] (28)	2.85 (0.25)	13.2 (11.4–15.3)	3.3 (2.5–4.4)	100
St George (G3)	6 May 2023	468	24.5 [§] (28)	1.68 (0.13)	17.1 (13.8–21.2)	4.3 (3.1–5.9)	98.8
Goondiwindi (G4)	25 July 2023	270	22.0 [§] (22)	1.38 (0.14)	8.1 (5.8–11.1)	2.0 (1.3–3.0)	100
Mungindi (2) (G5)	25 July 2023	410	27.7 [§] (28)	1.57 (0.13)	8.8 (6.9–11.1)	2.2 (1.6–3.1)	100
Moree (G5)	8 September 2023	697	38.2 [§] (28)	1.66 (0.10)	9.6 (8.0–11.5)	2.4 (1.8–3.2)	100
Burren Junction (G3)	27 June 2023	524	43.2 (23)	1.70 (0.18)	10.4 (7.7–14.03)	2.6 (1.8–3.7)	100
Warren (G4)	8 January 2023	592	49.9 (28)	1.91 (0.17)	13.8 (10.8–17.8)	3.4 (2.5–4.8)	100

Abbreviations: *n*, number of insects tested; df, degrees of freedom; SE, standard error; FL, fiducial limit; CI, confidence interval.

[†] Generation tested.

[‡] The resistance ratio (RR) is calculated by dividing the LC₅₀ of the field populations by LC₅₀ of the susceptible population (SU07-1).

[§] χ^2 test significant at *P* < 0.05, for heterogeneity scaling. Chi-square values report how well bioassay data fit the probit model.

High resistance was observed to thiamethoxam (RR = 45), moderate resistance to acetamiprid (RR = 11) and no resistance to dinotefuran, although a RR of 6 was calculated, a 100% mortality was observed at the diagnostic dose of 320 mg L⁻¹. Resistance to imidacloprid (RR = 22.7) and clothianidin (RR = 11.8) was observed in the systemic uptake assay but, for the remaining neonicotinoids, resistance was less evident, particularly for dinotefuran, given the MEC was 10 mg L⁻¹.

3.2 Species identification

The species identification of field collected *B. tabaci* is documented in the Supplementary data (Table S1). In two populations collected from Goondiwindi in 2016 and 2017, *B. tabaci* AUS1 was detected at a population frequency of 19.6 and 7.9%, respectively.¹⁰ In 2023, a partial mtCOI sequence was used to discriminate between different *B. tabaci* species, as described earlier.⁴¹ The mtCOI contigs were mapped to the MEAM1 mtCOI

database.⁴² The susceptible laboratory strain SU07-1 has previously been identified as MEAM1.¹⁰ The contigs from the resistant laboratory strain GR15-1R and 10 field-collected whitefly populations were mapped to a mtCOI database, which confirmed that these populations were comprised solely of *B. tabaci* MEAM1.

3.3 RNAseq and functional analysis

RNAseq analysis was performed on the susceptible population SU07-1 and the resistant population GR15-1R. The SNPs A58T and R79E, known to confer neonicotinoid resistance, were not observed in the nAChR *BTβ1* gene of either the SU07-1 or the GR15-1R population.²¹ Moreover, SNP A387G in the P450 gene *CYP6CM1*, previously reported to be associated with enhanced neonicotinoid resistance, was also absent in GR15-1R.²⁶ Furthermore, a previous study³⁰ found that the over-expression of *CYP6CM1* is linked to MAPK-mediated CREB activation. However, the CREB orthologue LOC109029782 was not differentially

Table 5. Toxicity of dinotefuran to *B. tabaci* MEAM1 field populations in a foliar leaf-dip bioassay

Population (G [†])	Bioassay date	<i>n</i>	χ^2 (df)	Slope (SE)	LC ₅₀ (mg L ⁻¹) (95% FL)	RR [‡] (95% CI)	Mortality (%) at 320 mg L ⁻¹
SU07-1	24 March 2016	341	60.2 (33)	1.58 (0.19)	12.3 (8.3–18.2)		100
Emerald (G6)	6 December 2023	437	35.4 (18)	2.04 (0.26)	8.3 (5.7–11.4)	0.7 (0.4–1.1)	100
Theodore (G7)	15 September 2023	435	16.7 [§] (18)	2.66 (0.30)	13.5 (11.1–16.1)	1.1 (0.7–1.7)	100
Chinchilla (G4)	18 July 2023	271	18.6 [§] (18)	1.76 (0.20)	9.4 (6.8–12.5)	0.8 (0.5–1.2)	100
Lockyer Valley (G6)	15 September 2023	433	40.5 (23)	2.10 (0.25)	23.8 (17.8–30.9)	1.9 (1.2–3.0)	100
St George (G4)	14 July 2023	390	45.9 (18)	2.08 (0.34)	12.5 (7.9–17.9)	1.0 (0.6–1.7)	100
Goondiwindi (G4)	21 July 2023	380	30.5 (18)	1.62 (0.20)	8.9 (5.9–12.7)	0.7 (0.4–1.2)	100
Mungindi (2) (G6)	29 August 2023	383	13.3 [§] (23)	2.10 (0.21)	20.6 (16.5–25.2)	1.7 (1.1–2.6)	98.7
Moree (G4)	8 April 2023	511	63.9 (23)	1.49 (0.18)	8.9 (5.6–13.2)	0.7 (0.4–1.2)	100
Burren Junction (G4)	8 April 2023	564	68.7 (23)	1.44 (0.18)	9.3 (5.8–13.8)	0.8 (0.4–1.3)	100
Warren (G4)	8 April 2023	656	36.3 (18)	2.43 (0.29)	9.5 (7.1–12.2)	0.8 (0.5–1.2)	100

Abbreviations: *n*, number of insects tested; df, degrees of freedom; SE, standard error; FL, fiducial limit; CI, confidence interval.

[†] Generation tested.

[‡] The resistance ratio (RR) is calculated by dividing the LC₅₀ of the field populations by LC₅₀ of the susceptible population (SU07-1).

[§] χ^2 test significant at *P* < 0.05, for heterogeneity scaling. Chi-square values report how well bioassay data fit the probit model.

Table 6. Toxicity of neonicotinoids to *B. tabaci* MEAM1 laboratory strain GR15-1R

Assay	Insecticide	<i>n</i>	χ^2 (df)	Slope (SE)	LC ₅₀ (mg L ⁻¹) (95% FL)	RR [†] (95% CI)	MEC [‡] (mg L ⁻¹)
Foliar	Imidacloprid	684	73.0 (33)	0.47 (0.11)	>1000	>377	-
	Clothianidin	672	29.0 (33)	0.40 (0.15)	>1000	>66	-
	Thiacloprid	855	153.3 (32)	0.34 (0.12)	>1000	>512	-
	Thiamethoxam	804	153.4 (33)	1.67 (0.28)	358.1 (234.2–623.6)	45.2 (25.7–79.7)	-
	Acetamiprid	680	60.4 (33)	1.60 (0.14)	13.6 (10.3–17.9)	11.4 (8.0–16.3)	1000
Systemic	Dinotefuran	636	81.7 (28)	2.34 (0.28)	17.8 (13.3–23.8)	6.3 (4.3–9.3)	320
	Imidacloprid	728	56.9 (33)	1.53 (0.15)	21.2 (16.1–28.6)	22.7 (14.4–36.0)	-
	Clothianidin	706	212.9 (33)	1.28 (0.34)	88.5 (42.3–498.1)	11.8 (4.3–32.3)	-
	Thiacloprid	783	113.6 (33)	1.86 (0.21)	4.9 (3.6–6.9)	2.8 (1.2–6.5)	100
	Thiamethoxam	803	94.1 (33)	2.62 (0.32)	5.8 (4.6–7.5)	6.7 (4.1–11.0)	100
	Acetamiprid	696	96.3 (33)	1.92 (0.22)	2.5 (1.8–3.4)	3.9 (2.3–6.7)	100
	Dinotefuran	506	123.9 (23)	2.14 (0.36)	1.1 (0.7–1.6)	4.9 (3.1–7.6)	10

Abbreviations: *n*, number of insects tested; df, degrees of freedom; SE, standard error; FL, fiducial limit; CI, confidence interval; –, 100% mortality not observed at any concentration.

[†] The resistance ratio (RR) was calculated by dividing the LC₅₀ of the GR15-1R by LC₅₀ of the susceptible population SU07-1.

[‡] Minimum effective concentration (MEC) that resulted in 100% mortality.

expressed in our *B. tabaci* MEAM1 GR15-1R dataset ($\log_2$ fold change = $\log_2$ FC) ($-1 < \log_2$ FC < 1 and *P* > 0.05).

Transcriptomic analysis was used to measure gene expression in adult whiteflies from the resistant strain GR15-1R before and after 72 h of clothianidin exposure. The cut-off for differentially expressed genes was chosen as $\log_2$ FC > 1 for up-regulated genes, and $\log_2$ FC < -1 for down-regulated genes, with *P* < 0.05. Compared to the SU07-1 susceptible strain, in the GR15-1R before exposure to clothianidin there were 705 genes differentially expressed (Supplementary data, Table S6), while after exposure to clothianidin, 1445 genes were differentially expressed (Supplementary data, Table S7). However, we found that only 179 genes were differentially expressed in GR15-1R survivors of clothianidin exposure relative to whiteflies of the same strain that were not exposed to clothianidin (Supplementary data, Table S8).

An enrichment analysis was performed based on differentially expressed genes from GR15-1R before clothianidin exposure,

relative to SU07-1 (Fig. 2). A total of 36 enriched GO terms were found across the categories of biological process (BP), cellular component (CC) and molecular function (MF). The most highly annotated terms include transmembrane transport (*n* = 490 genes, BP), integral component of membrane (*n* = 659 genes, CC) and transmembrane transporter activity (*n* = 556 genes, MF). We also investigated the transcriptional changes in response to clothianidin treatment in GR15-1R (Fig. 3) and observed 21 enriched GO terms. In response to the clothianidin exposure, the most highly annotated terms were proteolysis (*n* = 463 genes, BP), extracellular region (*n* = 76 genes, CC) and peptidase activity (*n* = 428 genes, MF).

3.4 Differentially expressed detoxification genes through RNAseq

We identified several genes that were previously reported to be associated with neonicotinoid resistance in the resistant strain

GR15-1R (Supplementary data, Table S9). To provide an overview of the detoxification response associated with neonicotinoid resistance, differentially expressed genes meeting the DESeq2 criteria ($|\log_2\text{FoldChange}| > 1$ and adjusted $P < 0.05$) were grouped into families, i.e., cytochrome P450 monooxygenases (P450s),²⁵ UDP-glucuronosyltransferases (UGTs),⁵⁴ glutathione S-transferases (GSTs),⁵⁵ esterases⁵⁶ and ATP-binding cassette (ABC) transporters⁵⁷ (Fig. 4). In the untreated resistant *B. tabaci* MEAM1 population, the P450 family contained the highest number of differentially expressed genes. In contrast, following clothianidin treatment, the UGT family had the highest number of differentially expressed genes.

Among the differentially expressed genes, the P450 gene *CYP6CM1* showed the greatest differential expression (Table 7). The expression levels of four other P450 genes, *CYP6EM1*, *CYP6CX4*, *CYP6CX2* and *CYP6DW4* (Table 7) were also higher in GR15-1R relative to SU07-1, but expression levels were far lower compared to *CYP6CM1*. The exposure of adult whiteflies of the resistant strain GR15-1R to clothianidin did not change *CYP6CM1* expression levels, suggesting that the gene is likely to be

constitutively overexpressed in the resistant population. We also compared the coding sequences of the *CYP6CM1* gene between the susceptible SU07-1 and the resistant GR15-1R strain and found them to be identical.

3.5 *CYP6CM1* expression linked with resistance to clothianidin in *B. tabaci* MEAM1 field populations

Among the P450 genes, *CYP6CM1* showed the greatest differential expression in the resistant strain GR15-1R compared with the susceptible strain SU07-1. Based on this strong association with resistance, the transcript level of *CYP6CM1* was measured using RT-qPCR in the 2023 field populations. We chose to measure the transcript level of cytochrome P450 *CYP6CM1* and use it as an indicator for neonicotinoid resistance due to several factors: (i) its constitutive expression, (ii) the highest fold change observed from our RNAseq result, (iii) its established association with neonicotinoid resistance^{24,25} and (iv) its stable expression.⁵²

CYP6CM1 expression level in GR15-1R was more than 50-fold higher compared to the susceptible SU07-1 strain. In the field populations, *CYP6CM1* was overexpressed 7.5–21.7-fold relative

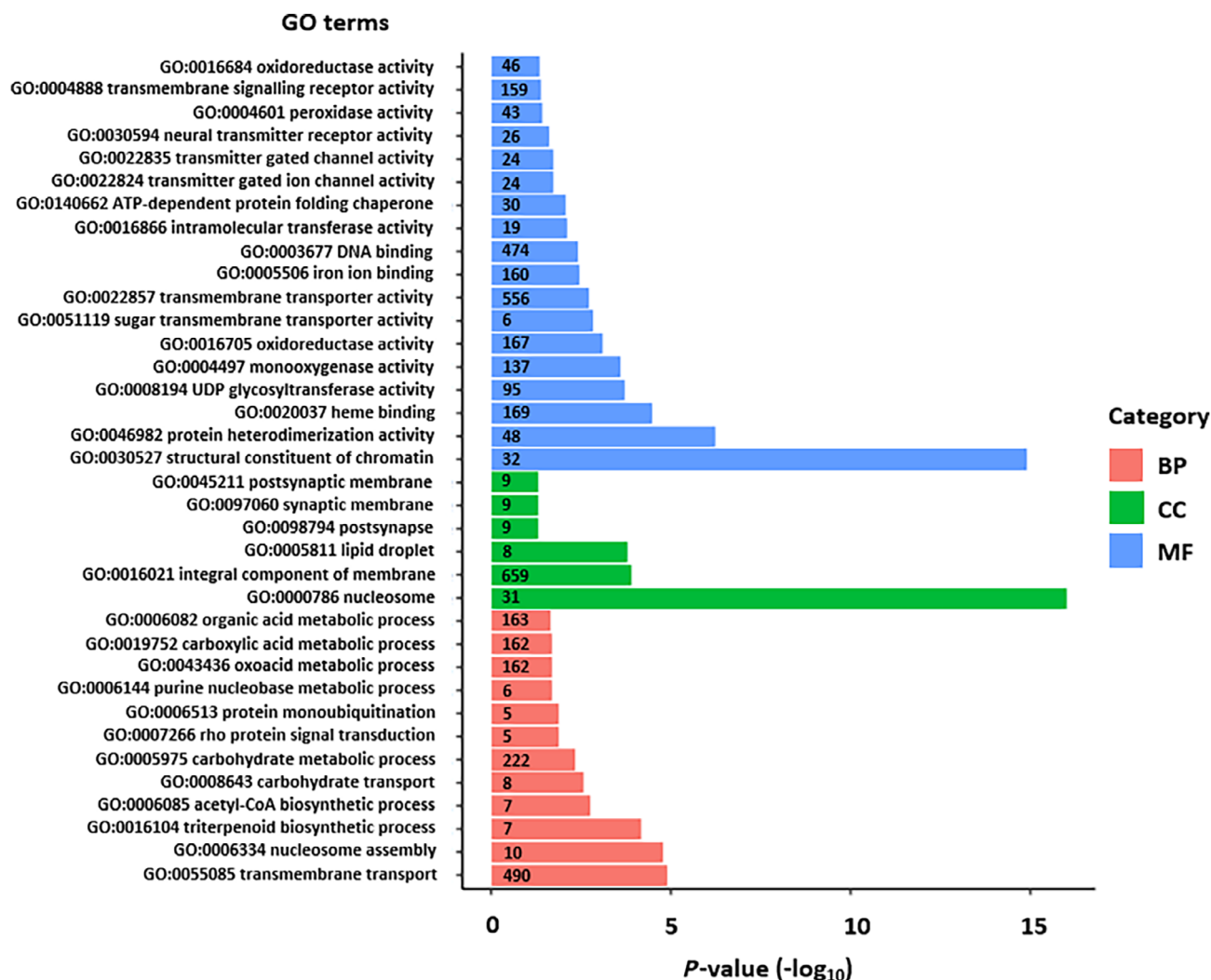


Figure 2. Functional analysis of differentially expressed genes in GR15-1 (resistant) relative to SU07-1 (susceptible). The horizontal axis shows the significance of GO term enrichment expressed as P -value ($-\log_{10}$), with higher values indicating stronger enrichment. The GO terms under the categories of biological process (BP), cellular component (CC) and molecular function (MF) are listed on the vertical axis. The number of annotated genes for each GO term is provided for each column, e.g., 490 genes for the GO term 'transmembrane transport'.

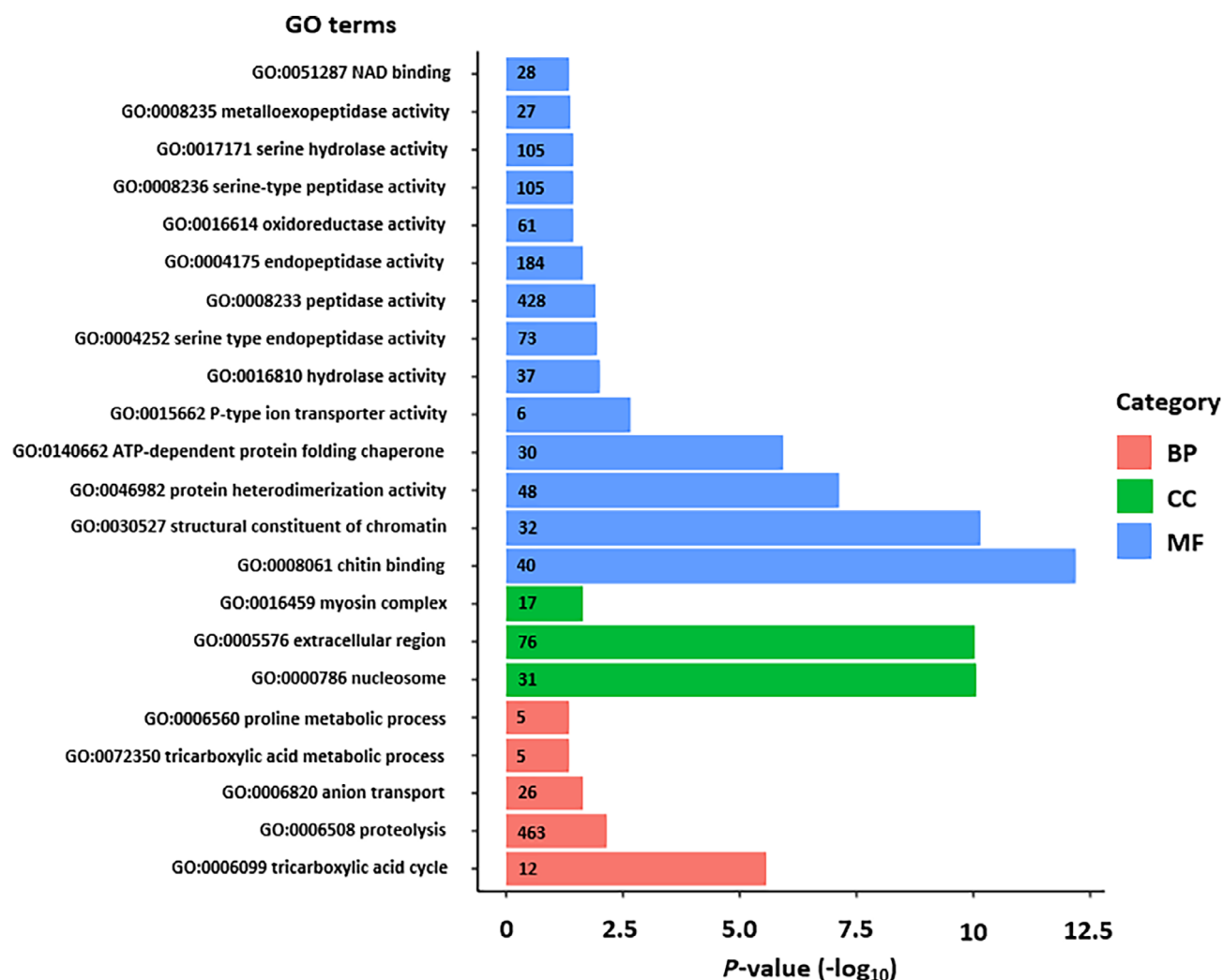


Figure 3. Functional analysis of differentially expressed genes in GR15-1R survivors after clothianidin exposure relative to GR15-1R without exposure. The horizontal axis shows the significance of GO term enrichment expressed as $-\log_{10}$ (P -value), with higher values indicating stronger enrichment. The GO terms under the categories of biological process (BP), cellular component (CC) and molecular function (MF) are listed on the vertical axis. The number of annotated genes for each GO term is provided for each column, e.g., 28 genes for the GO term 'NAD binding'.

to SU07-1. A one-way ANOVA performed using ΔC_t values from the field populations revealed significant differences in *CYP6CM1* expression among populations ($F_{9,20} = 6.683$, $P < 0.001$), indicating substantial inter-population variation in baseline *CYP6CM1* expression levels (Fig. 5).

Following exposure to clothianidin, *CYP6CM1* expression was higher in the field population compared to the susceptible SU07-1, with increases of 13.7–23.4-fold. However, when we compared the expression between clothianidin survivors and the corresponding untreated populations, t -tests revealed no significant differences ($P > 0.05$) in *CYP6CM1* expression for seven populations (Warren, Chinchilla, Moree, Emerald, Goondiwindi, St George and Mungindi); significant differences were only detected in whiteflies from Theodore, Burren Junction and Lockyer Valley (Table 8).

4 DISCUSSION

Field evolved neonicotinoid resistance in the invasive *B. tabaci* MEAM1 has recently become widespread throughout the cotton

growing regions of Eastern Australia. Cotton growers extensively rely on neonicotinoids such as thiamethoxam and, to lesser extent, imidacloprid, as seed treatments. Neonicotinoids are also used as foliar sprays against several pest bugs (aphids, mirids, shield bugs and whiteflies). Amongst these, clothianidin and acetamiprid are commonly used to control shield bugs and whiteflies, respectively. We think that resistance in *B. tabaci* MEAM1 has likely arisen from exposure to neonicotinoids through the annual, geographically widespread use of seed treatments and through inadvertent exposure to clothianidin as part of mid-late season shield bug foliar sprays. Despite the use of acetamiprid and dinotefuran to control *B. tabaci* MEAM1 since 2017, no evidence of resistance was detected in our sampled populations. This may simply reflect the limited use of these neonicotinoids by cotton growers in favor of other insecticides like pyriproxyfen and spirotetramat.¹³ Alternatively, resistance has not evolved, or if present, exists at very low frequencies and has therefore not yet been detected.

In both foliar and systemic uptake bioassays, the GR15-1R strain demonstrated partial cross-resistance to neonicotinoids, with high resistance observed to both imidacloprid and clothianidin,

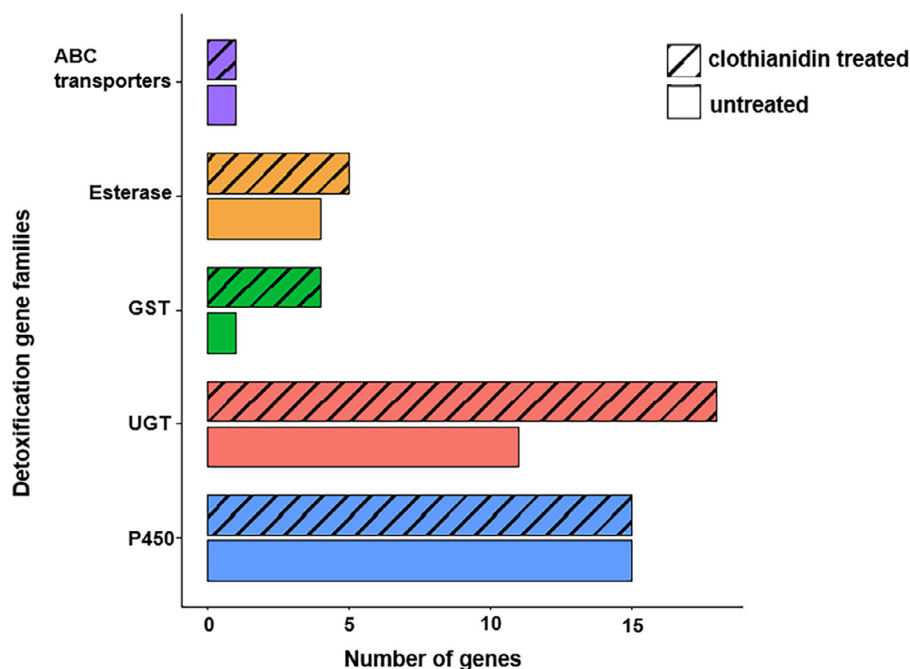


Figure 4. Differentially expressed detoxification gene families in untreated and clothianidin-treated *B. tabaci* MEAM1 populations. Genes were classified into five major detoxification gene families associated with insecticide resistance, including cytochrome P450 monooxygenases (P450s), UDP-glucuronosyltransferases (UGTs), glutathione S-transferases (GSTs), esterases, and ATP-binding cassette (ABC) transporters. Differentially expressed genes were identified using the criteria $|\log_2\text{FoldChange}| > 1$ and adjusted P -value (padj) < 0.05 . Bars represent the number of differentially expressed genes within each detoxification gene family. Solid bars indicate untreated populations, whereas striped bars indicate clothianidin-treated populations.

Table 7. Differentially expressed cytochrome P450 genes in GR15-1R pre and post treatment with clothianidin

Gene name	Gene ID	Expression (fold change) [†]	
		Pre exposure	Post exposure
<i>CYP6CM1</i>	LOC109039286	90.5	84.5
<i>CYP6EM1</i>	LOC109035029	5.3	9.8
<i>CYP6CX4</i>	LOC109029764	2.8	4
<i>CYP6CX2</i>	LOC109041191	2.6	5.3
<i>CYP6DW4</i>	LOC109030486	2.3	3

[†] Fold change in GR15-1R relative to SU07-1.

in line with previous studies,^{58,59} while we found no evidence of cross-resistance to dinotefuran, which again is in line with earlier findings.²² Despite our observation of resistance to thiacloprid, thiamethoxam and acetamiprid in the foliar assay, resistance was less evident in the systemic assay. Improved efficacy of neonicotinoids when applied systemically has been noted in other studies and is likely due to ingestion rather than contact exposure.^{32,33} We observed an approximate threefold improvement in efficacy in the susceptible strain when exposed to imidacloprid in a systemic assay (data not shown). Additionally, it is possible that observed differences between the two assays indicate reduced insecticide penetration due to changes in the insect cuticle,⁶⁰ but more experiments are required before this can be claimed.

Similar cross-resistance trends have been seen in whitefly populations globally. For example, in California, an imidacloprid-resistant *B. tabaci* MEAM1 strain did not exhibit cross-resistance to acetamiprid, dinotefuran or thiamethoxam in a systemic assay,

while the contrary was found in a strain from Guatemala that displayed moderate cross-resistance to acetamiprid and thiamethoxam.⁶¹ Our foliar assay results align with findings from a foliar assay that was used to test *B. tabaci* MEAM1 populations in Mexico and Guatemala. The data showing that high resistance to imidacloprid and thiamethoxam can occur with lower resistance to acetamiprid (RRs = 15–30).⁶²

The underlying mechanism responsible for a partial cross-resistance to neonicotinoids is not known, but there does not appear to be a clear linkage to neonicotinoid structural configurations. Imidacloprid, thiamethoxam, clothianidin and dinotefuran all belong to N-nitroguanidines⁶³ yet in GR15-1R, dinotefuran did not share cross-resistance with imidacloprid, thiamethoxam and clothianidin. Similarly, both acetamiprid and thiacloprid belong to N-cyanoamidines,⁶³ although, according to our foliar results, they had markedly different levels of resistance.

Previous studies demonstrated a correlation between *CYP6CM1* expression and resistance to neonicotinoids, particularly clothianidin

and imidacloprid.^{24–26} In the present study, *CYP6CM1* was constitutively overexpressed in the resistant strain GR15-1R relative to the susceptible strain SU07-1, with no evidence of further induction following clothianidin exposure. The functional role of *CYP6CM1* in neonicotinoid resistance has previously been demonstrated using complementary experimental approaches. Heterologous expression of *CYP6CM1* in transgenic *Drosophila melanogaster* conferred resistance to neonicotinoid insecticides.⁶⁴ Recombinant-enzyme studies have also demonstrated that *CYP6CM1* can metabolize imidacloprid and selected other neonicotinoids, while elevated *CYP6CM1* transcript and protein levels have been associated with imidacloprid resistance in laboratory and field-derived *B. tabaci* populations.^{24,65} Therefore, although the expression analyses conducted in the present study are correlative, they are supported by an established body of functional studies linking *CYP6CM1* to neonicotinoid resistance.

Our RT-qPCR data shows that *CYP6CM1* is over-expressed in all 10 field populations, and we found that the expression aligns with our bioassay results of moderate to high resistance to clothianidin

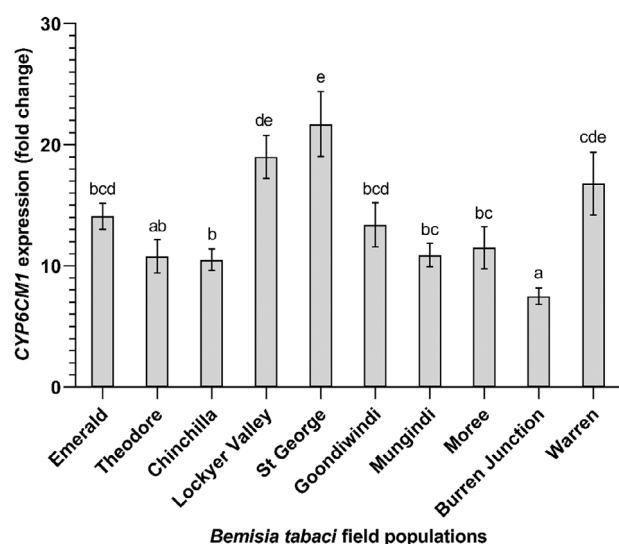


Figure 5. Expression $\pm$ SE of *CYP6CM1* in *Bemisia tabaci* MEAM1 populations relative to susceptible reference population ($2^{-\Delta\Delta C_t}$). Data (ΔC_t values) analyzed by one-way ANOVA, different letters above columns indicate significantly different expression between populations, Fisher's LSD test ($P > 0.05$).

and imidacloprid in each of these populations. This aligns with an earlier study that linked the expression of *CYP6CM1* with imidacloprid resistance.²⁴ However, our bioassay results showed no conclusive evidence of field resistance to either dinotefuran or acetamiprid, which is supported by evidence that *CYP6CM1* does not metabolize either compound.^{22,58} Similar to GR15-1R, exposure testing of field populations with clothianidin was associated with the constitutive overexpression of *CYP6CM1*, but in three of our populations there was evidence of an induced response, that has been observed in other insects to insecticides⁶⁶ and host plant chemicals.⁶⁷ Therefore, an induced response cannot be completely ruled out and the issue requires further investigation.

Beyond *CYP6CM1*, transcriptome analysis revealed differential expression across multiple detoxification gene families, including UGT and esterase genes. Both gene families have been shown to detoxify neonicotinoids;^{68,69} however, their specific contribution to neonicotinoid resistance in *B. tabaci* MEAM1 remains unclear. Additional cytochrome P450 genes, such as *CYP6EM1*, *CYP6CX2*, *CYP6CX4* and *CYP6DW4*, were also differentially expressed in GR15-1R, although expression was substantially lower than that of *CYP6CM1*. The *CYP6EM1* gene has been functionally linked to dinotefuran resistance in *B. tabaci*;²³ however, GR15-1R did not exhibit strong resistance to dinotefuran, and the present data do not provide conclusive evidence that *CYP6EM1* makes a major contribution to the observed resistance in this strain. Both *CYP6CX2* and *CYP6CX4* have been found to be overexpressed in *B. tabaci* MEAM1 selected for acetamiprid⁷⁰ and thiamethoxam⁷¹ resistance. Low resistance to acetamiprid was observed in the foliar assay of GR15-1R, which could be linked to overexpression of *CYP6CX2* and *CYP6CX4*, but this is not as conclusive as the link between neonicotinoid resistance and *CYP6CM1* expression. Functional studies are required to determine the potential role of *CYP6CX2* and *CYP6CX4* expression in acetamiprid resistance.

An overexpression of *CYP6CM1* has been linked to MAPK-mediated CREB activation in *B. tabaci* MED³⁰; however, the CREB orthologue LOC109029782 was not differentially expressed in GR15-1R, suggesting that alternative cis- and/or trans-acting regulatory mechanisms may underlie *CYP6CM1* upregulation in MEAM1. In addition, several amino acid substitutions previously associated with neonicotinoid resistance, including A58T,²¹ R79E²¹ and A387G²⁶ were absent in GR15-1R. This suggests that the resistance phenotype observed in Australian *B. tabaci* MEAM1 is unlikely to be explained by known changes in the receptor gene. Instead, our observations suggest that the resistance is

Table 8. Comparison of *CYP6CM1* expression in *Bemisia tabaci* MEAM1 before and after exposure to clothianidin

Population	$\Delta C_t \pm SD$		<i>P</i> value	Relative expression $2^{-\Delta\Delta C_t}$	
	Untreated	Treated		Untreated	Treated
Emerald	1.95 $\pm$ 0.05	2.08 $\pm$ 0.12	NS	14.1	20.4
Theodore	1.74 $\pm$ 0.32	2.55 $\pm$ 0.07	0.012	10.8	18.7
Chinchilla	2.20 $\pm$ 0.05	2.51 $\pm$ 0.25	NS	10.5	17.1
Lockyer Valley	1.87 $\pm$ 0.05	1.66 $\pm$ 0.12	0.043	19.0	21.5
St George	1.91 $\pm$ 0.47	1.47 $\pm$ 0.22	NS	21.7	21.0
Goondiwindi	2.47 $\pm$ 0.17	2.17 $\pm$ 0.25	NS	13.4	14.2
Mungindi	1.81 $\pm$ 0.40	2.45 $\pm$ 0.21	NS	10.9	22.4
Moree	1.75 $\pm$ 0.48	2.40 $\pm$ 0.06	NS	11.5	23.4
Burren Junction	2.17 $\pm$ 0.24	3.06 $\pm$ 0.02	0.023	7.5	13.7
Warren	1.97 $\pm$ 0.42	1.93 $\pm$ 0.06	NS	16.8	15.9

caused by changes in gene expression. Future work should investigate the regulatory basis of the *CYP6CM1* overexpression in *B. tabaci* MEAM1 (to determine whether changes to the promoter sequence, gene copy number or cis/trans-regulatory mechanisms are responsible for the observed resistance in GR15-1R).

In our study, *CYP6CM1* showed the strongest association with neonicotinoid resistance in both laboratory and field populations. Nevertheless, the differential expression of other P450 genes suggests that multiple resistance mechanisms may exist in *B. tabaci* MEAM1. Thus, measuring *CYP6CM1* expression alone may not capture the complexity of resistance mechanisms operating in field populations. However, *CYP6CM1* still appears to be a useful molecular marker for resistance to imidacloprid, clothianidin, thiacloprid and thiamethoxam. Incorporating additional detoxification genes may improve future resistance monitoring and provide a more comprehensive understanding of detoxification pathways in *B. tabaci* MEAM1, but it is likely bioassays will remain an effective tool for detecting novel cases of resistance.

Determining resistance in the field offers practical advantages, e.g., a lateral flow test was developed to rapidly detect *B. tabaci* metabolic resistance mediated by *CYP6CM1*,⁷² but this tool has yet to be commercialized. Comparing our results with the populations used to validate the lateral flow test, we would predict that, at a minimum, the test would have detected resistance in whiteflies from the Mungindi, St George and Goondiwindi populations. Thus, a test such as this could enable agronomists to avoid applying insecticides where there is a high likelihood of field failure. In our study, we quantified *CYP6CM1* transcript levels using RT-qPCR in *B. tabaci* MEAM1 and demonstrated that, when combined with bioassays, this approach provides a robust indicator of neonicotinoid resistance. While RT-qPCR is less convenient and requires more labor power and laboratory resources compared to a lateral flow test, it provides a readily available, reliable, rapid method for screening imidacloprid, clothianidin, thiacloprid and thiamethoxam resistance linked to *CYP6CM1* expression. While not easily deployable in the field, this type of analysis is valuable for formulating industry wide resistance management strategies and IPM extension messages.

From a cotton industry perspective, the detection of partial cross-resistance will have minimal direct impact on the current use of neonicotinoids to control *B. tabaci* as neither acetamiprid nor dinotefuran are efficiently detoxified by *CYP6CM1*. However, from an IPM perspective, the mid-to-late season use of neonicotinoids like clothianidin for shield bug control is of greater concern. Clothianidin applications may disrupt natural enemy populations, while resistance enables *B. tabaci* populations to increase unimpeded. Under such situations, due to the risk of honeydew contamination, additional insecticide applications to manage late season *B. tabaci* populations may be required. Future IPM research should focus on cotton industry extension regarding the use of clothianidin along with developing new management options for pest shield bugs.

5 CONCLUSION

Our results demonstrate that the P450 *CYP6CM1* gene is constitutively over-expressed in the resistant *B. tabaci* MEAM1 strain GR15-1R and in 10 Australian field populations collected in 2023. Bioassay results revealed varying levels of resistance to the neonicotinoids clothianidin and imidacloprid in these field populations, while resistance to acetamiprid and dinotefuran was not observed. The combination of molecular techniques with

bioassay data confirmed the presence of neonicotinoid resistance in *B. tabaci* MEAM1 populations in several Australian cotton-growing regions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

- 1 Dinsdale A, Cook L, Riginos C, Buckley YM and De Barro P, Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodidae: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. *Ann Entomol Soc Am* **103**:196–208 (2010).
- 2 Elfekih S, Etter P, Tay WT, Fumagalli M, Gordon K, Johnson E *et al.*, Genome-wide analyses of the *Bemisia tabaci* species complex reveal contrasting patterns of admixture and complex demographic histories. *PLoS One* **13**:1–18 (2018).
- 3 Vyskočilová S, Tay WT, Van Brunschot S, Seal S and Colvin J, An integrative approach to discovering cryptic species within the *Bemisia tabaci* whitefly species complex. *Sci Rep* **8**:1–14 (2018).
- 4 De Barro PJ and Coombs MT, Post-release evaluation of *Eretmocerus hayati* Zolnerowich and rose in Australia. *Bull Entomol Res* **99**:193–206 (2009).
- 5 De Barro PJ and Driver F, Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Aust J Entomol* **36**:149–152 (1997).
- 6 Sequeira RV and Naranjo SE, Sampling and management of *Bemisia tabaci* (Genn.) biotype B in Australian cotton. *Crop Prot* **27**:1262–1268 (2008).
- 7 Byrne DN and Bellows TS, Whitefly biology. *Annu Rev Entomol* **36**:431–457 (1991).
- 8 Ellsworth PC, Tronstad R, Leser J, Goodell PB, Godfrey LD *et al.*, *Sticky Cotton Sources and Solutions. IPM Series 13*. Cooperative Extension, University of Arizona, Tucson, AZ (1999).
- 9 Horowitz AR, Ghanim M, Roditakis E, Nauen R and Ishaaya I, Insecticide resistance and its management in *Bemisia tabaci* species. *J Pest Sci* **93**:893–910 (2020).
- 10 Fang C, Hopkinson JE, Balzer J, Frese M, Tay WT and Walsh T, Screening for insecticide resistance in Australian field populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) using bioassays and DNA sequencing. *Pest Manag Sci* **78**:3248–3259 (2022).

- 11 Hopkinson J, Pumpa S, van Brunschot S, Fang C, Frese M, Tay WT *et al.*, Insecticide resistance status of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australian cotton production valleys. *Austral Entomol* **59**:202–214 (2020).
- 12 Lueke B, Douris V, Hopkinson JE, Maiwald F, Hertlein G, Papapostolou KM *et al.*, Identification and functional characterization of a novel acetyl-CoA carboxylase mutation associated with ketoenol resistance in *Bemisia tabaci*. *Pestic Biochem Physiol* **166**: 104583 (2020).
- 13 Hopkinson J, Balzer J, Fang C and Walsh T, Insecticide resistance management of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australian cotton – pyriproxyfen, spirotetramat and buprofezin. *Pest Manag Sci* **79**:1829–1839 (2023).
- 14 Umina PA, McDonald G, Maino J, Edwards O and Hoffmann AA, Escalating insecticide resistance in Australian grain pests: contributing factors, industry trends and management opportunities. *Pest Manag Sci* **75**:1494–1506 (2019).
- 15 Lu W, Liu Z, Fan X, Zhang X, Qiao X and Huang J, Nicotinic acetylcholine receptor modulator insecticides act on diverse receptor subtypes with distinct subunit compositions. *PLoS Genet* **18**:e1009920 (2022).
- 16 Liu MY and Casida JE, High affinity binding of [3H]imidacloprid in the insect acetylcholine receptor. *Pestic Biochem Physiol* **46**:40–46 (1993).
- 17 Jeschke P, Nauen R, Schindler M and Elbert A, Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* **59**: 2897–2908 (2011).
- 18 Casida JE, Neonicotinoids and other insect nicotinic receptor competitive modulators: progress and prospects. *Annu Rev Entomol* **63**:125–144 (2018).
- 19 Nauen R and Denholm I, Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Arch Insect Biochem Physiol* **58**:200–215 (2005).
- 20 Elbert A, Haas M, Springer B, Thielert W and Nauen R, Applied aspects of neonicotinoid uses in crop protection. *Pest Manag Sci* **64**:1099–1105 (2008).
- 21 Yin C, O'Reilly AO, Liu S-N, Du T-H, Gong P-P, Zhang C-J *et al.*, Dual mutations in the whitefly nicotinic acetylcholine receptor $\beta 1$ subunit confer target-site resistance to multiple neonicotinoid insecticides. *PLoS Genet* **20**:e1011163 (2024).
- 22 Hamada A, Wahl GD, Nesterov A, Nakao T, Kawashima M and Banba S, Differential metabolism of imidacloprid and dinotefuran by *Bemisia tabaci* CYP6CM1 variants. *Pestic Biochem Physiol* **159**:27–33 (2019).
- 23 Huang M, Fu B, Yin C, Gong P, Liu S, Yang J *et al.*, Cytochrome P450 CYP6EM1 underpins dinotefuran resistance in the whitefly *Bemisia tabaci*. *J Agric Food Chem* **72**:5153–5164 (2024).
- 24 Karunker I, Benting J, Lueke B, Ponge T, Nauen R, Roditakis E *et al.*, Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Insect Biochem Mol Biol* **38**:634–644 (2008).
- 25 Nauen R, Vontas J, Kaussmann M and Wölfel K, Pymetrozine is hydroxylated by CYP6CM1, a cytochrome P450 conferring neonicotinoid resistance in *Bemisia tabaci*. *Pest Manag Sci* **69**:457–461 (2013).
- 26 Pym A, Mina JGM, Troczka BJ, Hayward A, Daum E, Elias J *et al.*, A single point mutation in the *Bemisia tabaci* cytochrome-P450 CYP6CM1 causes enhanced resistance to neonicotinoids. *Insect Biochem Mol Biol* **156**:103934 (2023).
- 27 Bass C, Carvalho RA, Oliphant L, Puinean AM, Field LM, Nauen R *et al.*, Overexpression of a cytochrome P450 monooxygenase, CYP6ERT, is associated with resistance to imidacloprid in the brown planthopper, Nilaparvata lugens. *Insect Mol Biol* **20**:763–773 (2011).
- 28 Puinean AM, Foster SP, Oliphant L, Denholm I, Field LM, Millar NS *et al.*, Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *PLoS Genet* **6**:e1000999 (2010).
- 29 Ilias A, Lagnel J, Kapantaidaki DE, Roditakis E, Tsiengenopoulos CS, Vontas J *et al.*, Transcription analysis of neonicotinoid resistance in Mediterranean (MED) populations of *B. Tabaci* reveal novel cytochrome P450s, but no nAChR mutations associated with the phenotype. *BMC Genomics* **16**:939 (2015).
- 30 Yang X, Deng S, Wei X, Yang J, Zhao Q, Yin C *et al.*, MAPK-directed activation of the whitefly transcription factor CREB leads to P450-mediated imidacloprid resistance. *Proc Natl Acad Sci USA* **117**:10246–10253 (2020).
- 31 Cahill M, Gorman K, Day S, Denholm I, Elbert A and Nauen R, Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull Entomol Res* **86**:343–349 (1996).
- 32 Castle SJ and Prabhaker N, Monitoring changes in *Bemisia tabaci* (Hemiptera: Aleyrodidae) susceptibility to neonicotinoid insecticides in Arizona and California. *J Econ Entomol* **106**:1404–1413 (2013).
- 33 Castle SJ, Merten P and Prabhaker N, Comparative susceptibility of *Bemisia tabaci* to imidacloprid in field- and laboratory-based bioassays. *Pest Manag Sci* **70**:1538–1546 (2014).
- 34 Hopkinson JE and Pumpa SM, Baseline susceptibility of *Bemisia tabaci* MEAM1 (Hemiptera: Aleyrodidae) in Australia to spirotetramat, cyantraniliprole and dinotefuran, with reference to pyriproxyfen cross-resistance. *Austral Entomol* **58**:762–771 (2019).
- 35 Abbott WS, A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
- 36 International VSN, *Genstat for Windows 21st Edition*. VSN International, Hemel Hempstead, UK (2021).
- 37 Finney DJ, *Probit Analysis*, 3rd edn. Cambridge University Press, Cambridge, UK (1971).
- 38 Herron G, Langfield B, Bogema D and Chen Y, Baseline susceptibility and cross-resistance in *Aphis gossypii* glover (Aphididae: Hemiptera) to phorate and sulfoxaflor. *Aust J Entomol* **53**:32–55 (2014).
- 39 Robertson JL, Jones MM, Olguin E and Alberts B, *Bioassays with Arthropods*, 3rd edn. CRC Press, Taylor and Francis Group, Boca Raton, FL (2017).
- 40 Torres-Vila LM, Rodríguez-Molina MC, Lacasa-Plasencia A, Bielza-Lino P and Rodríguez-del-Rincón Á, Pyrethroid resistance of *Helicoverpa armigera* in Spain: current status and agroecological perspective. *Agric Ecosyst Environ* **93**:55–66 (2002).
- 41 Tay WT, Court LN, Macfadyen S, Jacomb F, Vyskočilová S, Colvin J *et al.*, A high-throughput amplicon sequencing approach for population-wide species diversity and composition survey. *Mol Ecol Resour* **5**: 1706–1724 (2022).
- 42 Kunz D, Tay WT, Elfekih S, Gordon KHJ and De Barro PJ, Take out the rubbish – removing NUMTs and pseudogenes from the *Bemisia tabaci* cryptic species mtCOI database. *bioRxiv*:1–19 (2019). <https://www.biorxiv.org/content/10.1101/724765v1>.
- 43 Love M, Huber W and Anders S, Beginner's guide to using the DESeq2 package. Available: <https://bioconductor.statistik.tu-dortmund.de/packages/2.14/bioc/vignettes/DESeq2/inst/doc/beginner.pdf> (2014).
- 44 Pertea M, Kim D, Pertea GM, Leek JT and Salzberg SL, Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* **11**:1650–1667 (2016).
- 45 Chen S, Zhou Y, Chen Y and Gu J, Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**:i884–i890 (2018).
- 46 Chen W, Hasegawa DK, Kaur N, Klot A, Pinheiro PV, Luan J *et al.*, The draft genome of whitefly *Bemisia tabaci* MEAM1, a global crop pest, provides novel insights into virus transmission, host adaptation, and insecticide resistance. *BMC Biol* **14**:110 (2016).
- 47 Kim D, Paggi JM, Park C, Bennett C and Salzberg SL, Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol* **37**:907–915 (2019).
- 48 Liao Y, Smyth GK and Shi W, FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**:923–930 (2014).
- 49 Love MI, Huber W and Anders S, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:1–21 (2014).
- 50 Alexa A and Rahnenführer J, Gene topGO: Enrichment Analysis for Gene Ontology enrichment analysis with topGO. R package version 2.48.0, Bioconductor Improvements Available: <https://www.bioconductor.org/packages/2.7/bioc/vignettes/topGO/inst/doc/topGO.pdf> (2022).
- 51 Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M *et al.*, The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* **55**:611–622 (2009).
- 52 Liang P, Guo Y, Zhou X and Gao X, Expression profiling in *Bemisia tabaci* under insecticide treatment: indicating the necessity for custom reference gene selection. *PLoS One* **9**:e87514 (2014).
- 53 Livak KJ and Schmittgen TD, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25**:402–408 (2001).
- 54 Du T, Yin C, Gui L, Liang J, Liu S, Fu B *et al.*, Over-expression of UDP-glycosyltransferase UGT353G2 confers resistance to neonicotinoids in whitefly (*Bemisia tabaci*). *Pestic Biochem Physiol* **196**:105635 (2023).

- 55 Zhang R, Yang J, Hu J, Yang F, Liang J, Xue H *et al.*, Glutathione S-transferase directly metabolizes imidacloprid in the whitefly, *Bemisia tabaci*. *Pestic Biochem Physiol* **201**:105863 (2024).
- 56 Byrne FJ, Gorman KJ, Cahill M, Denholm I and Devonshire AL, The role of B-type esterases in conferring insecticide resistance in the tobacco whitefly, *Bemisia tabaci* (Genn). *Pest Manag Sci* **56**:867–874 (2000).
- 57 Wu C, Chakrabarty S, Jin M, Liu K and Xiao Y, Insect ATP-binding cassette (ABC) transporters: roles in xenobiotic detoxification and Bt insecticidal activity. *Int J Mol Sci* **20**:2829 (2019).
- 58 Roditakis E, Morou E, Tsagkarakou A, Riga M, Nauen R, Paine M *et al.*, Assessment of the *Bemisia tabaci* CYP6CM1vQ transcript and protein levels in laboratory and field-derived imidacloprid-resistant insects and cross-metabolism potential of the recombinant enzyme. *Insect Sci* **18**:23–29 (2011).
- 59 Karunker I, Morou E, Nikou D, Nauen R, Sertchook R, Stevenson BJ *et al.*, Structural model and functional characterization of the *Bemisia tabaci* CYP6CM1vQ, a cytochrome P450 associated with high levels of imidacloprid resistance. *Insect Biochem Mol Biol* **39**:697–706 (2009).
- 60 Balabanidou V, Grigoraki L and Vontas J, Insect cuticle: a critical determinant of insecticide resistance. *Curr Opin Insect Sci* **27**:68–74 (2018).
- 61 Prabhaker N, Castle S, Henneberry TJ and Toscano NC, Assessment of cross-resistance potential to neonicotinoid insecticides in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Bull Entomol Res* **95**:535–543 (2005).
- 62 Gorman K, Slater R, Blande JD, Clarke A, Wren J, McCaffery A *et al.*, Cross-resistance relationships between neonicotinoids and pymetrozine in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag Sci* **66**:1186–1190 (2010).
- 63 Jeschke P, Nauen R, Gutbrod O, Beck ME, Matthiesen S, Haas M *et al.*, Flupyradifurone (Sivanto™) and its novel butenolide pharmacophore: structural considerations. *Pestic Biochem Physiol* **121**:31–38 (2015).
- 64 Daborn PJ, Lumb C, Harrop TWR, Blasetti A, Pasricha S, Morin S *et al.*, Using *Drosophila melanogaster* to validate metabolism-based insecticide resistance from insect pests. *Insect Biochem Mol Biol* **42**:918–924 (2012).
- 65 Roditakis E, Tsagkarakou A and Vontas J, Identification of mutations in the para sodium channel of *Bemisia tabaci* from Crete, associated with resistance to pyrethroids. *Pestic Biochem Physiol* **85**:161–166 (2006).
- 66 Liu N, Li T, Reid WR, Yang T and Zhang L, Multiple cytochrome P450 genes: their constitutive overexpression and permethrin induction in insecticide resistant mosquitoes, *Culex quinquefasciatus*. *PLoS One* **6**:e23403 (2011).
- 67 Feyereisen R, Insect P450 enzymes. *Annu Rev Entomol* **44**:507–533 (1999).
- 68 Khan MM, Kaleem-Ullah RM, Siddiqui JA and Ali S, Insecticide resistance and detoxification enzymes activity in *Nilaparvata lugens* Stål against neonicotinoids. *J Agric Sci* **12**:24–36 (2020).
- 69 Huang J, Huang Z, Wu J, Ge F, Li F, Zhou W *et al.*, Uridine diphosphate-dependent glycosyltransferase (UGT)-mediated metabolic transformation mechanism of neonicotinoid insecticides in rice. *Environ Chem Ecotoxicol* **7**:2262–2275 (2025).
- 70 Zhou C, Cao Q, Li G and Ma D, Role of several cytochrome P450s in the resistance and cross-resistance against imidacloprid and acetamiprid of *Bemisia tabaci* (Hemiptera: Aleyrodidae) MEAM1 cryptic species in Xinjiang, China. *Pestic Biochem Physiol* **163**:209–215 (2020).
- 71 Yang J, Fu B, Gong P, Zhang C, Wei X, Yin C *et al.*, CYP6CX2 and CYP6CX3 mediate thiamethoxam resistance in field whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). *J Econ Entomol* **116**:1342–1351 (2023).
- 72 Nauen R, Wölfel K, Lueke B, Myridakis A, Tsakireli D, Roditakis E *et al.*, Development of a lateral flow test to detect metabolic resistance in *Bemisia tabaci* mediated by CYP6CM1, a cytochrome P450 with broad spectrum catalytic efficiency. *Pestic Biochem Physiol* **121**:3–11 (2015).