FINAL REPORT

For Public Release

Part 1 - Summary Details

CRDC ID: DAN1507

Project Title: The sustainable chemical control and resistance management of aphids, mites and mirids in Australian cotton 2014-2019

Project Start Date: 01 July 2014 **Project Completion Date:** 30 June 2019

Research Program: 1 Farmers

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Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report)

Background

1. Outline the background to the project.

Since the introduction of *Bt*-cotton secondary pests have become more prominent. Mirids have received targeted sprays since the 2003-2004 season and research continues to establish a resistance monitoring capability. Mites have a long history in Australian cotton of developing resistance that has led to control failures. Two-spotted mite has been the dominant mite species in cotton for three decades; however, mites in cotton appear to be changing.

The reasons for this are complex but likely relate to IPM being widely practiced with potential mite problems being controlled by beneficials. Additionally, the dominance of *Bt*-cotton and its consequent reduction in sprays has seen banana or strawberry mite becoming much more abundant giving anecdotal support to a change in the cotton-mite-complex. The implications of any change in the cotton-mite-complex are unknown and require quantification.

Aphids have been very problematic over the last two decades with control failures against the OPs and carbamates. More recently, control issues with cotton aphid and the newer neonicotinoids occurred. These failures can cause 'sticky cotton', a situation with a huge potential to damage Australian cotton's good reputation for high quality clean lint. Nonetheless neonicotinoid resistance remains a serious concern. The neonicotinoid insecticides are crucial to the Australian cotton industry and any compromise in efficacy due to resistance limits effective control options.

Recently there has been anecdotal evidence that cotton seedling thrips may not be being controlled as well as it once was by neonicotinoid seed dressings. In crops other than cotton, cotton seedling thrips is known neonicotinoid resistant so resistance is possible. Western flower thrips have a long history of developing resistance and their control in cotton is based singularly on spinetoram (Success® Neo) which has recently been associated with failures in stone fruit.

Control of green mirid requires targeted spraying and resistance is a possibility. Bioassay of mirids *per se* is not difficult but their fragile bodies and recalcitrant ability to establish into laboratory culture makes resistance confirmation problematic. A low-input rearing procedure has been recently developed making bioassay possible however currently we use a DNA based method to screen only for fipronil (Maestro® or Albatross®) resistance with other mechanisms unknown.

An increase in DNA based diagnostics is desirable but reference genomes are unavailable or fragmented. Additionally species and/or chemical specific resistance mechanism information is not centralised but spread widely within the scientific literature. This slows the development of DNA based diagnostics that would benefit from such centralization. The cotton industry would benefit from a planned transition from bioassay to essentially DNA based resistance detection technology.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

2.1 Objective: Resistance monitoring in two-spotted mite and cotton aphid against strategic chemicals. Milestone: Two-spotted mite and cotton aphid collected and established into culture. Performance indicator: Resistance monitoring data for two-spotted mite and cotton aphid generated.

> Objective, milestone and performance indicators met in full. Two-spotted mite and cotton aphid were collected annually from season 2014-2015 to season 2018-2019 and tested for resistance against chemicals used for their control (see Appendix A for specific seasonal testing details).

2.2 Objective: Appointment of Professional (Scientific) Officer. Milestone: Paper work completed and submitted to create and fill a Professional (Scientific) Officer position. Performance indicator: Professional (Scientific) Officer commences duty.

> Objective, milestone and performance indicators met in full. Paperwork and role description for a new Professional (Scientific) Officer position was established by the 30th December 2014 and recruitment finalised on 1st July 2015 with the appointment Kate L. Marshall.

2.3 Objective: New field based method to detect resistance in green mirid.

> 2.3.1 Milestone: Bioassay method identified. Performance indicator: Method finalised i.e. vial, tube or Petri dish and residue stability verification commenced.
>
> Objective, milestone and performance indicators met in full. An adult vial test was adapted by Miss Marshall from that used to detect resistance in *Lygus* spp. found in cotton growing regions in the USA.
>
> 2.3.2 Milestone: Bioassay method used. Performance indicator: Method used in the field and bioassay data generated.
>
> Objective, milestone and performance indicators met in full. Mirids were collected off lucerne using a sweep net. Encouragingly results for the vial test procedure were statistically equivalent to those obtained from the laboratory based Potter spray tower.

2.4 Objective: Strawberry and bean spider mite collected

2.4.1 Milestone: Mite collection data to determine relative species abundance. Performance indicator: Mite species abundance determined

Objective, milestone and performance indicators met in full. Mites were collected from cotton during season 2016-2017, 2017-2018 and 2018-2019. Random sampling returned some 75 strain over the three seasons and unexpectedly no bean spider mite was found. Two-spotted mite comprised approximately 25% of the collections and was restricted to NSW only. Banana or strawberry spider mite appeared to be now the dominant mite species in Australian cotton.

2.4.2 Millstone: Mites collected and established into culture. Performance indicator: Culture of strawberry and bean spider mite established.

Objective, milestone and performance indicators met in full. Bean spider mite was not found so couldn't be cultured but all banana or strawberry mite collected were cultured and baseline established.

2.4.3 Milestone: Mites bioassayed. Performance indicator: Populations of both species tested against diafenthiuron, propargite and abamectin.

Objective, milestone and performance indicators met in full. Bean spider mite was not found so couldn't be cultured but all banana or strawberry mite collected was used to establish diafenthiuron, propargite and abamectin baseline. Discriminating doses (DDs) of 0.0007g/L abamectin, 0.03 g/L diafenthiuron and 0.7 g/L propargite were established and used in season 2018-2019.

2.5 Objective: New molecular testing methods developed.

2.5.1 Milestone: Molecular methods to detect resistance in two-spotted mite. Performance indicator: Methods developed to detect organophosphate, pyrethroid and abamectin resistance causing mutations in two-spotted mite.

Objective, milestone and performance indicators met in full. DAN1507 was varied at the November 2014 progress report to include a significantly increased molecular diagnostic component. Initially *Helicoverpa* sp. was studied but subsequently three additional molecular based objectives 7 (thrips), 8 (*Helicoverpa* sp.) and 9 (identification) were included. Mite molecular diagnostics then concentrated on newer IPM chemicals with a DNA based test developed for two-spotted mite and etoxazole resistance which was subsequently and unexpectedly detected.

2.5.2 Milestone: DNA library. Performance indicator: DNA library containing all known target site causing mutations established.

Objective, milestone and performance indicators met in full. A library was established and used consistently through the study e.g. mirids and fipronil resistance, mites and etoxazole resistance, western flower thrips and spinetoram resistance and cotton seedling thrips and neonicotinoid resistance (See Appendix B for details).

2.6 Objective: Extension

2.6.1 Milestone: Dr Herron (or his replacement) attends the 17th 18th and 19th Australian Cotton Conferences. Performance indicator: Conference contribution.

Objective, milestone and performance indicators met in full. Dr Herron attended and contributed to all three conferences.

2.6.2 Milestone: Data made available to industry. Performance indicator: Dr Herron attends TIMS technical with current data then made available to industry via the *Cotton Pest Management Guide*.

Objective, milestone and performance indicators met in full. Dr Herron attended all five TIMS technical meetings where changes to the *Cotton Pest Management Guide* were discussed and implemented.

2.6.3 Milestone: Data made available to individuals. Performance indicator: All those individuals who forwarded strains for testing receive a written report.

Objective, milestone and performance indicators met in full. Anyone who contributed to the resistance testing received a written testing report summary that was additionally posted on the Crop Consultants Australia website site (see Appendix A for individual testing report details).

2.7 Objective: Resistance detection in cotton seedling thrips using both bioassay and molecular techniques

2.7.1 Milestone: Use resistance data base; check for known resistance causing mechanisms and screen current strains. Performance indicator: DNA library containing all known target site causing mutations established and strains screened.

Objective, milestone and performance indicators met in full. We looked for the mutation R81T in cotton seedling thrips known to confer neonicotinoid resistance in green peach aphid and cotton aphid. We sequenced the region of the nAChR $\beta 1$ subunit which harbours the R81T mutation and confirmed that the R81T mutation is absent from cotton seedling thrips.

2.7.2 Milestone: Cotton seedling thrips bioassayed. Performance indicator: Baseline data generation for cotton seedling thrips against strategic chemical controls.

Objective, milestone and performance indicators met in full. Cotton seedling thrips collected from Narrabri and Griffith and tested for dimethoate, λ-cyhalothrin and imidacloprid (as a model for all neonicotinoids) resistance. Against all chemicals resistant individuals were found. Neonicotinoid resistance confirmation corroborated grower concerns that neonicotinoid seed dressings were not working as well as they once did and a resistance warning was included into the 2018-2019 *Cotton Pest Management Guide*.

2.8 Objective: *Helicoverpa* sp. / indoxacarb resistance with the aim of finding the causal SNP and verified molecular diagnostic.

2.8.1 Milestone: Whole genome and transcriptome sequencing to further delineate causal SNP of indoxacarb resistance. Performance indicator: The causal SNP is identified or alternative resistance mechanism narrowed.

Objective, milestone and performance indicators met in full. The study generated more than 90GB of genome sequence for *Helicoverpa* sp. with results supporting the conclusion that the resistant causing gene is to be found on chromosome 16 with the resultant study published in *Pest Management Science* (see publications 18, 24).

2.8.2 Milestone: CRISPR gene editing technology used to verify causal SNP. Performance indicator: Molecular diagnostic developed and verified.

Objective, milestone and performance indicators met in full. A molecular diagnostic was developed and verified against field collected material but unexpectedly a second resistance mechanism not associated with chromosome 16 was found. Yizhou Chen liaised with Prof Wu Yidong at Nanjing Agricultural University regarding CRISPR gene editing technology. Prof Wu Yidong's group further studied the chromosome 16 region identified here using CRISPR confirming its involvement in P450 mediated indoxacarb resistance (see publication 24).

2.9 Objective: Development of a field DNA based kit for spider mite identification

2.9.1 Milestone: *Tetranychus* mite species identified via qPCR assay. Performance indicator: qPCR assay correctly delineates between *Tetranychus* mite species: *urticae*, *ludeni* and *lambi*.

Objective, milestone and performance indicators met in full. We have developed a diagnostic assay to identify *T. urticae*, *T. lambi* and *T. ludeni* in a single real-time PCR assay. The assay design was based on a universal primer pair to amplify the internal transcribed spacer of nuclear ribosomal DNA (ITS1) with three species-specific TaqMan probes. (see publication 28).

2.9.2 Milestone: Development of field based DNA kit for spider mite species identification. Performance indicator: Kit is validated in the field.

Objective, milestone and performance indicators met in full. An SOP was written for the DNA based spider mite identification kit that was passed over to Biosecurity Australia (AQIS) for field use. The kit correctly identified intercepts as *Tetranychus* mite species: *urticae*, *ludeni* or *lambi* (see publications list 28).

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

3.1 Resistance testing

3.1.1 Chemicals tested

Cotton aphid was treated with clothianidin (Shield®), diafenthiuron (Pegusus®), sulfoxaflor (Transform®), and thiamethoxam (Actara®). All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron (CGA-140408) was used. This is necessary because diafenthiuron (Pegasus®) is activated by exposure to UV light, which would not normally occur in the laboratory. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via DNA based methods.

Two-spotted mite was treated with abamectin (Agrimec®), bifenthrin (Talstar®), propargite (Comite®), and diafenthiuron (Pegasus® as CGA-140408). Etoxazole (Paramite® or Zeal®) resistance was evaluated via a DNA based method.

Banana or strawberry spider mite was treated with abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA-140408).

Cotton seedling thrips were tested against imidacloprid (Confidor®) and western flower thrips against spinetoram (Success® Neo) insecticides.

3.1.2 Aphid - collection and culturing

Cotton aphids were collected by researchers, Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden [Elizabeth McArthur Agricultural Institute (EMAI)] and each field strain was cultured separately on pesticide-free cotton at 25 ± 4 °C under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages.

Aphid - resistance detection

Via Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al.* 2001). Briefly, batches of thirty adult female aphids per leaf disc were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower (to yield percent insecticide susceptible). All tests were replicated (unless otherwise noted) and included a water-only sprayed control (that did not exceed 10%). After spraying, clear plastic film was used to cover the Petri dishes. Aphids were then maintained at 25 ± 0.1 °C in 16:8 L:D for 24 h after which mortality was assessed.

Molecular. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method. The qPCR method for estimating pyrethroid, OP

and pirimicarb (Pirimor®) resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. For each 200 aphid sample, pooled DNA is extracted and triplicate qPCRs are carried out using two TaqMan probes (one detects the resistance allele and the other detects the susceptible allele). The ratio of the fluorescence intensity produced for each qPCR reaction, is calculated along with a standard reference series, whose resistance allele frequency is known. The resistance allele frequency from field populations is then accurately estimated based on the ratio of fluorescence increase between the resistant and susceptible probe (Chen *et al*. 2014).

3.1.3 Two-spotted mite - collection and culturing

Strains of two-spotted mite were sourced from a range of cotton fields in NSW (none were found in Qld) and put into culture as above at EMAI.

Two-spotted mite – resistance detection

Via bioassay. The bioassay procedure required fifteen to twenty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites on leaf discs were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower as above. Each test was replicated (unless otherwise indicated) and included a water only sprayed control (that did not exceed 10%). After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Molecular. Genomic DNA was extracted using Chelex-100 resin (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions and screened for the presence of the I1017F mutation (Van Leeuwen *et al*. 2012) by direct sequencing of PCR amplicons performed at the Australian Genome Research Facility Ltd. Sequencing results were analysed using Sequencher version 5.2.4 (Gene Codes Corporation).

3.1.4 Banana or strawberry spider mite - collection and culturing

Strains of banana or strawberry spider mite were sourced from a range of cotton fields in NSW and Qld and put into culture as above for two-spotted mite at EMAI.

Banana or strawberry spider mite – resistance detection

Via bioassay. The bioassay procedure is as described above for two-spotted mite.

3.1.5 Mirid - collection

Green mirid was collected from cotton, pigeon pea refuges or mung beans in close proximity to cotton via beat sheet or sweep net and transported alive to the laboratory for processing.

Mirids – resistance detection

Molecular. Resistance to fipronil is detected via a mutation in the *Rdl* gene (known associated with fipronil (Maestro® or Albatross®) resistance in the ferment fly)(ffrench-

Constant *et al*. 1993). Genomic DNA was extracted from individual green mirids and PCR amplification performed for the *Rdl* gene. Amplified products were then sent to the Australian Genome Research Facility for fipronil sequencing.

3.1.6 Cotton seedling thrips and western flower thrips - collection and culturing

Strains of cotton seedling thrips and western flower thrips were sourced from a range of cotton fields in NSW and Qld and put into culture as above at EMAI. Cotton seedling thrips were maintained using methods described in detail in Herron *et al*. (2008) while western flower thrips were maintained using methods outlined in Herron and Gullick (1998).

Cotton seedling thrips and western flower thrips – resistance detection

Via bioassay. Cotton seedling thrips were bioassayed using methods described in detail in Herron *et al*. (2008) while western flower thrips were tested using methods outlined in Herron and Gullick (1998).

Molecular. For cotton seedling thrips we looked for the R81T mutation in D-loop region of the β1 subunit of the nicotinic acetylcholine receptor (nAChR) known to confer neonicotinoid resistance in green peach aphid and cotton aphid. (Bass *et al.* 2011 and Shi *et al.* 2012). We designed our own degenerated primers based on the homology of the aphids and western flower thrips to amplify the target mutation of R81T in cotton seedling thrips. Amplified products were then sent to the Australian Genome Research Facility for fipronil sequencing.

For western flower thrips we used the library to find the target site mutation G275E in *nAChR* α6 subunit known to cause spinosad resistance in western flower thrips (Chen *et al*. 2011) that should be applicable to spinetoram.

3.2 Banana or strawberry spider mite baseline and abundance

Collection and rearing

Mites were collected opportunistically from Australian cotton producing regions in New South Wales and Queensland and reared on insecticide free French bean (*Phaseolus vulgaris*) using methods described in detail in (Herron, Edge et al. 1998). Briefly, mites were housed in individual mite proof cages in an insectary running at 25±4°C and under natural light and ambient humidity.

Chemicals tested

Mites were exposed to abamectin (Vertimec® Miticide/Insecticide, Syngenta Australia Pty Ltd), propargite (Omite® 300 W Wettable Powder Miticide, Arysta Life Science Australia Pty Ltd) and diafenthiuron. All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus® Miticide/Insecticide, Syngenta Australia Pty Ltd) for which the UV activated technical grade carbodiimide derivative of diafenthiuron (CGA-140408) was tested instead.

Bioassay

The bioassay procedure required twenty to thirty young adult female mites to be transferred from culture to French bean leaf discs (Herron, Rophail et al. 2004). Briefly, mites on leaf discs were then sprayed with insecticide with the aid of a Potter spray tower. Banana or strawberry spider mite was exposed to serial insecticide concentrations to yield full dose responses. Each test was replicated and included a water only sprayed control that did not exceed 15%. After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality was assessed.

Analysis

Data were analysed via a stand-alone PC based probit program developed by Barchia (Barchia 2001). Analysis was done without replicate pooling to account for variability between replicates. LC_{50} and $LC_{99.9}$ values were estimated using the method of (Finney 1971) after control mortality correction (Abbott 1925). Minimum effective concentration (MEC) data was interpreted directly from the bioassay data being the lowest dose across all concentrations that achieved 100% mortality.

3.3 Cotton boll worm and indoxacarb resistance

Insect strains, crosses, and bioassays

We used two strains of cotton boll worm, a known laboratory strain, New GR established in the mid-1980s and is known to be susceptible to indoxacarb and . The resistant strain GY7-39 established from a single cotton boll worm moth from maize near Moree in 2013. Strains New GR and GY7-39 were crossed 5 times to generate F_6 progeny. Commercial insecticide as Steward® EC Insecticide (150 g/L indoxacarb) (DuPont Australia Ltd, Macquarie Park, Australia) was used for all testing as described by Bird (2016).

DNA extraction and Genotype-by-sequencing

DNA was extracted with the DNeasy® Blood & Tissue kit (Qiagen, Australia) according to the manufacturer's instructions from the head of adult or larvae (Woolley *et al.* 2017). Ninety-five DNA samples plus one negative control (no DNA) were placed in a 96-well plate. Samples included eight individuals from susceptible New GR (including two susceptible F_0 females), fourteen resistant GY7-39 (including two resistant F_0 males), and seventy-three resistant F_6 (one family with 35 and a second family with 38 F_6). Genotype-by-sequencing library construction and sequencing were performed at the Cornell genomic diversity facility (http://www.biotech.cornell.edu) using restriction enzyme *Pst*I (Elshire 2011).

Processing sequenced reads

Unique sequence tags (64 bp) were obtained by processing raw sequence reads using "sequence to Tag/TaxaDB" of GBSv2 in the TASSEL 5.0 (Glaubitz et al. 2014). For all the samples, each tag was ranked based on the normalized sequence reads and we consider the tag to be absent if the normalized read count was less than 0.1 of the maximum of the normalized read counts.

Tag mapping

The filtered Tags were first aligned to a cotton boll worm reference genome using HISAT2 Catchen *et al.* (2013) with default parameters and those tags found not aligned by HISAT2 were subject to Basic Local Alignment Search Tool (BLAST) (Ailschul *et al*. 1997). The full sequence of the tags that were significantly associated with indoxacarb resistance were extracted and re-aligned to a cotton boll worm reference genome using a HISAT2 and BLAST and alignments manually examined via IGV (Robinson *et al*. 2011).

3.4 DNA based kit for spider mite identification

Spider-mite populations

A total of 22 spider-mite populations were used belonging to three different species: two-spotted mite (*Tetranychus urticae), bean spider mite* (*T. ludeni)* and banana or strawberry spider mite (*T. lambi*).

DNA extraction

Genomic DNA was extracted from the individual mite with 5% (v/v) Chelex-100 (Bio-Rad, Hercules, CA, USA) based on to the manufacturer's instructions with some modification. In brief, single mite was placed in 1.5mL Eppendorf tube with 100 µL 5% (v/v) Chelex-100 resin. The sample mix was first heated for 10 minutes at 100 °C. After a second short 10 second vortex, the sample mix was heated for another 10 minutes at 100 °C and cooled on ice for 5 minutes.

Primer design and multiplex PCR for spider-mite identification

Here we used all available spider mite ITS sequences from National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; [1988] – [cited 2018June 2]. Available from: https://www.ncbi.nlm.nih.gov/. Multiple sequence alignment was carried out by ClusterW of Analysis Tool Web Service from the EMBI_EBI (McWilliam *et al.* 2013). PCR primers and probes used were designed by Primer3 based on 87 ITS spider mite sequence (Untergasser *et al.* 2012). The diagnostic assay constructed consisted of a universal forward primer (Mite-F) and reverse primer (Mite-R) that amplified the ITS1 region in the three spider mites two-spotted mite*, bean spider mite* and banana or strawberry spider mite. We also used a universal primer ITS1_y and Mite-R to amplify the longer ITS1 region.

Real-time PCR for the diagnostic assay

Primers and probes were synthesized by Biosearch Technologies Inc. (Biosearch Technologies Inc, Novato USA). Real-time PCR contained 9 µM forward primer and reverse primer, 0.2µM of Turti_P, 0.4µM of Lumbi_P and 0.8µM of Ludeni_P in a Taqman Genotyping Mastermixa (ThermoFisher Scientific, Australia) comprising a 25 µL reaction volume with 10 min at 95°C followed by 40 cycles of 15 seconds at 95°C and 1

min at 60°C. We used an ABI7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and a portable micPCR (Bio Molecular Systems Australia).

Sequencing the ITS1 region and COI gene

The ITS1 region and COI gene for all three mite species were sequenced by Sanger sequencing. PCR was performed in a 25µL reaction containing 1 x MyFi™ Mix (Bioline, Australia) master mix, ~20ng DNA and 1µM each primer. Thermal cycling conditions were: 95°C for 10 min followed by 35 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 60 s, with a final extension step for 5 min at 72°C. PCR product was gel extracted and purified using the Wizard SV PCR and Gel Clean-Up system (Promega, Australia). DNA sequencing was performed with ABI PRISM BigDye terminator cycle sequencing Version 2.0 at the Australian Genome Research Facility (AGRF). Sequence analysis was carried out via SEQUENCHER 5.4.6 (Gene Codes, Ann Arbor, MI, USA).

3.5 DNA based resistance detection in thrips

Cotton seedling thrips

The DNA library was used and we looked for the mutation R81T in cotton seedling thrips known to confer neonicotinoid resistance in green peach aphid and cotton aphid. (Bass *et al.* 2011; Shi *et al.* 2012).

Here we aligned the β1 protein sequence of cotton aphid (Chinese) with other insect species and found that the D-loop sequence is conserved among the species examined (Figure 3.5.1). However, there is currently no DNA sequence available for these potential nAChR genes in cotton seedling thrips to align against. Here we designed our own degenerated primers based on the homology of the aphids and western flower thrips creating new primers to amplify potential target site mutations in cotton seedling thrips.

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ETN60728_[A.darlingi]          FVQLINVNEKNQIMKSNVWLRLVWSDYQLQWDEADYGGIGVLRLPPDKVWKPDIVLFNNA   117
XP_309158_[A.gambiae]          FVQLINVNEKNQIMKSNVWLRLVWSDYQLQWDEADYGGIGVLRLPPDKVWKPDIVLFNNA   117
KFB39351_[A.sinensis]          FVQLINVNEKNQIMKSNVWLRLVWSDYQLQWDEADYGGIGVLRLPPDKVWKPDIVLFNNA   97
XP_025200812_[M.sacchari]      FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
XP_025414553_[S.flava]         FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
XP_015374884_[D.noxia]         FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
AFP55243_[A.glycines]          FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
XP_026814555_[R.maidis]        FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
AFH00994_[A.gossypii]          FVQLINVNEKSQIMKSNVWLTLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
XP_022165274_[M.persicae]      FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
BBA21165_[A.gossypii]          FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   120
XP_008184755_[A.pisum]         FVQLINVNEKSQIMKSNVWLRLVWRDYQLQWDEADYGGIQVLRLPPDKVWKPDIVLFNNA   72
                               **********.********* *** ************** ********************
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Figure 3.5.1. The protein sequence alignment among the insect species for AFH00094 carried the R81T mutation. [note: Interestingly, our previous work confirmed that Australian neonicotinoid resistant cotton aphid do not have this mutation]

Western flower thrips

Again from the library we know the target site mutation G275E in nAChR α6 subunit in western flower thrips is known to cause spinosad resistance western flower thrips, *Thrips palmi* and *Tuta absoluta (*Wen Xue *et al.* 2014*)* and so should then be applicable to spinetoram.

3.6 References

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Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

4.1 Resistance testing

4.1.1 *Cotton aphid*.

We noted a repeated detection of low frequency neonicotinoid (thiamethoxam and clothianidin) resistance and no subsequent increase in resistance (Table 4.1.1). From this we

concluded neonicotinoid survivors were likely high level vigour tolerant rather than low level resistant.

From season 2017-2018 discriminating doses were modified to account for such variation and better delineate tolerance and resistance. Consequently from 2017-2018 neonicotinoid resistance was no longer detected nor was pirimicarb (Pirimor®) and organophosphate resistance. The result highlights the advantage of the DNA based pirimicarb (Pirimor®) and organophosphate resistance detection that is not subject to interpretation as is a discriminating dose.

Consequently neonicotinoids, pirimicarb (Pirimor®) and organophosphates can be used with confidence against cotton aphid. Interestingly pyrethroid resistance was also monitored for using a DNA test and was often detected at low frequencies in some strains. This was surprising as cotton aphid is not directly targeted with pyrethroids in cotton. The perplexing result may be a consequence of aphids being selected in crops other than cotton and flying in or coincidental selection in cotton with sprays targeted at other species.

4.1.2 *Two-spotted mite and banana or strawberry spider mite*
Two-spotted mite abundance has been declining in cotton growing areas; however, despite this, two-spotted mite populations sourced off cotton remain resistant and especially to

Table 4.1.1 Cotton aphid strains collected off cotton between 2013-2017 that contained survivors via discriminating dose bioassay to thiamethoxam (T) and/or clothianidin (C)

	% susceptible at the discriminating dose against thiamethoxam (T)(0.02 g a.i. / L) and clothianidin (C) (0.05 g a.i. / L)							
Strain	**2013**		**2015**		**2016**		**2017**	
	T	**C**	**T**	**C**	**T**	**C**	**T**	**C**
1	99	99						
2	97	91						
3	99	100						
4			100	99				
5			99	100				
6					100	99		
7					99	100		
8					100	98		
9					99	100		
10					99	100		
11					99	100		
12							97	97
13							-	99
14							-	98
15							99	-

bifenthrin and abamectin (Figure 4.1.2). Three seasons ago in season 2016-2017 we introduced a DNA-based resistance screening capacity for the miticide, etoxazole (Paramite® or Zeal®) against TSM; the reason being etoxazole (Paramite® or Zeal®) had potential to reduce abamectin (Agrimec®) selection. Worryingly etoxazole (Paramite® or Zeal®) resistance was detected in three strains of TSM in the first season of monitoring but encouragingly for the last two seasons resistance has not been found.

Bifenthrin (Talstar®) resistance in two-spotted mite from cotton has been relatively common with strains often showing a few percent resistant mites. For 2014-2015 more than 80% strains contained bifenthrin (Talstar®) resistant two-spotted mite and one strain was essentially 100% resistant. The trend continued and in the final season of this study 2018-2019 strain Mor Col 3 comprised 95% resistant individuals. The reason for this sustained resistance frequency is not clear because pyrethroid use in cotton is limited and two-spotted mite do not fly (although they can aerially disperse via wind) so their immigration from sprayed crops other than cotton must be limited.

Similarly, abamectin (Agrimec®) resistance monitoring against two-spotted mite has rarely detected positive resistance results until season 2010-2011, when abamectin resistance was detected in three out of the four two-spotted mite strains tested. Again in 2011-2012 resistance was detected in a single two-spotted mite strain and in 2012-2013 abamectin resistance was again detected in two strains with 6 and 7% resistant two-spotted mite. During the 2013-2014 season abamectin was detected in six out of the eleven (54%) strains tested with one comprising 79% resistant two-spotted mite. Season 2014-2015 again saw resistance detected in six out of the nine (67%) strains with the highest frequency detected being 75% resistant with two others containing 74 and 72% resistant two-spotted mite. This trend continued up until and including season 2018-2019. The reason for this continuing detection of high frequency abamectin (Agrimec®) resistance is speculated to relate to the low price of abamectin. For that reason it can be applied as a prophylactic treatment in combination with mirid sprays. These mirid sprays are often disruptive to beneficials so the inclusion of abamectin reduces the risk of subsequent mite outbreaks. However, this usage pattern will ultimately lead to failure of abamectin against concurrent mites.

Having previously establishing a baseline (see results section 4.2) in season 2018-2019 we included banana or strawberry spider mite into the resistance testing. Results produced for banana or strawberry spider mite are encouraging (and in complete contrast to two-spotted mite) with no banana or strawberry spider mite resistance detected (Table 4.1.2).

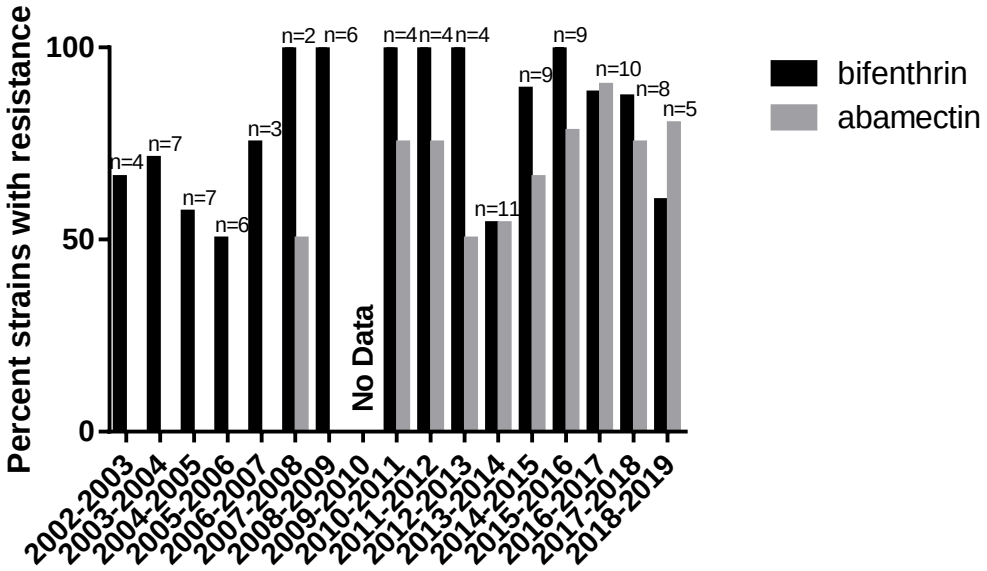

Figure 4.1.2 Percent two-spotted mite strains showing bifenthrin (Talstar®) or abamectin (Agrimec®) discriminating dose survivors.

4.1.3 *Mirids*

Green mirid was screened for fipronil resistance from season 2015-2016 using a molecular DNA based method but none was detected.

4.1.4 *Cotton seedling thrips and western flower thrips*

Australian cotton growers have anecdotally experienced control problems with cotton seedling thrips. Results presented here suggest dimethoate, λ cyhalothrin and imidacloprid resistance (Figures 4.1.4.1 and 4.1.4.2). The problem is most acute when cotton is establishing where seed dressings are often required to prevent damage. Using imidacloprid (Genero®, Confidor®) as a neonicotinoid model a comparison of the minimum dose required to kill all

Table 4.1.2. Percent susceptible SSM via bioassay against abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA-140408) as percent mortality at the discriminating dose (i.e. percent susceptible) for mites collected during season 2018-19.

Strain	Abamectin (Agrimec®)	Propargite (Comite®)	Diafenthiuron (Pegasus® as CGA140408)
Mor B1	100	100	100
Mor B2	100	100	100
War 22	100	100	100
Goon B	100	100	100
Kim 21	100	100	100*
Eum 3	100	100	*nt*
Mor G	100*	100*	*nt*
Kor 4	100*	100*	100

*not replicated, *nt* not tested

strain Spring 2016-2017 (0.001 g/L) to strain Griff 2015-2016 (0.1 g/L) produced a 100 fold difference (Figure 4.1.4.2). That means there is likely 100-fold imidacloprid (Genero®, Confidor®) resistance in strain Griff 2015-2016 meaning 100 fold resistance to neonicotinoids generally. As strains Spring 2015-16 and Pine 2015-2016 may not themselves be completely imidacloprid (Genero®, Confidor®) susceptible the resistance fold value is best case scenario and so may underestimate resistance. As there is known cross resistance between imidacloprid (Genero®, Confidor®) and thiamethoxam (Cruiser®, Cruiser Extreme®) anecdotal control issues experienced with cotton seedling thrips may be resistance related. For this reason the 2018-2019 Cotton Pest Management Guide was modified to include a resistance warning with thrips and neonicotinoids including seed dressings.

Where control issues with cotton seedling thrips have been experienced with neonicotinoid seed treatments, alternative at-planting treatment options are available including the organophosphate phorate (Thimet®) and the carbamate carbosulfan (Marshal®). In our experience, phorate (Thimet®) should provide robust protection against cotton seedling thrips as previous bioassay data showed phorate (Thimet®) had high efficacy against western flower thrips.

In season 2018-2019 thrips were collected but unexpectedly only one strain of cotton seedling thrips was forwarded with the remaining nine being western flower thrips (Figure 4.1.4.3). That single cotton seedling thrips strain was imidacloprid (Confidor®) susceptible but unexpectedly five of the western flower thrips were spinetoram (Success® Neo) resistant. Spinetoram (Success® Neo) is the only registered western flower thrips control for Australian cotton so the multiple detections are both worrisome and confusing. Spinosyn use to cause such widespread resistance would need to be high suggesting resistance may be being selected outside of cotton.

Despite the single strain of cotton seedling thrips collected being found imidacloprid (Confidor®) susceptible (via bioassay) we consider DNA based resistance detection possible. We aligned all available mRNA sequences of β1 subunit for cotton aphid and western flower thrips that was used to design multiple primers to amplify the D-loop region of β1 subunit known to cause resistance (Figure 4.1.4.4). We confirmed that our sequence for cotton seedling thrips also belonged to gene β1 subunit and further aligned that sequence against the western flower thrips isolate FOCC.00 unplaced genomic scaffold, Focc_2.1 scaffold 8.

As most thrips collected were western flower thrips and spinetoram resistant (confirmed via bioassay) molecular diagnostic verification was attempted. Sequencing of some bioassay control treatments showed resistant and susceptible thrips could be detected but sample size was too small for a meaningful comparison to the bioassay data (Table 4.1.4.1). Nonetheless results are encouraging but more verification is required.

Table 4.1.4.1. WFT collected from cotton during season 2018-2019 and tested for spinetoram resistance using both bioassay and DNA base methodology

Strain	Number SS homozygous via DNA	Number SR* heterozygous via DNA	Number RR homozygous via DNA	% susceptible via DNA	% mortality @ DD
Kulki Ku9	1	1	None	50	95%

Comet Argoon	1	None	None	100	99%
Springwell B1 WFT	1	3	1	80	92%
Hills K4	Not done	Not done	Not done	Not done	100%
Moree B2	Not done	Not done	Not done	Not done	100%
Goondi 10	Not done	Not done	Not done	Not done	100%
Griffith 35	Not done	Not done	Not done	Not done	99%
Carrathool	Not done	Not done	Not done	Not done	100%
Griffith 74	Not done	Not done	Not done	Not done	99%

*NB as resistance is recessive an SR heterozygote has the same phenotype as susceptible.

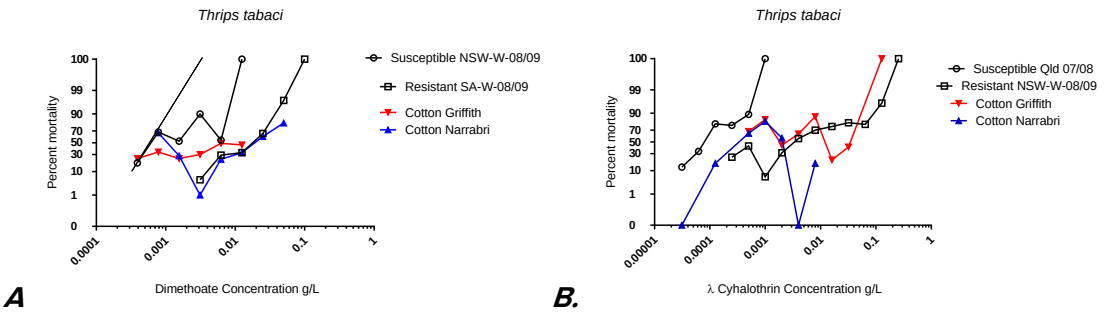

Figure 4.1.4.1 : A. Dose responses against dimethoate and B. λ-cyhalothrin for 'susceptible' and 'resistant' strains of cotton seedling thrips (*Thrips tabaci*) from onion overlaid with the response of field collected strain 'Cotton Griffith' and 'Cotton Narrabri'.

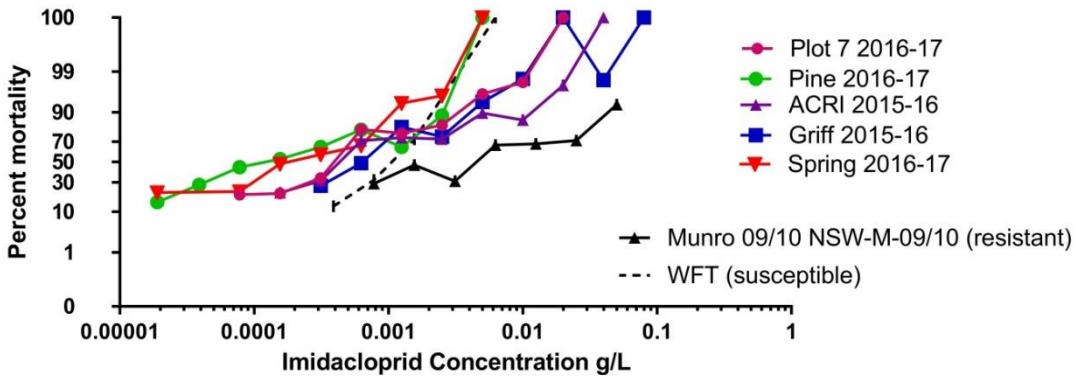

Figure 4.1.4.2 Dose response data for cotton seedling thrips collected during the 2015-16 and 2016-17 cotton seasons and tested against imidacloprid (Confidor®) plus a reference

resistant strain from horticulture [Munro 09/10 NSW-M-09/10 (resistant)] and a reference susceptible western flower thrips [WFT (susceptible)] included for comparison.

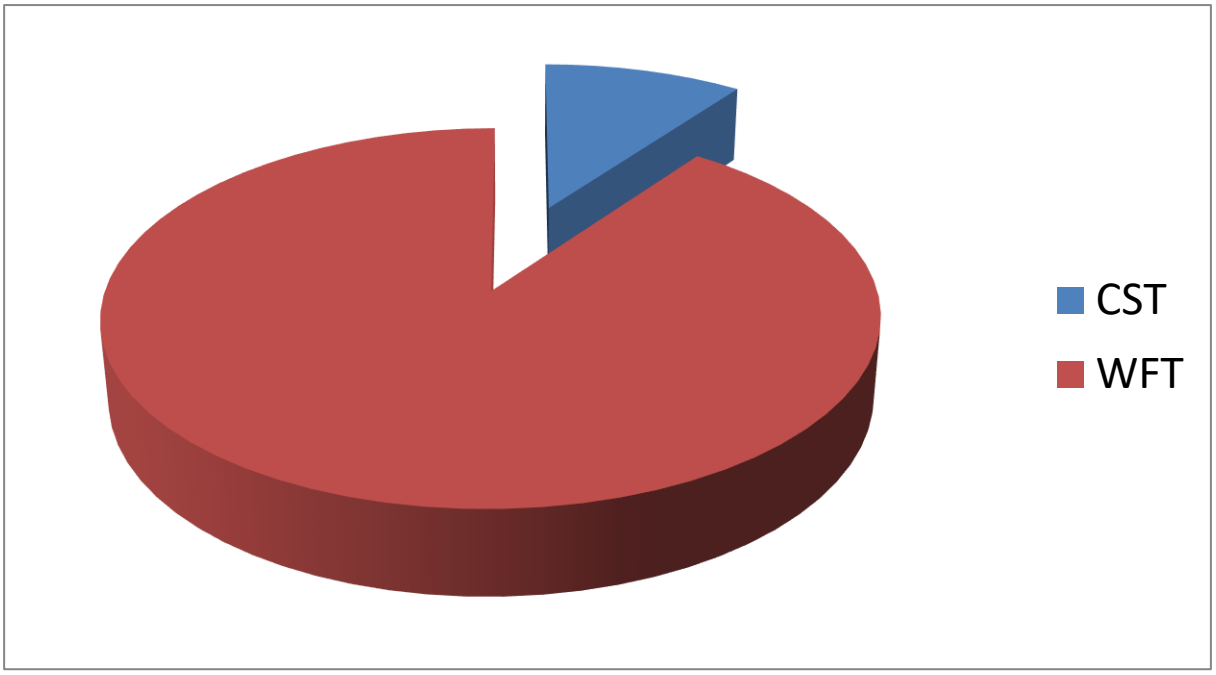

Figure 4.1.4.3. The relative abundance of cotton seedling thrips (CST) and western flower thrips (WFT) collected from Australian cotton in season 2018-2019.

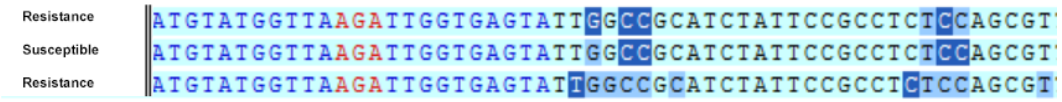

Figure 4.1.4.4. Sequence of D-loop of β1 subunit, both resistance and susceptible strain contain the same sequence at for the position R81T (red font) which coded amino acid arginine (R).

4.2 Banana or strawberry spider mite baseline and abundance

4.2.1 *Abundance*

Mites were gathered from New South Wales and Queensland cotton with 70 strains collected (Figure 4.2.1). Banana or strawberry spider mite was the most abundant species comprising 71% of the mites in 2016-2017, 67% in 2017-2018 and 75% in 2018-2019. Interestingly, bean spider mite was not collected at all nor was two-spotted mite found outside New South Wales (Figure 4.2.1).

The banana or strawberry spider mite result is both interesting and perplexing because two-spotted mite continue to develop resistance with the latest being abamectin. Despite a seemingly never ending ability to develop resistance, recent collection data suggest two-spotted mite is becoming less abundant. Collection data presented here support anecdotal grower observations that two-spotted mite is indeed not the dominant mite species in Australian cotton and has all but disappeared from Queensland (Figure 4.2.1).

The reason for this may relate to the progressive reduction in insecticide use in Australian cotton since the introduction of *Bt* cotton. Although two-spotted mite from Australian cotton remain resistant spraying in *Bt* cotton may have reduced to such an extent that two-spotted mite is not getting a selective advantage and is being competitively displaced by banana or strawberry spider mite. Interestingly bean spider mite was not collected at all even though two-spotted mite is declining and so remains absent from Australian cotton.

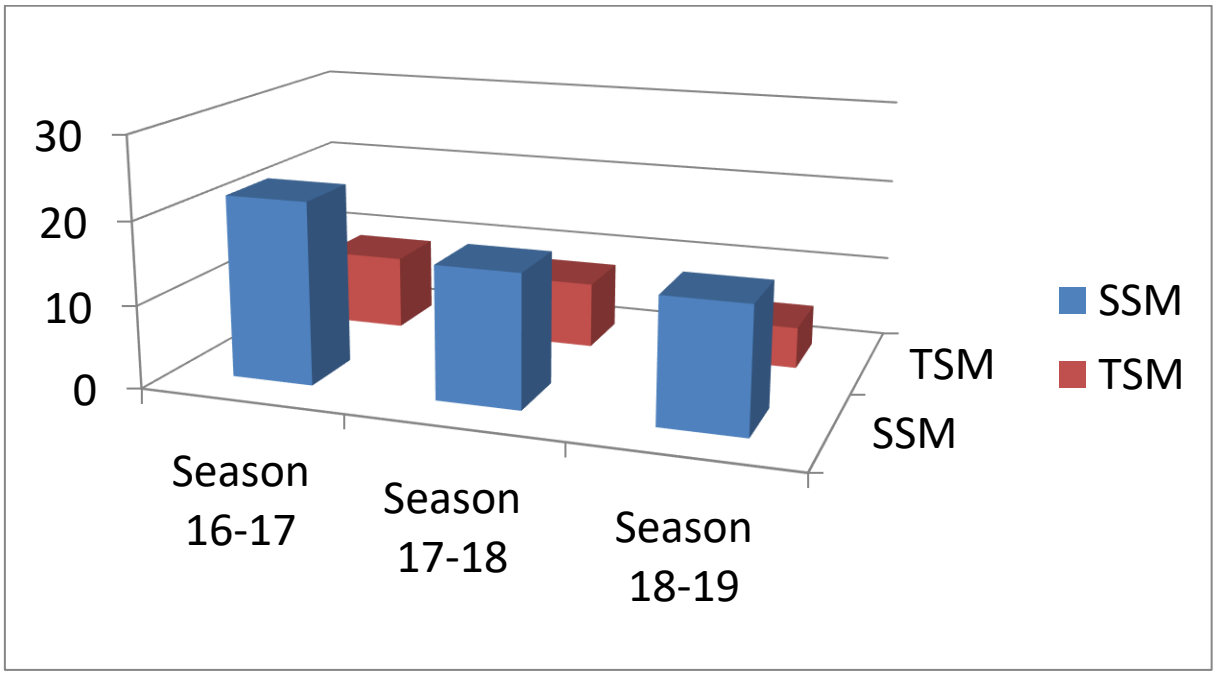

Figure 4.22. The abundance of banana or strawberry spider mite (SSM) and two-spotted spider mite (TSM) collected during Australian cotton seasons 2016-2017, 2017-2018 and 2018-2019 (N.B. TSM was found in NSW only).

Some may consider all good then for sucking pest control in Australian cotton but any change in the agroecosystem can significantly change pest and/or predator interaction. In 1993 western flower thrips was first detected in Western Australia and almost concurrently a 1993 study of thrips in Australian cotton found tomato thrips and cotton seedling thrips breeding and developing well, with the latter being the most important early season species. Times have changed and western flower thrips is a dominant species and known difficult to control due to resistance. Although sometimes considered a pest and at times requiring control thrips have demonstrated themselves an important spider mite predator. Any significant change in thrips abundance may unintentionally affect mite control.

4.2.2 *Banana or strawberry spider mite baseline*

Baseline data were established against abamectin (Figure 4.2.2.1), diafenthiuron (Figure 4.2.2.2) and propargite (Figure 4.2.2.3). Against propargite the calculated $LC_{99.9}$ ranged from 0.3-0.9 that was higher than the actual MEC that ranged from 0.125-0.25 g/L. Similarly, against diafenthiuron, the calculated $LC_{99.9}$ ranged from 0.01-0.03 that was again higher than the actual MEC of 0.005-0.01 g/L. Finally, the abamectin calculated $LC_{99.9}$ ranged from 0.0001-0.003 that was also higher than the observed MEC of 0.000125-0.00025 g/L. Based on

calculated and observed tolerance we consider robust DDs for abamectin, diafenthiuron and propargite would fall within the range of ~0.0005-0.001, ~0.02-0.04 and ~0.5-1.0 g/L respectively. Therefore we propose DDs of 0.0007g/L abamectin, 0.03 g/L diafenthiuron and 0.7 g/L propargite.

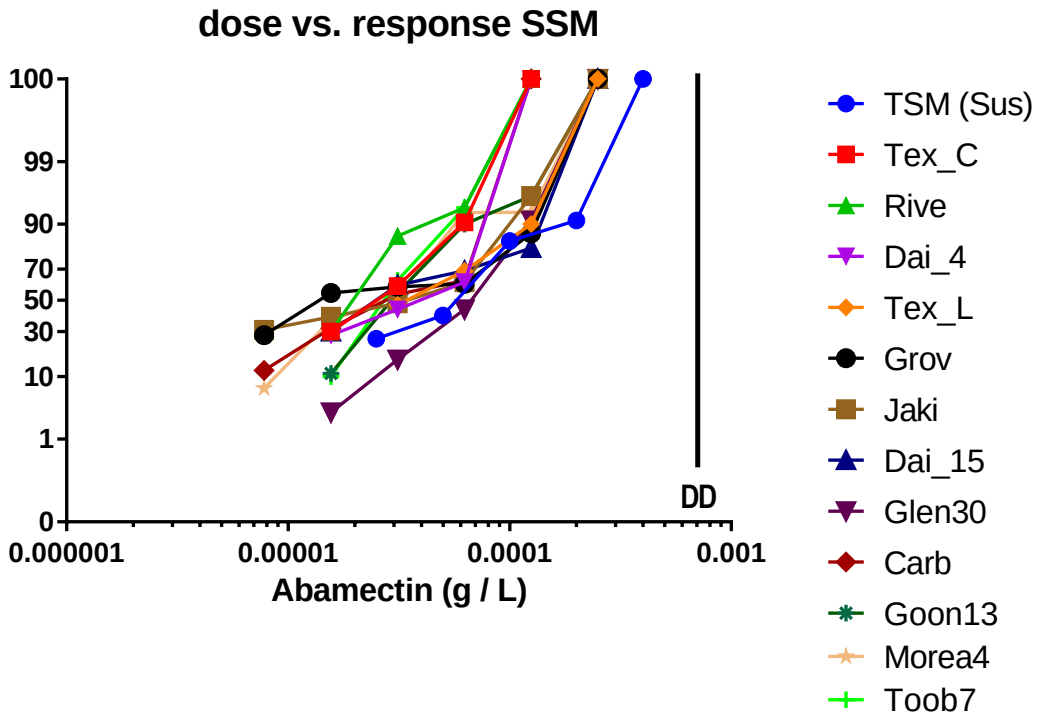

Figure 4.2.2.1 Dose response baseline data for banana or strawberry spider mite (SSM) collected from Australian cotton and tested against abamectin (Agrimec®) plus a reference susceptible two-spotted mite [TSM (Sus)] included for comparison.

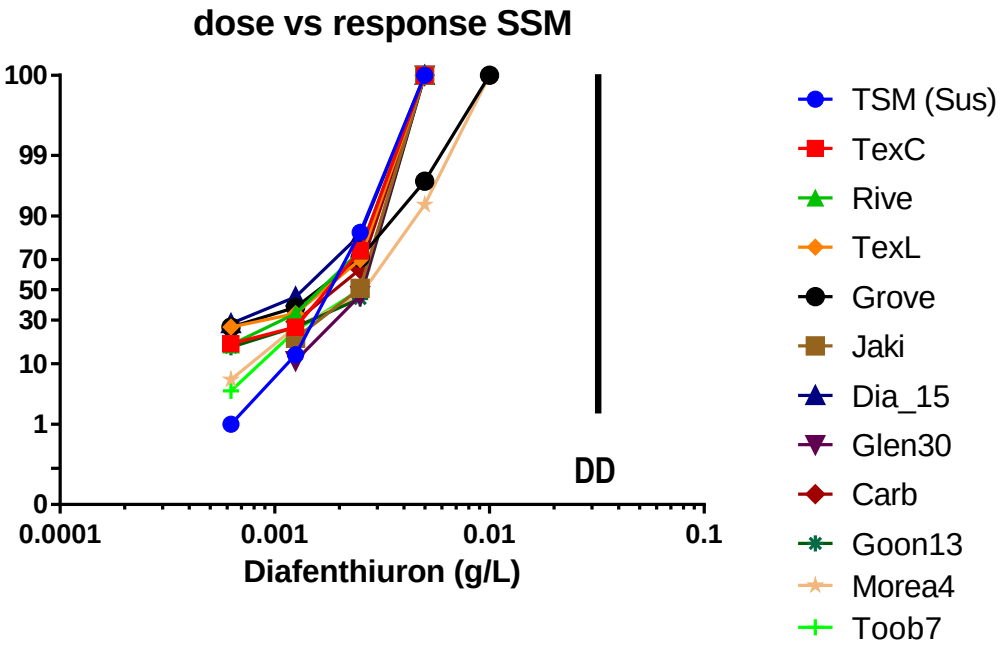

Figure 4.2.2.2. Dose response baseline data for banana or strawberry spider mite (SSM) collected from Australian cotton and tested against diafenthiuron (Pegasus®) plus a reference susceptible two-spotted mite [TSM (Sus)] included for comparison.

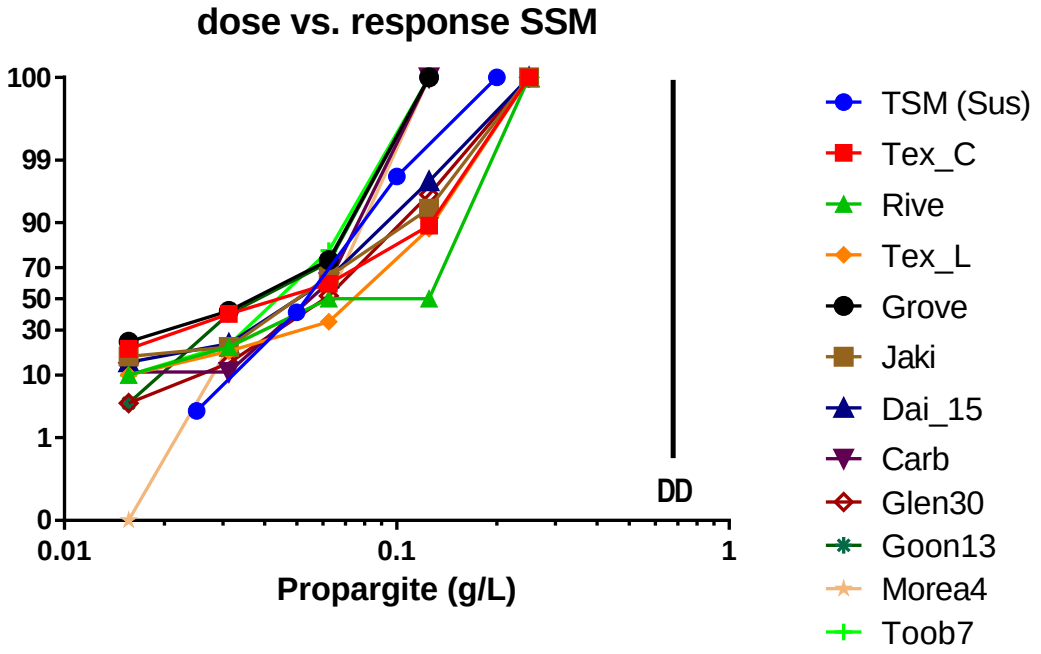

Figure 4.2.2.3. Dose response baseline data for banana or strawberry spider mite (SSM) collected from Australian cotton and tested against propargite (Comite®) plus a reference susceptible two-spotted mite [TSM (Sus)] included for comparison.

4.3 DNA based kit for spider mite identification

An appropriate technology platform was found to be the ‘MIC’ mico qPCR machine (Figure 4.3.1). This cutting edge hand-held PCR technology can used to delineate multiple spider mite samples in the field with a battery power supply.

Figure 4.3.1. Micro ‘MIC’ qPCR machine sitting on a bench at the EMAI before being filled with spider mites for identification.

The micro ‘MIC’ qPCR machine single PCR assay detected unambiguously the three species in this study: two-spotted mite (Figure 4.3.2 A), banana or strawberry spider mite (Figure 4.3.2 B) and bean spider mite (Figure 4.3.2 C).

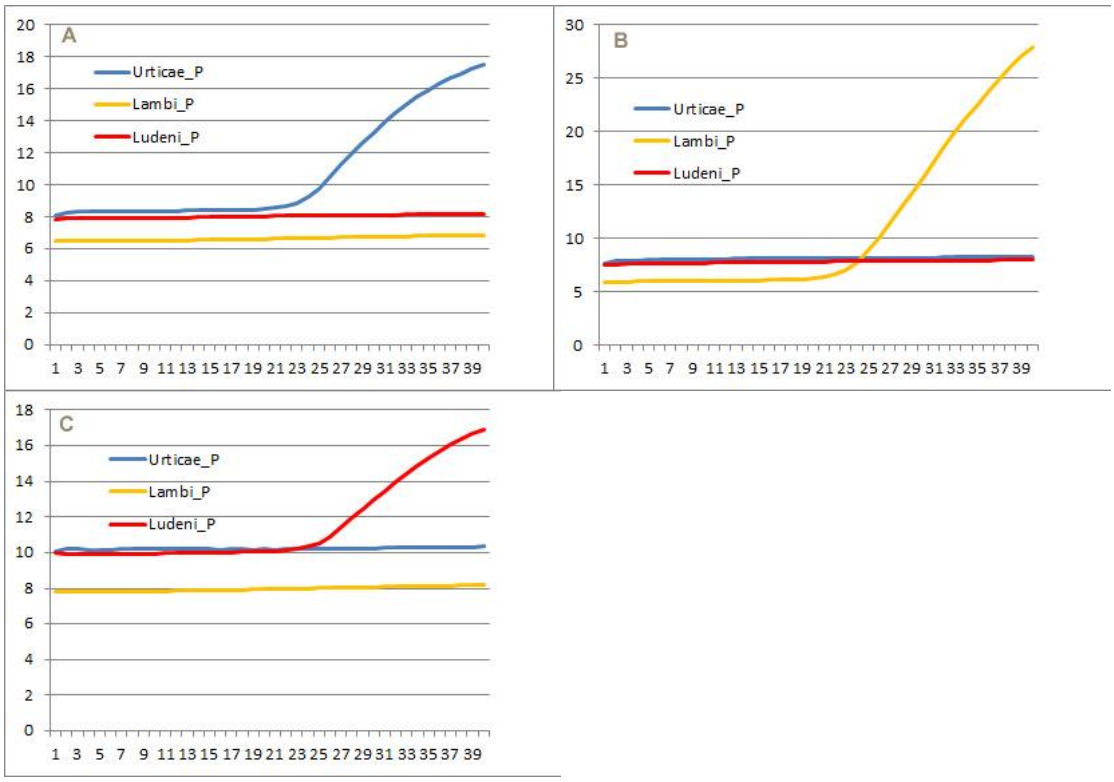

Fig. 4.3.2. Real-time PCR Amplification curve profiles of reference two-spotted mite (*Tetranychus urticae*), bean spider mite (*T. ludeni*) and banana or strawberry spider mite (*T. lambi*) and with micPCR. The assay has universal primers (Mite-F and Mite-R) and three TaqMan probe (Urticae_P, green, Lambi_P yellow and Ludeni_p red). A: *T. urticae* strain 'S', B: *T. lambi* strain 'Carb' and C: *T. ludeni* strain 'BSM_S'.

In blind testing of mite samples [provided via Biosecurity Australia (AQIS)] we successfully identified two-spotted mite, bean spider mite and strawberry spider mite including reference and positive control samples (Table 4.3.1). In addition, we confirmed a two-spotted mite sample previously unknown for Biosecurity Australia. Although there were two unconfirmed cases of two-spotted mite (AM18-41 and AM18-44), those were likely caused by low DNA quantity in the sample.

In all instances the 'MIC' correctly identified the mite species and as a result Biosecurity Australia was interested in obtaining the technology for their field use. The request to do that was agreed to by CRDC and NSW DPI with the final output to be publication (see publication 28).

Further we further tested an additional 26 mite strains, 5 of which are known reference collections and an additional 21 that had been collected from Australian cotton during seasons 2016-2017, 2017-2018 and 2018-2019 and maintained at EMAI in cages within a mass culture facility (table 4.3.1). As expected all 5 reference strains were 100% pure but many field collected strains that had been previously bioassayed for resistance and kept were not. It noteworthy here that bioassay requires each mite tested to be individually assessed twice (scored) under a stereo microscope by an operator familiar with their identification so significant strain contamination during bioassay is not possible. Nonetheless eight field

collected strains (F2020 Cole, Moree Colmlee 3, Griffith 35, Hills K4, Merk2, WhitNFOI, Walla and MoreA4) were

Table 4.3.1. Blind testing of Biosecurity Australia (AQIS) supplied mite DNA samples

Sample Code	***AQIS Result***	***EMAI Result***	***Comment***
T. urticae ref (EMAI)		*TSM*	
T. lambi ref (EMAI)		*SSM*	
T. ludeni (EMAI)		*BSM*	
T. urticae +ve control	*aliquot of DNA ex New Zealand*	*TSM*	
T. ludeni (MQ39)	*aliquot of DNA ex New Zealand*	*BSM*	
T. urticae (TLG146)	*aliquot of DNA ex New Zealand*	*TSM*	
T. urticae (TLR14a)	*aliquot of DNA ex New Zealand*	*TSM*	
T. urticae (as T. cinnabarinus) (MQ74)	*aliquot of DNA ex New Zealand*	*TSM*	
T. parakanzawai (MQ22)	*aliquot of DNA ex New Zealand*	*Negative*	
T. truncatus (MQ26)	*aliquot of DNA ex New Zealand*	*Negative*	
T. kanzawai (MQ29)	*aliquot of DNA ex New Zealand*	*Negative*	
T. puearicola (MQ30)	*aliquot of DNA ex New Zealand*	*Negative*	
T. collyerae (MQ71)	*aliquot of DNA ex New Zealand*	*Negative*	
T. truncatus (MQ87)	*aliquot of DNA ex New Zealand*	*Negative*	
T. turkestani (MQ93)	*aliquot of DNA ex New Zealand*	*Negative*	
T. kanzawai (NTK14a)	*aliquot of DNA ex New Zealand*	*Negative*	
T. neocaladonicus (T14-3298)	*aliquot of DNA ex New Zealand*	*Negative*	
T.urticae (AM18-49)	*Confirmed as T. urticae by qPCR and sequencing*	*TSM*	
(unknown) AM18-117	*PrepGem extraction- qPCR and ITS sequencing failed*	*TSM*	
(unknown) AM18-126	*Inconclusive on qPCR; NIL or Ct value >36*	*TSM*	
T.urticae (AM18-41)	*Confirmed as T. urticae by qPCR and sequencing*		*not tested*
T.urticae (AM18-44)	*Confirmed as T. urticae by qPCR and sequencing*	*Negative*	*No DNA left*
(Unknown) AM18-118	*PrepGem extraction- qPCR and ITS sequencing failed*	*negative*	

(Unknown) AM18-127	*Inconclusive on qPCR; NIL or Ct value >36*	*Negative*
(Unknown) AM18-27	*Inconclusive on qPCR; NIL or Ct value >36*	*Negative*
(Unknown) AM18-58	*Inconclusive on qPCR; NIL or Ct value >36*	*Negative*

mixtures of two-spotted mite and banana or strawberry mite with bean spider mite completely absent. The result shows the difficultly of trying to maintain pure cultures is close proximity in a mass culture facility. Many strains that were not regularly checked (such as reference strain) were heavily contaminated after a relatively short period in laboratory culture. For instance strain F2020 Cole that was collected during season 2018-2019 showed significant contamination while banana or strawberry spider mite and strain Glen 30 was completely overrun by two-spotted mite since its 2016-2017 season collection.

Table 4.3.1 Testing of reference and field collected cotton spider mite populations

Population	***No tested***	***PCR Result***	***Collection note***
S*	10	100% *T. urticae*	EMAI reference
Nashee*	10	100% *T. urticae*	EMAI reference
F2020 Cole	7	57% *T. urticae*, 43% *T. lambi*	TSM 18-19
Griffith 74	20	100% *T. urticae*	TSM 18-19
Moree Colmlee 3	20	90% *T. urticae* 10% *T. lambi*	TSM 18-19
Griffith 35	20	95% *T. urticae*, 5% *T. lambi*	TSM 18-19
Hills K4	9	56% *T. urticae*,44% *T. lambi*	TSM 18-19
Brai	10	100% *T. urticae*	TSM 17-18
Broo34	10	100% *T. urticae*	TSM 17-18
Broo36	10	100% *T. urticae*	TSM 17-18
Merk2	10	90% *T. urticae* 10% *T. lambi*	TSM 17-18
Warr	20	100% *T. urticae*	TSM 17-18
WhitP4	20	100% *T. urticae*	TSM 17-18
WhitNFOI	20	80% *T. urticae* 20% *T. lambi*	TSM 17-18
Glen30	20	100% *T. urticae*	SSM 16-17
Carb*	40	100% *T. lambi*	EMAI reference
Toob_2	20	100% *T. lambi*	SSM 16-17
Toob_7	20	100% *T. lambi*	SSM 16-17
TexCar	20	100% *T. lambi*	SSM 16-17
Jakins	20	100% *T. lambi*	SSM 16-17
Grove	20	100% *T. lambi*	SSM 16-17
Walla	9	56% *T. urticae*,44% *T. lambi*	SSM 17-18
MoreA4	20	25% *T. urticae*,75% *T. lambi*	SSM 17-18

Goon13	10	100% *T. lambi*	SSM 17-18
BSM_S*	pool	*T. ludeni*	*EMAI reference*
BSM_Melon*	pool	*T. ludeni*	*EMAI reference*

*EMAI known reference strain

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

5.1 Question: Resistance levels in aphids and two-spotted mite?

Expected output: Resistance monitoring data generated for aphids and mites to assess chemical resistance and if required modification to the IRMS.

Expected outcome: Resistance monitoring data fed directly into the *Cotton Pest Management Guide* at annual TIMS Technical review meetings with management strategies modified according to most recent data.

Management outcomes have been further communicated to industry via industry conferences (see publications 1, 2, 3, 5, 7) and industry publications (see publications 4, 12, 19), scientific conferences (see publications 9, 10, 14, 18, 21) and scientific publications (see publications 6, 8, 11, 15, 16, 17, 20, 22, 23, 24, 28).

5.2 Question: Is the cotton mite complex changing?

Expected output: The relative abundance of the three different species i.e. two-spotted, strawberry or bean spider mite quantified.

Expected outcome: Publication in a refereed scientific journal (see publication 26), the Australian CottonGrower (see publication 27) and industry conferences (see publication 13, 25).

5.3 Question: Potential chemical controls for strawberry and bean spider mite?

Expected output: Laboratory culturing methods for strawberry and bean spider mite plus new laboratory bioassay methodology based on modified two-spotted mite methodology.

Expected outcome: As above for 5.2, publication in a refereed scientific journal (see publication 26), the Australian CottonGrower (see publication 27) and industry conferences (see publication 13, 25).

5.4 Question: Field based resistance detection capacity for mirids?

Expected output: Baseline data established against mirids for dimethoate, fipronil and clothianidin.

Expected outcome: Publication in refereed scientific journals, the Australian CottonGrower, Cotton Tails and conferences.

Baseline data was established (see publication 13) but as the project evolved laboratory culture of mirids was developed and successfully implemented in season 2018-2019 making the field based methodology redundant.

5.5 Question: Transition of two-spotted mite resistance testing to molecular techniques?

Expected output: Molecular based methods to detect organophosphate, pyrethroid and abamectin resistance in two-spotted mite.

Expected outcome: Publication in refereed scientific journals, the Australian CottonGrower, Cotton Tails and conferences.

The molecular based methods component of the study was significantly expanded beyond two-spotted mite. It included other species and three more unplanned research questions requiring a significant project variation. Despite this 16 Australian two-spotted mite strains were screened for all target site mutations including organophosphate, pyrethroid and abamectin (see publication 8) and a specific molecular diagnostic developed for etoxazole (see publication 22).

5.6 Question: DNA Library developed?

Expected output: A DNA library containing the SNP sequences of known resistance causing mutations.

Expected outcome: Publication in refereed scientific journals the Australian CottonGrower, Cotton Tails and conferences.

The DNA library produced is significant (see appendix B) and although not a stand-alone publication it has been instrumental in multiple publication outputs and outcomes generated (see publications 8, 16, 18, 21, 22, 24, 28).

5.7 Question: Resistance detection in cotton seedling thrips using both bioassay and molecular techniques

Expected output: Baseline data generation for Cotton Seedling Thrips against key chemical controls.

Expected outcome: Publication in refereed scientific journals the Australian CottonGrower, Cotton Tails and conferences.

Dose response data was generated for dimethoate, λ cyhalothrin and imidacloprid confirming grower anecdotal observations that neonicotinoid seed dressings were not working as they once did due to resistance. A resultant warning was included into the 2018-2019 *Cotton Pest Management Guide* and industry publication (see publication 27). A molecular diagnostic was developed (see results 4.1.4 *Cotton seedling thrips and western*

flower thrips) but could not be used because western flower thrips displaced cotton seedling thrips in season 2018-2019 (see publication 27).

5.8 Question: *Helicoverpa* / indoxacarb resistance with the aim of finding the causal SNP and verified molecular diagnostic?

Expected output: Identification of the causal resistance mechanism and a molecular diagnostic.

Expected outcome: Publication in refereed scientific journals, the Australian CottonGrower, Cotton Tails and conferences.

The causal mechanism was found and a molecular diagnostic developed (see publications 18, 24) but study collaborator Lisa Bird found a second resistance mechanism making use of a single mechanism diagnostic impractical.

5.9 Question: Development of a field DNA based kit for spider mite identification.

Expected output: Development of a field-based kit to delineate spider mite species using DNA-methodology.

Expected output: Publication in refereed scientific journals the Australian CottonGrower, Cotton Tails and conferences.

A field based kit was developed (see methods section 3.4 DNA based kit for spider mite identification) but the envisaged field use was changed from cotton to biosecurity mite intercept identification (see publication 28). The methodology has been successfully used in the field by Biosecurity Australia and to date some 2,125 samples done (Gregory Chandler pers. comm.).

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

No patents or licenses have been granted or applied for.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

Genotype by sequencing has been used for the first time with cotton bollworm *Helicoverpa armigera* to isolate a causal SNP and produce a molecular diagnostic.

c) required changes to the Intellectual Property register.

The IP register has already been updated to account for the extra work done i.e. thrips, cotton bollworm *Helicoverpa armigera* and mite identification.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

Superficially management of the sucking pests in Australian cotton (excluding whitefly) seem to be in a reasonable situation. However, if the past is a predictor of the future this state of affairs is unlikely to continue for seasons to come as any change in the agroecosystem will significantly change pest and / or predator interaction.

For instance the agroecosystem is currently in drought making it exceedingly difficult for cotton aphid to overwinter. Bioassay in this study indicates cotton aphid has been pushed to its limits of neonicotinoid tolerance requiring discriminating dose adjustment. When the drought finally ends and aphids build in number and are again targeted specifically for control resistance including neonicotinoid resistance will undoubtedly return.

The study has shown the mite complex in Australian cotton is dynamic and subject to change. Banana or strawberry spider mite is currently dominant but remains insecticide susceptible. Any change to the agroecosystem that causes mites to be again sprayed will favour two-spotted mite as they remain resistant to many chemicals. If sprayed two-spotted mite will again quickly dominate causing economic damage.

The study has shown that some cotton seedling thrips are resistant to the neonicotinoid seed dressings used for their control. If the cotton agroecosystem changes and thrips become more abundant pressure on early season cotton by thrips would likely increase and could result in seeding dressing failures. Additionally the thrips complex in Australian cotton has a new contender in the form of western flower thrips. Worryingly western flower thrips is already resistant to many chemicals used for its control including the only registered control in Australian cotton spinetoram (Success Neo®). Such resistance makes targeted control against western flower thrips in Australian cotton potentially unreliable.

Mirids continue to be sprayed regularly in Australian cotton and the risk of insecticide resistance is ever present. If failures do eventuate in this study we established the technology to effectively transport live mirid samples from the field and establish them in laboratory culture. From these cultures fipronil, dimethoate or sulfoxaflor resistance can be diagnosed and appropriate management initiated.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:
(a) to further develop or to exploit the project technology.

Extension has already been actively pursued during the study with the latest resistance management results being fed directly into the *Cotton Pest Management Guide*.

(b) for the future presentation and dissemination of the project outcomes.

Project outputs and outcomes have already been well disseminated via Industry conferences or meetings (see publications 1, 2, 3, 5, 13, 25) and industry publications (see publications 4, 7, 19, 27).

(c) for future research.

The study has done much to transitions resistance detection from bioassay to more cost effective DNA based methodology. There is enormous scope to pursue this transition and turn routine resistance detection over to DNA based monitoring. This is particularly applicable to *Helicoverpa* sp. where recessive resistance(s) detection requires very time consuming crossing to produce homozygous resistant test subjects.

9. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

1. **Wilson, L., Downes, S., Herron, G., Sequeira, R., Grundy, P. and Macpherson I. (2014) Back to the future: IPM, whitefly & *Helicoverpa* including the history of resistance. *In*: Seminar Proceedings, Goondiwindi 16-17 July 2014. Crop Consultants Australia.**

2. **Herron, Grant A., Suann, Monica J., Woolley, Lauren K., Chen, Yizhou & Wilson, Lewis J. (2014) Resistance management of cotton aphid, two-spotted mite and mirids. In: *17th Australian Cotton Conference, 5 – 7 August 2014, Gold Coast Australia*. http://www.australiancottonconference.com.au/2014-presentations-papers/herron-grant-suann-monica-wooley-lauren-chen-yizhou-amp-wilson-lewis**

3. **Marshall, Kate. L., Yizhou, Chen., Herron, Grant. A. (2014) Neonicotinoid resistance in cotton aphid from Australia In: *17th Australian Cotton Conference, 5 – 7 August 2014, Gold Coast Australia.* http://www.australiancottonconference.com.au/2014-presentations-papers/marshall-kate-chen-yizhou-herron-grant**

4. **Grant Herron (2015) Resistance management of cotton aphid and two-spotted mite. pp. 26-27. In: Melanie Jenson Ed., *Spotlight on Cotton R&D*, *Summer 2014-15.* CRDC, Narrabri.**

5. **Grant A. Herron (2015) Resistance in cotton aphid, two-spotted mite and mirids pp. 63-66. In: *CCA Cropping Solutions Seminar*, Griffith, 12-13 May 2015. Crop Consultants Australia, Woombye.**

6. ***Monica Suann, Daniel R. Bogema, Yizhou Chen, Sarah Mansfield, Idris M. Barchia & Grant A. Herron (2015) A TaqMan qPCR method for detecting *kdr* resistance in *Aphis gossypii* demonstrates improved sensitivity compared to conventional PCR–RFLP. *Journal of Pest Science* 88, 785-791. J Pest Sci DOI 10.1007/s10340-015-0651-5.**

7. **Grant Herron (2015) Chemical control and chemical resistance, their relationship and management In*: Minor Use Education Symposium*, Conrad Jupiters & Casino, Gold Coast Australia, 27 June 2015. AusVeg, Camberwell.**

8. **Woolley LK, Chen Y and Herron GA (2015) Investigation of target site resistance mechanisms in sixteen Australian cultures of *Tetranychus urticae* (Tetranychidae: Acari). p 52. In: 2nd Australian Cotton Research Conference, Science Securing**

Cotton's Future, 8-10 September 2015, University of Southern Queensland, Toowoomba. The Association of Australian Cotton Scientists.

9. **Marshall KL & Herron GA (2015) The need for a field based method to detect to detect resistance in green mirid *Creontiades dilutes* (Stål) (Miridae: Hemiptera) from Australian cotton. p 57. In: 46th AGM and Scientific Conference, 27-30 September 2015. Australian Entomological Society.**

10. **Grant Herron (2015) Resistance detection and management of secondary sucking pest of Australian cotton. p. 12. In: The 3rd Australian Agrichemical Resistance Meeting, 12th November 2015, The University of Melbourne, Melbourne, Victoria.**

11. ***Marshall KL, Wilson LJ & Herron GA. 2015. Efficacy of two thiamethoxam pre germination seed treatments and a phorate alternative against neonicotinoid and pirimicarb resistant *Aphis gossypii* Glover (Hemiptera: Aphididae). *Austral Entomology* 54, 351-357.**

12. **Grant A. Herron and Lewis J. Wilson (2016) Mite resistance danger from over-use of abamectin. *The Australian Cottongrower February–March 2016* 37, 14-16.**

13. **Kate Marshall and Grant Herron (2016) The correct identification of spider mite species in cotton and development of a field based bioassay for resistance detection in mirids. In: *CCA Cropping Solutions Seminar 4-5 May 2016*. Crop Consultants Australia Ltd, Woombye QLD.**

14. **Herron GA and Wilson LJ (2016) Resistance management of *Aphis gossypii* Glover in Australian cotton; from a highly pesticide reliant system to an integrated IRM and IPM strategy. In: *XXV International Congress of Entomology 25th-30th September 2016*. Entomological Society of America.**

15. ***Herron GA and Wilson LJ (2017) Can resistance management strategies recover insecticide susceptibility in pests?: a case study with cotton aphid *Aphis gossypii* (Aphididae: Hemiptera) in Australian cotton. *Austral Entomology* 56, 1-13. DOI: 10.1111/aen.12236**

16. ***Sarah Tieu, Yizhou Chen, Lauren K. Woolley, Damian Collins, Idris Barchia, Nathan Lo and Grant A. Herron (2017) A significant fitness cost associated with *ACE*1 target site pirimicarb resistance in a field isolate of *Aphis gossypii* Glover from Australian cotton. *J Pest Sci*. 90:773-779. DOI 10.1007/s10340-016-0803-2.**

17. ***Lauren K Woolley, Yizhou Chen, Kate L Langfield and Grant A Herron (2017) High quality DNA extraction from *Helicoverpa armigera* for next generation sequencing. *General and Applied Entomology* 45, 59-64.**

18. **Yizhou Chen, Lauren Woolly, Kate Langfield, Lisa Bird and Grant Herron (2017) Linkage mapping of an indoxacarb resistance gene isolated from a field population of *Helicoverpa armigera* via genotype-by-sequencing. The Association of Australian Cotton Scientists, Cotton Research Conference 5-7 September 2017, CSIRO Discovery Centre, Canberra.**

19. **Whitehouse, Mary; Herron, Grant; Heimoana, Simone; Wilson, Lewis (2017) What is the value of IPM in cotton production systems? High Sustainable profit. *The Australian Cotton Grower*. *The Cotton Yearbook* 2017, Volume 38 (6): 154-159.**

20. ***Lewis J Wilson, Mary EA Whitehouse, Grant A Herron (2018) The Management of Insect Pests in Australian Cotton: An Evolving Story. *Annual Review of Entomology* 63, 215-237.**

21. **Yizhou Chen, Bogema D. Barchia I and Herron G. (2018) Insecticide resistance monitoring with qPCR and next generation sequencing. In: *Symposium: 2018 NGS, dPCR & qPCR - 24 & 25 May*, Harbourview Ballroom, Taronga Centre Sydney, Australia.**

22. ***Grant A Herron, Lauren K Woolley, Kate L Langfield and Yizhou Chen (2018) First detection of etoxazole resistance in Australian two-spotted mite *Tetranychus urticae* Koch (Acarina: Tetranychidae) via bioassay and DNA methods *Austral Entomology* 57, 365-368. Version of Record online: 5 JUL 2017 | DOI: 10.1111/aen.12290**

23. ***Kate L. Langfield, Lauren K. Woolley, Stewart Learmonth and Grant A. Herron (2018) Spinetoram resistance detected in western flower thrips *Frankliniella occidentalis* (Pergande) following a control failure. *General and Applied Entomology* 46, 43-45.**

24. ***Yizhou Chen, Lisa J Bird, Lauren K Woolley, Tom Walsh, Karl Gordon and Grant A Herron (2019) Linkage mapping an indoxacarb resistance locus in *Helicoverpa armigera* (Lepidoptera: Noctuidae) by genotype-by-sequencing. July 2019 *Pest Management Science* DOI: 10.1002/ps.5557.**

25. **Grant A Herron & Kate L Langfield (2019) The Changing Spider Mite Complex in Australian Cotton (& an Update on Thrips). In: CCA *Cropping Solutions Seminar*, Crossing Theatre Narrabri, 20-21 June 2019. Crop Consultants Australia.**

26. ***Kate L. Langfield and Grant A. Herron (2019) The evolving spider-mite complex in Australian transgenic *Bt*-cotton cotton necessitates a *Tetranychus lambi* Pritchard and Baker (Tetranychidae: Acarina) baseline study to aid sustainable chemical control. Currently with *Austral Entomology* for their consideration to publish.**

27. **Grant A. Herron (2019) Is the sucking pest complex in Australian cotton changing? Currently with the Australian CottonGrower Magazine for consideration to publish.**

28. ***The development and use of a single PCR assay to identify the three spider mite species *Tetranychus urticae*, *T. lambi* and *T. ludeni* (Acari: Tetranychidae) in Australia. Draft in preparation**
29.

NB * indicates refereed journal contribution

B. Have you developed any online resources and what is the website address?

No

Part 4 – Final Report Executive Summary

Provide a one-page summary of your research that is not commercial in confidence, and that can be published on the internet. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

- Cotton aphid, two-spotted mite (TSM), banana or strawberry spider mite (SSM), cotton seedling thrips, western flower thrips (WFT) and green mirid were collected from Australian cotton growing regions.
- TSM regularly showed discriminating dose survivors against bifenthrin (Talstar®) and abamectin (Agrimec®) but seldom propargite (Comite®) or etoxazole (Paramite® or Zeal®) and never diafenthiuron (Pegasus® as CGA140408).
- Bifenthrin (Talstar®) and abamectin (Agrimec®) resistance detected in TSM was often at high frequency so may fail. The reason for this is not clear because pyrethroid use in cotton is limited and TSM do not fly so their immigration from sprayed crops other than cotton must be limited. It is speculated that the continuing increase in abamectin (Agrimec®) resistance is due to its use as a preventative treatment with mirid sprays. Mirid sprays are disruptive to beneficials so the inclusion of abamectin reduces the risk of subsequent mite flare.
- SSM was the most found mite species in Australian cotton confirming anecdotal observations that the cotton mite complex has changed and is no longer TSM dominant. Additionally TSM was restricted to NSW only and bean spider mite (BSM) was not collected so remains absent from Australian cotton.
- To allow resistance monitoring SSM baseline data was established for abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA140408) and monitoring commenced but none was detected.
- A DNA based method to identify SSM, TSM and BSM was developed and successfully deployed by Biosecurity Australia where it has been used to identify more than 2000 quarantine mite intercepts.
- Cotton aphid was tested for pirimicarb (Pirimor®), OP-specific, pyrethroid, clothianidin (Shield®), diafenthiuron (Pegasus® as CGA140408), thiamethoxam (Actara or Cruiser®) and sulfoxaflor (Transform®) resistance. Interestingly pyrethroid resistance was often detected although it is not registered for this use in cotton and pirimicarb (Pirimor®) and OP-specific resistance was not detected so these chemicals can be used with confidence. Neonicotinoid survivors were detected in some strains but later thought vigour tolerant rather than resistant requiring discriminating dose adjustment to eliminate those false positives.
- Methods to transport and culture green mirid were developed making resistance detection possible with established laboratory based bioassay technology. Green

mirid was screened for fipronil (Maestro® or Albatross®) resistance using a molecular-based diagnostic and none was detected.

- Neonicotinoid resistance was detected in cotton seedling thrips confirming anecdotal consultant / grower observations that seed treatments may not be working as well as they did. Unexpectedly WFT was the most abundant thrips found and worryingly spinetoram (Success® Neo)(the only registered control in cotton) resistance was detected in some strains.
- Much effort was put into transitioning resistance detection away from conventional bioassay to DNA based techniques. To this end indoxacarb resistance in cotton bollworm *Helicoverpa armigera* was extensively studied via genotype-by-sequencing and a diagnostic developed. Unexpectedly a second resistance mechanism was found preventing practical use of that diagnostic.
- A molecular diagnostic was successfully developed and deployed against TSM and etoxazole (Paramite® or Zeal®).
- Molecular methods to detect neonicotinoid in cotton seedling thrips and spinetoram (Success Neo®) in WFT were developed but require further validation.

Appendix A

Annual Resistance Testing Summaries

Resistance testing summary for the 2014-2015 cotton season: cotton aphid *Aphis gossypii* and two-spotted mite *Tetranychus urticae*

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Summary

- Cotton aphid (*Aphis gossypii*) and two-spotted mite (TSM)(*Tetranychus urticae*) were collected from Australian cotton growing regions.
- TSM showed discriminating dose survivors against bifenthrin (Talstar®), abamectin (Agrimec®) and propargite (Comite®).
- More than 80% of TSM strains were bifenthrin (Talstar®) resistant with one strain comprising 96% resistant individuals.
- Similarly, abamectin (Agrimec®) resistance was detected in over 70% of TSM strains with one strain comprising 75% resistant TSM. As previously stated this may relate to abamectin being applied as a prophylactic treatment for mites in combination with mirids sprays.
- In 2010-2011 some 96% of cotton aphid strains tested showed some level of neonicotinoid resistance (ie Actara®, Cruiser® or Shield®) but that had fallen to 29% in season 2011-2012 and a similar 33% of strains in season 2012-2013. We speculated in 2013 that neonicotinoid resistance may settle at approximately 30% of populations tested, however, in season 2013-2014 neonicotinoid resistance was not detected in any strain collected off cotton. Interestingly, resistance has again been detected in 31% of strains collected in 2014-2015.
- Pirimicarb (Pirimor®) and OP-specific resistance was not detected in any aphid strain so these compounds can be used with confidence.

Introduction

With the introduction of transgenic cotton in Australia to control cotton bollworm *Helicoverpa armigera*, a reduction in chemical insecticide usage has occurred. Subsequently, there has been an increase in populations of sucking insect pests such as green mirid (*Creontiades dilutes*) and green vegetable bug (*Nezara viridula*). Control of these pests with broad-spectrum insecticides depletes beneficial insect populations and allows other secondary pests such as two-spotted mite (TSM)(*Tetranychus urticae*) and cotton aphid (*Aphis gossypii*) to increase in abundance and if controlled are inevitably selected for insecticide resistance. Dealing with the likelihood of such resistance development requires on-going monitoring to key pesticides if future control problems are to be averted.

Cotton aphid is resistant to a range of insecticides in many crops and countries. Some fifteen years ago high-level resistance to organophosphates (omethoate (Folimat®) and dimethoate (Rogor®)) and cross-resistance to some carbamates (pirimicarb (Pirimor®)) developed in cotton aphid strains in Australian cotton regions causing failures (Herron *et al*. 2001). Effective resistance management caused products to be

recovered by about season 2005-2006 allowing the pirimicarb/dimethoate/omethoate to once again be used effectively. Neonicotinoid resistance was detected in season 2007-2008 and increased in both level and abundance over the next 3-4 years again causing failures (Herron and Wilson 2011). The aphid resistance management strategy was modified to reduce selection yet neonicotinoid resistance in cotton aphid continued to increase and was detected in 96% of strains tested during season 2010-2011. From that season resistance declined to 33% in season 2012-2013 and was not detected at all in season 2013-2014.

TSM is notorious world-wide for developing resistance to insecticides and acaricides including in Australia. In Australian cotton, TSM has developed resistance to dimethoate (Folimat®) / omethoate (Folimat®) (1970's), monocrotophos (Nuvacron®) (1980's), profenofos (Curacron®) (1980's) and bifenthrin (Talstar®) (1990's). TSM resistance continues to evolve in Australian cotton and most recently caused chlorfenapyr (Intrepid®) resistance (Herron *et al*. 2004).

For both aphids and TSM, resistance management requires ongoing vigilance. Especially so because several key products used against aphids or TSM also target other species: diafenthiuron (Pegasus®) (aphids, mites, silverleaf whitefly (SLW)), abamectin (Agrimec®) (*H. punctigera* and mites) and spirotetramat (Movento®) (aphids and SLW). Continued insecticide resistance monitoring is essential for effective ongoing resistance management of these pests.

Here we present monitoring data for season 2014-2015.

Methods

Chemicals tested

Aphids were treated with clothianidin (Shield®), diafenthiuron (Pegusus®), sulfoxaflor (Transform®), and thiamethoxam (Actara®). All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron (CGA-140408) was tested. This is necessary because diafenthiuron (Pegusus®) is activated by exposure to UV light, which would not normally occur in the laboratory. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method.

TSM were treated with abamectin (Agrimec®), bifenthrin (Talstar®), propargite (Comite®), and diafenthiuron (as CGA-140408).

Aphid collection and culturing

Aphids were collected by researchers, CRC Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden (Elizabeth McArthur Agricultural Institute (EMAI)) and each field strain was cultured separately on pesticide-free cotton at 25 ± 4 °C under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages.

Aphids - resistance detection

Via Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001). Briefly, batches of thirty adult female aphids per leaf disc were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower (to yield percent insecticide susceptible). All tests were replicated (unless otherwise marked) and included a water-only sprayed control. After spraying, clear plastic film was used to cover the Petri dishes. Aphids were then maintained at 25 ± 0.1 °C in 16:8 L:D for 24 h after which mortality was assessed.

Molecular. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method. The qPCR method for estimating pyrethroid, OP and pirimicarb (Pirimor®) resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. For each 200 aphid sample, pooled DNA is extracted and triplicate qPCRs are carried out using two TaqMan probes (one detects the resistance allele and the other detects the susceptible allele). The ratio of the fluorescence intensity produced for each qPCR reaction, is calculated along with a standard reference series, whose resistance allele frequency is known. The resistance allele frequency from field populations is then accurately estimated based on the ratio of fluorescence increase between the resistance and susceptible probe (Chen *et al*. 2014).

Two-spotted mite

Strains of TSM were collected from a range of cotton fields in northern NSW and Qld and put into culture as above at EMAI. The bioassay procedure required fifteen to twenty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites and leaf discs were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower as above. Each test was replicated (unless otherwise indicated) and included a water only sprayed control. After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Results

Nine strains of TSM were put into culture and tested for resistance (Table 1). TSM showed discriminating dose survivors against all chemicals tested except diafenthiuron (Pegasus®). Survivors were restricted to a few in one strain against propargite (Comite®) but in contrast bifenthrin (Talstar®) and abamectin (Agrimec®) resistance was detected in 89 and 67% of strains tested respectively. One abamectin (Agrimec®) resistant strain known as Car comprised 75% resistant individuals with bifenthrin (Talstar®) resistance at 96% in strain Valo.

Thirteen strains of cotton aphid were put into culture and tested for resistance (Table 2). No Pirimicarb (Pirmor®), organophosphate specific, diafenthiuron (Pegasus®) or sulfoxaflor (Transform®) resistance was detected (Table 2). Four strains showed low 1% frequency neonicotinoid resistance with two showing clothianidin (Shield®) and a different two showing thiamethoxam (Actara or Cruiser®) survivors (Table 2). Although not registered for aphid control pyrethroid resistance was again detected in three Strains with strain Pamp comprising 36% resistant aphids (Table 2).

Discussion

In the 2007-2008 season neonicotinoid resistance was detected and then increased in both level and abundance during the following seasons, peaking at 96% of strains tested for season 2010-2011. However, over the next two seasons resistance decline to about 30% (29% for season 2011-2012 and 33% for season 2012-2013). This caused us to speculate that neonicotinoid resistance may stabilise at about 30% of strains showing resistance. Unexpectedly, neonicotinoid resistance was not detected in any strains the following season (2013-2014). However, it has again been detected in 31% of strains tested in 2014-2015 (although at a very low frequency in the individual strains).

Bifenthrin (Talstar®) resistance in TSM from cotton has been relatively common with strains often showing a few percent resistant mites. Last season one strain of TSM known to have been specifically targeted with bifenthrin (Talstar®) had 21% resistant individuals. Although a strain with 66% resistant individuals was seen in 2011-2012 it is still somewhat disturbing that in 2013-2014 60% of strains tested contained bifenthrin resistance with one strain of TSM essentially 100% resistant. For 2014-2015 more than 80% strains contained bifenthrin (Talstar®) resistant TSM and again with one strain essentially 100% resistant. It is noteworthy then that average bifenthrin use in Bollgard® cotton started to increase from 2005 and by 2010 was about 20 g ai ha^{-1} where it then decreased to about 3 g ai ha^{-1} the following year but went back to about 25 g ai ha^{-1} in 2013 and then remained under 4 g ai ha^{-1}.

Similarly, abamectin (Agrimec®) resistance monitoring against TSM has rarely detected positive resistance results until season 2010-2011, when abamectin resistance was detected in three out of the four TSM strains tested. Again in 2011-2012 resistance was detected in a single TSM strain and in 2012-2013 abamectin resistance was again detected in two strains with 6 and 7% resistant TSM. During the 2013-2014 season abamectin was detected in six out of the eleven (54%) strains tested with one comprising 79% resistant TSM. Season 2014-2015 again saw resistance detected in six out of the nine (67%) strains with the highest frequency detected being 75% resistant with two others containing 74 and 72% resistant TSM. The reason this continuing increase in abamectin (Agrimec®) resistance has been previously speculated to relate to the low price of abamectin. For that reason it can be applied as a prophylactic treatment in combination with mirid sprays. These mirid sprays are often disruptive of beneficials so the inclusion of abamectin reduces the risk of subsequent mite outbreaks. However, this usage pattern will ultimately lead

to failure of abamectin against concurrent mites so needs to be addressed. Finally, propargite (Comite®) is a mainstay control for TSM so it is of some concern that resistance to it in TSM continues to be consistently detected season to season (although at low frequency).

Acknowledgments
Dr Yizhou Chen is thanked for overseeing the technical aspects of the molecular PCR assays. Lauren Woolley assisted with the molecular testing and Monica Suann assisted with the aphid and mite bioassay plus their culturing. The many researchers, CRC Regional Extension Officers, consultants and growers who collected aphids and mites are thanked. This study is funded by the CRDC (DAN1203). Finally, Lewis Wilson is thanked for critically reading an early version of this report.

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Table 1. Percent mortality at the discriminating dose (ie percent susceptible) for various strains of TSM collected during season 2014-2015 and evaluated for resistance against bifenthrin (Talstar®), abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® (CGA-140408))

Strain	**Bifenthrin (Talstar®)**	**Abamectin (Agrimec®)**	**Propargite (Comite®)**	**Diafenthiuron (Pegasus® CGA140408)**
Bro	83%	100%	100%	100%*
Car	58%	25%	100%	100%
Dob	31%	26%	100%	100%
Loc	97%	100%	100%	100%
Pyj	28%	28%	100%	100%
Trin	44%	36%	99%	100%
Valo	4%	33%	100%	100%
War	52%	44%	100%	100%
Whit	100%	100%	100%	100%

* Discriminating dose survivors traced back to product anomaly.

Table 2. Percent pirimicarb / dimethoate (Pir / Dim), pyrethroid (Pyr) and organophosphate specific (profenofos / chlorpyrifos [Pro / Chl]) susceptible using molecular diagnosis plus bioassay determination of clothianidin (Clo)(Shield®), diafenthiuron (Dia)(CGA140408)(Pegasus®), and thiamethoxam (Thia) (Actara or Cruiser®) and sulfoxaflor (Sul)(Transform®) resistance via percent mortality at the discriminating dose (ie percent susceptible) for cotton aphid collected during season 2014-2015

Culture Name	Pir / dim qPCR S431F	Pro / Chl qPCR S302A	Pyr qPCR KDR	Dia 0.003%	Clo 0.005 %	Thia 0.002 %	Sul 0.001%
Hor	100	100	100	100	100	99	100
Jill	100	100	100	100	100	100	100
Spring	100	100	94	100	100	100	100
Curr	100	100	100	100*	99	100	100
Moon	100	100	100	100	100	100	100
West	100	100	100	100*	99	100	100
Plant	100	100	100	100	100	100	100
Tim	100	100	100	100	100	99	100
Vall	100	100	100	100*	100	100	100
Why	100	100	100	100	100	100	100
Toob	100	100	64	100	100	100	100
Pamp	100	100	83	100	100	100	100
Kog	100	100	100	100*	100	100	100

*Discriminating dose survivors traced back to product anomaly.

Resistance testing summary for the 2015-2016 cotton season: cotton aphid *Aphis gossypii* and two-spotted mite *Tetranychus urticae*

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Summary

- Cotton aphid (*Aphis gossypii*) and two-spotted mite (TSM)(*Tetranychus urticae*) were collected from Australian cotton growing regions.
- TSM showed discriminating dose survivors against bifenthrin (Talstar®), abamectin (Agrimec®) and propargite (Comite®).
- Future effective use of bifenthrin (Talstar®) against TSM may not be possible as 100% of strains tested contained resistant individuals.
- Abamectin (Agrimec®) resistance was detected in 78% of strains tested with one strain comprising 73% resistant individuals.
- Low frequency propargite resistance continues to be detected in some TSM strains.
- Etoxazole (Paramite®) resistance via the I1017F mutation was absent in all TSM strains tested. As a result, etoxazole (Paramite®) has potential to be incorporated into a resistance management strategy to reduce abamectin (Agrimec®) selection pressure against TSM.
- Pirimicarb (Pirimor®) and OP-specific resistance were not detected in any cotton aphid strain so these compounds can be used with confidence.
- Low frequency neonicotinoid resistance (<2%) was detected in 60% of the cotton aphid strains tested which is a worrying trend up.
- Green mirid were screened for resistance to fipronil using a molecular-based diagnostic. Fortunately no resistance was detected but future work should seek to develop a reliable bioassay technique to verify any future molecular result.

Introduction

With the introduction of transgenic cotton in Australia to control cotton bollworm *Helicoverpa armigera*, a reduction in chemical insecticide usage has occurred. Subsequently, there has been an increase in populations of sucking insect pests such as green mirid (*Creontiades dilutus*) and green vegetable bug (*Nezara viridula*). Control of these pests with broad-spectrum insecticides depletes beneficial insect populations and allows other secondary pests such as two-spotted mite (TSM)(*Tetranychus urticae*) and cotton aphid (*Aphis gossypii*) to increase in abundance and if controlled are inevitably selected for insecticide resistance. Dealing with the likelihood of such resistance development requires on-going monitoring to key pesticides if future control problems are to be averted.

Cotton aphid is resistant to a range of insecticides in many crops and countries. Some fifteen years ago high-level resistance to organophosphates (omethoate (Folimat®) and dimethoate (Rogor®)) and cross-resistance to some carbamates (pirimicarb

(Pirimor®)) developed in cotton aphid strains in Australian cotton regions causing failures (Herron *et al.* 2001). Effective resistance management caused products to be recovered by about season 2005-2006 allowing the pirimicarb/dimethoate/omethoate to once again be used effectively. Neonicotinoid resistance was detected in season 2007-2008 and increased in both level and abundance over the next 3-4 years again causing failures (Herron and Wilson 2011). The aphid resistance management strategy was modified to reduce selection yet neonicotinoid resistance in cotton aphid continued to increase and was detected in 96% of strains tested during season 2010-2011. From that season resistance declined to 33% in season 2012-2013 and was not detected at all in season 2013-2014.

TSM is notorious world-wide for developing resistance to insecticides and acaricides including in Australia. In Australian cotton, TSM has developed resistance to dimethoate (Rogor®) / omethoate (Folimat®) (1970's), monocrotophos (Nuvacron®) (1980's), profenofos (Curacron®) (1980's) and bifenthrin (Talstar®) (1990's). TSM resistance continues to evolve in Australian cotton and most recently caused chlorfenapyr (Intrepid®) resistance (Herron *et al.* 2004).

Control of green mirid requires targeted spraying and resistance is a possibility (Herron and Rophail 2008). Laboratory bioassay resistance detection was developed but significant issues remain unsolved (Herron and Rophail 2008). Bioassay of mirids *per se* is not difficult but their fragile bodies and recalcitrant ability to establish into laboratory culture makes resistance confirmation problematic (Herron 2011). For this reason mirid resistance monitoring will benefit enormously from molecular based testing and such a method has been developed to detect fipronil.

For both aphids and TSM, resistance management requires ongoing vigilance. Especially so because several key products used against aphids or TSM also target other species: diafenthiuron (Pegasus®) (aphids, mites, silverleaf whitefly (SLW)), abamectin (Agrimec®) (*H. punctigera* and mites) and spirotetramat (Movento®) (aphids and SLW). Continued insecticide resistance monitoring is essential for effective ongoing resistance management of these pests.

Here we present monitoring data for season 2015-2016.

Methods

Chemicals tested

Aphids were treated with clothianidin (Shield®), diafenthiuron (Pegusus®), sulfoxaflor (Transform®), and thiamethoxam (Actara®). All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron (CGA-140408) was tested. This is necessary because diafenthiuron (Pegusus®) is activated by exposure to UV light, which would not normally occur in the laboratory. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method.

TSM were treated with abamectin (Agrimec®), bifenthrin (Talstar®), propargite (Comite®), and diafenthiuron (as CGA-140408). Etoxazole resistance was evaluated via a DNA based method.

Aphid collection and culturing
Aphids were collected by researchers, CRC Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden (Elizabeth McArthur Agricultural Institute (EMAI)) and each field strain was cultured separately on pesticide-free cotton at 25 ± 4 °C under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages.

Aphids - resistance detection
Via Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001). Briefly, batches of thirty adult female aphids per leaf disc were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower (to yield percent insecticide susceptible). All tests were replicated (unless otherwise marked) and included a water-only sprayed control. After spraying, clear plastic film was used to cover the Petri dishes. Aphids were then maintained at 25 ± 0.1 °C in 16:8 L:D for 24 h after which mortality was assessed.

Molecular. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method. The qPCR method for estimating pyrethroid, OP and pirimicarb (Pirimor®) resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. For each 200 aphid sample, pooled DNA is extracted and triplicate qPCRs are carried out using two TaqMan probes (one detects the resistance allele and the other detects the susceptible allele). The ratio of the fluorescence intensity produced for each qPCR reaction, is calculated along with a standard reference series, whose resistance allele frequency is known. The resistance allele frequency from field populations is then accurately estimated based on the ratio of fluorescence increase between the resistance and susceptible probe (Chen *et al*. 2014).

Two-spotted mite
Except for strains of TSM collected from Australian Cotton Research Institute (ACRI) glasshouses mites were sourced from a range of cotton fields in northern NSW and Qld and put into culture as above at EMAI.

Via bioassay. The bioassay procedure required fifteen to twenty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites on leaf discs were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower as above. Each test was replicated (unless otherwise indicated) and included a water only sprayed control. After spraying, mites

on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Molecular. Genomic DNA was extracted using Chelex-100 resin (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions and screened for the presence of the I1017F mutation (Van Leeuwen *et al*. 2012) by direct sequencing of PCR amplicons performed at the Australian Genome Research Facility Ltd. Sequencing results were analysed using Sequencher version 5.2.4 (Gene Codes Corporation).

Mirids

Green mirids were collected from cotton, pigeon pea refuges or mung beans in close proximity to cotton via beat sheet or sweep net and put into analytical grade ethanol and transported to the laboratory for processing.

Molecular. Resistance to fipronil is detected via a mutation in the *Rdl* gene (known associated with fipronil resistance in the ferment fly)(ffrench-Constant *et al*. 1993-). Genomic DNA was extracted from individual green mirids and PCR amplification performed for the *Rdl* gene. Amplified products were then sent to the Australian Genome Research Facility for fipronil sequencing.

Results

Nine strains of TSM were put into culture and tested for resistance (Table 1). No etoxazole resistance was detected. TSM showed discriminating dose survivors against all chemicals tested except diafenthiuron (Pegasus®). Propargite (Comite®) resistance showed a slight increase from last season where only one strain with <1% resistance was detected. This season, three strains displayed resistance to propargite (Comite®) with resistance frequencies varying between 2-17% (Table 1). Bifenthrin (Talstar®) resistance was detected in 100% of strains tested with resistance frequencies varying between 2-39% (Table 1) while abamectin (Agrimec®) resistance was detected in 78% of strains tested with resistance frequencies varying between 12-73% (Table 1).

Ten strains of cotton aphid were put into culture and tested for resistance (Table 2). No pirimicarb (Pirimor®), organophosphate specific, diafenthiuron (Pegasus®) or sulfoxaflor resistance was detected (Table 2). Six strains showed low (<2%) frequency neonicotinoid resistance with four showing thiamethoxam survivors (Actara® or Cruiser®) and a different two showing clothianidin (Shield®) survivors (Table 2). Although not registered for aphid control pyrethroid resistance was detected in three strains with strain Fair comprising 19% resistant aphids.

Six strains of green mirid were collected but sequencing results found no evidence of the fipronil resistance causing point mutation (Table 3).

Discussion

Generally, TSM abundance has been declining in cotton growing areas; however, despite this, TSM populations sourced off cotton appear to be receiving increased selection pressure against registered miticides. Resistance to bifenthrin (Talstar®) was again detected at frequencies warranting concern and continued use should be carefully considered because it would be at best unreliable. Compared to 2014-2015, where eight out of nine (89%) of strains tested contained resistant individuals, this season 100% of strains tested were resistant to bifenthrin (Talstar®)(it should be remembered two strains were not field collected although their responses seem typical of field collected strains). The reason for this increase in frequency is not clear because pyrethroid use in cotton is limited and TSM do not fly (although they can aerially disperse via wind) so their immigration from sprayed crops other than cotton must be limited. Similarly, abamectin (Agrimec®) resistance in TSM has been increasing in frequency since its first detection in a single strain in season 2010-2011. In 2013-2014 season abamectin was detected in six out of the eleven (54%) strains tested and in 2014-2015, resistance detected in six out of the nine (67%) strains. This season, resistance was detected in seven out of the nine strains (78%) with one strain comprising 73% resistant individuals. It has previously been speculated that this continuing increase in abamectin (Agrimec®) resistance is due to its low cost and it being applied as a prophylactic (preventative) treatment in combination with mirid sprays. These mirid sprays are often disruptive of beneficials so the inclusion of abamectin reduces the risk of subsequent mite outbreaks. If continued, this usage pattern will ultimately lead to failure of abamectin against concurrent mites. Promoting beneficials through the use of IPM friendly chemistries will always be the best option. Finally, resistance to propargite (Comite®) continues to be consistently detected season to season (although at low frequency) and should be used judiciously.

In the 2007-2008 season neonicotinoid resistance in cotton aphid was detected and then increased in both level and abundance during the following seasons, peaking at 96% of strains tested for season 2010-2011. However, over the next two seasons neonicotinoid resistance in cotton aphid declined to about 30% (29% for season 2011-2012 and 33% for season 2012-2013). This caused us to speculate that neonicotinoid resistance may stabilise at about 30% of strains showing resistance. Unexpectedly, neonicotinoid resistance was not detected in any strains the following season (2013-2014). However, it has again been detected in 31% of strains tested in 2014-2015 (although at a very low frequency in the individual strains). For season 2015-2016 low frequency clothianidin resistance was again detected in 20% of aphid strains tested. Interestingly, low frequency thiamethoxam resistance was detected in 40% of strains tested that were different to those clothianidin resistant. Overall then 60% of cotton aphid strains contained a small proportion of neonicotinoid resistant cotton aphid strains which is a worrying trend.

This testing season our DNA based screening capacity was expanded to include the miticide, etoxazole (Paramite®); the reason being that etoxazole has the potential to reduce selection pressure against abamectin (Agrimec®). Fortunately, our results

illustrated that etoxazole may be confidently used as part of an integrated pest management strategy to control resistant TSM. Additionally, etoxazole has a low impact rating (Maas and Redfern, 2016) and could therefore be implemented where conservation of beneficial insects is desirable.

Laboratory based bioassay methodology has been successfully developed against green mirid but difficulty remains with establishing suspect resistant insects into culture and maintaining them prior to resistance testing. Mirids do not travel well because they are very fragile and in our experience most will die in transit. Those that do establish into culture will be slow and time consuming to breed and resistance may revert before it can be diagnosed. Consequently mirids were a species which we believed would benefit from molecular based testing and during 2015-2016 mirids were evaluated for resistance to fipronil using a PCR-based technique. Although no resistance to fipronil was detected, future resistance monitoring should seek to incorporate a bioassay method to verify the phenotypic expression of resistance. To that end, we are currently developing a field based bioassay as a more practical solution for detection of resistance in mirids.

Acknowledgments
Dr Yizhou Chen is thanked for overseeing the technical aspects of the molecular PCR assays. Lauren Woolley assisted with the molecular testing and Monica Suann assisted with the aphid and mite bioassay plus their culturing. The many researchers, CRC Regional Extension Officers, consultants and growers who collected aphids and mites are thanked. This study is funded by the CRDC (DAN1507). Finally, Lewis Wilson is thanked for critically reading an early version of this report.

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Table 1. Percent etoxazole (Paramite®) susceptible TSM via molecular diagnosis plus bioassay determination of bifenthrin (Talstar®), abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® (CGA-140408)) resistance via percent mortality at the discriminating dose (ie percent susceptible) for TSM collected during season 2015-2016.

Strain	**Etoxazole (Paramite® via PCR I1017F)**	**Bifenthrin (Talstar®)**	**Abamectin (Agrimec®)**	**Propargite (Comite®)**	**Diafenthiuron (Pegasus® CGA140408)**
Lie	100	63	41	100	100
Boo	100	89	100	100	100
A3	100	98	88	84	100
Mer	100	61	85	100	100
App	100	65	27	100	100
Mul	100	87	100	100	100
To 2	100	79	30	100	100
Old*	100	69	52	83	100
You*	100	78	44	98	100

* N.B. not field collected: ACRI glasshouse (ex Warwick Stiller)

Table 2. Percent pirimicarb / dimethoate (Pir / Dim), pyrethroid (Pyr) and organophosphate specific (profenofos / chlorpyrifos [Pro / Chl]) susceptible using molecular diagnosis plus bioassay determination of clothianidin (Clo)(Shield®), diafenthiuron (Dia)(CGA140408)(Pegasus®), thiamethoxam (Thia) (Actara or Cruiser®) and sulfoxaflor (Sul)(Transform®) resistance via percent mortality at the discriminating dose (ie percent susceptible) for cotton aphid collected during season 2015-2016.

Culture Name	Pir / dim qPCR S431F	Pro / Chl qPCR S302A	Pyr qPCR KDR	Dia 0.003%	Clo 0.005 %	Thia 0.002 %	Sul 0.001 %
Syl D	100	100	100	100	99	100	100
Thor	100	100	96	100	100	100	100
Yar P	100	100	100	100	100	99	100
Lem G	100	100	100	100	100	100	100
Riv	100	100	100	100+	100	100	100
Fair	100	100	81	100	98	100	100
Yar	100	100	100	100	100	99	100
Som	100	100	100	100	100	99	100
Cor	100	100	96	100	100	100	100
Tar	100	100	100	100	100	99	100

+Not replicated

Table 3. Percent fipronil susceptible via molecular diagnosis for green mirid collected during 2015-16.

Strain name	Fipronil A301S
Alch	100
Aus N	100
Cor	100
Thor	100
Curr	100
AW	100

Resistance testing summary for the 2016-17 cotton season: cotton aphid, two-spotted mite, strawberry spider mite, green mirid, brown mirid and cotton seedling thrips.

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Summary

- Cotton aphid, two-spotted mite (TSM), strawberry spider mite (SSM), green mirid, brown mirid and cotton seedling thrips were collected from Australian cotton growing regions.
- Bifenthrin (Talstar®) and abamectin (Agrimec®) resistance were detected in 90% of TSM strains tested so would be expected to fail if targeted against mites.
- Etoxazole (Paramite®) resistance detected via a known point mutation was present in three TSM strains. This is a serious concern because etoxazole (Paramite®) was thought to have potential to be incorporated into a resistance management strategy to reduce abamectin (Agrimec®) selection pressure against TSM.
- Quantitative abundance data for SSM suggests that this mite is now the dominant mite species in cotton.
- Resistance monitoring for abamectin (Agrimec®) against SSM can use a discriminating dose of 0.0008 g/L.
- Pirimicarb (Pirimor®) and organophosphate-specific resistance were not detected in any cotton aphid strain sourced from cotton so those compounds can be used with confidence.
- However, very high level organophosphate-specific and Pirimicarb (Pirimor®) resistance (94.7% and 100% resistant individuals, respectively) were detected in a single cotton aphid strain sourced from melons.
- Low frequency neonicotinoid survivors (<2%) were detected in many cotton aphid strains but we consider this may be vigour tolerance rather than resistance. Discriminating doses will be adjusted accordingly next season.
- Green and brown mirid were screened for resistance against fipronil (Regent®) using a molecular-based diagnostic and none was detected.
- Resistance monitoring for fipronil (Regent®) in green mirid via a Potter spray tower or treated glass vial method can use the same 0.004 g/L discriminating dose.
- Simple relative strain response comparison suggests there is likely 100-fold imidacloprid (Genero®, Confidor®) resistance in cotton seedling thrips supporting anecdotal grower concerns that neonicotinoid seed dressings may be compromised.

Introduction

Cotton aphid is resistant to a range of insecticides in many crops and countries. Previously, high-level resistance to organophosphates (omethoate (Folimat®) and dimethoate (Rogor®)) and cross-resistance to some carbamates (pirimicarb (Pirimor®)) was detected in cotton aphid strains in Australian cotton regions causing failures (Herron *et al*. 2001). Effective resistance management caused products to be recovered by about season 2005-06 allowing the pirimicarb/dimethoate/omethoate to once again

be used effectively. Neonicotinoid resistance was detected in season 2007-08 and increased in both level and abundance over the next 3-4 years again causing failures (Herron and Wilson 2011). The aphid resistance management strategy was modified to reduce selection against neonicotinoid insecticides and to date, no more control failures have been reported.

TSM is notorious world-wide for developing resistance to insecticides and acaricides including in Australia. In Australian cotton, TSM has developed resistance to dimethoate (Rogor®) / omethoate (Folimat®) (1970's), monocrotophos (Nuvacron®) (1980's), profenofos (Curacron®) (1980's) and bifenthrin (Talstar®) (1990's). TSM resistance continues to evolve in Australian cotton and most recently to chlorfenapyr (Intrepid®) (Herron *et al*. 2004). Since etoxazole (Paramite®) resistance was recently detected in horticulture molecular methods have been established to detect resistance in cotton (Herron *et al*. 2017).

The dominance of *Bt*-cotton and reduction in overall sprays has seen SSM and bean spider mite (BSM) become much more abundant, with SSM now likely the dominant mite species in cotton. Worryingly, although SSM is now anecdotally very common, its response to chemical control is completely unknown. Consequently, SSM need to be tested against newer miticides and baseline data established for resistance monitoring.

Control of green mirid requires targeted spraying and resistance is a possibility (Herron and Rophail 2008). Bioassay of mirids *per se* is not difficult but their fragile bodies and recalcitrant ability to establish into laboratory culture makes resistance confirmation problematic (Herron 2011). Development of a practical, robust and low-input rearing procedure would subsequently allow for routine bioassay of this pest. Alternatively, development of an in-field bioassay would remove the difficulties associated with laboratory rearing. Both field and laboratory assays to detect resistance in mirids have differing limitations so should complement each other.

Thrips [tobacco (cotton), tomato and western flower thrips] regularly occur on cotton. Thrips can cause early season damage to terminals, leaves and stems (Mass and Redfern 2017). Generally severe damage causing yield loss occurs occasionally so targeted sprays are infrequent. Thrips in cotton are usually managed via a seed treatment or at-planting insecticide (Mass and Redfern 2017). In Australia the only available seed treatments for cotton contain insecticides belonging to the neonicotinoid chemical class [(thiamethoxam: Cruiser® and Cruiser Extreme®) and (imidacloprid: Genero® and Gaucho®)] (InfoPest 2017). Recently Australian cotton growers have anecdotally experienced control problems with Cotton Seedling Thrips in the time period where seed treatments should be active.

For all sucking pests, resistance management requires ongoing vigilance. Especially so because key products target other species: thiamethoxam (Cruiser®, Cruiser Extreme®)(aphids, thrips and wireworm), diafenthiuron (Pegasus®) [aphids, mites, silverleaf whitefly (SLW)], abamectin (Agrimec®) (*H. punctigera* and mites) and spirotetramat (Movento®) (aphids and SLW). Continued insecticide resistance monitoring is essential for effective ongoing resistance management of these pests.

As part of CRDC funded project *DAN 1507*, routine resistance monitoring of key insect pests against chemicals registered for their control was completed for the 2016-17 cotton season. Here we present that monitoring data.

Methods

Chemicals tested

Aphids were treated with clothianidin (Shield®), diafenthiuron (Pegusus®), sulfoxaflor (Transform®), and thiamethoxam (Actara®). All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron (CGA-140408) was tested. This is necessary because diafenthiuron (Pegusus®) is activated by exposure to UV light, which would not normally occur in the laboratory. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance were detected via a DNA based method.

TSM were treated with abamectin (Agrimec®), bifenthrin (Talstar®), propargite (Comite®), and diafenthiuron (as CGA-140408). Etoxazole resistance was evaluated via a DNA based method.

SSM were treated with abamectin (Agrimec®) to produce baseline susceptibility data from which a discriminating dose was extrapolated.

Thrips were treated with imidacloprid (Confidor®) while fipronil resistance in mirids was screened via a DNA based method.

Aphid collection and culturing

Cotton aphid was collected by researchers, CRC Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden [Elizabeth McArthur Agricultural Institute (EMAI)] and each field strain was cultured separately on pesticide-free cotton at 25 ± 4 °C under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages.

Aphids - resistance detection

Via Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001). Briefly, batches of thirty adult female aphids per leaf disc were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower (to yield percent insecticide susceptible). All tests were replicated (unless otherwise marked) and included a water-only sprayed control. Sprayed dishes were covered with a clear plastic film containing ventilation holes and maintained at 25 ± 0.1 °C in 16:8 L:D for 24 h after which mortality was assessed.

Molecular. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance were detected via a DNA based method. The qPCR method for estimating pyrethroid, organophosphate and pirimicarb (Pirimor®) resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. For each 150 aphid sample, pooled DNA is extracted and triplicate qPCRs are carried out using two TaqMan probes (one detects

the resistance allele and the other detects the susceptible allele). The ratio of the fluorescence intensity produced for each qPCR reaction, is calculated along with a standard reference series, whose resistance allele frequency is known. The resistance allele frequency from field populations is then accurately estimated based on the ratio of fluorescence increase between the resistance and susceptible probe (Chen *et al*. 2014).

Two-spotted mite and strawberry spider mite collection and culturing
Except for strains of TSM collected from Australian Cotton Research Institute (ACRI) glasshouses, mites were sourced from a range of cotton fields in northern NSW and QLD and put into culture as above at EMAI with ten strains collected. SSM were also collected as above expect twenty-two strains were forwarded making them more abundant.

Two-spotted mite – resistance detection
Via bioassay. The bioassay procedure required twenty to thirty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites on leaf discs were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower as above. Each test was replicated (unless otherwise indicated) and included a water only sprayed control. After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Molecular. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Australia) according to the manufacturer's instructions and screened for the presence of the I1017F mutation (Van Leeuwen *et al*. 2012) by direct sequencing of PCR amplicons (performed at the Australian Genome Research Facility Ltd). Sequencing results were analysed using Sequencher version 5.2.4 (Gene Codes Corporation).

Strawberry spider mite – baseline susceptibility data
Via bioassay. The bioassay procedure required twenty to thirty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Mites and leaf discs were then sprayed with serial concentrations of formulated abamectin (Agrimec®) with the aid of a Potter spray tower producing 1.6±0.07 mg/cm^{-2} deposit. Each test was replicated and included a water only sprayed control. After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality was assessed. Bioassay data was analysed without replicate pooling using a stand-alone probit program developed by Barchia (2001) that ensured variability between replicates was taken into account. The LC_{50} and $LC_{99.9}$ values plus their 95% fiducial-limits (FL) were calculated using the method of Finney (1971) and included control mortality correction where appropriate (Abbott 1925).

Mirids collection and culturing
Green and brown mirids were collected from cotton, pigeon pea refuges or mung beans in close proximity to cotton via beat sheet or sweep net and put into analytical grade ethanol and transported to the laboratory for processing. Seven strains of green mirid and two strains of brown mirid were collected.

Fipronil baseline susceptibility testing (via potter spray tower and glass vial bioassay methodologies) from which a discriminating dose could be extrapolated was performed on mirids collected off lucerne at EMAI (Menangle).

To validate the discriminating dose generated, discriminating dose testing was performed on two strains: one collected off lucerne at EMAI (Menangle) and a second sourced off lucerne at Eugowra.

Mirids – resistance detection

Via Potter spray tower. Briefly, methods followed that previously employed by Herron and Rophail (2000) against cotton aphid. Adult mirids which had been anesthetised with CO_2 were tested by placing them in a in a 35 mm Petri dish on an excised 2 cm diameter section of green bean fixed in agar. Batches of 2-3 mirids were then sprayed with the aid of a Potter spray tower with serial dilutions of fipronil set to achieve 0 to 100% mortality. After spraying, each Petri dish was covered with its lid and maintained at 25 ± 0.1 °C under 16:8 L:D for 24 h after which mortality was assessed. Mirids were considered dead if they did not respond to gentle probing or could not right themselves if turned upside down. All tests were replicated and included a water-only sprayed control which did not exceed 10%. Bioassay data was analysed as above (Barchia 2001) as were LC_{50} and $LC_{99.9}$ values plus their 95% fiducial-limits (FL) (Finney 1971) and control mortality correction (Abbott 1925).

Via Glass Vial Bioassay. Methodology was based on that of Snodgrass (1996) with modification. Briefly, glass scintillation vials (20 mL) with un-lined lids were coated with 0.5 mL of serially diluted technical grade fipronil in acetone or acetone only (controls). Immediately after the solution was added the vial was placed onto a rotating commercial hot dog roller with heating element disconnected until the acetone evaporated. When dry, a 1 cm section of washed retail green bean was added to each evenly fipronil coated vial. Finally, one healthy green mirid was placed into each vial and the lid replaced loosely to allow air exchange. Vials were maintained at 25°C ± 0.1°C for 24 h after which mortality was recorded using the same criteria as for the Potter spray tower above. Controls were placed in vials coated with acetone only. Bioassay data was analysed exactly as above.

Molecular. Fipronil resistance is detected via a mutation in the *Rdl* gene (known associated with fipronil resistance in the ferment fly)(ffrench-Constant *et al*. 1993). Genomic DNA was extracted from individual green mirids and PCR amplification performed for the *Rdl* gene. Amplified products were then sent to the Australian Genome Research Facility for fipronil sequencing.

Thrips collection and culturing

Thrips strains were collected from Australian cotton during both 2015-2016 and 2016-2017 seasons from southern and northern NSW. Five strains of cotton seedling thrips, two from the 2015-16 season and three from the 2016-17 were screened for imidacloprid (Genero®, Confidor®) resistance.

Thrips – resistance detection

Via bioassay. Thrips were maintained as discrete populations on sprouted garlic cloves in 150 mm soil filled pots contained within thrips proof cages. The method required ca. 25 CO_2 anesthetised adult thrips to be placed on a broad bean leaf disc set in agar in a Petri dish (Herron *et al*. 2011). The Petri dish was then sprayed with 4 mL of a serially diluted insecticide or water (control) via a Potter spray tower. Each replicate (two or more) test comprised several serial concentrations plus control. Sprayed dishes were covered with a clear plastic film containing ventilation holes and maintained at 25 ± 0.1 °C under 16:8 L: D for 48 h before mortality was assessed. Bioassay data was analysed as above.

Results

Ten strains of TSM were put into culture and tested for resistance (Table 1). No propargite (Comite®) resistance was detected. TSM showed discriminating dose survivors against bifenthrin (Talstar®) and abamectin (Agrimec®) with most strains tested having resistance (Table 1). One strain showed diafenthiuron (Pegasus®) survivors that lived <24h and did not reproduce. Alarmingly, three strains showed etoxazole (Paramite®) resistance via the I1017F mutation (Table 1).

Twenty two strains of SSM were collected, but due to logistical constraints only five maintained in laboratory culture and used for abamectin (Agrimec®) baseline susceptibility data. The ratio of the LC_{50} responses of all strains was not significant as indicated by overlapping fiducial limits (Table 2). The minimum effective dose (MED) to kill all mites was 0.00025 g/L with the exception of strain Dai_4 that required 0.000125 g/L. In contrast, $LC_{99.9}$ estimates suggest doses of 0.0004 – 0.001 g/L would be required to control the SSM tested.

Fourteen strains of cotton aphid were put into culture and tested for resistance (Table 3). No pirimicarb (Pirimor®)(S431F), organophosphate specific (S302A), or sulfoxaflor (Transform®) resistance was detected in any strain sourced from cotton (Table 3). A single strain collected off melons contained very high organophosphate specific (S302A) and pirimicarb (Pirimor®)(S431F) resistance (Table 3). Four strains showed diafenthiuron (Pegasus®) survivors that lived <24h and did not reproduce. Many strains showed low (<5%) frequency neonicotinoid survivors with seven showing thiamethoxam survivors (Actara® or Cruiser®) and six clothianidin (Shield®) survivors (Table 3). Although not registered for aphid control pyrethroid resistance was detected in four strains with strain Broo_29 comprising 89% resistant aphids.

Sequencing results found no evidence of the fipronil (Regent®) resistance causing point mutation in green or brown mirid (Table 4).

Dose responses for green mirid against fipronil (Regent®) produced steep (3.1-3.2) and statistically equivalent regression slopes so either method should reflect good sensitivity of the assay to increasing dose (Table 5). As for slope, the LC_{50} and LC_{99} level responses treated glass vials and potter spray tower methodologies generated, were not significantly different (as indicated by overlapping 95% FLs).

Simple relative strain response comparison suggests there is likely 100-fold imidacloprid (Genero®, Confidor®) resistance in cotton seedling thrips strain Griff 2015-16 (Figure 1).

Discussion

Anecdotally TSM abundance has been declining in cotton growing areas; however, despite this, TSM populations sourced off cotton remain resistant so must still receive significant selection pressure against registered miticides. Resistance to bifenthrin (Talstar®) was again detected at frequencies warranting concern and use would be at best unreliable. This season, nine out of ten (90%) strains contained bifenthrin (Talstar®) resistant individuals, and five of those nine strains contained a frequency of >30% resistant individuals. This continued detection is surprising because pyrethroid use in cotton is limited and TSM do not fly (although they can aerially disperse via wind) so their immigration from sprayed crops other than cotton must be limited. Abamectin (Agrimec®) is favoured due to its low cost and is routinely applied as a prophylactic (preventative) treatment in combination with mirid sprays. Not surprisingly then, last season we reported that abamectin (Agrimec®) resistance in TSM was steadily increasing in frequency since its first detection in a single strain in season 2010-2011. This trend continues this season, with eight out of ten strains (80%) tested containing resistant individuals. Alarmingly, six strains contained resistance frequencies greater than 50%. Finally, resistance to propargite (Comite®) was not detected in any strain tested this season but should still be used judiciously.

TSM has been the dominant mite species in cotton for three decades; however, the cotton-mite-complex appears to be changing. Anecdotal reports suggest that TSM are decreasing in abundance and SSM is now the dominant mite species in cotton. Although this is our first season of documenting SSM abundance, data produced thus far confirms these anecdotal reports with 10 TSM strains versus 22 SSM strains collected. Reasons for this are complex but likely relate to IPM being widely practiced with potential mite problems being controlled by beneficials. The dominance of *Bt*-cotton and the subsequent reduction in chemical sprays has seen SSM become much more abundant. Consequently, baseline susceptibility data was produced for five field-collected strains of SSM against abamectin (Agrimec®). When compared to the response of a reference abamectin-susceptible TSM strain, SSM strain responses were slightly less tolerant but similar and suggest that no SSM resistance to abamectin (Agrimec®) exists in Australian cotton. The result is particularly interesting when the abundance data is considered because although TSM clearly has an advantage against abamectin (Agrimec®) use SSM still dominates. Clearly if abamectin (Agrimec®) use is reduced and replaced by etoxazole (Paramite®) again frequencies may change dependant on resistance status (see below). For monitoring, a suitable theoretical discriminating dose for SSM against abamectin (Agrimec®) is approximately 0.0008 g/L; being a compromise between the calculated $LC_{99.9}$ estimate and the MED of all strains tested.

Last season we introduced a DNA-based resistance screening method for etoxazole (Paramite®); the reason being that etoxazole has potential to reduce abamectin (Agrimec®) selection pressure against TSM. Although no strains tested positive for

resistance via the I1017F mutation during 2015-16, this season three strains of TSM contained resistant individuals. Therefore, addition of etoxazole to an integrated resistance management strategy to control abamectin (Agrimec®) resistant TSM must be carefully managed. It should be noted that these strains were geographically distinct, and located between northern (Moree, Narrabri) and southern NSW (Griffith).

In season 2014-15, low frequency neonicotinoid [thiamethoxam (Actara® or Cruiser®) and clothianidin (Shield®)] resistance was detected in 30% of cotton aphid strains tested at very low frequencies (often <3% resistant individuals). Similarly, in season 2015-16 low frequency clothianidin (Shield®) resistance was detected in 20% of strains tested, while low frequency thiamethoxam Actara® or Cruiser® resistance was detected in 40% of strains tested. This season, ten out of fourteen strains (71%) tested comprised very low frequency neonicotinoid resistant individuals (always <5%). Of these strains, five were resistant to each compound tested, four to thiamethoxam Actara® or Cruiser® only, and one resistant to clothianidin (Shield®). We now consider that the repeated detection of low level resistance between seasons 2014-15 and 2016-17 is more likely vigour tolerance than resistance. Given that all cotton seed is treated with a neonicotinoid insecticide, it is unlikely that continued selection pressure wouldn't result in increasing frequency of neonicotinoid resistant individuals, which we have not seen. Discriminating doses will be adjusted accordingly next season.

Although no resistance to fipronil (Regent®) has been detected over the last three seasons using molecular based testing, a glass vial bioassay technique to verify the phenotypic expression of resistance (if detected via PCR) was developed. From the baseline susceptibility data produced, a discriminating dose of 0.004 g/L fiponil (Regent®) for monitoring in green mirid was extrapolated; being a compromise between the calculated $LC_{99.9}$ estimates of both glass vial bioassay and potter spray tower methodologies. This testing can now be incorporated into resistance monitoring of mirids in Australian cotton if required (e.g. growers perceive reduced control when PCR is not detecting resistance).

Recently Australian cotton growers have anecdotally experienced control problems with cotton seedling thrips in the time period where seed treatments should be active. There are numerous reasons why such control issues could happen with resistance but one possible cause. The ratio comparison of the minimum dose required to kill all strain Spring 2016-17 (0.001 g/L) to strain Griff 2015-16 (0.1 g/L) produced a 100 fold difference. That means there is likely 100-fold imidacloprid (Genero®, Confidor®) resistance in strain Griff 2015-16. As strains Spring 2015-16 and Pine 2015-16 may not themselves be completely imidacloprid (Genero®, Confidor®) susceptible the resistance fold value is best case scenario and so may underestimate resistance. As there is known cross resistance between imidacloprid (Genero®, Confidor®) and thiamethoxam (Cruiser®, Cruiser Extreme®) anecdotal control issues experienced with cotton seedling thrips may be resistance related.

Where control issues with thrips have been experienced with neonicotinoid seed treatments, alternative at-planting treatment options are available including the organophosphate phorate (Thimet®) and the carbamate Carbosulfan (Marshal®). In our

experience, phorate should provide robust protection against cotton seedling thrips as previous bioassay data showed phorate had high efficacy against western flower thrips. As always, when choosing your first foliar spray, consider previous insecticide use and rotate chemical groups.

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Table 1. Percent* etoxazole (Paramite®) susceptible TSM via molecular diagnosis plus bioassay determination of bifenthrin (Talstar®), abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® (CGA-140408)) resistance via percent mortality at the discriminating dose (ie percent susceptible) for TSM collected during season 2016-17.

Strain	Etoxazole* (Paramite® via PCR I1017F)	Bifenthrin (Talstar®)	Abamectin (Agrimec®)	Propargite (Comite®)	Diafenthiuron (Pegasus® CGA140408)
ACRI_C	resistant	67	39	100	100
ACRI_S	susceptible	48	47	100	100
CORN	susceptible	52	31	100	100
F35B	susceptible	97	99	100	100
SAF4	susceptible	50	44	100	100
SAF9_ SW	susceptible	55	42	100	100
SAF9_SE	resistant	82	40	100	100
GTSM	susceptible	98	100	100	99**
Dai4TSM	resistant	100	100	100	100
G25D	susceptible	96	94	100	100

* N.B. Etoxazole test comprised a pool of 40 individuals that could not be distinguished so frequency data is not available.
** N.B. Survived <24h post treatment on an untreated host so not resistant.

Table 2. Abamectin baseline susceptibility data for field sourced strawberry spider mite (SSM).

Strain	Chi-Square (df)	Slope (se)	LC_{50}* (95% FL)	$LC_{99.9}$* (95% FL)	MED#*
Tex_L	31.55 (13)	1.59 (0.38)	0.000002 (0.000001-0.000003)	0.0004 (0.00008-0.051)	0.00025
Dai_15	41.66 (20)	1.27 (0.19)	0.000002 (0.000001-0.000002)	0.001 (0.0003-0.022)	0.00025
Jaki	145.47 (40)	1.32 (0.17)	0.000002 (0.000001-0.000002)	0.001 (0.0003-0.009)	0.00025
Grov	52.32 (21)	1.36 (0.23)	0.000002 (0.000001-0.000003)	0.0009 (0.0002-0.019)	0.00025
Dai_4	38.50 (9)	1.36 (0.37)	0.000002 (0.000001-0.000004)	0.001 (0.0001-26.1)	0.000125

* g ai / L.
# Minimum effective dose to kill all test insects.

Table 3. Percent pirimicarb / dimethoate (Pir / Dim), pyrethroid (Pyr) and organophosphate specific (profenofos / chlorpyrifos [Pro / Chl]) susceptible using molecular diagnosis plus bioassay determination of clothianidin (Clo)(Shield®), diafenthiuron (Dia)(CGA140408)(Pegasus®), thiamethoxam (Thia)(Actara or Cruiser®) and sulfoxaflor (Sul)(Transform®) resistance via percent mortality at the discriminating dose (ie percent susceptible) for cotton aphid collected during season 2016-17.

Strain	Pir / dim qPCR S431F	Pro / Chl qPCR S302A	Pyr qPCR KDR	Dia 0.003%	Clo 0.005 %	Thia 0.002 %	Sul 0.001 %
Field_28	100	100	100	99**	97	97	100
Broo	100	100	66	99**	99	97	100
Field_26	100	100	100	100	98	99	100
Hill	100	100	100	98**	99	100	100
Merr_H9	100	100	21	100	99	100	100
Newt	100	100	100	99**	100	99	100
Road	100	100	100	100	99	100	100
Merr	100	100	73	100	100	99	100
Broo_29	100	100	11	100	100	100	100
Broo_20	100	100	100	100	100	99	100
Broo_28	100	100	100	100	100	100	100
WesP	100	100	100	100	100	100	100
Melo_WA	5.3	0	100	100	100	95	100
Shar_17	+	+	+	100	100	100	100

+Not tested

** N.B. Survived <24h post treatment on an untreated host so not resistant.

Table 4. Percent fipronil (A301S) susceptible using molecular diagnosis and bioassay determination of fipronil (Regent®) resistance via percent mortality at the discriminating dose (ie percent susceptible) for green mirid; and molecular diagnosis (A301S) for brown mirid collected during 2016-17.

Strain	Green mirid PCR A302S	Bioassay Glass Vial / Potter spray tower	Brown mirid PCR A302S
Burg	100	NT	NA
Kato	100	NT	NA
Kato2	100	NT	NA
WesB_B	100	NT	100
RanB_B	100	NT	100
WesB_G	100	NT	NA
RanB_G	100	NT	NA
Choubra (Eugowra)	100	100	NA
EMAI_Lucerne	100	100	NA

NA – not collected
NT - not tested

Table 5. Fipronil response of green mirid collected off EMAI lucerne using Potter spray tower or treated glass vial methodology.

Method tested	Population	Slope ± SE	LC_{50}* (95%FL)	LC_{99}* (95% FL)
Spray tower	EMAI lucerne	3.1 ± 0.75	0.00043 (0.00030-0.00064)	0.0044 (0.0019-0.044)
Glass vial	EMAI lucerne	3.2 ± 0.75	0.00026 (0.00018-0.00038)	0.0036 (0.0015-0.035)

*g a.i. / L;
SE - standard error;
FL - fiducial limits;
EMAI - Elizabeth Macarthur Agricultural Institute.

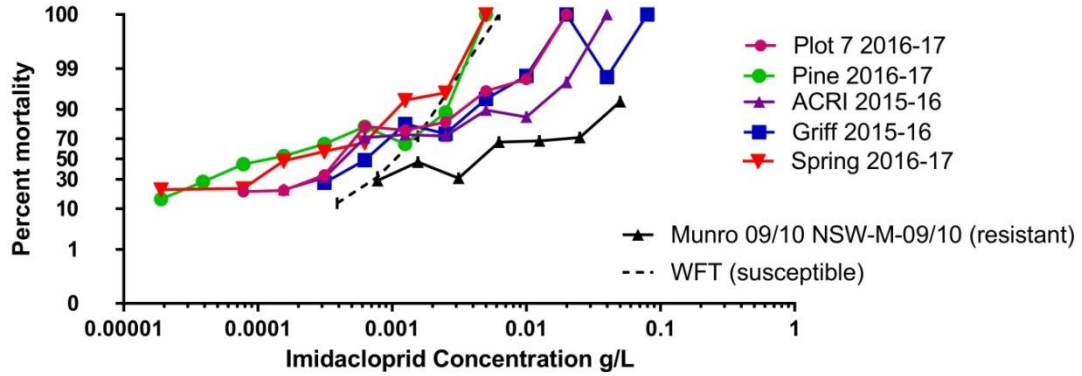

Figure 1. Dose response data for cotton seedling thrips collected during the 2015-16 and 2016-17 cotton seasons and tested against imidacloprid (Confidor®) plus a reference resistant strain from horticulture [Munro 09/10 NSW-M-09/10 (resistant)] and a reference susceptible western flower thrips [WFT (susceptible)] included for comparison.

Resistance testing summary for the 2017-18 cotton season: cotton aphid, two-spotted mite and green mirid.

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Summary

- Cotton aphid, two-spotted mite (TSM) and green mirid were collected from Australian cotton growing regions.
- TSM showed discriminating dose survivors against bifenthrin (Talstar®), propargite (Comite®) and abamectin (Agrimec®) but not etoxazole (Paramite® or Zeal®) or diafenthiuron (Pegasus®).
- Bifenthrin (Talstar®) and abamectin (Agrimec®) resistance detected in TSM was sometimes at high frequency so would be expected to fail.
- Pirimicarb (Pirimor®), OP-specific resistance, clothianidin (Shield®), diafenthiuron (Pegasus®), thiamethoxam (Actara or Cruiser®) and sulfoxaflor (Transform®) were not detected so can be used with confidence.
- Pyrethroid resistance was detected in a single cotton aphid strain only but at a frequency that would likely compromise control.
- Green mirid was screened for resistance against fipronil (Maestro® or Albatross®) using a molecular-based diagnostic and none was detected.

Introduction

Cotton aphid is resistant to a range of insecticides in many crops and countries. Previously, high-level resistance to organophosphates [omethoate (Folimat®) and dimethoate (Rogor®)] and cross-resistance to some carbamates [pirimicarb (Pirimor®)] was detected in cotton aphid strains in Australian cotton regions causing failures (Herron *et al*. 2001). Effective resistance management caused products to be recovered by about season 2005-06 allowing the pirimicarb/dimethoate/omethoate to once again be used effectively. Neonicotinoid resistance was detected in season 2007-08 and increased in both level and abundance over the next 3-4 years again causing failures (Herron and Wilson 2011). The aphid resistance management strategy was modified to reduce selection against neonicotinoid insecticides and to date, no control failures have been reported.

TSM is notorious world-wide for developing resistance to insecticides and acaricides including in Australia. In Australian cotton, TSM has developed resistance to dimethoate (Rogor®) / omethoate (Folimat®) (1970's), monocrotophos (Nuvacron®) (1980's), profenofos (Curacron®) (1980's) (Herron *et al*. 1998) and bifenthrin (Talstar®) (1990's) (Herron *et al*. 2001). TSM resistance continued to evolve in Australian cotton to include chlorfenapyr (Intrepid®) (Herron *et al*. 2004) and most recently etoxazole (Paramite® or Zeal®)(Herron *et al*. 2018)

Control of green mirid requires targeted spraying and resistance is a possibility (Herron and Rophail 2008). Bioassay of mirids *per se* is not difficult but their fragile bodies and recalcitrant ability to establish into laboratory culture makes resistance confirmation problematic (Herron 2011). Development of a practical, robust and low-input rearing procedure would subsequently allow for routine bioassay of this pest. Alternatively, development of an in-field bioassay would remove the difficulties associated with laboratory rearing. Currently we use a DNA based method to screen for fipronil (Maestro® or Albatross®) resistance.

For all sucking pests, resistance management requires ongoing vigilance. Especially because key products target other species: thiamethoxam (Cruiser®, Cruiser Extreme®)(aphids, thrips and wireworm), diafenthiuron (Pegasus®) (aphids, mites, silverleaf whitefly (SLW)), abamectin (Agrimec®) (*H. punctigera* and mites) and spirotetramat (Movento®) (aphids and SLW)(Mass and Redfern 2017). Continued insecticide resistance monitoring is essential for effective ongoing resistance management of these pests.

For the 2017-18 cotton season (as part of CRDC DAN1507) resistance monitoring of key sucking insect pests was completed and here we present that data.

Methods

Chemicals tested

Aphids were treated with clothianidin (Shield®), diafenthiuron (Pegusus®), sulfoxaflor (Transform®), and thiamethoxam (Actara®). All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron (CGA-140408). This is necessary because diafenthiuron (Pegusus®) is activated by exposure to UV light, which would not normally occur in the laboratory. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via DNA based methods.

TSM were treated with abamectin (Agrimec®), bifenthrin (Talstar®), propargite (Comite®), and diafenthiuron (as CGA-140408). Etoxazole (Paramite® or Zeal®) resistance was evaluated via a DNA based method.

Aphid collection and culturing

Aphids were collected by researchers, CRC Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden [Elizabeth McArthur Agricultural Institute (EMAI)] and each field strain was cultured separately on pesticide-free cotton at 25 ± 4 °C under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages.

Aphids - resistance detection

Via Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al.* 2001). Briefly, batches of thirty adult

female aphids per leaf disc were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower (to yield percent insecticide susceptible). All tests were replicated (unless otherwise marked) and included a water-only sprayed control. After spraying, clear plastic film was used to cover the Petri dishes. Aphids were then maintained at 25 ± 0.1 °C in 16:8 L:D for 24 h after which mortality was assessed.

Molecular. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method. The qPCR method for estimating pyrethroid, OP and pirimicarb (Pirimor®) resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. For each 200 aphid sample, pooled DNA is extracted and triplicate qPCRs are carried out using two TaqMan probes (one detects the resistance allele and the other detects the susceptible allele). The ratio of the fluorescence intensity produced for each qPCR reaction, is calculated along with a standard reference series, whose resistance allele frequency is known. The resistance allele frequency from field populations is then accurately estimated based on the ratio of fluorescence increase between the resistance and susceptible probe (Chen *et al*. 2014).

Two-spotted mite collection and culturing
Strains of TSM were sourced from a range of cotton fields in northern NSW and Qld and put into culture as above at EMAI.

Two-spotted mite – resistance detection
Via bioassay. The bioassay procedure required fifteen to twenty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites on leaf discs were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower as above. Each test was replicated (unless otherwise indicated) and included a water only sprayed control. After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Molecular. Genomic DNA was extracted using Chelex-100 resin (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions and screened for the presence of the I1017F mutation (Van Leeuwen *et al*. 2012) by direct sequencing of PCR amplicons performed at the Australian Genome Research Facility Ltd. Sequencing results were analysed using Sequencher version 5.2.4 (Gene Codes Corporation).

Mirids collection
Green mirids were collected from cotton, pigeon pea refuges or mung beans in close proximity to cotton via beat sheet or sweep net and put into analytical grade ethanol and transported to the laboratory for processing.

Mirids – resistance detection

Molecular. Resistance to fipronil is detected via a mutation in the *Rdl* gene (known associated with fipronil (Maestro® or Albatross®) resistance in the ferment fly)(ffrench-Constant *et al*. 1993). Genomic DNA was extracted from individual green mirids and PCR amplification performed for the *Rdl* gene. Amplified products were then sent to the Australian Genome Research Facility for fipronil sequencing.

Results

Eight strains of TSM were put into culture and tested for resistance (Table 1). No etoxazole (Paramite® or Zeal®) and diafenthiuron (Pegasus®) resistance was detected. Propargite (Comite®) resistance was detected in a single strain only at a very low frequency. TSM showed discriminating dose survivors against bifenthrin (Talstar®) and abamectin (Agrimec®) with many strains tested having resistance at high frequency (Table 1).

Four strains of cotton aphid were put into culture and tested for resistance (Table 2). No pirimicarb (Pirimor®)(S431F), organophosphate specific (S302A), sulfoxaflor (Transform®), diafenthiuron (Pegasus®), thiamethoxam (Actara® or Cruiser®) or clothianidin (Shield®) resistance was detected (Table 2). Although not registered for aphid control pyrethroid resistance was detected in strain RedH comprising 85% resistant aphids.

Five strains of green mirid were tested for fipronil resistance but none was detected (Table 3).

Discussion

TSM abundance has been declining in cotton growing areas; however, despite this, TSM populations sourced off cotton remain resistant to some chemicals. Two seasons ago we introduced a DNA-based resistance screening capacity for the miticide, etoxazole (Paramite® or Zeal®) against TSM; the reason being etoxazole (Paramite® or Zeal®) had potential to reduce abamectin (Agrimec®) selection. Worryingly last season three strains of TSM contained resistant individuals and encouragingly this season no resistance was detected.

Resistance to bifenthrin (Talstar®) was again detected at frequencies warranting concern and use would be at best unreliable. This season, all but one strain contained bifenthrin (Talstar®) resistant individuals with one strain comprising approximately 60% resistant individuals. For several seasons now we have reported abamectin (Agrimec®) resistance in TSM since its first detection in season 2010-2011. This trend continues this season, with six out of eight strains (75%) tested containing resistant individuals. It is now understood that abamectin (Agrimec®) resistance is likely due to its low cost and being applied as a prophylactic (preventative) treatment in combination with mirid sprays. Propargite (Comite®) was detected at a low frequency in a single strain which is consistent with past detections of resistance (again at low frequency) in previous seasons e.g. 2014-15 and 2015-16 but not 2016-17.

In season 2014-15, low frequency neonicotinoid (thiamethoxam and clothianidin) resistance was detected in 30% of cotton aphid strains tested. Similarly, in season 2015-16 low frequency clothianidin resistance was detected in 20% of strains tested, while low frequency thiamethoxam resistance was detected in 40% of strains tested (strains distinct to those containing clothianidin resistant individuals). During season 2016-17 ten out of fourteen strains (71%) tested comprised very low frequency neonicotinoid resistance (always <5%). We considered that the low level resistance being detected was more likely vigour tolerance rather than resistance. This season discriminating doses were adjusted accordingly to account for such vigour tolerance and no survivors were found supporting a vigour tolerance conclusion. Consequently neonicotinoids can be used with confidence for aphid control.

Acknowledgments
Drs Yizhou Chen and Kate Langfield are thanked for overseeing the methodological aspects of the molecular PCR and bioassay respectively. Dr Lauren Woolley and Risha Gupta assisted with the molecular testing and Dr Duong Nguyen assisted with the aphid bioassay plus their culturing. Damian Aiken assisted with mite bioassay and its culturing. The many researchers, CRC Regional Extension Officers, consultants and growers who collected aphids and mites are thanked. This study is funded by the CRDC (Project ID: DAN1507).

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23 October 2018

Table 1. Percent etoxazole (Paramite® or Zeal®) susceptible TSM via molecular diagnosis plus bioassay determination of bifenthrin (Talstar®), abamectin (Agrimec®), propargite (Comite®) and diafenthiuron [Pegasus® (CGA-140408)] resistance via percent mortality at the discriminating dose (i.e. percent susceptible) for mites collected during season 2017-18.

Strain	Etoxazole (Paramite® via PCR I1017F)	Bifenthrin (Talstar®)	Abamectin (Agrimec®)	Propargite (Comite®)	Diafenthiuron (Pegasus® CGA140408)
Brai	100	100	64	100	100
Broo34	100	99	62	100	100
Broo36	100	97	43	100	100
Broo37(Hill)	100	95	47	99	100
Warr	100	99	95	100	100
MerrK2	100	92	94	100	100
WhitP4	100	98	100	100	100
WhitNFOI	100	42	100	100	100

Table 2. Percent pirimicarb / dimethoate (Pir / Dim), pyrethroid (Pyr) and organophosphate specific (profenofos / chlorpyrifos [Pro / Chl]) susceptible using molecular diagnosis plus bioassay determination of clothianidin (Clo)(Shield®), diafenthiuron (Dia)(CGA140408)(Pegasus®), thiamethoxam (Thia)(Actara or Cruiser®) and sulfoxaflor (Sul)(Transform®) resistance via percent mortality at the discriminating dose (i.e. percent susceptible) for cotton aphid collected during season 2017-18.

Strain	Pir / dim qPCR S431F	Pro / Chl qPCR S302A	Pyr qPCR KDR	Dia 0.006%	Clo 0.010 %	Thia 0.004 %	Sul 0.002 %
Koro3	100	100	100	100	100	100	100
RedH	100	100	15	100	100	100	100
Koro9	100	100	100	100	100	100	100
Neil2	100	100	100	100	100	100	100

Table 3. Percent fipronil (A301S) susceptible green mirid using molecular diagnosis to detect resistance in 2017-18.

Strain	Green mirid PCR A302S
IslandBLN	100
WallaLUC	100
WallaPIG	100
Brook	100
KywPIG	100

Resistance testing summary for cotton season 2018-19: cotton aphid (*Aphis gossypii*), two-spotted mite (*Tetranychus urticae*), banana or strawberry spider mite (*Tetranychus lambi*), cotton seedling thrips (*Thrips tabaci*), western flower thrips (*Frankliniella occidentalis*) and green mirid (*Crontiades dilutus*).

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Summary

- Cotton aphid, two-spotted mite (TSM), banana or strawberry spider mite (SSM), cotton seedling thrips, western flower thrips and green mirid were collected from Australian cotton growing regions.
- TSM showed discriminating dose survivors against bifenthrin (Talstar®) and abamectin (Agrimec®) but not propargite (Comite®), etoxazole (Paramite® or Zeal®) or diafenthiuron (Pegasus® as CGA140408).
- Bifenthrin (Talstar®) and abamectin (Agrimec®) resistance detected in TSM was sometimes at high frequency so may fail.
- For the first time banana or strawberry spider mite (SSM) was tested for abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA140408) resistance but none was detected.
- Pirimicarb (Pirimor®), OP-specific resistance, pyrethroid, clothianidin (Shield®), diafenthiuron (Pegasus® as CGA140408), thiamethoxam (Actara or Cruiser®) and sulfoxaflor (Transform®) resistance was not detected in cotton aphid so all can be used with confidence.
- Green mirid was screened for resistance against fipronil (Maestro® or Albatross®) using a molecular-based diagnostic and none was detected.
- Neonicotinoid resistance was not detected in the single strain of cotton seedling thrips tested but unexpectedly WFT was the most abundant thrips found and worryingly spinetoram (Success® Neo)(the only registered control in cotton) resistance was detected in many strains.

Introduction

Cotton aphid is resistant to a range of insecticides in many crops and countries. Previously, high-level resistance to organophosphates [omethoate (Folimat®) and dimethoate (Rogor®)] and cross-resistance to some carbamates [pirimicarb (Pirimor®)] was detected in cotton aphid strains in Australian cotton regions causing failures (Herron *et al*. 2001a). Effective resistance management caused products to be recovered by about season 2005-06 allowing the pirimicarb/dimethoate/omethoate to once again be used effectively. Neonicotinoid resistance was detected in season 2007-08 and increased in both level and abundance over the next 3-4 years again causing failures (Herron and Wilson 2011). The aphid resistance management strategy was modified to reduce selection against neonicotinoid insecticides and to date, no control failures have been reported (Herron and Wilson 2017).

TSM is notorious world-wide for developing resistance to insecticides and acaricides including in Australia. In Australian cotton, TSM has developed resistance to dimethoate (Rogor®) / omethoate (Folimat®) (1970's), monocrotophos (Nuvacron®) (1980's), profenofos (Curacron®) (1980's) (Herron *et al*. 1998) and bifenthrin (Talstar®) (1990's) (Herron *et al*. 2001b). TSM resistance continued to evolve in Australian cotton to include chlorfenapyr (Intrepid®) (Herron *et al*. 2004) and most recently etoxazole (Paramite® or Zeal®)(Herron *et al*. 2018). Banana or strawberry spider mite (SSM) is anecdotally abundant in Australian cotton but these mites have not previously been tested for resistance against key chemicals that could potentially be used for their control.

Control of green mirid requires targeted spraying and resistance is a possibility (Herron and Rophail 2008). Bioassay of mirids *per se* is not difficult but their fragile bodies and recalcitrant ability to establish into laboratory culture makes resistance confirmation problematic (Herron 2011). A low-input rearing procedure has been recently developed in this current study making bioassay possible however currently we use a DNA based method to screen only for fipronil (Maestro® or Albatross®) resistance.

Recently there has been anecdotal evidence that cotton seedling thrips may not be being controlled as well as they were by neonicotinoid seed dressings. In crops other than cotton, cotton seedling thrips is known neonicotinoid resistant (Herron *et al*. 2011) so resistance is possible. Western flower thrips (WFT) have a long history of developing resistance and their control in cotton is based singularly on spinetoram (Success® Neo) which has recently been associated with failures in stone fruit (Langfield *et al*. 2018).

For all sucking pests, resistance management requires ongoing vigilance. Especially because key products target other species: thiamethoxam (Cruiser®, Cruiser Extreme®)(aphids, thrips and wireworm), diafenthiuron (Pegasus®) (aphids, mites, silverleaf whitefly (SLW)), abamectin (Agrimec®) (*H. punctigera* and mites) and spirotetramat (Movento®) (aphids and SLW)(Mass and Redfern 2018). Continued insecticide resistance monitoring is essential for effective ongoing resistance management of these pests.

For the 2018-2019 cotton season (as part of CRDC DAN1507) resistance monitoring of key sucking insect pests was completed and here we present those data.

Methods

Chemicals tested

Cotton aphid was treated with clothianidin (Shield®), diafenthiuron (Pegusus®), sulfoxaflor (Transform®), and thiamethoxam (Actara®). All were proprietary commercial insecticide formulations except diafenthiuron (Pegasus®) for which the UV activated carbodiimide derivative of diafenthiuron (CGA-140408) was used. This is necessary because diafenthiuron (Pegasus®) is activated by exposure to UV light,

which would not normally occur in the laboratory. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via DNA based methods.

TSM were treated with abamectin (Agrimec®), bifenthrin (Talstar®), propargite (Comite®), and diafenthiuron (Pegasus® as CGA-140408). Etoxazole (Paramite® or Zeal®) resistance was evaluated via a DNA based method. SSM were treated with abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA-140408).

Cotton seedling thrips were tested against imidacloprid (Confidor®) and WFT against spinetoram (Success® Neo) insecticides.

Aphid collection and culturing
Aphids were collected by researchers, Regional Extension Officers, consultants and growers from commercial cotton fields or cotton plants in the vicinity of commercial crops. They were sent to the bioassay laboratory at Camden [Elizabeth McArthur Agricultural Institute (EMAI)] and each field strain was cultured separately on pesticide-free cotton at 25 ± 4 °C under natural light. Strain integrity was assured by maintaining populations in purpose built insect proof cages.

Aphids - resistance detection
Via Bioassay. Aphids were tested by placing them in a 35 mm Petri dish on an excised cotton plant leaf disc fixed in agar (Herron *et al*. 2001a). Briefly, batches of thirty adult female aphids per leaf disc were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower (to yield percent insecticide susceptible). All tests were replicated (unless otherwise marked) and included a water-only sprayed control (that did not exceed 10%). After spraying, clear plastic film was used to cover the Petri dishes. Aphids were then maintained at 25 ± 0.1 °C in 16:8 L:D for 24 h after which mortality was assessed.

Molecular. Pirimicarb (Pirimor®), organophosphate and pyrethroid resistance was detected via a DNA based method. The qPCR method for estimating pyrethroid, OP and pirimicarb (Pirimor®) resistance allele frequency is based on Taqman probes for resistant and susceptible alleles in one PCR reaction with DNA extracted from pooled (hundreds) aphids. For each 200 aphid sample, pooled DNA is extracted and triplicate qPCRs are carried out using two TaqMan probes (one detects the resistance allele and the other detects the susceptible allele). The ratio of the fluorescence intensity produced for each qPCR reaction, is calculated along with a standard reference series, whose resistance allele frequency is known. The resistance allele frequency from field populations is then accurately estimated based on the ratio of fluorescence increase between the resistance and susceptible probe (Chen *et al*. 2014).

Two-spotted mite collection and culturing

Strains of TSM were sourced from a range of cotton fields in NSW (none were found in Qld) and put into culture as above at EMAI.

Two-spotted mite – resistance detection
Via bioassay. The bioassay procedure required fifteen to twenty young adult female mites to be transferred from culture to French bean leaf discs (Herron *et al*. 2004). Briefly, mites on leaf discs were then sprayed with a discriminating dose of insecticide with the aid of a Potter spray tower as above. Each test was replicated (unless otherwise indicated) and included a water only sprayed control (that did not exceed 10%). After spraying, mites on leaf discs were maintained at 28 ± 0.1 °C in constant light for 48 h after which mortality is assessed.

Molecular. Genomic DNA was extracted using Chelex-100 resin (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions and screened for the presence of the I1017F mutation (Van Leeuwen *et al*. 2012) by direct sequencing of PCR amplicons performed at the Australian Genome Research Facility Ltd. Sequencing results were analysed using Sequencher version 5.2.4 (Gene Codes Corporation).

Banana or strawberry spider mite (SSM) collection and culturing
Strains of SSM were sourced from a range of cotton fields in NSW and Qld and put into culture as above for TSM at EMAI.

Banana or strawberry spider mite – resistance detection
Via bioassay. The bioassay procedure is as described above for TSM.

Mirid collection
Green mirids were collected from cotton, pigeon pea refuges or mung beans in close proximity to cotton via beat sheet or sweep net and transported alive to the laboratory for processing.

Mirids – resistance detection
Molecular. Resistance to fipronil is detected via a mutation in the *Rdl* gene (known associated with fipronil (Maestro® or Albatross®) resistance in the ferment fly)(ffrench-Constant *et al*. 1993). Genomic DNA was extracted from individual green mirids and PCR amplification performed for the *Rdl* gene. Amplified products were then sent to the Australian Genome Research Facility for fipronil sequencing.

Results
Five strains of TSM were tested for resistance and no etoxazole (Paramite® or Zeal®) Propargite (Comite®) or diafenthiuron (Pegasus® as CGA-140408) resistance was detected (Table 1). TSM showed discriminating dose survivors against bifenthrin (Talstar®) and abamectin (Agrimec®) with strain Mor Col 3 having the highest frequency of both survivors (Table 1). In contrast, some 14 strains of SSM were collected and approximately half tested against abamectin (Agrimec®), propargite

(Comite®) and diafenthiuron (Pegasus® as CGA140408) but no resistance was detected (Table 2).

Similarly, three strains of cotton aphid were put into culture and tested for resistance but no pirimicarb (Pirimor®)(S431F), organophosphate specific (S302A), pyrethroid (KDR), sulfoxaflor (Transform®), diafenthiuron (Pegasus® as CGA-140408), thiamethoxam (Actara® or Cruiser®) or clothianidin (Shield®) resistance was detected (Table 3).

Fifteen strains of green mirid were collected with ten tested for fipronil resistance but none was detected (Table 4).

One strain of cotton seedling thrips was tested for imidacloprid (Confidor®) resistance but none was detected (Table 5). In contrast, nine strains of WFT were tested for spinetoram (Success® Neo) resistance that was surprisingly detected in five (Table 6).

Discussion

TSM abundance has been declining in cotton growing areas; however, despite this, TSM populations sourced off cotton remain resistant. Three seasons ago we introduced a DNA-based resistance screening capacity for the miticide, etoxazole (Paramite® or Zeal®) against TSM; the reason being etoxazole (Paramite® or Zeal®) had potential to reduce abamectin (Agrimec®) selection. Worryingly etoxazole (Paramite® or Zeal®) resistance was detected in three strains of TSM in the first season of monitoring but encouragingly for the last two seasons resistance has not been found.

Resistance to bifenthrin (Talstar®) was again detected at frequencies warranting concern with strain Mor Col 3 comprising 95% resistant individuals. For several seasons now we have reported abamectin (Agrimec®) resistance in TSM since its first detection in season 2010-2011. The trend continues this season, with four out of five strains (80%) tested containing resistant individuals.

For the first time we have included SSM into the resistance testing with some fourteen strains collected. Testing would normally conclude by September – October but funding beyond June 30 was not available causing testing to be stopped with approximately half the strains complete and some tests not replicated. Even so, results produced for SSM are encouraging (and in complete contrast to TSM) with no SSM resistance detected.

Again, for the first time, we returned live green mirid from the field to the laboratory rather than preserving straight into RNA*later*®. The reasoning was to have scope to verify any fipronil positive results (that never happened) via bioassay as a double check on the methodology. Initially strains arrived in such poor condition that not

even a DNA testing could be done but with fine tuning of the methodology all strains were arriving alive and in good condition by seasons end.

In season 2014-15, low frequency neonicotinoid (thiamethoxam and clothianidin) resistance was detected in 30% of cotton aphid strains tested. Similarly, in season 2015-16 low frequency clothianidin resistance was detected in 20% of strains tested, while low frequency thiamethoxam resistance was detected in 40% of strains tested (strains distinct to those containing clothianidin resistant individuals). During season 2016-17 ten out of fourteen strains (71%) tested comprised very low frequency neonicotinoid resistance (always <5%). We considered that the low level resistance being detected was more likely vigour tolerance rather than resistance. For this reason last season the discriminating doses were adjusted accordingly to account for such vigour tolerance and no survivors were found supporting a vigour tolerance conclusion. Again testing this season continued with the adjusted discriminating doses and again no survivors were found suggesting neonicotinoids can be used with confidence for aphid control.

In season 2016-2017 cotton seedling thrips were tested for the first time and imidacloprid (Confidor®) resistance detected in three out of the five strains. As neonicotinoid cross resistance is known it supported anecdotal grower concerns that neonicotinoid resistance was likely affecting thiamethoxam (Cruiser®) seed dressing efficacy. Consequently resistance warnings were included into the 2018-2019 *Cotton Pest Management Guide*.

This season we again wanted to study resistance in thrips from cotton but unexpectedly only one strain of cotton seedling thrips was collected with the remainder (nine) being WFT. That single cotton seedling thrips strain was imidacloprid (Confidor®) susceptible but unexpectedly many of the WFT were spinetoram (Success® Neo) resistant. Spinetoram (Success® Neo) is the only registered WFT control for Australian cotton so the multiple detections are both worrisome and confusing. Spinosyn use to cause such widespread resistance would need to be high suggesting resistance may be being selected outside of cotton.

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Table 1. Percent etoxazole (Paramite® or Zeal®) susceptible TSM via molecular diagnosis plus bioassay determination of bifenthrin (Talstar®), abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA-140408) resistance via percent mortality at the discriminating dose (i.e. percent susceptible) for mites collected during season 2018-2019.

Strain	Etoxazole (Paramite® via PCR I1017F)	Bifenthrin (Talstar®)	Abamectin (Agrimec®)	Propargite (Comite®)	Diafenthiuron (Pegasus® as CGA140408)
Col F2020	100	79	91	100	100
Grif 74	100	100	93	100	100
Mor Col 3	100	5	71	100	100
Grif 35	100	61	74	100	100
Hill K4	100	100	100	100	100

Table 2. Percent susceptible SSM via bioassay against abamectin (Agrimec®), propargite (Comite®) and diafenthiuron (Pegasus® as CGA-140408) as percent mortality at the discriminating dose (i.e. percent susceptible) for mites collected during season 2018-19.

Strain	Abamectin (Agrimec®)	Propargite (Comite®)	Diafenthiuron (Pegasus® as CGA140408)
Mor B1	100	100	100
Mor B2	100	100	100
War 22	100	100	100
Hill K4	not tested	not tested	not tested
Goon 10	not tested	not tested	not tested
Goon 15	not tested	not tested	not tested
Goon B	100	100	100
Kim 21	100	100	100*
Kor 1	not tested	not tested	not tested
Eum 3	100	100	not tested
Mor G	100*	100*	not tested
Kor 1 M	not tested	not tested	not tested
Kor 2	not tested	not tested	not tested
Kor 4	100*	100*	100

*not replicated

Table 3. Percent pirimicarb / dimethoate (Pir / Dim), pyrethroid (Pyr) and organophosphate specific (profenofos / chlorpyrifos [Pro / Chl]) susceptible using molecular diagnosis plus bioassay determination of clothianidin (Clo)(Shield®), diafenthiuron (Dia)(Pegasus® as CGA140408), thiamethoxam (Thia)(Actara or Cruiser®) and sulfoxaflor (Sul)(Transform®) resistance via percent mortality at the discriminating dose (i.e. percent susceptible) for cotton aphid collected during season 2018-2019.

Strain	**Pir / dim qPCR S431F**	**Pro / Chl qPCR S302A**	**Pyr qPCR KDR**	**Dia**	**Clo**	**Thia**	**Sul**
Nar	100	100	100	100	100	100	100
Loom	100	100	100	100	100	100	100
Arm	100	100	100	100	100	100	100

Table 4. Percent fipronil (A301S) susceptible green mirid using molecular diagnosis to detect resistance in 2018-2019.

Strain	Green mirid PCR A302S
Spr B1	not tested DOA
Ill B1	not tested DOA
War A7	not tested DOA
Mor 2	not tested DOA
Cash	100
Har 4	100
Kim 22	not tested DOA
Mor W	100
Broo	100
Con	100
Hil	100
Grif	100
Gus M R	100
Mor Ant	100
Nar 17	100

DOA dead on arrival

Table 5. Percent susceptible cotton seedling thrips via bioassay against imidacloprid (Confidor®)

Strain	imidacloprid (Confidor®)
Spring B1	100

Table 6. Percent susceptible WFT via bioassay against spinetoram (Success® Neo)

Strain	Spinetoram (Success® Neo)
Kul K 9	95
Com Arg	99
Spring B1	92
Hill K4	100
Mor B2	100
Goon 10	100
Grif 35	99
Car	100
Grif 74	99

Appendix B

DNA Library (Insecticide Resistance Database)

Active Ingredient (chemical group)	Point mutation	Location of mutation	GenBank accession from susceptible strain	GenBank accession from resistant strain	Genus Species	Common name(s)	Host	Reference
Abamectin (avermectin)	A309V	N-terminus of the third transmembrane helix (M3) of the glutamate-gated chloride channel	JX014231	JX014232.1	*Plutella xylostella*	Diamondback moth	Crucifers, nasturtium	Wang, X., Wang, R., Yang, Y., Wu, S., O'Reilly, A., and Wu, Y. (2015). A point mutation in the glutamate-gated chloride channel of *Plutella xyostella* is associated with resistance to abamectin. Insect Molecular Biology.
Abamectin (avermectin)	G323D	Glutamate-gated chloride channel	See sequence in Figure 2 (no accession number provided).	See sequence in Figure 2 (no accession number provided).	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Kwon, D.H., Yoon, K.S., Clark, J.M., and Lee, S.H. (2010). A point mutation in a glutamate-gated chloride channel confers abamectin resistance in the

								two-spotted spider mite, Tetranychus urticae Koch. Insect Molecular Biology.
Abamectin								
Azinophosmethyl (organophosphate)	Serine to glycine mutation for which the position corresponds to Val 238 of the Torpedo AChE	*Ace*	No accession number provided.	No accession number provided.	*Leptinotarsa decemlineata*	Colorado potato beetle	Eggplant, pepper, potato, tomato	Zhu, K.Y., Lee, S.H. and Clark, J.M. (1996). A point mutation of acetylcholinesterase associated with azinphosmethyl resistance and reduced fitness in colorado potato beetle. Pesticide Biochemistry and Physiology, 55, 100-108.
Bifenthrin (pyrethroid)	L925I and T929V	Domain IIS4-IIS6 of the para sodium channel gene	AJ440727.1	AY094601.1 (not the study reference strain, but cited in text and has L925I mutation). See Table 3 for mutations.	*Bemisia tabaci*	Sweetpotato whitefly	Cotton	Roditakis, Emmanouil, Tsagkarakou, Anastasia, Vontas, John. (2006). Identification of

								mutations in the para sodium channel of Bemisia tabaci from Crete, associated with resistance to pyrethroids. Pesticide Biochemistry and Physiology 85, 85 161-166.
Bifenthrin (pyrethroid)	F1538I	Voltage-gated sodium channel gene			*Tetranychus urticae*	Two spotted spider mite		Piraneo, T.G., Bull, J., Morales, M.A., Lavine, L.C., Walsh, D.B., Zhu, F. (2015). Molecular mechanisms of Tetranychus urticae chemical adaption in hop fields. Scientific Reports, 5:
Bifenthrin (pyrethroid)	*kdr* mutation (L1014F) and MENTIONED NOT	Transmembrane segment IIS6	AJ131759	AJ131760	*Myzus persicae*	Green peach aphid	Flower, crops, fruit, trees, grains, tobacco,	Martinez-Torres, D., Foster, S.P., Field, L.M., Devonshire, A.L., and Williamson, M.S. (1999). A

	FOUND: *super-kdr* mutation (M918T)						vegetables	sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, Myzus persicae (Sulzer) (Hemiptera: Aphididae). Insect Molecular Biology, 8 339-346.
Bifenthrin (pyrethroid)	F1538I and A1215D (Other mutations ID with unknown role in resistance: L151V, G178S, A182G, G189D, S253T, M1016)	Intracellular linker connecting domains II and III. Mutations with unknown role in resistance occur within the II/III linker, except M1016 which occurs in IIS6 transmembrane domain.	Alignment performed against susceptible *Musca domestica* (U38813)-see Figure 1.	FJ906804-FJ906811	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Tsagkarakau, A., Leeuwent, T., Khajehali, J., Ilias, A., Grispou, M., Williamson, M., Tirry, L., and Vontas, J. (2009). Identification of pyrethroid resistance associated mutations in the para sodium channel of the two-spotted

								spider mite Tetranychus urticae (Acari: Tetranychidae). Insect Molecular Biology, 18 583-593.
Bifenthrin (pyrethroid)	M918L, L925I and T929I	*para*-type voltage gated sodium channel (section including domain-II S4-6)	No accession number provided; see supporting information for sequences.	No accession number provided; see supporting information for sequences.	*Trialeurodes vaporariorum*	Greenhouse whitefly	Cucumber, tomato, ornamentals	Karatolos, N., Gorman, K., Williamson, M. S., and Denholm, I. (2012). Mutations in the sodium channel associated with pyrethroid resistance in the greenhouse whitefly, Trialeurodes vaporariorum. Pest Management Science, 68 834-838.
Bifenazate	G126S	Cytochrome b gene			*Tetranychus urticae*	Two-spotted spider mite		Piraneo, T.G., Bull, J., Morales, M.A., Lavine, L.C., Walsh, D.B., Zhu, F. (2015).

Bifenazate (hydrazine carbazate)	G126S, I136T, S141F (a combination of at least two mutations necessary to confer pronounced resistant phenotype); P262T mutation alone confers a highly resistant phenotype.	Cytochrome b Q(o) pocket (cd1 helix; ef helix)	EU345430	FJ196444, FJ196445	*Tetranychus urticae* Koch	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Molecular mechanisms of Tetranychus urticae chemical adaption in hop fields. Scientific Reports, 5: Nieuwenhuyse, P.V., Leeuwen, T.V., Khajehali, J., Vanholme, B., and Tirry, L. (2009). Mutations in the mitochondrial cytochrome b of Tetranychus urticae Koch (Acari: Tetranychidae) confer cross-resistance between bifenazate and acequinocyl. Pest Management Science, 65, 404-412

Bifenazate (hydrazine carbazate)	G126S, S141F, D161G	Cytochrome b (mutations located in the Qo oxidation site-in the cd1 helix aligning the enzyme pocket)	EU345430 (ACA97098.1)	EU556754 (ACA97098.1)	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Van Leeuwen, T., Vanholme, B., Van Pottelberge, S., Van Nieuwenhuyse, P., Nauen, R., Tirry, L. and Denholm, I. (2008). Mitochondrial heteroplasmy and the evolution of insecticide resistance: non-mendelian inheritance in action. Proceedings of the National Academy of Sciences of the United States of American, 105, 5980-5985.
Chlorantraniliprole (diamides)	Deletion of 14 amino acids (Q4546-S4559); G4946E	Carboxyl-terminal region of PxRyR (ryanodine receptor)	JN801028.	No accession number given-mutation stated in text only.	*Plutella xylostella*	Diamond-back moth	Crucifers, nasturtium	Gong, W., Yan, H., Gao, L., Guo, Y., Xue, C. (2014). Chlorantraniliprole Resistance in the

	mutation in resistant field strains; G4946E substitution in resistant strain				Diamondback Moth (Lepidoptera: Plutellidae). Journal of Economic Entomology, 107(2) 806-814.
Chlorantraniliprole	G4946E, E1338D, Q4595L and I4790M	Ryanodine receptor	*Plutella xylostella*	Diamondback moth	Guo. L., Liang, P., Zhou, X., Gao, X. (2014). Novel mutations and mutation combinations of ryanodine receptor in a chlorantraniliprole resistant population of Plutella xylostella (L.). Scientific Reports, 4.
Chlorantraniliprole	G4946E	Ryanodine receptor	*Plutella xylostella*	Diamondback moth	Troczka, B., Zimmer, C.T., Elisa, J., Schorn, C., Bass, C., Davies, T.G., Field, L.M., Williamson, M.S.,

						Slater, R., Nauen, R. (2012). Resistance to diamide insecticides in diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae) is associated with a mutation in the membrane-spanning domain of the ryanodine receptor. Insect Biochemistry and Molecular Biology, 42: 873-880.
Chlorantraniliprole	Duplication of ryanodine receptor gene sequences occurs and that the susceptible strain	Ryanodine receptor		*Plutella xylostella*	Diamondback moth	Genotyping for the G4946E site of ryanodine receptor gene in Plutella xylostella (Lepidoptera: Yponomeutidae) considering gene duplication. Applied

	has the G4946E mutation but that the ryanodine receptor gene sequences of the susceptible strain are intronless. This suggests that the mutation encoded in intronless sequences does not function as a resistant factor.								Entomology and Zoology, 51: 195-204.
Clofentezine (tetrazine)	I1017F	Chitin synthase 1	JQ613274.	JQ613276-JQ613281.	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts,	Demaeght, P., Osborne, E.J., Odman-Naresh, J., Grbić, M., Nauen, R.,	

							ornamentals	Merzendorfer, H., Clark, R.M., and Van Leeuwen, T. (2014). High resolution genetic mapping uncovers chitin synthase-1 as the target-site of the structurally diverse mite growth inhibitors clofentezine, hexythiazox and etoxazole in Tetranychus urticae. Insect Biochemistry and Molecular Biology, 51, 52-61.
Cypermethrin (pyrethroid)	C190A	Domain II S4-5 linker from the para-type sodium channel gene	AF134216.2 (not the study reference strain, but primers used in this study are based on this strains sequence).	No accession number provided. See Table 3.	*Rhipicephalus microplus*	Southern/tropical cattle tick	Cattle	Domingues, L.N., Santos Alves Figueiredo Brasil, B.d., Paiva Bello, A.C.P.d., Leite, R.C., Silaghi, C. Pfister, K., and Passos, L.M.F. (2012). Survey of

								pyrethroid and organophosphate resistance in Brazilian field populations of Rhipicephalus (Boophilus) microplus: Detection of C190A mutation in domain II of the para-type sodium channel gene. Veterinary Parasitology, 189 327-332.
Cypermethrin (pyrethroid)	F1550I	Sodium channel gene	AF134216.2 (not the study reference strain, but primers used in this study are based on this strain's sequence).	No accession number provided.	*Rhipicephalus microplus*	Southern/tropical cattle tick	Cattle	Rodriguez-Vivas, R. I., Hodgkinson, J. E., Rosado-Aguilar, J. A., Villegas-Perez, S. L., and Trees, A. J. (2012). The prevalence of pyrethroid resistance phenotype and genotype in Rhipicephalus (Boophilus)

								microplus in Yucatan, Mexico. Veterinary Parasitology, 184 221-229.
Cypermethrin (pyrethroid)	T929I (absence of L1014F and M918T substitutions)	Domain IIS5 of the sodium channel gene	No accession number provided (study did not include a susceptible strain). *Thrips palmi* gene for sodium channel, complete cds, isolate: Kochi-strain GenBank: AB849921.1.	AB742424	*Thrips palmi*	Melon thrips	Melon, various vegetables, fruits, legumes, asters	Bao, W., Sonoda, S. (2012). Resistance to cypermethrin in melon thrips, Thrips palmi (Thysanoptera: Thripidae), is conferred by reduced sensitivity of the sodium channel and CYP450-mediated detoxification. Applied Entomology and Zoology, 47 443-448.
Cypermethrin (pyrethroid)	M918T, T929I and L1014F	A section of the *para*-orthologous sodium channel α-subunit spanning the IIS3-IIS6 region.	AB499848	AB499850.1, AB499851.1	*Thrips tabaci*	Onion thrips	Onion	Toda, S., and Morishita, M. (2009). Identification of three point mutations on the

								sodium channel gene in pyrethroid-resistant Thrips tabaci (Thysanoptera: Thripidae). Insecticide Resistance and Resistance Management, 102, 2296-2300.
Cypermethrin (pyrethroid)	S431F; A302S; Super-*kdr* mutation (M918L)	*ace*-1 gene; *ace*-1 gene; *para* gene	No accession number provided. *Aphis gossypii* clone *ace*-1 acetylcholine sterase mRNA GenBank: AF502081.1	No accession number provided.	*Aphis gossypii*	Cotton-melon aphid	Cotton, vegetables	Carletto, J., Martin, T., Vanlerberghe-Masutti, F., Brevault, T. (2010). Insecticide resistance traits differ among and within host races in Aphis gossypii. Pest Management Science, 66 301-307.
Beta-cypermethrin (pyrethroid)	L1014F	*para-sodium channel* (IIS4-IIS6)	No accession number provided.	JN002364	*Anopheles sinensis*	mosquito		Tan, W-L., Wang, Z-M., Li, C-X., Chu, H-L., Xu, Y.,

							Dong, Y-D., Wang, Z-C., Chen, D-Y., Liu, H., Liu, D-P., Liu, N., Sun, J. and Zhao, T. (2012). First report on co-occurrence knockdown resistance mutations and susceptibility to beta-cypermethrin in Anopheles sinensis from Jiangsu province, China. PLoS ONE,
Deltamethrin (pyrethroid)	V1016I mutation	Exon 21 of the *kdr* gene	No accession number provided.	No accession number provided. See text for primers.	*Aedes aegypti*	Yellow fever mosquito	Human Maestre-Serrano, R., Gomez-Camargo, D., Ponce-Garcia, G., and Flores, A. E. (2014). Susceptibility to insecticides and resistance mechanisms in Aedes aegypti

								from the Colombian Caribbean Region. Pesticide Biochemistry and Physiology.
Deltamethrin (pyrethroid)	L1035F/S (corresponds to position L1014F in the house fly)	Sodium channel gene	AB453977.1	No accession number provided. See Tables 3-6 in text for point mutation summary.	*Culex pipiens quinquefasciatus*	Mosquito	Human, birds	Zhao, M., Dong, Y., Ran, X., Guo, X., Xing, D., Zhang, Y., Yan, T., Zhu, X., Su, J., Zhang, H., Wang, G., Hou, W., Wu, Z., Li, C., Zhao, T. (2014). Sodium channel point mutations associated with pyrethroid resistance in Chinese strains of Culex pipiens quinquefasciatus (Diptera: Culicidae). Parasites & Vectors, 7.
Deltamethrin (pyrethroid)	L1014F (confers low level	Domains IIS2-IIS6 of the *para*-like voltage-sensitive	AJ408849-AJ408850	AJ408851-AJ408858	*Frankliniella occidentalis* (Pergande)	Western Flower Thrips	Range of flowering plants	Forcioli, D., Frey, B., and Frey, J.E. (2002). High

	resistance); M929V/T/I	sodium channel gene.					including weeds, vegetable crops and fruit trees	nucleotide diversity in the para-like voltage-sensitive sodium channel gene sequence in the Western Flower Thrips (Thysanoptera: Thripidae). Insecticide Resistance and Resistance Management, 95, 838-848.
Deltamethrin (pyrethroid)	L1014F	LdVssc1 gene	Did not have access to full text. *Leptinotarsa juncta* voltage-gated sodium channel (Vssc) gene, partial cds GenBank: HQ589185.1	Did not have access to full text.	*Leptinotarsa decemlineata*	Colorado potato beetle	Eggplant, pepper, potato, tomato	Jiang, W., Guo, W., Lu, W., Shi, X., Xiong, M., Wang, Z., and Li, G. (2011). Target site insensitivity mutations in the AChE and LdVssc1 confer resistance to pyrethroids and carbamates in Leptinotarsa decemlineata in northern Xinjiang

								Uygur autonomous region. Pesticide Biochemistry and Physiology, 100 74-81.
Deltamethrin (pyrethroid)	C190A	Domain IIS4-5 linker of para-sodium channel gene	Did not have access to full text.	Did not have access to full text.	*Rhipicephalus* (*Boophilus*) *microplus*	Southern/tropical cattle tick	Cattle	Kuman, R., Nagar, G., Kumar Sharma, A., Kuman, S., Ray, D.D., Chaudhuri, P., and Ghosh, S. (2013). Survey of pyrethroids resistance in Indian isolates of Rhipicephalus (Boophilus) microplus: Identification of C190A mutation in the domain II of the para-sodium channel gene. Acta Tropica, 125 237-245.
Deltamethrin (pyrethroid)	F to I (not sure of amino acid position)	S6 transmembrane segment of domain III (para-	No accession number provided.	No accession number provided. See primers and Table 1 in text.	*Rhipicephalus* (*Boophilus*) *microplus*	Southern cattle tick	Cattle	Rosario-Cruz, R., Guerrero, F.D., Miller, R.J., Rodriguez-Vivas,

		type sodium channel gene)					R.I., Dominguez-Garcia, D.I., Cornel, A.J., Hernandez-Ortiz, R., George, J.E. (2005). Roles Played by Esterase Activity and by a Sodium Channel Mutation Involved in Pyrethroid Resistance in Populations of Boophilus microplus (Acari: Ixodidae) Collected from Yucatan, Mexico. Journal of Medical Entomology, 42(6) 1020-1025.
Deltamethrin (pyrethroid)	L1014F	*para*-sodium channel gene	Unique DNA haplotype sequences (not sure if susceptible or resistant at	Unique DNA haplotype sequences (not sure if susceptible or resistant at this stage) were	*Culex pipiens pallens*	Northern house mosquito,	Chen, L., Zhong, D., Zhang, D., Shi, L., Zhou, G., Gong, M., Zhou, H., Sun, H., Ma, L., He, J., Hong,

			this stage) were deposited into GenBank, accession numbers: GU198929 - GU198944, GU325775 - GU325777, GU339219 - GU339221	deposited into GenBank, accession numbers: U198929 - GU198944, GU325775 - GU325777, GU339219 - GU339221				S., Zhou, D., Xiong, C., Chen, C., Zou, P., Zhu, C. and Yan, G. (2010). Molecular ecology of pyrethroid knockdown resistance in Culex pipiens pallens mosquitoes. PLoS ONE,
DDT (organochlorine)	L1014F/S. L1014F/W substitution coexisted in the 1014 allele. N1013 (co-existed with L1014F).	α-subunit of the *para*-type sodium channel gene (domain VI).	No accession number provide, however, positions of the amino acids was numbered according to the *An. gambiae* sodium channel sequence (GenBank accession no.	No accession number provided. See Figure 5 in text for deduced nucleotide and amino acid sequences around L1014.	*Anopheles sinensis*	Mosquito	Cattle, buffalo, human	Tan, W.L., Li, C.X., Wang, Z.M., Liu, M.D., Dong, Y.D., Feng, X.Y., Wu, Z.M., Guo, X.X., Xing, D., Zhang, Y.M., Wang, Z.C., and Zhao, T.Y. (2012). First detection of multiple knockdown resistance (kdr)-like mutations in voltage-gated sodium channel using three new

			XM_316809.4)					genotyping methods in Anopheles sinensis from Guangxi province, China. Journal of Medical Entomology. 49: 1012-1020.
Dimethoate (organophosphorus)	F331W, T280A (A201S mutation uncertain role)	Acetylcholinesterase1	GQ461336-GQ461338	GQ461345-GQ461347	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Khajehali, J., Van Leeuwen, T., Grispou, M., Morou, E., Alout, H., Weill, M., Tirry, L., Vontas, J., Tsagkarakou, A. (2010). Acetylcholinesterase point mutations in European strains of Tetranychus urticae (Acari: Tetranychidae) resistant to organophosphates. Pest Management

					Science, 66 220-228
Dimethoate	A302S (equivalent of A201), S431F (equivalent of F331) and G221A	Ace2	*Aphis gossypii*	Melon aphid	Lokeshwari, D., Krishna Kumar N.K., Manjunatha, H. (2016). Multiple mutations on the second acetylcholinesterase gene associated with dimethoate resistance in the melon aphid, Aphis gossypii (Hemiptera: Aphididae). Journal of Economic Entomology, 109: 887-897.
Dimethoate	G448S (equivalent of G396)	Acetylcholinesterase	*Bactrocera oleae*	Olive fruit fly	Vontas, J.G., Hejazi, M.J., Hawkes, N.J., Cosmidis, N., Loukas, M., Hemingway, J. (2002). Resistance-

								associated point mutations of organophosphate insensitive acetylcholinesterase, in the olive fruit fly Bactrocera oleae. Insect Molecular Biology, 11: 329-336.
Endosulfan (cyclodiene)	A302G/S	γ-aminobutyric acid (GABA) receptor subunit	No accession number provided.	No accession number provided, see Figure 1 in text.	*Myzus persicae*	Green peach aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Anthony, N., Unruh, T., Ganser, D. and ffrench-Constant, R. (1998). Duplication of the Rdl GABA receptor subunit gene in an insecticide-resistant aphid, Myzus persicae. Molecular Genetics and Genomics, 260, 165-175.
Endosulfan	Ala302Ser	Rdl gene (codes for GABA gated			*Hypothenemus hampei*	Coffee berry borer		ffrench-Constant, R.H., Steichen,

		chloride ion channel)						J.C., Brun, L.O. (1994). A molecular diagnostic for endosulfan insecticide resistance in the coffee berry borer Hypothenemus hampei (Coleoptera: Scolytidae). Bulletin of Entomological Research, 84: 11-16.
Etoxazole (diphenyloxazoline)	I1017F	Chitin synthase 1	JQ613274-JQ613277	JQ613278-JQ613281.	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Demaeght, P., Osborne, E.J., Odman-Naresh, J., Grbić, M., Nauen, R., Merzendorfer, H., Clark, R.M., and Van Leeuwen, T. (2014). High resolution genetic mapping uncovers chitin synthase-1 as the

								target-site of the structurally diverse mite growth inhibitors clofentezine, hexythiazox and etoxazole in Tetranychus urticae. Insect Biochemistry and Molecular Biology, 51, 52-61.
Etoxazole (diphenyloxazoline)	I1017F	C-terminal transmembrane domain of chitin synthase 1	JQ613274	JQ613278-JQ613281	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Van Leeuwen, T., Demaeght, P., Osborne, E.J., Dermauw, W., Gohlke, S., Nauen, R., Grbić, Tirry, L., Merzendorfer, H. and Clark, R.M. (2012). Population bulk segregant mapping uncovers resistance mutations and the mode of

								action of a chitin synthesis inhibitor in arthropods. Proceedings of the National Academy of Sciences of the United States of America, 109, 4407-4412.
Fenopropathrin (pyrethroid) + acephate (organophosphate) mixture	M918V, L925I	IIS4-5 linker of the para-type sodium channel	AJ440727.1	AJ440728.1 (M918V mutation only)	*Bemisia tabaci*	Sweetpotato whitefly	Cotton	Morin, S., Williamson, M.S., Goodson, S.J., Brown, J.K., Tabashnik, B.E., and Dennehy, T.J. (2002). Mutations in the Bemisia tabaci para sodium channel gene associated with resistance to a pyrethroid plus organophosphat e mixture. Insect Biochemistry and Molecular

								Biology, 32, 178-1791.
Fipronil (phenyl pyrazole)	A2'N	RDL γ-aminobutyric acid (GABA) receptor (M2 membrane-spanning region)	AB253526	AB594844	*Laodelphax striatellus*	Small brown planthopper	Rice	Nakao, T., Kawase, A., Kinoshita, A., Abe, R., Hama, M., Kawahara, N., and Hirase, K. (2011). The A2'N Mutation of the RDL γ-Aminobutyric Acid Receptor Conferring Fipronil Resistance in Laodelphax striatellus (Hemiptera: Delphacidae). Journal of Economic Entomology, 104 646-652.
Fipronil	A302S; R300Q	Γ-aminobytyruc acid receptor	Rdl nucleotide sequence: KC841916	AGK30293 Note this study also included alignment ofRdl amino acid sequences from	*Nilaparvata lugens*	Brown planthopper		Zhang, Y., Meng, X., Yang, Y., Li, H., Wang, X., Yang, B., Zhang, J., Li, C., Millar, S., Liu, Z. (2016).

various insect species and highlighted the positions of arginine and alanine corresponding to the R300Q and A302S mutations. Accession numbers for the sequences are: *Aedes aegypti*, AAA68961; *Apis mellifera*, AAC63381; *Blattella germanica*, AAB33733; *Ceratitis capitata*, AAD51101; *Drosophila melanogaster*, P25123; *Drosophila simulans*, AAK00512;

Synergistic and compensatory effects of two point mutations conferring target-site resistance to fipronil in the insect GABA receptor RDL. Scientific Reports: Doi: 10.1038/srep32335

			Lucilia cuprina, AAB81966 ; *Musca domestica*, AAC23602			
Fipronil	A2'N	GABA receptor		*Sogatella furcifera*	Whitebacked planthopper	Nakao, T., Naoi, A., Kawahara, N., Hirase, K. (2010). Mutation of the GABA receptor associated with fipronil resistance in the whitebacked planthopper, Sogatella furcifera. Pesticide Biochemistry and Physiology. 97: 262-266.
Fipronil	A302S	Rdl (GABA gated chloride channel) allele		*Musca domestica*	House fly	Gao, J-R., Kozaki, T., Leichter, C.A., Rinkevich, F.D., Shonom T., Scott, J.G. (2007). The A302S mutation in Rdl that confers resistance to

							cyclodienes and limited cross-resistance to fipronil is undetectable in field populations of house flies from the USA. Pesticide Biochemistry and Physiology. 88: 66-70.
Fipronil	A302S	Within the M2 transmembrane region of the GABA receptor subunit termed Rdl		*Ctenocephalides felis*	Cat flea	Cats	Bass, C., Schroeder, I., Turberg, A., Field, L.M., Williamson, M.S. (2004). Identification of the Rdl mutation in laboratory and field strains of the cat flea, Ctenocephalides felis (Siphonaptera: Pulicidae). Pest Management Science. 60: 1157-1162

Fipronil	A302S and Met360Ile	Rdl. Said that two nonsynonymous polymorphisms were present in a duplication site encompassing Rdl: A302S=insecticide resistance site and Met360Ile at an RNA-edited site.			*Drosophila melanogaster*	Fruit fly		Remnant, E.J., Good, R.T., Schmidt, J.M., Lumb, C., Robin, C., Daborn, P.J., Batterham, P. (2013). Gene duplication in the major insecticide target site, Rdl, in Drosophila melanogaster. PNAS. 110: 14705-14710.
Flumethrin (pyrethroid)	L190I, G214T	Domain II S4-5 linker region of the *para*-sodium channel gene	Partial sodium channel gene of *R. microplus* sequences GenBank: AF134216	No accession number provided. See Table 2 in text for sequence.	*Rhipicephalus microplus*	Cattle tick	Cattle	Jonsson, N.N., Cutullè, C., Corley, S.W., and Seddon, J.M. (2010). Identification of a mutation in the para-sodium channel gene of the cattle tick Rhipicephalus microplus associated with resistance to flumethrin but not to

								cypermethrin. International Journal for Parasitology, 40, 1659-1664.
Hexythiazox (unclassified)	I1017F	Chitin synthase 1	JQ613274.	JQ613276-JQ613281.	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Demaeght, P., Osborne, E.J., Odman-Naresh, J., Grbić, M., Nauen, R., Merzendorfer, H., Clark, R.M., and Van Leeuwen, T. (2014). High resolution genetic mapping uncovers chitin synthase-1 as the target-site of the structurally diverse mite growth inhibitors clofentezine, hexythiazox and etoxazole in Tetranychus urticae. Insect Biochemistry and Molecular

								Biology, 51, 52-61.
Imidacloprid (neonicotinoids)	R81T	The loop D region of the nicotinic acetylcholine receptor β1 subunit	No accession number provided. See Table 4 in text. *Myzus persicae* mRNA for putative nicotinic acetylcholine receptor subunit (β1 gene) GenBank: AJ251838.1	No accession number provided. See Table 4 in text.	*Myzus persicae*	Green peach aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Bass, C., Puinean, A. M., Andrews, M., Cutler, P., Daniels, M., Elias, J., Paul, V. L., Crossthwaite, A. J., Denholm, I., Field, L. M., Foster, S. P., Lind, R., Williamson, M. S., and Slater, R. (2011). Mutation of a nicotinic acetylcholine receptor B subunit is associated with resistance to neonicotinoid insecticides in the aphid Myzus persicae. Biomed Central Neuroscience, 12 51.

Imidacloprid (neonicotinoids)	R81T	The loop D region of the nicotinic acetylcholine receptor β1 subunit	No accession number provided. *Myzus persicae* mRNA for putative nicotinic acetylcholine receptor subunit (β1 gene) GenBank: AJ251838.1	No accession number provided. See Table 3 for primers.	*Myzus persicae*	Green peach aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Panini, M., Dradi,D., Marani, G., Butturini, A., and Mazzoni, E. (2014). Detecting the presence of target-site resistance to neonicotinoids and pyrethroids in Italian populations of Myzus persicae. Pest Management Science, 70 931-938.
Imidacloprid (neonicotinoids)	Y151S (only ever been found in laboratory strain)	A conserved position in two nAChR subunits, Nlα1 and Nlα3	No accession number provided.	AY378698-AY378700, AY378702	*Nilaparvata lugens*	Brown planthopper		Liu, Z., Williamson, M.S., Lansdell, S.J., Denholm, I., Han, Z. and Millar, N.S. (2005). A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in

								Nilaparvata lugens (brown plant hopper). PNAS, 102, 8420-8425.
Indoxacarb (oxadiazine)	F1845Y and V1848I (mutations were never found to co-exist in the same allele of *PxNa$_v$*)	In the sixth segment of domain IV of the PxNav protein (Sodium channel gene)	KM027335	No accession number provided. See Figure 1 in text.	*Plutella xylostella*	Diamond-back moth	Crucifers, nasturtium	Wang, X.L., Su, W., Zhang, J.H., Yang, Y.H., Dong, K., and Wu, Y.D. (2015). Two novel sodium channel mutations associated with resistance to indoxacarb and metaflumizone in the diamondback moth, Plutella xylostella. Insect Science 1-9.
Indoxacarb (oxadiazine)	L1014F	Domain IIS6 of the sodium channel gene.	No accession number provided. See Figure 3 in text. *Spodoptera exigua* GABA-gated chloride	No accession number provided. See Figure 3 in text.	*Spodoptera exigua*	Beet army worm, lesser army worm	Cotton, tomato, celery, lettuce, cabbage, alfalfa	Gao, M., Mu, W., Wang, W., Zhou, C., and Li, X. (2014). Resistance mechanisms and risk assessment regarding indoxacarb in the beet armyworm,

			channel a2 subunit mRNA, complete cds GenBank: EF535530.1					Spodoptera exigua. Phytoparasitica, 42, 585-594.
Lambda-cyhalothrin (pyrethroid)	L1015F	Voltage-sensitive sodium channel gene (named *AIVSSC*)	No accession number provided.	KR139855. See Figure 3 in text.	*Apolygus lucorum* (Meyer-Dür)	Green mirid bug	Cotton, Lucerne, fruits, dates	Zhen, C., and Gao, X. (2015). A point mutation (L1015F) of the voltage-sensitive sodium channel gene associated with lambda-cyhalothrin resistance in Apolygus lucorum (Meyer-Dur) population from the transgenic Bt cotton field of China. Pesticide Biochemistry and Physiology.
Lambda-cyhalothrin (pyrethroid)	M918L; V1010A	Transmembrane segments IIS4-IIS6 of the para-orthologous	No accession number provided. See text for primers and	No accession number provided. See text for primers and Figure 2 and 3 for sequences.	*Thrips tabaci*	Onion thrips	Onion	Wu, M., Gotoh, H., Waters, T., Walsh, D. B., and Lavine, L. C. (2013).

		sodium channel gene.	Figure 2 and 3 for sequences. *Thrips tabaci* gene for sodium channel, partial cds, strain: TypeI GenBank: AB499848	*Thrips tabaci* gene for sodium channel, partial cds, GenBank: AB499850.1 (strain: TypeIII), AB499851.1 (strain: TypeIV)			Identification of an alternative knockdown resistance (kdr)-like mutation, M918L, and a novel mutation, V1010A, in the Thrips tabaci voltage-gated sodium channel gene. Pest Management Science, 70 977-981.
Malathion (organothiophosphate)	F368Y	*Ace*	No accession number provided, see Figure 3 in text.	No accession number provided, see Figure 3 in text.	*Drosophilia melanogaster*	Fruit fly	Fournier, D., Bride, J-M., Hoffmann, F. and Karch, F. (1992). Acetylcholinesterase: Two types of modifications confer resistance to insecticides. The Journal of Biological Chemistry, 267, 14270-14274.

Methomyl (carbamate)	P59L, A85S; P8L, S182-183F, W185L	P450 CYP6C gene; P450 CYP9F gene	No accession number provided. See Figure 2 and 3 in text.	P450 6C gene accession numbers for B, Cv and Q biotype Bemisia tabaci are FJ025798, FJ025799 and FJ025800 respectively. P450 9F gene accession numbers for B, Cv and Q biotype *Bemisia tabaci* are EU723679, EU723680 and EU723681respectively.	*Bemisia tabaci*	Sweetpotato whitefly	Cotton	Qiu, B., Liu, L., Mathur, V., Qin, Z., Ren, S. (2009). Genetic mutations associated with chemical resistance in the cytochrome P450 genes of invasive and native Bemisia tabaci (Hemiptera: Aleyrodidae) populations in China. Insect Science, 1 237-245.
Methomyl (carbamate)	F331W, T280A (A201S mutation uncertain role)	Acetylcholinesterase1	GQ461336-GQ461338	GQ461351-GQ461343, GQ461345-GQ461347, GQ461339-GQ461341	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Khajehali, J., Van Leeuwen, T., Grispou, M., Morou, E., Alout, H., Weill, M., Tirry, L., Vontas, J., Tsagkarakou, A. (2010). Acetylcholinesterase point mutations in

								European strains of Tetranychus urticae (Acari: Tetranychidae) resistant to organophosphates. Pest Management Science, 66 220-228.
Monocrotophos (organophosphate)	A1753T (location corresponds to unpublished AChE cDNA sequence)	Between the β_9 and β_{10} sheets in AChE gene	AF369793	No accession number provided. See Table 3 in text.	*Helicoverpa armigera*	Cotton bollworm	Cotton, corn, sorghum, tomato	Ren, X., Z. Han, and Y. Wang. (2002). Mechanisms of Monocrotophos resistance in Cotton Bollworm, Helicoverpa armigera (Hubner). Archives of Insect Biochemistry and Physiology, 51 103-110.
Nodulisporic acid (indole diterpenes)/ivermectin (avermectin)	P299S	Glutamate-gated chloride channel subunit	U58776.1	AF297500, AF297501	*Drosophila melanogaster*	Fruit fly		Kane, N.S., Hirschberg, B., Qian, S., Hunt, D., Thomas, B., Brochu, R.,

							Ludmerer, S.W., Zheng, Y., Smith, M., Arena, J.P., Cohen, C.J., Schmatz, D., Warmke, J., and Cully, D.F. (2000). Drug-resistant Drosophila indicate glutamate-gated chloride channels are targets for the antiparasitics nodulisporic acid and ivermectin. PNAS, 13949-13954.
Permethrin (pyrethroid)	L1014H	*para*-type sodium channel gene	X96668	No accession number provided. See sequences in Figure 1.	*Musca domestica*	Housefly	Liu, N., and Pridgeon, J.W. (2002). Metabolic detoxification and the kdr mutation in pyrethroid resistant house flies, Musca

								domestica (L.). Pesticide Biochemistry and Physiology, 73, 157-163.
Permethrin (pyrethroid) and DDT	L1014S	Voltage-gated sodium channel	Y13592	No accession number provided. See Table 1 in text.	*Anopheles gambiae*	African malaria mosquito		Ranson, H., Jensen, B., Vulule, J.M., Wang, X., Hemingway, J. and Collins, F.H. (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethoids. Insect Molecular Biology, 9, 491-497.
Pirimicarb (carbamate)	S431F	Acetylcholinesterase1	AF502082	No accession number provided. See Figure 1 in text.	*Aphis gossypii* Glover	Cotton-melon aphid	Cotton, vegetables	Benting, J., and Nauen, R. (2004). Biochemical

								evidence that an S431F mutation in acetylcholinesterase-1 of Aphis gossypii mediates resistance to pirimicarb and omethoate. Pest Management Science, 60, 1051-1055.
Pirimicarb (carbamate)	S431P	Acetylcholinesterase active site	No accession number provided.	No accession number provided. See Table 2 in text.	*Myzus persicae*	Green Peach Aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Fuentes-Contreras, E., Figueroa, C., Silva, A., Bacigalupe, L., Briones, L., Foster, S., and Unruh, T. (2013). Survey of Resistance to Four Insecticides and their Associated Mechanisms in Different Genotypes of the Green Peach

								Aphid (Hemiptera: Aphididae) from Chile. Journal of Economic Entomology, 106 400-407.
Pirimicarb (carbamate)	S431P	MpAChE2	No access to full text	No access to full text	*Myzus persicae*	Green peach aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Nabeshima, T., Kozaki, T., Tomita, T. and Kono, Y. (2003). An amino acid substitution on the second acetylcholinesterase in the pirimicarb-resistant strains of the peach potato aphid, Myzus persicae. Biochemical and Biophysical Research Communications, 307, 15-22.
Prothiofos (organophosphorus)	Ala201Ser, Gly227Ala, Ala441Gly	*pxace1*	AAV65825.2	No accession number provided. See Figure 3 in text.	*Plutella xylostella*	Diamondback moth	Crucifers, nasturtium	Baek, J.H., Kim, J II., Lee, D-W., Chung, B.K., Miyata, T. and

								Lee, S.H. (2005). Identification and characterisation of ace1-type acetylcholinesterase likely associated with organophosphate resistance in Plutella xylostella. Pesticide Biochemistry and Physiology, 81, 164-175.
Omethoate (organophosphate)	S413F	Acetylcholinesterase-1	AF502082	No accession number provided. See Figure 1 in text.	*Aphis gossypii* Glover	Cotton-melon aphid	Cotton, vegetables	Benting, J., and Nauen, R. (2004). Biochemical evidence that an S431F mutation in acetylcholinesterase-1 of Aphis gossypii mediates resistance to pirimicarb and omethoate. Pest Management

								Science, 60, 1051-1055.
Thiamethoxam (neonicotinoids)	R81T	The loop D region of the nicotinic acetylcholine receptor β1 subunit	No accession number provided. See Table 4 in text. *Myzus persicae* mRNA for putative nicotinic acetylcholine receptor subunit (β1 gene) GenBank: AJ251838.1	No accession number provided. See Table 4 in text.	*Myzus persicae*	Green peach aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Bass, C., Puinean, A. M., Andrews, M., Cutler, P., Daniels, M., Elias, J., Paul, V. L., Crossthwaite, A. J., Denholm, I., Field, L. M., Foster, S. P., Lind, R., Williamson, M. S., and Slater, R. (2011). Mutation of a nicotinic acetylcholine receptor B subunit is associated with resistance to neonicotinoid insecticides in the aphid Myzus persicae. Biomed Central Neuroscience, 12, 51.

Unspecified organophosphate (no access to full text)	F331(439)C	Acetylcholinesterase	No access to full text	No access to full text	*Tetranychus urticae*	Two-spotted spider mite	Cotton, fruits, vegetables, walnuts, ornamentals	Anazawa, Y., Tomita, T., Aiki, Y., Kozaki, T, and Kono, Y. (2003). Sequence of a cDNA encoding acetylcholinesterase from susceptible and resistant two-spotted spider mite, Tetranychus urticae. Insect Biochemistry and Molecular Biology, 33, 509-514.
Unspecified organophosphate (no access to full text)	F139L (corresponding to F115S in *Drosophilia*); A302S	*Ace*2; *Ace*1	No access to full text	No access to full text	*Aphis gossypii* Glover	Cotton aphid	Cotton, vegetables	Li, F, and Han, Z. (2004). Mutations in acetylcholinesterase associated with insecticide resistance in the cotton aphid, Aphis gossypii Glover. Insect Biochemistry and Molecular

							Biology, 34, 397-405.
Unspecified organophosphate (no access to full text)	F455W	*Ace*2	No access to full text	No access to full text	*Culex tritaeniorhynchus*	Mosquito	Nabeshima, T., Mori, A., Kozaki, T., Iwata, Y., Hidoh, O., Harada, S., Kasai, S., Severson, D.W., Kono, Y. and Tomita, T. (2004). An amino acid substitution attributable to insecticide-insensitivity of acetylcholinesterase in a Japanese encephalitis vector mosquito, Culex tritaeniorhynchus. Biochemical and Biophysical Research Communications, 313, 794-801.
Unspecified pyrethroid (DDT?)	L1014F/S, V1010L	IIS6 transmembrane segments of	No accession number provided.	No accession number provided.	*Anopheles culicifacies*	Anopheles mosquito	Singh, O.M., Dykes, C.L., Das, M.K., Pradhan, S.,

		voltage-gated sodium channel	*Anopheles culicifacies* voltage-gated sodium channel gene, partial cds GenBank: FJ968792.1				Bhatt, R.M., Agrawal, O.P., and Adak, T. (2010). Presence of two alternative kdr-like mutations, L1014F and L1014S, and a novel mutation, V1010L, in the voltage gated Na+ channel of Anopheles culicifacies from Orissa, India. Malaria Journal, 9.
Unspecified pyrethroid (no access to full text)	L1014F; M918T	Domain II of the para-type sodium channel gene (IIS6 transmembrane; intracellular IIS4-5 loop)	No accession number as no access to full text. *Musca domestica* sodium channel protein para-like (para-like), mRNA	No accession number as no access to full text.	*Musca domestica*	Housefly	Williamson, M.S., Martinez-Torres, D., Hick, C.A., and Devonshire, A.L. (1996). Identification of mutations in the housefly para-type sodium channel gene associated with knockdown

			locus NM_0012868 85				resistance (kdr) to pyrethroid insecticides. Molecular and General Genetics, 252, 51-60.
Unspecified pyrethroid (no access to full text)	F1550I	S6 transmembrane segment of the domain III in the sodium channel	No access to full text. *Boophilus microplus* putative sodium channel mRNA, partial cds GenBank: AF134216.2	No access to full text.	*Rhipicephalus microplus*	Southern/tropical cattle tick	Cattle	He, H., Chen, A.C., Davey, R.B., Ivie, G.W., and George, J.E. (1999). Identification of a point mutation in the para-type sodium channel gene from a pyrethroid-resistance cattle tick. Biochemical and Biophysical Research Communication, 261, 558-561.
Unspecified pyrethroid (no accession to full text)	V421M	IS6 of the hscp voltage-gated sodium channel	No access to full text. *Heliothis virescens* voltage-	No access to full text.	*Heliothis virescens* F.	Tobacco budworm		Park, Y., Taylor, M.F., and Feyereisen R. (1997). A valine421 to methionine

			sensitive sodium channel alpha subunit (hscp) gene, partial cds GenBank: U24236.1				mutation in IS6 of the hscp voltage-gated sodium channel associated with pyrethroid resistance in Heliothis virescens F. Biochemical and Biophysical Research Communications, 3: 688-691.
Unspecified pyrethroids (no access to full text)	L993L	*para* sodium channel genes	No access to full text.	Sequences from study deposited in the Genbank database accession numbers U73583 and U73584	*Blattella germanica*	German cockroach	Dong, K. A single amino acid change in the para sodium channel protein is associated with knockdown-resistance (kdr) to pyrethroid insecticides in German cockroach. Insect Biochemistry and Molecular

							Biology, 27: 93-100.
Unspecified pyrethroid	L1014S/F	DIIS6 region of the *para*-type sodium channel gene	No accession number provided.	No accession number provided.	*Anopheles gambiae* and *Anopheles arabiensis*	African malaria mosquito	Verhaeghen. K., Van Bortel, W., Roelants, P., Backeljau, T. and Coosemans, M. (2006). Detection of the East and West African kdr mutation in Anopheles gambiae and Anopheles arabiensis from Uganda using a new assay based on FRET/Melt Curve analysis. Malaria Journal, 5.
Unspecified pyrethroids (no access to full text)	L190I	Domain II S4-5 linker of the para-sodium channel	No access to full text	No access to full text	*Rhipicephalus (Boophilus) microplus*	Cattle tick	Morgan, J.A., Corley, S.W., Jackson, L.A., Lew-Tabor, A.E., Moolhuijzen, P.M. and Jonsson, N.N. (2009).

						Identification of a mutation in the para-sodium channel gene of the cattle tick Rhipicephalus (Boophilus) microplus associated with resistance to synthetic pyrethroid acaricides. International Journal for Parasitology, 39, 775-779.
Unspecified pyrethroids (no access to full text)	L1014F and M918T	*Vssc1*	No access to full text	No access to full text	*Musca domestica*	House fly	Lee, S.H., Smith, T.J., Knipple, D.C. and Soderlund, D.M. (1999). Mutations in the house fly Vssc1 sodium channel gene associated with super-kdr resistance abolish the pyrethroid sensitivity of

								Vssc1/tipE sodium channels expressed in Xenopus oocytes. Insect Biochemistry and Molecular Biology. 29, 185-194.
Various pyrethroids (including deltamethrin, cypermethrin, lambda-cyhalothrin, tau-fluvalinate, bifenthrin, permethrin and tefluthrin)	L1014F and M918T	S6 of the domain II region of the sodium channel	No accession number provided.	AM711603	*Myzus persicae* (Sulzer)	Peach-potato aphid	Flower, crops, fruit, trees, grains, tobacco, vegetables	Eleftherianos, I., Foster, S.P., Williamson, M.S., and Denholm, I. (2008). Characterisation of the M918T sodium channel gene mutation associated with strong resistance to pyrethroid insecticides in the peach-potato aphid, Myzus persicae (Sulzer). Bulletin of Entomological Research, 98: 183-191.

Combination of carbamates (carbaryl and propoxur) and organophosphorous (malaoxon and paraoxon)	F115S, I199V/T, G303A, F368Y	*Ace*	No accession number provided	No accession number provided	*Drosophila melanogaster*	Fruit fly	Mutero, A., Pralavorio, M., Bride, J-M. and Fournier, D. (1994). Resistance-associated point mutations in insecticide-insensitive acetylcholinesterase. 91, Proceedings of the National Academy of Sciences, USA, 5922-5926

Sequence alignments (using Clustal Omega)

Glutamate-gated chloride channel and GABA receptor alignment

Abamectin resistance in *Plutella xylostella*- A309V mutation site (JX014231 and JX014232.1).

Abamectin resistance in Tetranychus urticae – **G**323**D** (TuGlCl_AbaS and TuGl_AbaR). Note sequence for TuGlCl_AbaS/R was hand-copied from journal article so double check before using.
Nodulisporic acid (indole diterpenes)/ivermectin (avermectin) resistance in *Drosophilia melanogaster* – **P**299**S** mutation site (AF297500, AF297501 and U58776.1).
Fipronil resistance in *Laodelphax striatellus* –**A**2'**N** mutation (AB253526 and AB594844).

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AB253526        ---------MRRALALVWLAVTTTLLRPADRLPFVLAGTGGGSMLGDVNISAILDSF--S
AB594844        ------------------------------------------------------------
TuGlCl_AbaS      MFNYLNLVNMSDPLTT-IFS-IVLIVASY-----STLVNGSAS-F-REAEKKILDRIIGK
TuGlCl_AbaR      MFNYLNLVNMSDPLTT-IFS-IVLIVASY-----STLVNGSAS-F-REAEKKILDRIIGK
JX014231        ----MDVLRPSCALFV-LFLLYCA---HL-----TECVNAKIN-F-REKEKQILDQILGP
JX014232.1       ----MDVLRPSCALFV-LFLLYCA---HL-----TECVNAKIN-F-REKEKQILDQILGP
AF297501         ---------MGSGHYF-WAILYFASLCSA-----SLANNAKIN-F-REKEKKVLDQILGA
U58776.1        ---------MGSGHYF-WAILYFASLCSA-----SLANNAKVN-F-REKEKKVLDQILGA
AF297500         ---------MGSGHYF-WAILYFASLCSA-----SLANNAKVN-F-REKEKKVLDQILGA


AB253526         VSYDKRVRPNY------GGPPVEVGVTMYVLSISSLSEVKMDFTLDFYFRQFWTDPRLAF
AB594844         ------------------------------------------------------------
TuGlCl_AbaS       GVYDPRIRPSGANATVDGDEPCIVKVNIYIRSISRIDDVTMEYATQITFREEWRDSRLVF
TuGlCl_AbaR       GVYDPRIRPSGANATVDGDEPCIVKVNIYIRSISRIDDVTMEYATQITFREEWRDSRLVF
JX014231        GRYDARIRPSGINGT--GDAPTLVRVNLYLRSISKIDDYKMEYSVQLTFREQWLDERLKF
JX014232.1       GRYDARIRPSGINGT--GDAPTLVRVNLYLRSISKIDDYKMEYSVQLTFREQWLDERLKF
AF297501         GKYDARIRPSGINGT---DGPAVVRVNIFVRSISKIDDVTMEYSVQLTFREQWTDERLKF
U58776.1        GKYDARIRPSGINGT---DGPAIVRINLFVRSIMTISDIKMEYSVQLTFREQWTDERLKF
AF297500         GKYDARIRPSGINGT---DGPAIVRINLFVRSIMTISDIKMEYSVQLTFREQWTDERLKF


AB253526         RKRPGVETLSVGSEFIKNIWVPDTFFVNEKQSYFHIATTSNEFIRIHHSGSITRSIRLTI
AB594844         ------------------------------------------------------------
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TuGlCl_AbaS     DDMGGRIKFLVL-TDPEKLWKPDLFFSNEKNGHFHDIIMPNVLLRIFPNGDILYSIRISL
TuGlCl_AbaR     DDMGGRIKFLVL-TDPEKLWKPDLFFSNEKNGHFHDIIMPNVLLRIFPNGDILYSIRISL
JX014231       NNLGGRLKYLTL-TEANRVWMPDLFFSNEKEGHFHNIIMPNVYIRIFPNGNVLYSIRISL
JX014232.1      NNLGGRLKYLTL-TEANRVWMPDLFFSNEKEGHFHNIIMPNVYIRIFPNGNVLYSIRISL
AF297501        DDIQGRLKYLTL-TEANRVWMSDLFFSNEKEGHFHNIIMPNVYIRIFPNGSVLYSIRISL
U58776.1       DDIQGRLKYLTL-TEANRVWMPDLFFSNEKEGHFHNIIMPNVYIRIFPNGSVLYSIRISL
AF297500        DDIQGRLKYLTL-TEANRVWMPDLFFSNEKEGHFHNIIMPNVYIRIFPNGSVLYSIRISL


AB253526        TASCPMNLQYFPMDRQLCHIEIESFGYTMRDIRYKWNEGPNSVGVSNEVSLPQFKVLGHR
AB594844        ------------------------------------------------------------
TuGlCl_AbaS     NLFCPMDLKYFPLDIQNCSISMASYGYTTEDLVFLWKAGDP-VQITKSLHLPRFTLMKYL
TuGlCl_AbaR     NLFCPMDLKYFPLDIQNCSISMASYGYTTEDLVFLWKAGDP-VQITKSLHLPRFTLMKYL
JX014231       TLSCPMNLKLYPLDKQTCSLRMASYGWTTDDLVFLWKEGDP-VQVVKNLHLPRFTLEKFL
JX014232.1      TLSCPMNLKLYPLDKQTCSLRMASYGWTTDDLVFLWKEGDP-VQVVKNLHLPRFTLEKFL
AF297501        TLACPMNLKLYPLDRQICSLRMASYGWTTNDLVFLWKEGDP-VQVVKNLHLPRFTLEKFL
U58776.1       TLACPMNLKLYPLDRQICSLRMASYGWTTNDLVFLWKEGDP-VQVVKNLHLPRFTLEKFL
AF297500        TLACPMNLKLYPLDRQICSLRMASYGWTTNDLVFLWKEGDP-VQVVKNLHLPRFTLEKFL


AB253526        QRAMEISLTTGNYSRLACEIQFVRSMGYYLIQIYIPSGLIVIISWVSFWLNRNATPARV**A**
AB594844        ------------------------YYLIQIYIPSGLIVIISWVSFWLNRNATPARV**N**
TuGlCl_AbaS     TSYCTSKTNTGEYSCLKVELVFKREFSYYLFLIYVPCCMLVIVSWVSFWIDPNSAAARV**L**
TuGlCl_AbaR     TSYCTSKTNTGEYSCLKVELVFKREFSYYLFLIYVPCCMLVIVSWVSFWIDPNSAAARV**L**
JX014231       TDYCNSKTNTGEYSCLKVDLLFKREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARV**S**
JX014232.1      TDYCNSKTNTGEYSCLKVDLLFKREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARV**S**
AF297501        TDYCNSKTNTGEYSCLKVDLLFKREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARV**S**
U58776.1       TDYCNSKTNTGEYSCLKVDLLFRREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARV**S**
AF297500        TDYCNSKTNTGEYSCLKVDLLFRREFSYYLIQIYIPCCMLVIVSWVSFWLDQGAVPARV**S**
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AB253526        LGVTTVLTMTTLMSSTNAALPKISYVKSIDVYLGTCFVMVFASLLEYATVGYMAKRIQMR
AB594844        LGVTTVLTMTTLMSSTNAALPKISYVKSIDVYLGTCFVMVFASLLEYATV----------
TuGlCl_AbaS      LGVTCLLTMSRQISGINASLPPVSYTKAVDIWTGCCLIFVFGALIEFAIVNYVSRTDTIK
TuGlCl_AbaR      LGVTCLLTMSRQISGINASLPPVSYTKAVDIWTDCCLIFVFGALIEFAIVNYVSRTDTIK
JX014231        LGVTTLLTMATQSSGINASLPPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSD---
JX014232.1       LGVTTLLTMATQSSGINASLPPVSYTKVIDVWTGVCLTFVFGALLEFALVNYASRSD---
AF297501        LGVTTLLTMATQTSGINASLSPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSGS--
U58776.1        LGVTTLLTMATQTSGINASLPPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSGSNK
AF297500        LGVTTLLTMATQTSGINASLSPVSYTKAIDVWTGVCLTFVFGALLEFALVNYASRSGSNK
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AB253526        KNRFMAIQKIAEQKQKQGMEAHAGHPPGVPGGGDPADHAPKQTATRYKTLDSKGHYKSGT
AB594844        ------------------------------------------------------------
TuGlCl_AbaS      ADKHRRRR----PQGIV------------GARRKFDTAKDSGI------ESS-DVEDGP
TuGlCl_AbaR      ADKHRRRR----PQGIV------------GARRKFDTAKDSGI------ESS-DVEDGP
JX014231        --MHRENM----KKTRREMEAAA----SMDAASDLLDTDSNATF------AMK-PLMRGA
JX014232.1       --MHRENM----KKTRREMEAAA----SMDAASDLLDTDSNATF------AMK-PLMRGA
AF297501        ------------------------------------------------------------
U58776.1        ANMHKENM----KKKRRDLEQ-A----SLDAASDLLDTDSNATF------AMK-PLVRHP
AF297500        ANMHKENM----KKKRRDLEQ-A----SLDAASDLLDTDSNATF------AMK-PLVRHP

AB253526        LDSRTYGRPDKEAPAPPPPPPPPEL--NRSERELNKMCGISASDIDKYSRIMFPVCFICF
AB594844        ------------------------------------------------------------
TuGlCl_AbaS      ---IGYAKALGSTKLPRKKPPNRGIFSNWLSRFHTR-----SKKIDVTSRIVFPFLFAIF
TuGlCl_AbaR      ---IGYAKALGSTKLPRKKPPNRGIFSNWLSRFHTR-----SKKIDVTSRIVFPFLFAIF
JX014231        V---LDSK-MRQCEVHMA-PPRKNCCRLWMSKFPTR-----SKRIDVISRITFPLVFALF
JX014232.1       V---LDSK-MRQCEVHMA-PPRKNCCRLWMSKFPTR-----SKRIDVISRITFPLVFALF
AF297501        ------------------------------------------------------------
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U58776.1        GDPLALEK-RLQCEVHMQAPKRPNCCKTWLSKFPTRQCS-RSKRIDVISRITFPLVFALF
AF297500         GDPLALEK-RLQCEVHMQAPKRPNCCKTWLSKFPTRQCS-RSKRIDVISRITFPLVFALF


AB253526         NLMYWIIYLHISDIVADDIVMLEPDK
AB594844         --------------------------
TuGlCl_AbaS      NAFYWTKYLLRDELMEL---------
TuGlCl_AbaR      NAFYWTKYLLRDELMEL---------
JX014231        NLAYWSTYLFRDEDEED---------
JX014232.1       NLAYWSTYLFRDEDEED---------
AF297501         --------------------------
U58776.1        NLVYWSTYLFREEEDE----------
AF297500         NLVYWSTYLFREEEDE----------
```

Acetylcholinesterase alignment
A302**S** mutation-
S431**F** mutation-pirimicarb and omethoate resistance in *Myzus persicae* (AY147797) and *Aphis gossypii* (AF502082)

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AF50281.1       ------------------------------------------MSV-----DCVYTS-
GQ461339         MVPMFNHNININHFNNVIVTTLTHHQYTNSRCNSNNNVIKRITNSILKSVTVFTVKTLWNHL
GQ461338         MVPMFNHNININHFNNVIVTTLTHHQYTNSRCNGNNNVIKSITNSILKSVTVFTVKTLWNHL
GQ461345         MVPMFNHNININHFNNVIVTTLTHHQYTNSRCNGNNNVIKSITNSILKSVTVFTVKTLWNHL
GQ461347        MVPMFNHNININHFNNVIVTTLTHHQYTNSRCNSNNNVIKRITNSILKSVTVFTVKTLWNHL
GQ461342         MVPMFNHNININHFNNVIVTTLTHHQYTNSRCNSNNNVIKRITNSILKSVTVFTVKTLWNHL
GQ461336         MVPMFNHNININHFNNVIVTTLTHHQYTNSRCNSNNNVIKRITNSILKSVTVFTVKTLWNHL
AAV65825.2       ----------------------------------------------------------
AF502082         ----MDQWLLWFGYLVAST-----YGLSLRHARHQ-----------SVGTPT----AEEI
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AY147797       ----MDQWLLWFSYLVAST-----YGLSLRHARHQ----------SVGTPT----AEEI


AF50281.1      ---------AVTLLL-CCSAVLGR-PSSNGGADAGGGGGGGGGAGGAGGGGAGGGGGGGS
GQ461339        LVPIVVI-----LLFQSSANVFSSA--------LPHS--EINSFYTDGPSSSSSFNSEHH
GQ461338        LVPIVVI-----LLFQSSANVFSSA--------LPHS--EINSLYADGPSFSSSFNSEHH
GQ461345        LVPIVVI-----LLFQSSANVFSSA--------LPHS--EINSLYADGPSFSSSFNSEHH
GQ461347        LVPIVVI-----LLFQSSANVFSSA--------LPHS--EINSFYADGPSSSSSFNSEHH
GQ461342        LVPIVVI-----LLFQSSANVFSSA--------LPHS--EINSFHADGPSSSSSFNSEHH
GQ461336        LVPIVVI-----LLFQSSANVFSSA--------LPHS--EINSFHADGPSSSSSFNSEHH
AAV65825.2      ------------------------------------------------------------
AF502082        LEPQILIEDTDHVFRQRASDMFAQEPEYTEKRNLNHR--RRSEFSGN-QDTNFESSGATY
AY147797       LEPQILIEDTDHVFRQRALDMFAQEPEYTEKRNLNHR--RRSEFSGN-QDNDFESSGETY


AF50281.1      AVDDTDEIPVVVTSTGLVQGYTKII-ANREVRVYTGIPFAKPPVGQLRFRRPVPVDPWTG
GQ461339        HHHHHNDPLVVLTKKGYVRGRSVVSPTGKPVDAFLGIRYAKPPTGKFRFRHPKPIDSWQG
GQ461338        HHHHHNDPLVVLTKKGYVRGRSVVSPTGKPVDAFLGIRYAKPPTGKFRFRRPKPIDSWQG
GQ461345        HHHHHNDPLVVLTKKGYVRGRSVVSPTGKPVDAFLGIRYAKPPTGKFRFRRPKPIDSWQG
GQ461347        HHHHHNDPLVVLTKKGYVRGRSVVSPTGKPVDAFLGIRYAKPPTGKFRFRHPKPIDSWQG
GQ461342        HHHHHNDPLIVLTKKGYVRGRSVVSPTGKPVDAFLSIRYAKPPTGKFRFRHPKPIDSWQG
GQ461336        HHHHHNDPLVVLTKKGYVRGRSVVSPTGKPVDAFLGIRYAKPPTGKFRFRHPKPIDSWQG
AAV65825.2      ------------------------TGKKVDAWFGIPYAQKPIGDLRFRHPRPVENWGD
AF502082        SAYTSDDPLIIHTNKGKIRGITQEATTGKLVDAWLGIPYAKKPIGDLRFRHPRPIDRWDT
AY147797       SAYKSDDPLVIHTNKGKIRGITQAASTGKLVDAWLGIPYAKKPIGDLRFRHPRPIDRWDN
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AF50281.1      -----VLNATRLPNTCYQERYEYFPGFVGEEMWSPNTKLSEDCLYLNIWIPKKQRTRHHS
GQ461339        -----IFNATSFSGACYQVNDTFFGNFMGATEWNPNVPLDEDCLSVNIWVPRPRP-----
GQ461338        -----IFNATSFPGACYQVNDTFFGNFMGATEWNPNVPLDEDCLSVNIWVPRPRP-----
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GQ461345       -----IFNATSFPGACYQVNDTFFGNFMGATEWNPNVPLDEDCLSVNIWVPRPRP-----
GQ461347       -----IFNATSFSGACYQVNDTFFGNFMGATEWNPNVPLDEDCLSVNIWVPRPRP-----
GQ461342       -----IFNATSFSGACYQVNDTFFGNFMGATEWNPNVPLDEDCLSVNIWVPRPRP-----
GQ461336       -----IFNATSFSGACYQVNDTFFGNFMGATEWNPNVPLDEDCLSVNIWVPRPRP-----
AAV65825.2      ----EILNATTLPHSCVQIIDTVFGDFPGAMMWNPNTDMQEDCLYINIVTPRPRP-----
AF502082       TSPETILNCTTPPNTCVQIFDTLFGDFPGATMWNPNSPVSEDCLYINVVVPRPRP-----
AY147797      TNPETILNCTTPPNTCVQIFDTLFGDFPGATMWNPNSPVSEDCLYINVVVPKPRP-----
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AF50281.1      NNAHHAKIPVLVWIYGGGYMSGTSTLDIYDGDLLAATFDVMIASMQYRLGAFGSLYLTPE
GQ461339       -----KSAAVLLWIYGGGFWSGSSSLDFYDGSVLAGEESIIFVSINYRVASLGFIFF---
GQ461338       -----KSAAVLLWIYGGGFWSGSSSLDFYDGSVLAGEESIIFVSINYRVASLGFIFF---
GQ461345       -----KSAAVLLWIYGGGFWSGSSSLDFYDGSVLAGEESIIFVSINYRVASLGFIFF---
GQ461347       -----KSAAVLLWIYGGGFWSGSSSLDFYDGSVLAGEESIIFVSINYRVASLGFIFF---
GQ461342       -----KSAAVLLWIYGGSFWSGSSSLDFYDGSVLAGEESIIFVSTNYRVASLGFIFF---
GQ461336       -----KSAAVLLWIYGGGFWSGSSSLDFYDGSVLAGEESIIFVSINYRVASLGFIFF---
AAV65825.2      -----KNAAVMLWVFGGGFYSGTATLDVYDPKILVSEEKIVYVSMQYRVASLGFLFF---
AF502082       -----QNAAVMVWIFGGGFYSGSATLDIYDPKILVSEENVILVSMQYRVASLGFLYF---
AY147797       -----QNAAVMVWIFGGGFYSGSATLDIYDPKVLVSEENVILVSMQYRVASLGFLYF---
                .  *:.*:.**.: **:.**.** .:*..  .:: .* :**: .:* :::

AF50281.1      LPEDSDDAPGNMGLWDQALAIKWIKENAAAFGADPETITLFGESAGGGSVSVHLISPETG
GQ461339       ---DTSDAPGNAGLFDQLMAMEWIRENIAAFGGNPANITIFGESAGAVSAALHLLSPLSR
GQ461338       ---DTSDAPGNAGLFDQLMAMEWIRENIAAFGGNPANITIFGESAGAVSAALHLLSPLSR
GQ461345       ---DTSDAPGNAGLFDQLMAMEWIRENIAAFGGNPANITIFGESAGAVSAALHLLSPLSR
GQ461347       ---DTSDAPGNAGLFDQLMAMEWIRENIAAFGGNPANITIFGESSGAVSAALHLLSPLSR
GQ461342       ---DTSDAPGNAGLFDQLMAMEWIRENIAAFGGNPANITIFGESAGAVSAALHLLSPLSR
GQ461336       ---DTSDAPGNAGLFDQLMAMEWIRENIAAFGGNPANITIFGESAGAVSAALHLLSPLSR
AAV65825.2      ---DTPDVPGNAGLFDQLMALQWVKDNIQYFGGNPHNVTLFGESAGAVSVSLHLLSPLSR
AF502082       ---DTEDVPGNAGLFDQLMALQWVHENIKLFGGNPNNVTLFGESAGAVSVSLHLLSPLSR
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AY147797       ---DTEDVPGNAGLFDQLMALQWVHENIKLFGGNPNNVTLFGESAGAVSVSLHLLSPLSR
                  *: * .*** **.** .*..*...*    **.* .*.*.****.*. * ..**.** :

AF50281.1      GMVKRGIIQSGTVNAPWSYMTGERAVEIAKKLLDDCNCNSTSLDSNPIGTMSCMRPVDAS
GQ461339        NVFSQAILQSGSATCPWAISDRKKAYQRSPALAQAVGCGSTSTR-SVHAIIECMQSIPAS
GQ461338        NVFSQAILQSGSATCPWAISDRKKAYQRSLALAQAVGCGSTSTR-SVHAIIECMQSIPAS
GQ461345        NVFSQAILQSGSATCPWAISDRKKAYQRSLALAQAVGCGSTSTR-SVHAIIECMQSIPAS
GQ461347        NVFSQAILQSGSATCPWAISDRKKAYQRSLALAQAVGCGSTSTR-SVHAIIECMQSIPAS
GQ461342        NVFSQAILQSGSATCPWAISDRKKAYQRSLALAQAVGCGSTSTR-SVHAIIECMQSIPAS
GQ461336        NVFSQAILQSGSATCPWAISDRKKAYQRSLALAQAVGCGSTSTR-SVHAIIECMQSIPAS
AAV65825.2       NMFSQAIMQSGAASAPWAIISREESVIRGIRLAEAVHCPHSK-T-DMGPMIECLRKKSAD
AF502082        NLFNQAIMESGSSTVPWAILSREESFSRGLKLAKAMGCPDDR-N-EIHKTVECLRKVNSS
AY147797       NLFNQAIMESGSSTAPWAILSREESYSRGLRLARAMGCPDDR-N-EIHKTVECLRKANSS
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AF50281.1      TISKKQWNSYSGILGFPSAPTVDGILLPEHPLDMLAKANFSDIDILIGSNLNEGTYFLLY
GQ461339        ELVAQEEAT-TGVVEFAFIPIVDGSFLDEDPEVSLRTENFKHTPILTGSNRDEATYWLVY
GQ461338        ELVAQEETT-TGVVEFAFIPIVDGSFLDEDPEVSLRTKNFKHTPILTGSNRDEGTYFLVY
GQ461345        ELVAQEETT-TGVVEFAFIPIVDGSFLDEDPEVSLRTKNFKHTPILTGSNRDEGTYFLVY
GQ461347        ELVAQEETT-TGVVEFAFIPIVDGSFLDEDPEVSLRTKNFKHTPILTGSNRDEATYWLVY
GQ461342        ELVAQEETT-TGVVEFAFIPIVDGSFLDEDPEVSLRTKNFKHTPILTGSNRDEGTYYLVY
GQ461336        ELVAQEETT-TGVVEFAFIPIVDGSFLDEDPEVSLRTKNFKHTPILTGSNRDEGTYFLVY
AAV65825.2       ELVNNEWGT-LGICEFPFVPIIDGSFLDEMPIRSLAHQNFKKTNILMGSNTEEGYYFILY
AF502082        AMVEKEWDH-VAMCFFPFVPVVDGAFLDDHPQKSLSTNNFKKTNILMGSNSEEGYYFIFY
AY147797       TMVEKEWDH-VAICFFPFVPVVDGAFLDDYPQKSLSTNNFKKTNILMGSNSEEGYYSIFY
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AF50281.1      DFVDFFDRTSATALPREKFVQIVNVIFKDRTQLERDAIIYQYSGWEKKEVDDIYSNQKQL
GQ461339        HSPHIFNLSEGIYISRSEFQSLIRIIYPHLSPLAQEAVIQEYTHWINP--DDQIENREAT
GQ461338        HSPHIFNLSEGIYISRSEFQSLIRIIYPHLSPLAQEAVIQEYTHWINP--DDQIENREAT
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GQ461345       HSPHIFNLSEGIYISRSEFQSLIRIIYPHLSPLAQEAVIQEYTHWINP--DDQIENREAT
GQ461347       HSPHIFNLSEGIYISRSEFQSLIRIIYPHLSPLAQEAVIQEYTHWINP--DDQIENREAT
GQ461342       HSPHIFNLSEGIYISRSEFQSLIRIIYPHLSPLAQEAVIQEYTHRINP--DDQIENREAT
GQ461336       HSPHIFNLSEGIYISRSEFQSLIRIIYPHLSPLAQEAVIQEYTHWINP--DDQIENREAT
AAV65825.2      YLTELFPKEENVGISREQYLQAVRELNPYVNDVARQAIVFEYTDWLNP--DDPIRNRNAL
AF502082       YLTELFKKEENVVVSRENFIKAIGQLNPNADAAVKSAIEFEYTDWFNP--NDPEKNRNAL
AY147797       YLTELFKKEENVVVSRENFVKAIGQLNPNADAAVKSAIEFEYTDWFSP--NDPEKNRNAL
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AF50281.1      SDVVADYFFVCPTNLFANIVSSRGARVYYYFFTHRTDSHLWGDWMGVLHGDEMQYVFGHL
GQ461339       DKFVGDYHSTCPVNEMSYRYALYGNDVWTYHFTHRSSKSFWPSWMGVIHGEEIKFVLGEP
GQ461338       DKFVGDYHFTCPVNEMSYRYALYGNDVWTYHFTHRSSKSFWPSWMGVIHGEEIKFVLGEP
GQ461345       DKFVGDYHFTCPVNEMSYRYALYGNDVWTYHFTHRSSKSFWPSWMGVIHGEEIKFVLGEP
GQ461347       DKFVGDYHFTCPVNEMSYRYALYGNDVWTYHFTHRSSKSFWPSWMGVIHGEEIKFVLGEP
GQ461342       DKFVGDYHFTCPVNEMSYRYALYGNDVWTYHFTHRSSKSFWPSWMGVIHGEEIKFVLGEP
GQ461336       DKFVGDYHFTCPVNEMSYRYALYGNDVWTYHFTHRSSKSFWPSWMGVIHGEEIKFVLGEP
AAV65825.2      DKMVGDYHFTCGVNEFAHRYAETGNNVYTYYYKHRSKNNPWPSWTGVMHADEINYVFGEP
AF502082       DKMVGDYQFTCNVNEFAHKYALTGNNVYMYYFKHRSLNNPWPKWTGVMHGDEISYVFGDP
AY147797       DKMVGDYQFTCNVNEFAHKYALTGNNVYMYYFKHRSLNNPWPKWTGVMHGDEISYVFGDP
                 ...*.**  .* .* ::  :  *  *: *.:.**: .  * .* **:*.::*:.:*:.*.

AF50281.1      LNMSMPYNARERDLSIRIMEAFTRFSLTGTPVS--------DDIDWPLYNESKPIYHVWN
GQ461339       LDPVHGYTPAEVQLSKRIMRYWANFARTGNPNKQFPDGDDTESIVWPEYTAHEKEYLVIS
GQ461338       LDPVHGYTPAEVQLSKRIMRYWANFARTGNPNKQFPDGDDTESIVWPEYTAHEKEYLVIS
GQ461345       LDPVHGYTPAEVQLSKRIMRYWANFARTGNPNKQFPDGDDTESIVWPEYTAHEKEYLVIS
GQ461347       LDPVHGYTPAGVQLSKRIMRYCANFARTGNPNKQFPDGDDTESIVWPEYTAHEKEYLVIS
GQ461342       LDPVHGYTPAEVQLSKRIMRYWANFARTGNPNKQFPDGDDTESIVWPEYTAHEKEYLVIS
GQ461336       LDPVHGYTPAEVQLSKRIMRYWANFARTGNPNKQFPDGDDTESIVWPEYTAHEKEYLVIS
AAV65825.2      LNPGKNYSPEEVEFSKTIMRYWANFARTGNPSQSPNG--ELTKVHWPVHTAFGREYLSLS
AF502082       LNPNKRYEIEEIELSKKMMRYWTNFAKTGNPSKTLEG--SWVTPKWPVRTAYGKEFLTLD
```

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AY147797       LNPNKRYEIEEIELSKKMMRYWTNFAKTGNPSKTLK---SWVTRQWPVHTAYGKEFLTLD
               *:   *    ::* :*. :.*: **.* .          **  .    :  .

AF50281.1      AAE-MHVGYGPRAAECQFWNGFFPKIAQALKET-SKTTCEDYPDSMPTINENCTFTSSFA
GQ461339        TND-SSIGRGLRAKQCAFWKNFLPKLINALENR-HNSTCTSHSNQIGSSNW--------S
GQ461338        TND-SSIGRGLRAKQCAFWKNFLPKLINALENR-HNSTCTSHSNQIGSSNW--------S
GQ461345        TND-SSIGRGLRAKQCAFWKNFLPKLINALENR-HNSTCTSHSNQIGSSNW--------S
GQ461347        TND-SSIGRGLRAKQCAFWKNFLPKLINALENR-HNSTCTSHSNQIGSSNW--------S
GQ461342        TND-SSIGRGLRAKQCAFWKNFLPKLINALENR-HNSTCTSHSNQIGSSNW--------S
GQ461336        TND-SSIGRGLRAKQCAFWKNFLPKLINALENR-HNSTCTSHSNQIGSSNW--------S
AAV65825.2       VNSSGTVGHGLRVKQCAFWQKYLPQLIAATSKPEPPKNCTSSAEAPRASYH--------V
AF502082         TNN-TSIGVGPRLEQCAFWKNYVPDLTVISKSMKSDKNCTTISGGTKTNMI--------K
AY147797        TNN-TSIACKTR--QCA----------------------------------------
               . .  ::   * :*

AF50281.1      TVNPQISFTIIFIFVLPAYGLF
GQ461339        LAISLISLIMCFLPSLR-----
GQ461338        LAISLISLIMCFLPSLR-----
GQ461345        LAISLISLIMCFLPSLR-----
GQ461347        LAISLISLIMCFLPSLR-----
GQ461342        LAISLISLIMCFLPSLR-----
GQ461336        LAISLISLIMCFLPSLR-----
AAV65825.2       LGLA---VVAAVSFSQK-----
AF502082         LSVWTIVMTTAVLML-------
AY147797        ----------------------
```

BIFENAZATE **RESISTANCE:**

```
FJ196444(R)       MKKILNNIFFIPTPSNISLMWNFGSLLGLSMVIQSISGIFVSMHYCNNTLLAFNSYIFLS
FJ196445.1(R)     MKKILNNIFFIPTPSNISLMWNFGSLLGLSMVIQSISGIFVSMHYCNNTLLAFNSYIFLS
EU556754.1(R)       MKKILNNIFFIPTPSNISLMWNFGSLLGLSMVIQSISGIFVSMHYCNNTLLAFNSYIFLS
EU345430.1(S)       MKKILNNIFFIPTPSNISLMWNFGSLLGLSMVIQSISGIFVSMHYCNNTLLAFNSYIFLS
                    ************************************************************

FJ196444(R)       KIIENGMILQMTQAHFSSIIFIIMYIHIMKSLLNKSFNKKQMWISGNLMLFMIMGSAFIG
FJ196445.1(R)     KIIENGMILQMTHAHFSSIIFIIMYIHIMKSLLNKSFNKKQMWISGNLMLFMIMGSAFIG
EU556754.1(R)       KIIENGMILQMTHAHFSSIIFIIMYIHIMKSLLNKSFNKKQMWISGNLMLFMIMGSAFIG
EU345430.1(S)       KIIENGMILQMTHAHFSSIIFIIMYIHIMKSLLNKSFNKKQMWISGNLMLFMIMGSAFIG
                    ************.***********************************************

FJ196444(R)       YVLPWGQMSFWGATVITNILSSIPFLGKKIVFWVWGSFSVDNPTLNRFFSLHFLMPLMIL
FJ196445.1(R)     YVLPWGQMSFWGATVITNILSSIPFLGKKIVFWVWGSFSVDNPTLNRFFSLHFLMPLMIL
EU556754.1(R)       YVLPWSQMSFWGATVITNILFSIPFLGKKIVFWVWGSFSVGNPTLNRFFSLHFLMPLLIL
EU345430.1(S)       YVLPWGQMSFWGATVITNILSSIPFLGKKIVFWVWGSFSVDNPTLNRFFSLHFLMPLLIL
                    *****.************** ****************** ****************.**

FJ196444(R)       AMSMIHLSILHEKGSSNQMGLNSSKDKIYFNKSFMFKDLISLMLMVMFYCLMLSFFIDFH
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FJ196445.1(R)      AMSMIHLSILHEKGSSNQMGLNSSKDKIYFNKSFMFKDLISLMLMVMFYCLMLSFFIDFH
EU556754.1(R)      AMSMIHLSILHEKGSSNQMGLNSSKDKIYFNKSFMFKDLISLMLMVMFYCLMLSFFIDFH
EU345430.1(S)      AMSMIHLSILHEKGSSNQMGLNSSKDKIYFNKSFMFKDLISLMLMVMFYCLMLSFFIDFH
                   ************************************************************

FJ196444(R)        FSMAKENFFPADPLNTPLHIKPEWYFMFAYAILRSVPSKIGGILSLLILFLLFILLMFNK
FJ196445.1(R)      FSMAKENFFPADPLNTPLHIKTEWYFMFAYAILRSVPSKIGGILSLLILFLLFILLMFNK
EU556754.1(R)      FSMAKENFFPADPLNTPLHIKPEWYFMFAYAILRSVPSKIGGILSLLILFLLFILLMFNK
EU345430.1(S)      FSMAKENFFPADPLNTPLHIKPEWYFMFAYAILRSVPSKIGGILSLLILFLLFILLMFNK
                   ********************* ***********************************

FJ196444(R)        SNYSKFFFKKKMLIFIFLTCFIILTNMGYKLIEYPFTEISVFFGMLIILILPLM
FJ196445.1(R)      SNYSKFFFKKKMLIFIFLTCFIILTNMGYKLIEYPFTEISLFFGMLIILILPLM
EU556754.1(R)      SNYSKFFFKKKMLIFIFLTCFIILTNMGYKLIEYPFTEISLFFGMLMILILPLM
EU345430.1(S)      SNYSKFFFKKKMLIFIFLTCFIILTNMGYKLIEYPFTEISLFFGMLMILILPLM
                   ****************************************.******.*******
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DELTAMETHRIN RESISTANCE:

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AJ408857(R)        ------------------------------------------------------------
AJ408858(R)        ------------------------------------------------------------
AJ408849(S)        ------------------------------------------------------------
AJ408850(S)        ------------------------------------------------------------
AJ408854(R)        ------------------------------------------------------------
AJ408855(R)        ------------------------------------------------------------
AJ408856(R)        ------------------------------------------------------------
AJ408851(R)        ------------------------------------------------------------
AJ408852(R)        ------------------------------------------------------------
AJ408853(R)        ------------------------------------------------------------
GU198929           ------------------------------------------------------------
GU325775           ------------------------------------------------------------
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GU339221          ------------------------------------------------------------
GU198944          ------------------------------------------------------------
AB453977.1(S)     IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD
GU339219          ------------------------------------------------------------


AJ408857(R)       ------------------------------------------------------------
AJ408858(R)       ------------------------------------------------------------
AJ408849(S)       ------------------------------------------------------------
AJ408850(S)       ------------------------------------------------------------
AJ408854(R)       ------------------------------------------------------------
AJ408855(R)       ------------------------------------------------------------
AJ408856(R)       ------------------------------------------------------------
AJ408851(R)       ------------------------------------------------------------
AJ408852(R)       ------------------------------------------------------------
AJ408853(R)       ------------------------------------------------------------
GU198929          ------------------------------------------------------------
GU325775          ------------------------------------------------------------
GU339221          ------------------------------------------------------------
GU198944          ------------------------------------------------------------
AB453977.1(S)     NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN
GU339219          ------------------------------------------------------------


AJ408857(R)       --ISVISPTYNLIANRQNQPIETTQKALAACS----------------------------
AJ408858(R)       --ISVISPTYNLIANRQNQPIETTQKALAACS----------------------------
AJ408849(S)       ------------------------------------------------------------
AJ408850(S)       ------------------------------------------------------------
AJ408854(R)       ------------------------------------------------------------
AJ408855(R)       ------------------------------------------------------------
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AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
GU339221         ----------------------------------------------------------
GU198944         ----------------------------------------------------------
AB453977.1(S)    LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS
GU339219         ----------------------------------------------------------


AJ408857(R)      --DNDRNNWVYYLNLPQGTAQYAIYELNIQ----------DSTSAPTVYSG---------
AJ408858(R)      --DNDRNNWVYYLNLPQGTAQYAIYELNIQ----------DSTSAPTVYSG---------
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
GU339221         ----------------------------------------------------------
GU198944         ----------------------------------------------------------
AB453977.1(S)    PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESVSEITRTTAPTATAAGTAKARKVS
GU339219         ----------------------------------------------------------
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AJ408857(R)      ----------------------------------------------------------
AJ408858(R)      ----------------------------------------------------------
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
GU339221         ----------------------------------------------------------
GU198944         ----------------------------------------------------------
AB453977.1(S)    AGVAAAFQKASLSLPGSPFNLRRGSRGSHQFTIRNGRGRFVGVPGSDRKPLVLSTYLDAQ
GU339219         ----------------------------------------------------------

AJ408857(R)      ----------PTPS---GNSNLAAVYFSPNKDRFIIFSNT------------------
AJ408858(R)      ----------PTPS---GNSNLAAVYFSPNKDRFIIFSNT------------------
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
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GU339221        ----------------------------------------------------------
GU198944        ----------------------------------------------------------
AB453977.1(S)    EHLPYADDSNAVTPMSEENGAIIVPVYYANLGSRHSSYTSHQSRISYTSHGDLLGGMTKE
GU339219        ----------------------------------------------------------


AJ408857(R)      ----------------------------------------------------------
AJ408858(R)      ----------------------------------------------------------
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
GU339221         ----------------------------------------------------------
GU198944         ----------------------------------------------------------
AB453977.1(S)    SRLRSRTQRNTNHSIVPPANMAASAASVTGAGSGAPNMSYVDTNHKGQQRDFDQSQDYTD
GU339219        ----------------------------------------------------------


AJ408857(R)      ----------------------------------------------------------
AJ408858(R)      ----------------------------------------------------------
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
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AJ408856(R)      ----------
AJ408851(R)      ----------
AJ408852(R)      ----------
AJ408853(R)      ----------
GU198929         ----------
GU325775         ----------
GU339221         ----------
GU198944         ----------
AB453977.1(S)    DAGKIKHNDNPFIEPSQTQTVVDMKDVMVLNDIIEQAAGRHSRASDHGVSVYYFPTEDDD
GU339219         ----------

AJ408857(R)      ----------
AJ408858(R)      ----------
AJ408849(S)      ----------
AJ408850(S)      ----------
AJ408854(R)      ----------
AJ408855(R)      ----------
AJ408856(R)      ----------
AJ408851(R)      ----------
AJ408852(R)      ----------
AJ408853(R)      ----------
GU198929         ----------
GU325775         ----------
GU339221         ----------
GU198944         ----------
AB453977.1(S)    EDGPTFKDKAVEFGMRMIDIFCVWDCCWVWLKFQEWVSFIVFDPFVELFITLCIVVNTLF
GU339219         ----------
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AJ408857(R)      ------------------------------------------------------
AJ408858(R)      ------------------------------------------------------
AJ408849(S)      ----------------------------------------------WNIFDFIIVALSL
AJ408850(S)      ----------------------------------------------WNIFDFIIVALSL
AJ408854(R)      ----------------------------------------------WNIFDFIIVALSL
AJ408855(R)      ----------------------------------------------WNIFDFIIVALSL
AJ408856(R)      ----------------------------------------------WNIFDFIIVALSL
AJ408851(R)      ----------------------------------------------WNIFDFIIVALSL
AJ408852(R)      ----------------------------------------------WNIFDFIIVALSL
AJ408853(R)      ----------------------------------------------WNIFDFIIVAMSL
GU198929         ------------------------------------------------------
GU325775         ------------------------------------------------------
GU339221         ------------------------------------------------------
GU198944         ------------------------------------------------------
AB453977.1(S)    MALDHHDMNPDMERALKSGNYFFTATFAIEATMKLIAMSPKWYFQEGWNIFDFIIVALSL
GU339219         ------------------------------------------------------


AJ408857(R)      ------------------------------------------------------
AJ408858(R)      ------------------------------------------------------
AJ408849(S)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLCFVLCIIIFIF
AJ408850(S)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLCFVLCIIIFIF
AJ408854(R)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLCIIIFIF
AJ408855(R)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLCIIIFIF
AJ408856(R)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLIFVLCIIIFIF
AJ408851(R)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLVFVLCIIIFIF
AJ408852(R)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLVFVLCIIIFIF
AJ408853(R)      LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLVFVLCIIIFIF
GU198929         ------------------------------------------------------
GU325775         ------------------------------------------------------
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GU339221        ------------------------------------------------------------
GU198944        ------------------------------------------------------------
AB453977.1(S)    LELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLCIIIFIF
GU339219        ------------------------------------------------------------


AJ408857(R)      ------------------------------------------------------------
AJ408858(R)      ------------------------------------------------------------
AJ408849(S)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
AJ408850(S)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCG---------------
AJ408854(R)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
AJ408855(R)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
AJ408856(R)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
AJ408851(R)     AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
AJ408852(R)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
AJ408853(R)      AVMGMQLFGKNYYDNVDKFPGGEMPRWNFINFMHSFMIVFRVLCGEWIESMWDCMLVGDW
GU198929        ----------LLTWPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDV
GU325775        ----------LLTWPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDV
GU339221        ----------LLTWPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDV
GU198944        ----------DLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDV
AB453977.1(S)    AVMGMQLFGKNYIDNVDRFPDKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDV
GU339219        ----------WNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDV


AJ408857(R)      ------------------------------------------------------------
AJ408858(R)      ------------------------------------------------------------
AJ408849(S)      SCIPFFLAAVVIGN**F**VVLNLFLALLLSNF------------------------------
AJ408850(S)      ------------------------------------------------------------
AJ408854(R)      SCIPFFLAAVVIGN**F**VVLNLFLALLLSNF------------------------------
AJ408855(R)      SCIPFFLAAVVIGN**F**VVLNLFLALLLSNF------------------------------
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AJ408856(R)      SCIPFFLAAVVIGNFVVLNLFLALLLSNF----------
AJ408851(R)      SCIPFFLATVVIGNLVVLNLFLALLLSNF----------
AJ408852(R)      SCIPFFLATVVIGNLVVLNLFLALLLSNF----------
AJ408853(R)      SCIPFFLAAVVIGNLVVLNLFLALLLSNF----------
GU198929         SCIPFFLATVVIGNLVVLNLFFALLLSKAA----------
GU325775         SCIPFFLATVVIGNLVVLNLFFALLLSKAA----------
GU339221         SCIPFFLATVVIGNLVVLNLFFALLLSKAA----------
GU198944         SCIPFFLATVVIGNFVVLNLFLALLLSK----------
AB453977.1(S)    SCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSAPTADNETNKIAEAFNRISRFSNWI
GU339219         SCIPFFLATVVIGNSVVLNLFLAL----------


AJ408857(R)      ----------
AJ408858(R)      ----------
AJ408849(S)      ----------
AJ408850(S)      ----------
AJ408854(R)      ----------
AJ408855(R)      ----------
AJ408856(R)      ----------
AJ408851(R)      ----------
AJ408852(R)      ----------
AJ408853(R)      ----------
GU198929         ----------
GU325775         ----------
GU339221         ----------
GU198944         ----------
AB453977.1(S)    KANIAAALKFVKNKLTSQIASVQPAGEQHNHLSWIWSEGKGVCPCISAEHGENELELTPD
GU339219         ----------
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AJ408857(R)      ----------
AJ408858(R)      ----------
AJ408849(S)      ----------
AJ408850(S)      ----------
AJ408854(R)      ----------
AJ408855(R)      ----------
AJ408856(R)      ----------
AJ408851(R)      ----------
AJ408852(R)      ----------
AJ408853(R)      ----------
GU198929         ----------
GU325775         ----------
GU339221         ----------
GU198944         ----------
AB453977.1(S)    DILADGLLKKGVKEHNQLEVAIGDGMEFTIHGDLKNKGKKNKQLMNNSKVIGNSISNHQD
GU339219         ----------

AJ408857(R)      ----------
AJ408858(R)      ----------
AJ408849(S)      ----------
AJ408850(S)      ----------
AJ408854(R)      ----------
AJ408855(R)      ----------
AJ408856(R)      ----------
AJ408851(R)      ----------
AJ408852(R)      ----------
AJ408853(R)      ----------
GU198929         ----------
GU325775         ----------
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GU339221        ------------------------------------------------------------
GU198944        ------------------------------------------------------------
AB453977.1(S)    NKLEHELNHRGMSLQDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKEDLGI
GU339219        ------------------------------------------------------------


AJ408857(R)      ----------------------------------------------DTRHYLYW
AJ408858(R)      ----------------------------------------------DTRHYLYW
AJ408849(S)      ------------------------------------------------------------
AJ408850(S)      ------------------------------------------------------------
AJ408854(R)      ------------------------------------------------------------
AJ408855(R)      ------------------------------------------------------------
AJ408856(R)      ------------------------------------------------------------
AJ408851(R)      ------------------------------------------------------------
AJ408852(R)      ------------------------------------------------------------
AJ408853(R)      ------------------------------------------------------------
GU198929         ------------------------------------------------------------
GU325775         ------------------------------------------------------------
GU339221         ------------------------------------------------------------
GU198944         ------------------------------------------------------------
AB453977.1(S)    DEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQGW
GU339219        ------------------------------------------------------------


AJ408857(R)      VNSTLQSG----------------------------------------------
AJ408858(R)      VNSTLQSG----------------------------------------------
AJ408849(S)      ------------------------------------------------------------
AJ408850(S)      ------------------------------------------------------------
AJ408854(R)      ------------------------------------------------------------
AJ408855(R)      ------------------------------------------------------------
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AJ408856(R)      ------------------------------------------------------
AJ408851(R)      ------------------------------------------------------
AJ408852(R)      ------------------------------------------------------
AJ408853(R)      ------------------------------------------------------
GU198929         ------------------------------------------------------
GU325775         ------------------------------------------------------
GU339221         ------------------------------------------------------
GU198944         ------------------------------------------------------
AB453977.1(S)    GNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIFF
GU339219         ------------------------------------------------------

AJ408857(R)      ----------------------------------NRIAGTGSVMSAS---------P-
AJ408858(R)      ----------------------------------NRIAGTGSVMSAS---------P-
AJ408849(S)      ------------------------------------------------------
AJ408850(S)      ------------------------------------------------------
AJ408854(R)      ------------------------------------------------------
AJ408855(R)      ------------------------------------------------------
AJ408856(R)      ------------------------------------------------------
AJ408851(R)      ------------------------------------------------------
AJ408852(R)      ------------------------------------------------------
AJ408853(R)      ------------------------------------------------------
GU198929         ------------------------------------------------------
GU325775         ------------------------------------------------------
GU339221         ------------------------------------------------------
GU198944         ------------------------------------------------------
AB453977.1(S)    LEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRPL
GU339219         ------------------------------------------------------
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AJ408857(R)      ----------------------------------------------------------
AJ408858(R)      ----------------------------------------------------------
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
GU339221         ----------------------------------------------------------
GU198944         ----------------------------------------------------------
AB453977.1(S)    RAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTTL
GU339219         ----------------------------------------------------------


AJ408857(R)      ----------------------------------------------LAATTITNV
AJ408858(R)      ----------------------------------------------LAATTITNV
AJ408849(S)      ----------------------------------------------------------
AJ408850(S)      ----------------------------------------------------------
AJ408854(R)      ----------------------------------------------------------
AJ408855(R)      ----------------------------------------------------------
AJ408856(R)      ----------------------------------------------------------
AJ408851(R)      ----------------------------------------------------------
AJ408852(R)      ----------------------------------------------------------
AJ408853(R)      ----------------------------------------------------------
GU198929         ----------------------------------------------------------
GU325775         ----------------------------------------------------------
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GU339221          ------------------------------------------------------
GU198944          ------------------------------------------------------
AB453977.1(S)     SHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRDIGKQ
GU339219          ------------------------------------------------------

AJ408857(R)       QTRSMT--IFLYYM---DVN--TLLNRIVGKVTDN----------EVHWYA
AJ408858(R)       QTSSMT--IFLYYM---DVN--TLLNRIVGKVTDN----------EIHWYA
AJ408849(S)       ------------------------------------------------------
AJ408850(S)       ------------------------------------------------------
AJ408854(R)       ------------------------------------------------------
AJ408855(R)       ------------------------------------------------------
AJ408856(R)       ------------------------------------------------------
AJ408851(R)       ------------------------------------------------------
AJ408852(R)       ------------------------------------------------------
AJ408853(R)       ------------------------------------------------------
GU198929          ------------------------------------------------------
GU325775          ------------------------------------------------------
GU339221          ------------------------------------------------------
GU198944          ------------------------------------------------------
AB453977.1(S)     PIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYYN
GU339219          ------------------------------------------------------

AJ408857(R)       NQVVEGAP----------------------------------------------
AJ408858(R)       NQVVEGAP----------------------------------------------
AJ408849(S)       ------------------------------------------------------
AJ408850(S)       ------------------------------------------------------
AJ408854(R)       ------------------------------------------------------
AJ408855(R)       ------------------------------------------------------
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AJ408856(R) ----------
AJ408851(R) ----------
AJ408852(R) ----------
AJ408853(R) ----------
GU198929 ----------
GU325775 ----------
GU339221 ----------
GU198944 ----------
AB453977.1(S) AMKKMGSKKPLKAIPRPKWRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQTETF
GU339219 ----------

SODIUM CHANNEL

EF535530.1 ----------
AJ408858.1 ----------
FJ906804 ----------
FJ906811 ----------
U38813 MTEDSDSISEEERSLFRPFTRESLLQIEQRIAEHEK-QKELERKRAA---------EGEQ
X96668 MTEDSDSISEEERSLFRPFTRESLLQIEQRIAEHEK-QKELERKRAA---------EGEQ
NM_001286885 MTEDSDSISEEERSLFRPFTRESLLQIEQRIAEHEK-QKELERKRAA---------EGEQ
KM027335 MSEDLDSISEEEPSLFRPFTRESLAAIEARIADELARQKENEKKRAEG----------E
Y13592 ----------
JN002364 ----------
AB453977.1 MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETGFGRKKKKKE
AF134216.2 ----------
AF134216 ----------
AJ131759 ----------
AJ131760 ----------
AJ408849 ----------
AJ408850 ----------
AB499851.1 ----------

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AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          MSDDSSSISEEERSLFRPFTRESLAAIEARIAEEYAKQKELEKKRAEG----------E
U73584          MSDDSSSISEEERSLFRPFTRESLAAIEARIAEEYAKQKELEKKRAEG----------E
KR139855        MSVISDYSSEEERSIFQPFTRESLAAIELRIAEEYAKQKELEKKRAEGE-GFGRKKKKKE
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      ----------------------------------------------------------
AJ408858.1      ----------------------------------------------------------
FJ906804        ----------------------------------------------------------
FJ906811        ----------------------------------------------------------
U38813          IRYDDEDEDEGPQPDPTLEQGVPIPVRMQGSFPPELASTPLEDIDPFYSNVLTFVVISKG
X96668          IRYDDEDEDEGPQPDPTLEQGVPIPVRMQGSFPPELASTPLEDIDPFYSNVLTFVVISKG
NM_001286885    IRYDDEDEDEGPQPDPTLEQGVPIPVRMQGSFPPELASTPLEDIDPFYSNVLTFVVISKG
KM027335        VRYEDEDEDEGPQPDATLEQGLPVPVRMQGSFPPELASTPLEDIDPYYHNQKTFVVVSRG
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG
AF134216.2      ----------------------------------------------------------
AF134216        ----------------------------------------------------------
AJ131759        ----------------------------------------------------------
AJ131760        ----------------------------------------------------------
AJ408849        ----------------------------------------------------------
AJ408850        ----------------------------------------------------------
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AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          VRYDDEDEDEGPQPDATLEQGAPIPVRMQGLFPPELASTPLEDIDPFYHNQRTFVVVSKG
U73584          VRYDDEDEDEGPQPDATLEQGAPIPVRMQGLFPPELASTPLEDIDPFYHNQRTFVVVSKG
KR139855        VRYEDEDEDEGPQPDTTLEQGAPVPVRLQGSFPPELASIPLEDIDPFYHNMRTFVVVSKG
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        ------------------------------------------------------------
FJ906811        ------------------------------------------------------------
U38813          KDIFRFSASKAMWLLDPFNPIRRVAIYILVHPLFSLFIITTILTNCILMIMPTTPTVEST
X96668          KDIFRFSASKAMWLLDPFNPIRRVAIYILVHPLFSLFIITTILTNCILMIMPTTPTVEST
NM_001286885    KDIFRFSASKAMWLLDPFNPIRRVAIYILVHPLFSLFIITTILTNCILMIMPTTPTVEST
KM027335        RDIFRFSATDALWMLDPFNPIRRVAIYILVHPLFSFFIITTILVNCILMIMPSTPTVEST
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
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AJ408850          ----------------------------------------------------------
AB499851.1        ----------------------------------------------------------
AJ408851          ----------------------------------------------------------
AB499848          ----------------------------------------------------------
AB742424          ----------------------------------------------------------
AB849921.1.       ----------------------------------------------------------
AB499850.1        ----------------------------------------------------------
U73583           KDIFRFSATDAMWILDPFNPIRRVAIYILVHPLFSLFIITTILTNCIFMIMPTTPTIEST
U73584           KDIFRFSATDAMWILDPFNPIRRVAIYILVHPLFSLFIITTILTNCIFMIMPTTPTIEST
KR139855          RDIYRFSATDAMYILDPFNPIRRVAIYILVHPIFSLFIIFTILFNCVLMIMPSSPTIEST
AY094601.1        ----------------------------------------------------------
AJ440727.1        ----------------------------------------------------------
AJ440728.1        ----------------------------------------------------------


EF535530.1        ----------------------------------------------------------
AJ408858.1        ----------------------------------------------------------
FJ906804          ----------------------------------------------------------
FJ906811          ----------------------------------------------------------
U38813           EVIFTGIYTFESAVKVMARGFILCPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
X96668            EVIFTGIYTFESAVKVMARGFILCPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
NM_001286885      EVIFTGIYTFESAVKVMARGFILCPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
KM027335          EVIFTGIYTFESAVKVMARGFILQPFTYLRDVWNWLDFVVIALAYVTMGIDLGNLAALRT
Y13592           ----------------------------------------------------------
JN002364          ----------------------------------------------------------
AB453977.1        EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
AF134216.2        ----------------------------------------------------------
AF134216          ----------------------------------------------------------
AJ131759          ----------------------------------------------------------
AJ131760          ----------------------------------------------------------
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AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
U73584          EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
KR139855        EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        ------------------------------------------------------------
FJ906811        ------------------------------------------------------------
U38813          FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
X96668          FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
NM_001286885    FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
KM027335        FRVLRALKTVAIIPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
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AJ131760          ----------------------------------------------------------
AJ408849          ----------------------------------------------------------
AJ408850          ----------------------------------------------------------
AB499851.1        ----------------------------------------------------------
AJ408851          ----------------------------------------------------------
AB499848          ----------------------------------------------------------
AB742424          ----------------------------------------------------------
AB849921.1.       ----------------------------------------------------------
AB499850.1        ----------------------------------------------------------
U73583            FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
U73584            FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
KR139855          FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC
AY094601.1        ----------------------------------------------------------
AJ440727.1        ----------------------------------------------------------
AJ440728.1        ----------------------------------------------------------


EF535530.1        ----------------------------------------------------------
AJ408858.1        ----------------------------------------------------------
FJ906804          ----------------------------------------------------------
FJ906811          ----------------------------------------------------------
U38813            IKRFPLDGSWGNLTDENWFLHNSNSSNWFTENDGESYPVCGNVSGAGQCGEDYVCLQGFG
X96668            IKRFPLDGSWGNLTDENWFLHNSNSSNWFTENDGESYPVCGNVSGAGQCGEDYVCLQGFG
NM_001286885      IKRFPLDGSWGNLTDENWFLHNSNSSNWFTENDGESYPVCGNVSGAGQCGEDYVCLQGFG
KM027335          VKSFPEDGSWGNLTDENWERFCQNETNWYFE-NG-AYPLCGNSSGAGQCEPGYVCLQGYG
Y13592            ----------------------------------------------------------
JN002364          ----------------------------------------------------------
AB453977.1        IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETG-DTPLCGNSSGAGQCEEGYVCLQGFG
AF134216.2        ----------------------------------------------------------
AF134216          ----------------------------------------------------------
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AJ131759        ----------------------------------------------------------
AJ131760        ----------------------------------------------------------
AJ408849        ----------------------------------------------------------
AJ408850        ----------------------------------------------------------
AB499851.1      ----------------------------------------------------------
AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          IKNFPINGSWGELNDENWHAFCSNNTNWYFPEGAPEVPLCGNSSGAGTCPPDYTCLQGFG
U73584          IKNFPINGSWGELNDENWHAFCSNNTNWYFPEGAPEVPLCGNSSGAGTCPPDYTCLQGFG
KR139855        VKNFPEDGSAGNMSDENWFKHCSNDSNWEGEEG--NYPLCGNSSGAGQCSPGYTCLQGFG
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      ----------------------------------------------------------
AJ408858.1      ----------------------------------------------------------
FJ906804        ----------------------------------------------------------
FJ906811        ----------------------------------------------------------
U38813          PNPNYDYTSFDSFGWAFLSAFRLMTQDFWEDLYQHVLQAAGPWHMLFFIVIIFLGSFYLV
X96668          PNPNYDYTSFDSFGWAFLSAFRLMTQDFWEDLYQHVLQAAGPWHMLFFIVIIFLGSFYLV
NM_001286885    PNPNYDYTSFDSFGWAFLSAFRLMTQDFWEDLYQHVLQAAGPWHMLFFIVIIFLGSFYLV
KM027335        PNPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGSWHVLFFVVIIFLGSFYLV
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      DNPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLV
AF134216.2      ----------------------------------------------------------
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AF134216          ----------------------------------------------------------
AJ131759          ----------------------------------------------------------
AJ131760          ----------------------------------------------------------
AJ408849          ----------------------------------------------------------
AJ408850          ----------------------------------------------------------
AB499851.1        ----------------------------------------------------------
AJ408851          ----------------------------------------------------------
AB499848          ----------------------------------------------------------
AB742424          ----------------------------------------------------------
AB849921.1.       ----------------------------------------------------------
AB499850.1        ----------------------------------------------------------
U73583            ENPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLV
U73584            ENPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLV
KR139855          QNPNYGYTSFDSFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLV
AY094601.1        ----------------------------------------------------------
AJ440727.1        ----------------------------------------------------------
AJ440728.1        ----------------------------------------------------------


EF535530.1        ----------------------------------------------------------
AJ408858.1        ---ISVISPTYNLIANRQNQPIETTQKAL-----------------------------
FJ906804          ----------------------------------------------------------
FJ906811          ----------------------------------------------------------
U38813            NLILAIVAMSYDELQKKAEEEEAAEEEAIREAEEAAAAKAAKLEERANVAAQAA-----Q
X96668            NLILAIVAMSYDELQKKAEEEEAAEEEAIREAEEAAAAKAAKLEERANVAAQAA-----Q
NM_001286885      NLILAIVAMSYDELQKKAEEEEAAEEEAIREAEEAAAAKAAKLEERANVAAQAA-----Q
KM027335          NLILAIVAMSYDELQKKAEEEEQAEEEALREAEQKAAARADKQEAREAHAREVA-----A
Y13592            ----------------------------------------------------------
JN002364          ----------------------------------------------------------
AB453977.1        NLILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAA----------
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AF134216.2      ----------
AF134216        ----------
AJ131759        ----------
AJ131760        ----------
AJ408849        ----------
AJ408850        ----------
AB499851.1      ----------
AJ408851        ----------
AB499848        ----------
AB742424        ----------
AB849921.1.     ----------
AB499850.1      ----------
U73583          NLILAIVAMSYDELQKKAEEEEAAEEEALREAEEAALAKEAKKLRQADKLA-------AQ
U73584          NLILAIVAMSYDELQKKAEEEEAAEEEALREAEEAALAKEAKKLRQADKLA-------AQ
KR139855        NLILAIVAMSYDELQKKAEEEEAAEEEALREAEEAAQARENRRAAHAAAHADRAAERAAA
AY094601.1      ----------
AJ440727.1      ----------
AJ440728.1      ----------


EF535530.1      ----------
AJ408858.1      ----------
FJ906804        ----------
FJ906811        ----------
U38813          DAADAAAAALHPEMAKSPTYSCISYELFVGGEKGNDDN-NKEKMSIRSVEVESESVSVIQ
X96668          DAADAAAAALHPEMAKSPTYSCISYELFVGGEKGNDDN-NKEKMSIRSVEVESESVSVIQ
NM_001286885    DAADAAAAALHPEMAKSPTYSCISYELFVGGEKGNDDN-NKEKMSIRSVEVESESVSVIQ
KM027335        AAEA--AAYAEAHPAKSPSFSCQSYELFVNQERGNQDDNTRERMSLRSDPFQDSVSTQPA
Y13592          ----------
JN002364        ----------
```

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AB453977.1      ---A--AAANPEIAKSPSDFSCHSYELFVGQEKGNDDN-NKEKMSIRSEGLESVSEITRT
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          ELAAAQELAGANLAKSPSGSSSRSYELFINQKDGNND-NKRENMSIRSEGGDSISEHKGR
U73584          ELAAAQELAGANLAKSPSGSSSRSYELFINQKDGNND-NKRENMSIRSEGGDSISEHKGR
KR139855        AEAAAEAAEAGSMAKSPSDFSAHSYELFIGQEKGNVDDNNREKMSIRSDSLS-----QGR
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------AAC
FJ906804        ------------------------------------------------------------
FJ906811        ------------------------------------------------------------
U38813          RQPAPTTAPA--------TKVRKVSTTSLSLPGSPFNLRRGSRSSHKYTIRNGRGRF-GI
X96668          RQPAPTTAPA--------TKVRKVSTTSLSLPGSPFNLRRGSRSSHKYTIRNGRGRF-GI
NM_001286885    RQPAPTTAPA--------TKVRKVSTTSLSLPGSPFNLRRGSRSSHKYTIRNGRGRF-GI
KM027335        HKPDPHS---------EARRQRKVSMASLSLPGSPFNLRRGSRGSHQMALRPN-GRPRYP
Y13592          ------------------------------------------------------------
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JN002364          ------------------------------------------------------------
AB453977.1        TAPTATAAGTAKARKVSAGVAAAFQKASLSLPGSPFNLRRGSRGSHQFTIRNGRGRFVGV
AF134216.2        ------------------------------------------------------------
AF134216          ------------------------------------------------------------
AJ131759          ------------------------------------------------------------
AJ131760          ------------------------------------------------------------
AJ408849          ------------------------------------------------------------
AJ408850          ------------------------------------------------------------
AB499851.1        ------------------------------------------------------------
AJ408851          ------------------------------------------------------------
AB499848          ------------------------------------------------------------
AB742424          ------------------------------------------------------------
AB849921.1.       ------------------------------------------------------------
AB499850.1        ------------------------------------------------------------
U73583          ----VGANGT-AIR--------KVSAASLSLPGSPFNHRRGSQGSHHFTIRNGRGRFVGP
U73584          ----VGANGT-AIR--------KVSAASLSLPGSPFNHRRGSQGSHHFTIRNGRGRFVGP
KR139855         ----PATAGS-RNRK---VSADNQTEASLSLPGSPFNLRRGSRGSHQFTIRNGRSRF---
AY094601.1        ------------------------------------------------------------
AJ440727.1        ------------------------------------------------------------
AJ440728.1        ------------------------------------------------------------


EF535530.1        ----------------------------------------MHTCRPRGVHSIALVLAL
AJ408858.1        SDNDRNN--------------------------------WVYYLNLPQGTAQYAIYEL
FJ906804          ------------------------------------------------------------
FJ906811          ------------------------------------------------------------
U38813          PGSDRKPLVLQTYQDAQQHLPYADDSNAVTPMSEENGAIIVPAYYCNLGSRHSSYTSHQS
X96668           PGSDRKPLVLQTYQDAQQHLPYADDSNAVTPMSEENGAIIVPAYYCNLGSRHSSYTSHQS
NM_001286885      PGSDRKPLVLQTYQDAQQHLPYADDSNAVTPMSEENGAIIVPAYYCNLGSRHSSYTSHQS
KM027335         PGADRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIIPVYYANLGSRHSSYTSHQS
```

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Y13592          ----------------------------------------------------------
JN002364         ----------------------------------------------------------
AB453977.1       PGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIVPVYYANLGSRHSSYTSHQS
AF134216.2       ----------------------------------------------------------
AF134216         ----------------------------------------------------------
AJ131759         ----------------------------------------------------------
AJ131760         ----------------------------------------------------------
AJ408849         ----------------------------------------------------------
AJ408850         ----------------------------------------------------------
AB499851.1       ----------------------------------------------------------
AJ408851         ----------------------------------------------------------
AB499848         ----------------------------------------------------------
AB742424         ----------------------------------------------------------
AB849921.1.      ----------------------------------------------------------
AB499850.1       ----------------------------------------------------------
U73583          PGGDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIVVPVYYASLGSRHSSYTSHAS
U73584          PGGDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIVVPVYYASLGSRHSSYTSHAS
KR139855         PGGDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAMVVPLYTTGLGSRHSSYTSHAS
AY094601.1       ----------------------------------------------------------
AJ440727.1       ----------------------------------------------------------
AJ440728.1       ----------------------------------------------------------


EF535530.1       AIAWLPHADHAAGAGGGG--------------------------------------
AJ408858.1       N----------------------------------------------------------
FJ906804         ----------------------------------------------------------
FJ906811         ----------------------------------------------------------
U38813          RISYTSHGDLLGGMAAMGASTMTKESKLRSRNTRNQS--IGA----------ATNGGSS
X96668           RISYTSHGDLLGGMAAMGASTMTKESKLRSRNTRNQS--IGA----------ATNGGSS
NM_001286885      RISYTSHGDLLGGMAAMGASTMTKESKLRSRNTRNQS--IGA----------ATNGGSS
```

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KM027335        RLSYTSHGDLLGGLGKAQT----KEAKLRNRSASR-NHSVTSQPHAYPLP-----RQDS-
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      RISYTSHGDLLGGMTKESR----L----RSRTQRNTNHSIVP-PANMAASAASVTGAGSG
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          RISYTSHGDLLGAG--NKS--QTKINQLRARSVRNNPSQVPNST----------------
U73584          RISYTSHGDLLGAG--NKS--QTKINQLRARSVRNNPSQVPNST----------------
KR139855        RISYTSHADLLSGIGGPRA--PTKESKLRSRSSRNSSVTVTHQPLQD-------------
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ----MFGDVNISAILDSLS--------------------------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        ------------------------------------------------------------
FJ906811        ------------------------------------------------------------
U38813          TAGGGYPDANH-KEQRDYEM-GQDYTDEAGKI--KHHDNPFIEPVQTQTVVDMKDVMVLN
X96668          TAGGGYPDANH-KEQRDYEM-GQDYTDEAGKI--KHHDNPFIEPVQTQTVVDMKDVMVLN
```

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NM_001286885      TAGGGYPDANH-KEQRDYEM-GQDYTDEAGKI--KHHDNPFIEPVQTQTVVDMKDVMVLN
KM027335          --------SLASRPLREYEMSTAECTDDAGKVLKPSNDNPFIESSEQPNVVDMRDVMVLN
Y13592            ------------------------------------------------------------
JN002364          ------------------------------------------------------------
AB453977.1        APNMSYVDTNHKGQQRDFD-QSQDYTDDAGKI--KHNDNPFIEPSQTQTVVDMKDVMVLN
AF134216.2        ------------------------------------------------------------
AF134216          ------------------------------------------------------------
AJ131759          ------------------------------------------------------------
AJ131760          ------------------------------------------------------------
AJ408849          ------------------------------------------------------------
AJ408850          ------------------------------------------------------------
AB499851.1        ------------------------------------------------------------
AJ408851          ------------------------------------------------------------
AB499848          ------------------------------------------------------------
AB742424          ------------------------------------------------------------
AB849921.1.       ------------------------------------------------------------
AB499850.1        ------------------------------------------------------------
U73583            ----------PYM------NASADSDDGAVKAKHTDNPFIEQMQQTTIVDMNDVMVLN
U73584            ----------PYM------NASADSDDGAVKAKHTDNPFIEQMQQTTIVDMNDVMVLN
KR139855          ----------KAFLRD----FDTSDLEDGLPKVKHQDNPFIEPAQQQAVVDMKDVMVLN
AY094601.1        ------------------------------------------------------------
AJ440727.1        ------------------------------------------------------------
AJ440728.1        ------------------------------------------------------------


EF535530.1        ------------------VSYDKRVRPNYGGPPVDVGAT---MHVLSISS--------
AJ408858.1        ------------------------------------------------------------
FJ906804          ------------------------------------------------------------
FJ906811          ------------------------------------------------------------
U38813            DIIEQAAGRHSRA-SERG--------EDDDEDGPTFKDIALEYILKGIEIFCVWDCCWVW
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X96668          DIIEQAAGRHSRA-SERG--------EDDDEDGPTFKDIALEYILKGIEIFCVWDCCWVW
NM_001286885    DIIEQAAGRHSRA-SERG--------EDDDEDGPTFKDIALEYILKGIEIFCVWDCCWVW
KM027335        EIIEQ-AGRQSRT-SDQNVSVYYFPTAEDDEDGPTMKEKLLECLMKGIDIFCVWDCCWLW
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      DIIEQAAGRHSRA-SDHGVSVYYFPTEDDDEDGPTFKDKAVEFGMRMIDIFCVWDCCWVW
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          DIIEQAAGQQSRA-SEHGVSIYYFPTDED-DEGPTVKEKVLAICMRGIDIFCVWDCCWLW
U73584          DIIEQAAGQQSRA-SEHGVSIYYFPTDED-DEGPTVKEKVLAICMRGIDIFCVWDCCWLW
KR139855        DIIEQAAGRQSRTSGEHGVSVYYFPTEED-DDEPTFKEKAVAVCLQGIDIFCVWDCCTPW
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        ------------------------------------------------------------
FJ906811        ------------------------------------------------------------
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U38813          LKFQEWVSFIVFDPFVELFITLCIVVNTMFMAMDHHDMNPELEKVLKSGNYFFTATFAIE
X96668          LKFQEWVSFIVFDPFVELFITLCIVVNTMFMAMDHHDMNPELEKVLKSGNYFFTATFAIE
NM_001286885    LKFQEWVSFIVFDPFVELFITLCIVVNTMFMAMDHHDMNPELEKVLKSGNYFFTATFAIE
KM027335        LEFQKYVALLVFDPFVELFITLCIVVNTLFMALDHHDMDRDMEKALKSGNYFFTATFAIE
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      LKFQEWVSFIVFDPFVELFITLCIVVNTLFMALDHHDMNPDMERALKSGNYFFTATFAIE
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          LKFQEYVALLVFDPFVELFITLCIVVNTLFMALDHHDMNKDMDKALKSGNYFFTATFAIE
U73584          LKFQEYVALLVFDPFVELFITLCIVVNTLFMALDHHDMNKDMDKALKSGNYFFTATFAIE
KR139855        LKFQELIALIVFDPFVELFITLCIVVNTLFMALDHHDMDKELEKALKSGNYFFSATFGIE
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      LSEVKMDFTLDFYFRQFWTDPRLAYKK-------RTGVETLS------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        ------------------------------------------------------------
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```
FJ906811        ------------------------------------------------------------
U38813          ASMKLMAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
X96668          ASMKLMAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
NM_001286885    ASMKLMAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
KM027335        ATLKLIAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------WP
AB453977.1      ATMKLIAMSPKWYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AF134216.2      ----------YFREGWNIFDFLIVALSLIELSLENVQGLSVLRSFRLLRVFKLAKSWP
AF134216        ----------YFREGWNIFDFLIVALSLIELSLENVQGLSVLRSFRLLRVFKLAKSWP
AJ131759        ------------------------------------------------------------SWP
AJ131760        ------------------------------------------------------------SWP
AJ408849        ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AJ408850        ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AB499851.1      ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AJ408851        ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AB499848        ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AB742424        ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AB849921.1.     ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
AB499850.1      ----------WNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
U73583          ATLKLIAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
U73584          ATLKLIAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWP
KR139855        AAFKLIAMSPKYYFQEGWNIFDFIIVALSLLELSLEGVQGLSVLRSFRLLRVFKLAKSWP
AY094601.1      ------------------------------------------------------AKSWP
AJ440727.1      ------------------------------------------------------AKSWP
AJ440728.1      ------------------------------------------------------AKSWP


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------
```

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FJ906804          TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPNAEIPRWNFK
FJ906811          TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPNAEIPRWNFK
U38813           TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYIDHKDRFKDHELPRWNFT
X96668            TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYIDHKDRFKDHELPRWNFT
NM_001286885      TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYIDHKDRFKDHELPRWNFT
KM027335          TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYVDHVDRFPDGDLPRWNFT
Y13592           -LNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYVDNVDRFPDHDLPRWNFT
JN002364          TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYTDNVDRFPDGDLPRWNFT
AB453977.1        TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYIDNVDRFPDKDLPRWNFT
AF134216.2        TLNLLISIMGKTIGALGNLTFVLGIIIFIFAVMGMQLFGKNYEESKHKFKDNMVPRWNFV
AF134216          TLNLLISIMGKTIGALGNLTFVLGIIIFIFAVMGMQLFGKNYEESKHKFKDNMVPRWNFV
AJ131759          TLNLLISIMGRTIGALGNLTFVLCIIIFIFAVMGMQLFGKNYTEKMYMFKDHELPRWNFT
AJ131760          TLNLLISIMGRTIGALGNLTFVLCIIIFIFAVMGMQLFGKNYTEKMYMFKDHELPRWNFT
AJ408849          TLNLLISIMGRTMGALGNLCFVLCIIIFIFAVMGMQLFGKNYYDNVDKFPGGEMPRWNFI
AJ408850          TLNLLISIMGRTMGALGNLCFVLCIIIFIFAVMGMQLFGKNYYDNVDKFPGGEMPRWNFI
AB499851.1        TLNLLISIXGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYFDNVDKFPGGEMPRWNFI
AJ408851          TLNLLISIMGRTMGALGNLVFVLCIIIFIFAVMGMQLFGKNYYDNVDKFPGGEMPRWNFI
AB499848          TLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYFDNVDKFPGGEMPRWNFI
AB742424          TLNLLISIMGRTMGALGNLIFVLCIIIFIFAVMGMQLFGKNYYDNVDKFPGGEMPRWNFI
AB849921.1.       TLNLLISIMGRTMGALGNLIFVLCIIIFIFAVMGMQLFGKNYYDNVDKFPGGEMPRWNFI
AB499850.1        TLNLLISIMGRTMGALGNLIFVLCIIIFIFAVMGMQLFGKNYFDNVDKFPGGEMPRWNFI
U73583           TLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYYDNVERFPDGDMPRWNFT
U73584           TLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYYDNVERFPDGDMPRWNFT
KR139855          TLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYVDNVDRFPGGELPRWNFT
AY094601.1        TLNLLISIMGRTVGAIGNLTFVLCIIIFIFAVMGMQLFGKNYT----------------
AJ440727.1        TLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYTDNVDRFPGGELPRWNFT
AJ440728.1        TLNLLISIVGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYTDNVDRFPGGELPRWNFT


EF535530.1        ----------VGSEFIRNIWVPD--------TFFVNE---------------KQSYFH
```

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AJ408858.1      ------------------------------------------------------------
FJ906804         DFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLFLALLLSSFG
FJ906811        DFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLFLALLLSSFG
U38813          DFMHSFMIVFRVLCGEWIESMWDCMYVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
X96668           DFMHSFMIVFRVLCGEWIESMWDCMYVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
NM_001286885     DFMHSFMIVFRVLCGEWIESMWDCMYVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
KM027335         DFMHSFMIVFRVLCGEWIESMWDCILVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
Y13592          DFMHSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
JN002364         DFMHSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
AB453977.1       DFMHSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
AF134216.2      DFMHSFMIVFRVLCGEWIQSMWDCMWVSGWPCIPFFLATVVIGNLVVLNLFLALLLSSFG
AF134216         DFMHSFMIVFRVLCGEWIQSMWDCMWVSGWPCIPFFLATVVIGNLVVLNLFLALLLSSFG
AJ131759        DFLHSFMIVFRVLCGEWIESMWDCLHVGEPTCIPFFLATVVIGNLVVLNLF---------
AJ131760        DFLHSFMIVFRVLCGEWIESMWDCLHVGEPTCIPFFLATVVIGNFVVLNLF---------
AJ408849         NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLAAVVIGNFVVLNLFLALLLSNF-
AJ408850         NFMHSFMIVFRVLCG-------------------------------------------
AB499851.1       NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNXVVLNLFLALLLS---
AJ408851        NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNLVVLNLFLALLLSNF-
AB499848         NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNLVVLNLFLALLLS---
AB742424         NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNLVVLNLFLALLLS---
AB849921.1.      NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNLVVLNLFLALLLS---
AB499850.1       NFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNLVVLNLFLALLLS---
U73583          DFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNLVVLNLFLALLLSNFG
U73584          DFMHSFMIVFRVLCGEWIESMWDCMLVGDWSCIPFFLATVVIGNFVVLNLFLALLLSNFG
KR139855         DFMHSFMIVFRVLCGEWIESMWDCMHVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFG
AY094601.1       ------------------------------------------------------------
AJ440727.1       DFMHSFMIVFRVLCGEWIESMWDCMHVGDVSCIPFFLATVVIGYLVVLNLFLALLLSNFG
AJ440728.1       DFMHSFMIVFRVLCGEWIESMWDCMHVGDVSCIPFFLATVVIGYLVVLNLFLALLLSNFG
```

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EF535530.1       IAT----------TSNEFIRIHHSGSITRSIRLTITASCPMDPQYFPMDRQLCNI-E
AJ408858.1       ------------------------------------------------------
FJ906804         ASNLSSPTSESADTKKLQEAFDRFGRAHKW---LKNRILTGL------KQLRSKTRNQIR
FJ906811        ASNLSSPTSESADTKKLQEAFDRFGRAHKW---VKNRILTGL------KQLRSKTRNQIR
U38813          SSSLSAPTADN-DTNKIAEAFNRIARFKNW---VKRNIADCF------KLIRNKLTNQIS
X96668           SSSLSAPTADN-DTNKIAEAFNRIARFKNW---VKRNIADCF------KLIRNKLTNQIS
NM_001286885     SSSLSAPTADN-DTNKIAEAFNRIARFKNW---VKRNIADCF------KLIRNKLTNQIS
KM027335         SSSLSTPTADQ-ETNKIAEAFNRISRFNEW---VKKSIADFL------KILKNKLTNQIA
Y13592          SSSLSAPTADN-DTNKIA---------------------------------------
JN002364         ------------------------------------------------------
AB453977.1       SSSLSAPTADN-ETNKIAEAFNRISRFSNW---IKANIAAAL------KFVKNKLTSQIA
AF134216.2       ASNLSQANPDSGDTKKLQEAIDRFHRASRW---IKSNSMKLF------KSFRRKPRNQIG
AF134216         ASNLSQANPDSGDTKKLQEAIDRFHRASRW---IKSNSMKLF------KSFRRKPRNQIG
AJ131759         ------------------------------------------------------
AJ131760         ------------------------------------------------------
AJ408849         ------------------------------------------------------
AJ408850         ------------------------------------------------------
AB499851.1       ------------------------------------------------------
AJ408851         ------------------------------------------------------
AB499848         ------------------------------------------------------
AB742424         ------------------------------------------------------
AB849921.1.      ------------------------------------------------------
AB499850.1       ------------------------------------------------------
U73583          SSNLSAPTADN-ETNKIAEAFERFSRFFNW---IKRSALNVA------KMLRAKLTNQIS
U73584          SSNLSAPTADN-ETNKIAEAFERFSRFFNW---IKRSALNVA------KMLRAKLTNQIS
KR139855         SSSLSAPTADS-ETNKIAEAFDRIGRFSAW---IKRNIMLGA------KAIRAKLTNQIS
AY094601.1       ------------------------------------------------------
AJ440727.1       SSSLSAPTADN-ETNK-----------------------------------------
AJ440728.1       SSSLSAPTADN-ETNK-----------------------------------------
```

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EF535530.1       I-ESFGYTMRDIRYKWNEGPN----------SVGVPSEVSLPQFK----------VLG
AJ408858.1       ------------------------------------------------------------
FJ906804         DTAQWPGRGGAS-----SGGMVGRSMMLG--SESVLDEGDIIMMDGLNASGLIRDKKLLA
FJ906811         DTAQWPSRGGGS-----SGGMVDRSMMLG--SESVLDEGDIIMMDGLNASGLIRDKKLLA
U38813           DQ----------------------PSEHGDNELELGHDEIMGDGLIKKGM--------
X96668           DQ----------------------PSEHGDNELELGHDEIMGDGLIKKGM--------
NM_001286885     DQ----------------------PSEHGDNELELGHDEIMGDGLIKKGM--------
KM027335         IHAPGLKAA----------LCGRCVSSDRVDNELELGTD--LEDTVLYKDK--------
Y13592           ------------------------------------------------------------
JN002364         ------------------------------------------------------------
AB453977.1       SVQPAGEQHNHLSWIWSEGKGVCPCISAEHGENELELTPDDILADGLLKKGV--------
AF134216.2       DQTTDIRGGGA----G----------EEL-EA-DPGVAGEVVLLDGRVPMRD-----RKP
AF134216         DQTTDIRGGGA----G----------EEL-EA-DPGVAGEVVLLDGRVPMRD-----RKP
AJ131759         ------------------------------------------------------------
AJ131760         ------------------------------------------------------------
AJ408849         ------------------------------------------------------------
AJ408850         ------------------------------------------------------------
AB499851.1       ------------------------------------------------------------
AJ408851         ------------------------------------------------------------
AB499848         ------------------------------------------------------------
AB742424         ------------------------------------------------------------
AB849921.1.      ------------------------------------------------------------
AB499850.1       ------------------------------------------------------------
U73583           DQTPDAHE--------------------RDTD-LDLTADEILADGIVYRDK-----KSP
U73584           DQTPDAHE--------------------RDTD-LDLTADEILADGIVYRDK-----KSP
KR139855         DQTTGEAPASS----W----------KQGRDRDLDDLGPDEIRADGMIYRDK-----K--
AY094601.1       ------------------------------------------------------------
AJ440727.1       ------------------------------------------------------------
AJ440728.1       ------------------------------------------------------------
```

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EF535530.1       HRQ--------RAMEIS----------------------------------------
AJ408858.1       ------------------------------------------------------
FJ906804          AAAYGETVVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIE
FJ906811         AAAYGETVVGPDGLEYSLTESGKVTLKSTAQTVLNSVKLSQAVKDLSSDKLILTESALIE
U38813           KGETQLEVAIGDGMEFTIHGDMKN----------------------------------
X96668           KGETQLEVAIGDGMEFTIHGDMKN----------------------------------
NM_001286885      KGETQLEVAIGDGMEFTIHGDMKN----------------------------------
KM027335          KLKDQVEVAIGDGMEFTIPVNGIISGD-------------------------------
Y13592           ------------------------------------------------------
JN002364          ------------------------------------------------------
AB453977.1       KEHNQLEVAIGDGMEFTIHGDLK----------------------------------
AF134216.2       QHNNDLEVVVGDGLDIAIQGDGKAVKM-------------------------------
AF134216         QHNNDLEVVVGDGLDIAIQGDGKAVKM-------------------------------
AJ131759         ------------------------------------------------------
AJ131760         ------------------------------------------------------
AJ408849          ------------------------------------------------------
AJ408850          ------------------------------------------------------
AB499851.1       ------------------------------------------------------
AJ408851         ------------------------------------------------------
AB499848          ------------------------------------------------------
AB742424          ------------------------------------------------------
AB849921.1.      ------------------------------------------------------
AB499850.1        ------------------------------------------------------
U73583           KEQTQLEVAIGDGMEFTIHGDLKNKLK-------------------------------
U73584           KEQTQLEVAIGDGMEFTIHGDLKNKLK-------------------------------
KR139855          ---DQLEVAIGDGMEFTIHGDLKSKMN-------------------------------
AY094601.1       ------------------------------------------------------
AJ440727.1       ------------------------------------------------------
```

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AJ440728.1      ----------

EF535530.1      ----------
AJ408858.1      ----------
FJ906804        KLSPSVTNHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNN
FJ906811        KLSPSVTNHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNN
U38813          ----------NKPKKSKFMNNTTM----------IGNS-INHQ
X96668          ----------NKPKKSKFINNTTM----------IGNS-INHQ
NM_001286885    ----------NKPKKSKFINNTTM----------IGNS-INHQ
KM027335        ----------NKYKKGKILMNNIN----------AITDNHR
Y13592          ----------
JN002364        ----------
AB453977.1      ----------NKGKKNKQLMNNSKV----------IGNSISNHQ
AF134216.2      ----------KLKNNSKPVMNS-VW----------VGPMIEPKN
AF134216        ----------KLKNNSKPVMNS-VW----------VGPMIEPKN
AJ131759        ----------
AJ131760        ----------
AJ408849        ----------
AJ408850        ----------
AB499851.1      ----------
AJ408851        ----------
AB499848        ----------
AB742424        ----------
AB849921.1.     ----------
AB499850.1      ----------
U73583          ----------KDK----LMMNSTKV----------IGNSLNHK-
U73584          ----------KDK----LMMNSTKV----------IGNSLNHK-
KR139855        ----------KVK----------NH----------IGNSIGNH-
AY094601.1      ----------
```

```
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ---------------------IQDSTSAPTVYSGPTPSGNSNLAAVYFSPNKDRFIIFS-
FJ906804        VNKVKPSLHD---ANGCSPFPLDDNHHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNN
FJ906811        VNKVKPSLHD---ANGCSPFPLDDNHHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNN
U38813          DNRL-------EHELNHRGLSI---------------QDDDTASINSYGSHKNRPF-----
X96668          DNRL-------EHELNHRGLSI---------------QDDDTASINSYGSHKNRPF-----
NM_001286885    DNRL-------EHELNHRGLSI---------------QDDDTASINSYGSHKNRPF-----
KM027335        DNRL-------ECELNHHGYPL---------------QDDDTISQKSYGSHKIRSF-----
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      DNKL-------EHELNHRGMSL---------------QDDDTASIKSYGSHKNRPF-----
AF134216.2      KQLEKDNKEKEKEAQGNKVYPQ---------------KDEDTLSEKSASSPKEKVL-----
AF134216        KQLEKDNKEKEKEAQGNKVYPQ---------------KDEDTLSEKSASSPKEKVL-----
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          ---------DNRIESGDYLHNR---------------QDEDTLSTGSYGSHKNRPY-----
U73584          ---------DNRIESGDYLHNR---------------QDEDTLSTGSYGSHKNRPY-----
KR139855        ---------QDNRLDTDYIRNR---------------YDEDSISNKSYGSHKHRPL-----
```

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AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        NVSNNNDSSAQQSSSLSVTPSHHLNHPSSHPTA-HHYNASQLSKVHPAGGPVPISFQHSY
FJ906811        NVSNNNDSSAQQSSSLSVTPSHHLNHPSSHPTA-HHYNASQLSKVHPAGGPVPISFQHSY
U38813          --------------------KDESHKGS-AETIEGEEKRDVSKE---------------
X96668          --------------------KDESHKGS-AETIEGEEKRDVSKE---------------
NM_001286885    --------------------KDESHKGS-AETIEGEEKRDVSKE---------------
KM027335        --------------------KDESHKGS-ADTIDGEEKKDASKE---------------
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      --------------------KDESHKGS-AETLEGEEKRDASKE---------------
AF134216.2      ---------------LGNKPSKDLSNSSLYLGNNLEEEKKDASKE---------------
AF134216        ---------------LGNKPSKDLSNSSLYLGNNLEEEKKDASKE---------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          --------------------KDDSHKGS-AETMDGEEKKDASKE---------------
U73584          --------------------KDDSHKGS-AETMDGEEKKDASKE---------------
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KR139855          ----------KDESHKGS-MESLDQEEKKDLSKE----------
AY094601.1        ------------------------------------------------------------
AJ440727.1        ------------------------------------------------------------
AJ440728.1        ------------------------------------------------------------


EF535530.1        ---------------------------------------------LTTGNYSRLACEI
AJ408858.1        ------------------------------------------------------------
FJ906804          SSLNRLAPSPLNHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCC-
FJ906811          SSLNRLDPSPLNHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCC-
U38813           -DLGL--DEELDEEAEGDEGQLDG---DIIIHAQNDDEIIDDYPADCFPDSYYKKFPILA
X96668           -DLGL--DEELDEEAEGDEGQLDG---DIIIHAQNDDEIIDDYPADCFPDSYYKKFPILA
NM_001286885     -DLGL--DEELDEEAEGDEGQLDG---DIIIHAQNDDEIIDDYPADCFPDSYYKKFPILA
KM027335         -ELGL--EEEIEEEEDKLEGLK------DIIVAAD-EDVVDDSPAECCPDKCYKQFPFLA
Y13592           ------------------------------------------------------------
JN002364         ------------------------------------------------------------
AB453977.1       -DLGI--DEELDDECEGEEGPLDG---EMIIHAEE-DEVIEDAPADCFPDNCYKRFPALA
AF134216.2       -DLGT--KEGEEAPTEEPINPDTEDVDTDKLETATSDIIIPEMPADCCPDWCYTRFAFAC
AF134216         -DLGT--KEGEEAPTEEPINPDTEDVDTDKLETATSDIIIPEMPADCCPDWCYTRFAFAC
AJ131759         ------------------------------------------------------------
AJ131760         ------------------------------------------------------------
AJ408849         ------------------------------------------------------------
AJ408850         ------------------------------------------------------------
AB499851.1       ------------------------------------------------------------
AJ408851         ------------------------------------------------------------
AB499848         ------------------------------------------------------------
AB742424         ------------------------------------------------------------
AB849921.1.      ------------------------------------------------------------
AB499850.1       ------------------------------------------------------------
U73583           -DLDQ--EGEGEE-DGEGEGPLEE---DMVLDAGTEDVMMSEYPADCCPDHCYKRFPFLA
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```
U73584          -DLDQ--EGEGEE-DGEGEGPLEE---DMVLDAGTEDVMMSEYPADCCPDHCYKRFPFLA
KR139855         -DLEH--VPDI----------EEE---DIVIEGGTEDAMLADYPADCCPDNCYKRFPFLA
AY094601.1       ------------------------------------------------------------
AJ440727.1       ------------------------------------------------------------
AJ440728.1       ------------------------------------------------------------


EF535530.1       QFVRS-----------------------------------------------------MG
AJ408858.1       --NTDTRHYLYWVNSTLQSGNRIAG--------TGSVMSASPLAATTITNVQTSSMTIFL
FJ906804         --LEETAFWIKWREVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSL
FJ906811        --LEETAFWIKWREVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSL
U38813           G-DEDSPFWQGWGNLRLKTFQLIENKYFETAVITMILMSSLALALEDVHLPDRPVMQDIL
X96668           G-DEDSPFWQGWGNLRLKTFQLIENKYFETAVITMILMSSLALALEDVHLPDRPVMQDIL
NM_001286885     G-DEDSPFWQGWGNLRLKTFQLIENKYFETAVITMILMSSLALALEDVHLPDRPVMQDIL
KM027335         G-DDESPFWQGWGMLRLKTFRLIENTYFETAVITMILLSSLALALEDVHLPHRPILQDIL
Y13592          ------------------------------------------------------------
JN002364         ------------------------------------------------------------
AB453977.1       G-DDDAPFWQGWGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVL
AF134216.2       FFDENKIFWQRYKIVRTKAYALVEHKYFETIVVVLILTSSLALALEDVNLKDRPTLKAVL
AF134216         FFDENKIFWQRYKIVRTKAYALVEHKYFETIVVVLILTSSLALALEDVNLKDRPTLKAVL
AJ131759         ------------------------------------------------------------
AJ131760         ------------------------------------------------------------
AJ408849         ------------------------------------------------------------
AJ408850         ------------------------------------------------------------
AB499851.1       ------------------------------------------------------------
AJ408851         ------------------------------------------------------------
AB499848         ------------------------------------------------------------
AB742424         ------------------------------------------------------------
AB849921.1.      ------------------------------------------------------------
AB499850.1       ------------------------------------------------------------
```

```
U73583          G-DEDSPFWQGWGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDIL
U73584          G-DEDSPFWQGWGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDIL
KR139855         G-DDDAPFWQGWANLRLKTFQLIENKYFETAVITMIMLSSLALALEDVHLAQRPILQDIL
AY094601.1       ------------------------------------------------------------
AJ440727.1       ------------------------------------------------------------
AJ440728.1       ------------------------------------------------------------


EF535530.1       YYLIQ---------------------------------------------------
AJ408858.1       YYMDVNT-------------------------------------------------
FJ906804         KYIDQFFTIIFTCEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSALNLGAEFAGLAKIQAF
FJ906811        KYIDQFFTIIFTCEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSLINFTVGQLGFSNIPAF
U38813          YYMDRIFTVIFFLEMLIKWLALGFKVYFTNAWCWLDFVIVMLSLINLVAVWSGLNDIAVF
X96668           YYMDRIFTVIFFLEMLIKWLALGFKVYFTNAWCWLDFVIVMLSLINLVAVWSGLNDIAVF
NM_001286885      YYMDRIFTVIFFLEMLIKWLALGFKVYFTNAWCWLDFVIVMLSLINLVAVWSGLNDIAVF
KM027335         YYMDRIFTVIFFLEMLIKWLALGFQKYFTNAWCWLDFIIVMVSLINFVAALCGAGGIQAF
Y13592          ------------------------------------------------------------
JN002364         ------------------------------------------------------------
AB453977.1       YYMDRIFTVIFFLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAF
AF134216.2       TYMDKTFTVIFFFEMMLKWLAFGFKKYFTNAWCWLDFVIVLVSFFNMAVAMMGYGRIPAF
AF134216         TYMDKTFTVIFFFEMMLKWLAFGFKKYFTNAWCWLDFVIVLVSFFNMAVAMMGYGRIPAF
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849         ------------------------------------------------------------
AJ408850         ------------------------------------------------------------
AB499851.1       ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848         ------------------------------------------------------------
AB742424         ------------------------------------------------------------
AB849921.1.      ------------------------------------------------------------
```

```
AB499850.1      ------------------------------------------------------------
U73583          YYMDRIFTVIFFIEMLIKWLALGFKKYFTNAWCWLDFIIVMVSLINFVASLVGAGGIQAF
U73584          YYMDRIFTVIFFIEMLIKWLALGFKKYFTNAWCWLDFIIVMVSLINFVASLVGAGGIQAF
KR139855        YYMDRIFTVIFFLEMLIKWLALGFRKYFTNAWCWLDFIIVMVSLINFTASMLGAGGIQAF
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ----------------------------IYIPSGLIVIISWVSFWLNRNATPARVSLGV
AJ408858.1      ------------------------------------------------------------
FJ906804        KTMRTLRAFRPLRAMSRSKGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGK
FJ906811        KTMRTLRALRPLRAMSRLEGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGK
U38813          RSMRTLRALRPLRAVSRWEGMKVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
X96668          RSMRTLRALRPLRAVSRWEGMKVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
NM_001286885    RSMRTLRALRPLRAVSRWEGMKVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
KM027335        KTMRTLRALRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      KTMRTLRALRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
AF134216.2      KTMRTLRALRPLRAMSRLEGMRVVVNALVQAIPAIFNVLLVCLIFWLIFSIMGVQMLAGK
AF134216        KTMRTLRALRPLRAMSRLEGMRVVVNALVQAIPAIFNVLLVCLIFWLIFSIMGVQMLAGK
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
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AB849921.1.      ------------------------------------------------------
AB499850.1       ------------------------------------------------------
U73583          KTMRTLRALRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
U73584          KTMRTLRALRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
KR139855         KTMRTLRALRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGK
AY094601.1       ------------------------------------------------------
AJ440727.1       ------------------------------------------------------
AJ440728.1       ------------------------------------------------------


EF535530.1       ------------------------------------------------------TTV----
AJ408858.1       ------------------------------------------------------
FJ906804         FSYCRDRNTEEKSDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWT
FJ906811        FSYCRDRNTEEKSDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWT
U38813          YFKCKDGNDTVLSH-EIIPNRNACKS--ENYTWENSAMNFDHVGNAYLCLFQVATFKGWI
X96668           YFKCKDGNDTVLSH-EIIPNRNACKS--ENYTWENSAMNFDHVGNAYLCLFQVATFKGWI
NM_001286885      YFKCKDGNDTVLSH-EIIPNRNACKS--ENYTWENSAMNFDHVGNAYLCLFQVATFKGWI
KM027335         YFKCVDLNHTTLSH-EIIPDRNACIL--ENYTWENSPMNFDHVGKAYLCLFQVATFKGWI
Y13592          ------------------------------------------------------
JN002364         ------------------------------------------------------
AB453977.1       YFKCVDTNKTTLSH-EIIPDVNACIA--ENYTWENSPMNFDHVGKAYLCLFQVATFKGWI
AF134216.2       FYRCVDGNGTRLNS-THVPNRKACEA--NNFTWDNPMINFDNVLNAYLALFQVATFKGWT
AF134216         FYRCVDGNGTRLNS-THVPNRKACEA--NNFTWDNPMINFDNVLNAYLALFQVATFKGWT
AJ131759        ------------------------------------------------------
AJ131760        ------------------------------------------------------
AJ408849         ------------------------------------------------------
AJ408850         ------------------------------------------------------
AB499851.1       ------------------------------------------------------
AJ408851        ------------------------------------------------------
AB499848         ------------------------------------------------------
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AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          YHKCVDSNSTTLSH-EIIPDRNACIA--ENYTWENSPMNFDHVGKAYLCLFQVATFKGWI
U73584          YHKCVDSNSTTLSH-EIIPDRNACIA--ENYTWENSPMNFDHVGKAYLCLFQVATFKGWI
KR139855        YYKCVDQNKTTLSH-EIIPDRNVCEA--ENYTWENSPMNFDHVGKAYLCLFQVATFKGWI
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      LTMTTLMSSTNAALPKISYVKSIDVYLGTCFVMVFASLLEYAT-VGYMAKRIQMR-----
AJ408858.1      ------------------------------------LLNRIVGKVTDNE--------
FJ906804        IIMDHAIDSREV-HQQPVYENSILMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKGG
FJ906811        IIMDHAIDSREV-HQQPVYENSILMYLYFVFFIIFGSFITLNLFIGVIIDNFNEQKKKGG
U38813          QIMNDAIDSREV-DKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
X96668          QIMNDAIDSREV-DKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
NM_001286885    QIMNDAIDSREV-DKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
KM027335        QIMNDAIDSREV-GRQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      QIMNDAIDSRDI-GKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
AF134216.2      DIMDNAIDSRGGKEDQPEYEANIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
AF134216        DIMDNAIDSRGGKEDQPEYEANIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
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AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          QIMNDAIDSREL-HKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
U73584          QIMNDAIDSREL-HKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
KR139855        QIMNDAIDSREI-GKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAG
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ----------KQRFTAVQKMAAEKKMQIDGPPGTSEPLPPPRTSTLTRPPPPSRSSEVR
AJ408858.1      ----------IHWYANQVVEGAP---------------------------------
FJ906804        GSREMLMTEDQKKYLNAMKKMGSKKPM---------KAIPRPR----------FKLQAII
FJ906811        GSREMLMTEDQKKYLNAMKKMGSKKPM---------KAIPRPR----------FKLQAII
U38813          GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPR----------WRPQAIV
X96668          GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPR----------WRPQAIV
NM_001286885    GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPR----------WRPQAIV
KM027335        GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPK----------WRPQAIV
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPK----------WRPQAIV
AF134216.2      GSLEMFMTEDQKKYYNAMKKMGSKKPA---------KAIPRPR----------FKLQAMV
AF134216        GSLEMFMTEDQKKYYNAMKKMGSKKPA---------KAIPRPR----------FKLQAMV
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
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AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPK----------WRPQAIV
U73584          GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPK----------WRPQAIV
KR139855        GSLEMFMTEDQKKYYNAMKKMGSKKPL---------KAIPRPR----------WRPQAIV
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      FKVHDPKAYSKGGTLENTINGS-------R-----------------------------
AJ408858.1      ----------------------------------------------------------
FJ906804        FDIVTNKKFDMLIMLFIMLNMFVMSLDHYQASAFMEHILEMCNLFFIAVFTAECMLKIFA
FJ906811        FDIVTNKKFDMLIMLFIMLNMFVMSLDHYQASAFMEHILEMCNLFFIAVFTAECMLKIFA
U38813          FEIVTDKKFDIIIMLFIGLNMFTMTLDRYDASEAYNNVLDKLNGIFVVIFSGECLLKIFA
X96668          FEIVTDKKFDIIIMLFIGLNMFTMTLDRYDASEAYNNVLDKLNGIFVVIFSGECLLKIFA
NM_001286885    FEIVTDKKFDIIIMLFIGLNMFTMTLDRYDASEAYNNVLDKLNGIFVVIFSGECLLKIFA
KM027335        FEIVTDKKFDMIIMLFIGLNMLTMTLDHYQQAESFSVVLDYLNMIFIVIFSSECMSKIFA
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      FEICTNKKFDMIIMLFIGFNMLTMTLDHYKQTETFSAVLDYLNMIFICIFSSECLMKIFA
AF134216.2      FDLTTNKMFDMAIMIFIVLNMTVMALDHYKQSRLFESILERLNIFFIAVFTAECLLKIFA
AF134216        FDLTTNKMFDMAIMIFIVLNMTVMALDHYKQSRLFESILERLNIFFIAVFTAECLLKIFA
AJ131759        ----------------------------------------------------------
AJ131760        ----------------------------------------------------------
AJ408849        ----------------------------------------------------------
AJ408850        ----------------------------------------------------------
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AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          FEICTDKKFDMIIMLFIGFNMLTMTLDHYQQSKQFSDVLDYLNMIFIVIFSSECLMKIFA
U73584          FEICTDKKFDMIIMLFIGFNMLTMTLDHYQQSKQFSDVLDYLNMIFIVIFSSECLMKIFA
KR139855        FEIVTDKKFDMIIMLFIGLNMLTMTLDHYQQTEMFSFVLDMLNMIFIVIFSSECLLKIFA
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ----------------------G---------PAPAPVPAAPQPDEEAGPPPHLIHASK
AJ408858.1      ------------------------------------------------------------
FJ906804        LRFHYFREPWNVFDFVIVILSIASSALKDFVENYLISPTLLRVVRVVKIGRVLRLVKGAR
FJ906811        LRFHYFREPWNVFDFVIVILSIASSALKDFVENYLISPTLLRVVRVVKIGRVLRLVKGAR
U38813          LRYHYFKEPWNLFDVVVVILSILGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
X96668          LRYHYFKEPWNLFDVVVVILSILGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
NM_001286885    LRYHYFKEPWNLFDVVVVILSILGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
KM027335        LRYHYFVDPWNWFDFVVVMFSILSLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      LRYHYFIEPWNLFDFVVVILSILGLVLSDLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
AF134216.2      LRWHYFREPWNMFDFVVVILSILGTVLKDLIAAYFVSPTLLRVVRVVKVGRVLRLVKGAR
AF134216        LRWHYFREPWNMFDFVVVILSILGTVLKDLIAAYFVSPTLLRVVRVVKVGRVLRLVKGAR
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
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AJ408850        ----------------------------------------------------------
AB499851.1      ----------------------------------------------------------
AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          LRYHYFKEPWNLFDFVVVILSILGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
U73584          LRYHYFKEPWNLFDFVVVILSILGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
KR139855        LRYHYFKEPWNLFDFVVVILSMLGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAK
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      GINKLLGTTPSDIDKYSRIVFPVCFVCFNLMYWIIY------LHVSDVVADDL-------
AJ408858.1      ----------------------------------------------------------
FJ906804        GIRTLLFALAMS----LPALFNICLLLFLVMFIYAIFGMSFFMNVKQRYGLDETFNFGTF
FJ906811        GIRTLLFALAMS----LPALFNICLLLFLVMFIYAIFGMSFFMNVKQRYGLDETFNFGTF
U38813          GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVKEKSGINAVYNFKTF
X96668          GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVKEKSGINAVYNFKTF
NM_001286885    GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVKEKSGINAVYNFKTF
KM027335        GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVKNKGGLDDVYNFKTF
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVKDKSGLDDVYNFKTF
AF134216.2      GIRTLLFALAMS----LPALFNICLLLFLVMFIYAIFGMSFFMHVKHRYGVDENFNFETF
AF134216        GIRTLLFALAMS----LPALFNICLLLFLVMFIYAIFGMSFFMHVKHRYGVDENFNFETF
AJ131759        ----------------------------------------------------------
AJ131760        ----------------------------------------------------------
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AJ408849        ----------------------------------------------------------
AJ408850        ----------------------------------------------------------
AB499851.1      ----------------------------------------------------------
AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVRDKGGLDDVYNFKTF
U73584          GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVRDKGGLDDVYNFKTF
KR139855        GIRTLLFALAMS----LPALFNICLLLFLVMFIFAIFGMSFFMHVKDKSGLDDVYNFKTF
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      ----------------------------------------------------------
AJ408858.1      ----------------------------------------------------------
FJ906804        FRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-------------------------
FJ906811        FRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-------------------------
U38813          GQSMILLFQMSTSAGWDGVLDAIINEEDCDPPDNDKGYPGNCGSATVGITFLLSYLVISF
X96668          GQSMILLFQMSTSAGWDGVLDAIINEEDCDPPDNDKGYPGNCGSATVGITFLLSYLVISF
NM_001286885    GQSMILLFQMSTSAGWDGVLDAIINEEDCDPPDNDKGYPGNCGSATVGITFLLSYLVISF
KM027335        VQSMILLFQMSTSAGWDGVLDGIINEEECDLPDNERGYPGNCGSATIGITYLLSYLVISF
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      GQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDNDKGYPGNCGSATIGITYLLAYLVISF
AF134216.2      GQSMILLFQMCTSAGWDGVLAAIMDEHDCNRPTDE--SEGNCGKRGIAVAYLVSYLIISF
AF134216        GQSMILLFQMCTSAGWDGVLAAIMDEHDCNRPTDE--SEGNCGKRGIAVAYLVSYLIISF
AJ131759        ----------------------------------------------------------
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AJ131760        ----------------------------------------------------------
AJ408849        ----------------------------------------------------------
AJ408850        ----------------------------------------------------------
AB499851.1      ----------------------------------------------------------
AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          GQSMILLFQMSTSAGWDGVLDGIMNEEDCNKPNSEIGYPGDCGSATVGIAFLLSYLVISF
U73584          GQSMILLFQMSTSAGWDGVLDGIMNEEDCNKPNSEIGYPGDCGSATVGIAFLLSYLVISF
KR139855        GQSMILLFQMSTSAGWDGVLDGIINEENCDKPDPEMGSPGDCGSATIGITFLLSYLVISF
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      ----------------------------------------------------------
AJ408858.1      ----------------------------------------------------------
FJ906804        ----------------------------------------------------------
FJ906811        ----------------------------------------------------------
U38813          LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPEGTQYIRYDQLSEFLDV
X96668          LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPEGTQYIRYDQLSEFLDV
NM_001286885    LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPEGTQYIRYDQLSEFLDV
KM027335        LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQRFDPDGTQYIRYDQLSDFLDV
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPDGTQYIRYDQLSDFLDV
AF134216.2      LVIINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPKGTQYVAYSNLTNFVNA
AF134216        LVIINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPKGTQYVAYSNLTNFVNA
```

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AJ131759        ----------------------------------------------------------
AJ131760        ----------------------------------------------------------
AJ408849        ----------------------------------------------------------
AJ408850        ----------------------------------------------------------
AB499851.1      ----------------------------------------------------------
AJ408851        ----------------------------------------------------------
AB499848        ----------------------------------------------------------
AB742424        ----------------------------------------------------------
AB849921.1.     ----------------------------------------------------------
AB499850.1      ----------------------------------------------------------
U73583          LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPDGTQYIRYDQLSDFLDV
U73584          LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPDGTQYIRYDQLSDFLDV
KR139855        LIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPDGTQYIRYDQLSEFLDV
AY094601.1      ----------------------------------------------------------
AJ440727.1      ----------------------------------------------------------
AJ440728.1      ----------------------------------------------------------


EF535530.1      ----------------------------------------------------------
AJ408858.1      ----------------------------------------------------------
FJ906804        ----------------------------------------------------------
FJ906811        ----------------------------------------------------------
U38813          LEPPLQIHKPNKYKIISMDMPICRGDMMYCVDILDALTKDFFARKGNPIEETGEIGEIA-
X96668          LEPPLQIHKPNKYKIISMDMPICRGDMMYCVDILDALTKDFFARKGNPIEETGEIGEIA-
NM_001286885    LEPPLQIHKPNKYKIISMDMPICRGDMMYCVDILDALTKDFFARKGNPIEETGEIGEIA-
KM027335        LEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILDALTKDFFARK----------
Y13592          ----------------------------------------------------------
JN002364        ----------------------------------------------------------
AB453977.1      LEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILDALTKDFFARKGNPIEDSAEMGEVQ-
AF134216.2      LEEPLQIPKPNKYKLIALDIPICKDDMVYCVDILDALTRDFFARKGHAIEEPPRFFSLPR
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AF134216        LEEPLQIPKPNKYKLIALDIPICKDDMVYCVDILDALTRDFFARKGHAIEEPPRFFSLPR
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          LEPPLQIHKPNKYKIVSMDIPICKGDLMFCVDILDALTKDFFARKGNPIEESAELGEVQP
U73584          LEPPLQIHKPNKYKIVSMDIPICKGDLMFCVDILDALTKDFFARKGNPIEESAELGEVQP
KR139855        LEPPLQIHKPNKYKIVSMDIPICKGDLMFCVDILDALTKDFFARKGNPIEESAELGEVAP
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------------------------------------------------
AJ408858.1      ------------------------------------------------------------
FJ906804        ------------------------------------------------------------
FJ906811        ------------------------------------------------------------
U38813          ARPDTEGYDPVSSTLWRQREEYCAKLIQNAWRRYKNGPPQEGDEGEAAGGEDGAEGGEGE
X96668          ARPDTEGYDPVSSTLWRQREEYCAKLIQNAWRRYKNGPPQEGDEGEAAGGEDGAEGGEGE
NM_001286885    ARPDTEGYDPVSSTLWRQREEYCAKLIQNAWRRYKNGPPQEGDEGEAAGGEDGAEGGEGE
KM027335        ------------------------------------------------------------
Y13592          ------------------------------------------------------------
JN002364        ------------------------------------------------------------
AB453977.1      QRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKERGGV----------GGGGGG
```

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AF134216.2       QK----------------------------------------------------
AF134216         QK----------------------------------------------------
AJ131759         ----------------------------------------------------
AJ131760         ----------------------------------------------------
AJ408849         ----------------------------------------------------
AJ408850         ----------------------------------------------------
AB499851.1       ----------------------------------------------------
AJ408851         ----------------------------------------------------
AB499848         ----------------------------------------------------
AB742424         ----------------------------------------------------
AB849921.1.      ----------------------------------------------------
AB499850.1       ----------------------------------------------------
U73583           GRPDEVGYEPVSSTLWRQREEYCARLIQNAWRKHKQQRQG----------------
U73584           GRPDEVGYEPVSSTLWRQREEYCARLIQNAWRKHKQQRQG----------------
KR139855         GRPDEVGYEPVSSTLWRQREEYCARLIQNAWRKHKQARAG----------------
AY094601.1       ----------------------------------------------------
AJ440727.1       ----------------------------------------------------
AJ440728.1       ----------------------------------------------------


EF535530.1       ----------------------------------------------------
AJ408858.1       ----------------------------------------------------
FJ906804         ----------------------------------------------------
FJ906811         ----------------------------------------------------
U38813           GGSGG--GGGDDGGSATGATA---AAGATSPSDPDAG------EADGASVGGPLSPGCV-
X96668           GGSGGGGGGGGDDGGSATGATA--AAAGATSPSDPDAG------EADGASVGGPLSPGCV-
NM_001286885     GGSGGGGGGGGDDGGSATGATA--AAAGATSPSDPDAG------EADGASVGGPLSPGCV-
KM027335         ----------------------------------------------------
Y13592           ----------------------------------------------------
JN002364         ----------------------------------------------------
```

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AB453977.1      GGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGGGSIAGGGSQANL-GPPSPKESP
AF134216.2      ------------------------------------------------------------
AF134216        ------------------------------------------------------------
AJ131759        ------------------------------------------------------------
AJ131760        ------------------------------------------------------------
AJ408849        ------------------------------------------------------------
AJ408850        ------------------------------------------------------------
AB499851.1      ------------------------------------------------------------
AJ408851        ------------------------------------------------------------
AB499848        ------------------------------------------------------------
AB742424        ------------------------------------------------------------
AB849921.1.     ------------------------------------------------------------
AB499850.1      ------------------------------------------------------------
U73583          ----APGEDSDEA-------------------------------------------G
U73584          ----APGEDSDEA-------------------------------------------G
KR139855        ----GGPDS-----------------------------------------------
AY094601.1      ------------------------------------------------------------
AJ440727.1      ------------------------------------------------------------
AJ440728.1      ------------------------------------------------------------


EF535530.1      ------------------V-------LLGEEN----------
AJ408858.1      ----------------------------------------
FJ906804        ----------------------------------------
FJ906811        ----------------------------------------
U38813          --SGGSNGRQTAVLVESDGFVTKNGHKVVIHSRSPSITSRTADV
X96668          --SGGSNGRQTAVLVESDGFVTKNGHKVVIHSRSPSITSRTADV
NM_001286885    --SGGSNGRQTAVLVESDGFVTKNGHKVVIHSRSPSITSRTADV
KM027335        ----------------------------------------
Y13592          ----------------------------------------
```

JN002364 ----------
AB453977.1 DGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV
AF134216.2 ----------
AF134216 ----------
AJ131759 ----------
AJ131760 ----------
AJ408849 ----------
AJ408850 ----------
AB499851.1 ----------
AJ408851 ----------
AB499848 ----------
AB742424 ----------
AB849921.1. ----------
AB499850.1 ----------
U73583 DDPELQDRHQTAVLVESDGFVTKNGHRVVIHSRSPSVTSRSTDV
U73584 DDPELQDRHQTAVLVESDGFVTKNGHRVVIHSRSPSVTSRSTDV
KR139855 --PGSPDAC------DTTVLVEKNGHKVVIHSRSPSTTSRLADV
AY094601.1 ----------
AJ440727.1 ----------
AJ440728.1 ----------

ETOXAZOLE RESISTANCE:

London MARETVKKWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613274(S) MARETVKKWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613279.1 ----------PPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613280.1 ------KKWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL

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JQ613281.1      MARETVKKWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613275.1      -------KWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613276.1      -------KWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613277.1      -------KWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
JQ613278        -------KWDVFVESPPPDDDETSSSEWIDVILKILKLCAYVITFIVVLACSVLSKGLVL
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London          FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613274(S)     FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613279.1      FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613280.1      FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613281.1      FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613275.1      FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613276.1      FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613277.1      FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
JQ613278        FMTSIIKPNRTGLIICSHGIPSLDRDKKYEVVLNLSDPERVAWIWALIGVLIVPELMTLF
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London          RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613274(S)     RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613279.1      RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613280.1      RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613281.1      RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613275.1      RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613276.1      RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613277.1      RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
JQ613278        RAARICTFKSIRRPSKAVFSLIFIVETLHTIGIVMLVFLILPSLDVTKGVILTNCFCFIP
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London          GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
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JQ613274(S)     GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613279.1      GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613280.1      GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613281.1      GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613275.1      GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613276.1      GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613277.1      GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
JQ613278        GCLSLFSRHSGEAGRGYKTLLDLLSVGAQLSGLILWSVAESTDNPVAIYIPITSLLISIG
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London          WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613274(S)     WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613279.1      WWENYIDKKSPFRAIQRLAQVKESLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613280.1      WWENYIDKKSPFRAIQRLAQVKESLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613281.1      WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613275.1      WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613276.1      WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613277.1      WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
JQ613278        WWENYIDKKSPFRAIQRLAQIKEGLQKSRYFIYIFISAWKIILIFIATIILRLLVDGSAL
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London          YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613274(S)     YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613279.1      YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613280.1      YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613281.1      YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613275.1      YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613276.1      YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613277.1      YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
JQ613278        YLFTQFKSAFTSHKILIIRDRSDLSKSLSDSNVGIESEWLEMPASTSAPIWMLILQISAS
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London          WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613274(S)      WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613279.1       WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613280.1       WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613281.1      WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613275.1       WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613276.1       WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613277.1       WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
JQ613278         WFCYVFGKFACKICIQRISFASPLILSVPVTAMTLAQFCVLNFENSCSLNRFLPRYLFWS
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London          CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613274(S)      CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613279.1       CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613280.1       CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613281.1      CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKRERLATTEKLFVNPMYCGLLI
JQ613275.1       CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613276.1       CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613277.1       CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
JQ613278         CPSADTFFTDGVFYNLHGIIWVIMYISQFWITFHIFNPKCERLATTEKLFVNPMYCGLLI
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London          DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613274(S)      DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613279.1       DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613280.1       DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613281.1      DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613275.1       DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
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JQ613276.1      DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613277.1      DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
JQ613278        DASMILNRRRDDKEVIKAGDIDKSVRDPDNVQDPSLHYETISEHPDDKKSTVQSTDFITK
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London          ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613274(S)     ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613279.1      ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613280.1      ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613281.1      ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613275.1      ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613276.1      ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613277.1      ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
JQ613278        ILVCATMWHETSEEMIQMLKSVFRMDFDQSARHKAQKYLRVVDPDYYEFEVHILFDDAFE
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London          LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYVLPGGNKL
JQ613274(S)     LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYVLPGGNKL
JQ613279.1      LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYVLPGGNKL
JQ613280.1      LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYVLPGGNKL
JQ613281.1      LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYILPGGNKL
JQ613275.1      LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYILPGGNKL
JQ613276.1      LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYILPGGNKL
JQ613277.1      LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYILPGGNKL
JQ613278        LSDDNDDYQVVNRFVKQFIEVIDTAASNIHQCEIRLKSPAKYPTPYGGKLEYILPGGNKL
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London          HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613274(S)     HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613279.1      HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
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JQ613280.1      HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613281.1      HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613275.1      HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613276.1      HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613277.1      HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
JQ613278        HVHLKDKMKIRHRKRWSQVMYMYYLLGHRLMELPIDVNRKATMAENTYILTLDGDINFRP
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London          EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613274(S)     EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613279.1      EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613280.1      EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613281.1      EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613275.1      EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613276.1      EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613277.1      EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
JQ613278        EAVQLLVDLMKKNKNLGAACGRIHPVGSGLMAWYQKFEYAVGHWLQKATEHMIGCVLCSP
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London          GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613274(S)     GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613279.1      GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613280.1      GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613281.1      GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613275.1      GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613276.1      GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613277.1      GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
JQ613278        GCFSLFRAKALMDDNVMRKYTTRSDEALHYVQYDQGEDRWLCTLLLQRGYRVEYSAAADA
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London          YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613274(S)      YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613279.1       YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613280.1       YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613281.1      YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613275.1       YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613276.1       YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613277.1       YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
JQ613278         YTHCPEGFGEFYTQRRRWAPSTMANILDLLGDYKRTVAVNDHISLLYIVYQGMLMVGTIL
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London          GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613274(S)      GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613279.1       GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613280.1       GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613281.1      GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613275.1       GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613276.1       GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613277.1       GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
JQ613278         GPGTIFLMLVGAMVAVFRISNWDSFLFNLIPILIFIVICFTCKNDIQILVAQIMSACYAL
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London          LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSIPCM
JQ613274(S)      LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSIPCM
JQ613279.1       LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSFPCM
JQ613280.1       LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSFPCM
JQ613281.1      LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSFPCM
JQ613275.1       LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSIPCM
JQ613276.1       LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSIPCM
JQ613277.1       LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSIPCM
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JQ613278        LMMAVFVGTAIQMAEDGVTSPSAVFFIALSGSFVVAALLHPQEFHCLYPCLLYFLSFPCM
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London          YLLLMIYSLVNLNVVTWGTREVQSKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613274(S)      YLLLMIYSLVNLNVVTWGTREVQSKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613279.1       YLLLMIYSLVNLNVVTWGTREVQSKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613280.1       YLLLMIYSLVNLNVVTWGTREVQSKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613281.1      YLLLMIYSLVNLNVVTWGTREVQTKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613275.1       YLLLMIYSLVNLNVVTWGTREVQTKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613276.1       YLLLMIYSLVNLNVVTWGTREVQTKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613277.1       YLLLMIYSLVNLNVVTWGTREVQTKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
JQ613278         YLLLMIYSLVNLNVVTWGTREVQTKKTKAELEEEKKAVEEIKKGNLLSFLNLNPNAKEEE
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London          GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGALEKYLDPLGGPRRKGS
JQ613274(S)      GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGALEKYLDPLGGPRRKGS
JQ613279.1       GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGALEKYLDPLGGPRRKGS
JQ613280.1       GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGALEKYLDPLGGPRRKGS
JQ613281.1      GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGSLEKYLDPLGGPRRKGS
JQ613275.1       GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGSLEKYLDPLGGPRRKGS
JQ613276.1       GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGSLEKYLDPLGGPRRKGS
JQ613277.1       GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGSLEKYLDPLGGPRRKGS
JQ613278         GSIEFSLANLFRCSFCTYPKPNDEKIHLLKIEQHLSEMTDKLGSLEKYLDPLGGPRRKGS
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London          SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDED
JQ613274(S)      SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDED
JQ613279.1       SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDED
JQ613280.1       SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDED
JQ613281.1      SIGRNARFSDNLSTVTENEEHEDMDSLGSGSQTDDMSIKDNEEVAPRFDEDHPYWIEDKD
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JQ613275.1      SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDKD
JQ613276.1      SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDKD
JQ613277.1      SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDKD
JQ613278        SIGRNARFSDNLSTVTENEEHEDMDSIGSESQTDDMSIKDNEEVAPRFDEDHPYWIEDKD
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London          LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613274(S)      LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613279.1       LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613280.1       LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613281.1      LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613275.1       LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613276.1       LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613277.1       LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
JQ613278         LRDGEIKQLAENEIAFWKELISKYLYPIDQNKDHQARVAVELKELRNRVVFSFFMLNALF
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London          VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613274(S)      VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613279.1       VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613280.1       VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613281.1      VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613275.1       VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613276.1       VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613277.1       VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
JQ613278         VLVVLILQLNKDILHVDWPYGIRENITFIPETNEIRIDKEYLEMEPIGLVFVGFFGLILL
            ************************************************************

London          IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613274(S)      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
```

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JQ613279.1      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613280.1      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613281.1      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613275.1      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613276.1      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613277.1      IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
JQ613278        IQLIGMLFHRFGTLSHMLASVNLFQSKRDDISGEDDLKRNAIDIARQLQKLQGFNDGESS
            ************************************************************

London          EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613274(S)     EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613279.1      EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613280.1      EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613281.1      EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613275.1      EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613276.1      EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613277.1      EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
JQ613278        EDNTYGIAARKTIQKLEMRGRQTIKTGTLDVAFREKFMAILEAQEEGTTTPVLGGKRNRE
            ************************************************************

London          TITALIKRSKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613274(S)     TITALIKRSKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613279.1      TITALIKRSKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613280.1      TITALIKRSKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613281.1      TITALIKRRKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613275.1      TITALIKRRKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613276.1      TITALIKRRKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613277.1      TITALIKRRKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
JQ613278        TITALIKRRKNILGYDDHVGMQTLGIKNEFVRNPARSTLDSKQRRPPNPYGTNGMVNQAF
            ******** **************************************************
```

```
London          DGLSSDEELEPPEVPMSTYRGINRKNLNGNSSQQL
JQ613274(S)      DGLSSDEELEPPEVPMSTYRGINRKNLNGNSSQQL
JQ613279.1       DGLSSDEELEPPEVPMSTYRGINRKNLNG------
JQ613280.1       DGLSSDEELEPPEVPMSTYRGINRKNLNG------
JQ613281.1      DGLSSDEELEPPEVPMSTYRGINRQNLNGNSSQQL
JQ613275.1       DGLSSDEELEPPEVPMSTYRGINRKNLN-------
JQ613276.1       DGLSSDEELEPPEVPMSTYRGINRQNLNG------
JQ613277.1       DGLSSDEELEPPEVPMSTYRGINRQNLNG------
JQ613278         DGLSSDEELEPPEVPMSTYRGINRQNLNG------
            ************************.***
                                    .
```

IMIDACLOPRID **RESISTANCE:**

```
AY378699.1(R)     ---------------------------------------------MTTNYKRLIRPVNVS
AY378702(R)       MAPDCCTKLMRTCPLILLILIILLLLCETVDGAAANPDSKRL-GDLLSNYNRLIRPVTNS
AJ251838.1(S)     ---------MNT--SVGLLMAVFFVCSQFIRGCWCSEDEERLVRDLFRGYNKLIRPVQNM
AY378698.1(R)     ---------------------------------------------MSNYNRLIRPVSNN
AY378700.1        ---------MAAGMPPPMLLLLFFLL--LTHPADANPGAKRLYDDLLSNYNKLIRPVGNN
                                           *..*****
                                            ..

AY378699.1(R)     DRNHTQQSRGKMGLRLQL---INLKNQI---NVWVWNDTKFGWQSDK------GNNDDYG
AY378702(R)       TDT--VLGSGKLGLRLSQLIDVNSKNQIMGTNVWVDLGHHSEWQDHKSSGFWNPSDDEYG
AJ251838.1(S)     TEK--VN--VQFGLAFVQLINVNEKSQIMKSNVWLRL----VWRDY--QLQWDEA--DYG
AY378698.1(R)     SDK--LT--SKMGLRLSQLIDVNLKNQIMTTNVWVQQ----QWGDY--KLKWNPD--DYG
```

```
AY378700.1       SDR--LL--VKLGLKLSQLIDVNGKNQIKVTNVWLEV----TWYDY--KGHWNPA--SYG
                 ::** :    :* *.**   ***:      * .          .**

AY378699.1(R)     GV---------LHVPWLPDIVLN-NADGVTIMT----KANNTLHHTGG-VWDPPAIFGSF
AY378702(R)       GVGSSTELAVPSEHIWDPDIVLYNNADGKYAVGGSMTATKAYLHYGGGVWWSPPAIYGSS
AJ251838.1(S)     GI---QVLRLPPDKVWKPDIVLFNNADGNYEVRYK---SNVLIRPNGELLWIPPAIYQSS
AY378698.1(R)     GV---DTLHVPSEHIWIPDIVLYNNADGNYVVTTK---AV--LHHTGKVVWKPPAIYKSF
AY378700.1        GV---DDLYVPLLLHWGPDIVLYNNADGKYEVVTY---GT--AGILHKVEWKPPAIYKSF
                  *:          * *****  ****   :              * ****: *

AY378699.1(R)     CEIDV--FPFDQK--CMKFGSWT**Y**NGEQGHVDARHMHNSPDSWTISPEFIDLQDCVGLSV
AY378702(R)       CEIDVEYFPFDEQGCYMKFGSWT**Y**DGDQFGRLKHFINQKEQIR-----EFDKKVKVGQKL
AJ251838.1(S)     CTIDVTYFPFDQQTCIMKFGSWT**F**NGDQVSLALYNDKQFVDL---------SDYW---KS
AY378698.1(R)     CEIDVRYFPFDQQKCFMKFGSWT**Y**DGNHVDLRHMSQSPDSDTIDVGIDL--QDYYLSV--
AY378700.1        CEIDVEYFPFDEQKCVMKFGSWT**Y**NGAQVDLLKHRIDQNYDDNKVSPEFYDKEYIVGIKM
                * ***  ****::   *******::* :          :         .

AY378699.1(R)     ----EWDITWYVPSVEWDEVYSCCGEEYP-IIFNIGMRRKTLFYTVNLI-PCVISGLL--
AY378702(R)       RENYEQDI-LWVP-ERHEDY-YCCGEPYI--DQNIGMRRKTLFYTVNLIIPCMGISFLTI
AJ251838.1(S)     GT---WDI-IEVPAYLN--VYQE-SPTQTDITFYIVIRRKTLFYTVNLILPTVLISFLCV
AY378698.1(R)     -E---WDI-MRVPAVRYEKFYSCCEEPYPDIIFNITLRRKTLFYTVNLIIPCVGISFLCV
AY378700.1        RE---HYT-WLVPSERHEKYYTCCAEWYP-IFFNIGMRRKTLFYTVNLIIPCVGISFLTI
                  **                 * :*********** * :  .:*

AY378699.1(R)     --VFLPS-------DEKVSLLDISILLSFG----LLDAIIPGTS-----LPLLGKL--FT
AY378702(R)       LVECLPGLLKSDGSGEKV-ALCISILLSLTVFFLLIAFIIPKGHSSVGAVPLLGKYLLFT
AJ251838.1(S)     LVFYLPA-----EAGEKV-TLGISILLSLVVFLLLVSKILPPTS---LVLPLIAKYLLFT
AY378698.1(R)     LVFYLPS-----DSGEKV-ALCISILLSQTMFFLLLAEIIPPTS---LTVPLLGKYLLFT
AY378700.1        LVFYLPK-----DSNEKV-GLSISILLSLGVFFLLITGIIPPTS---LVVPLIDKFVLFT
                **        ***  * ******      *:  *:*       :**: *   **
```

```
AY378699.1(R)     MVLLS--------VVVTL---NV-----NF---RTHRMRPWFI----QMLP--LLMERPK
AY378702(R)       MVLVKHHLASDLSVVVTILILNDGLHSSAFRKPVTHKMDPWVKGGS----RSLLFIKRDK
AJ251838.1(S)     FIMNT------VSILVTVIIINW-----NFRGPRTHRMPPWIRTVFLYYLPACMFMKRPK
AY378698.1(R)     MVLVG------LSVVITIGILNI-----NFRSPVTHRMRPWVHRLFIQMLPKVLLIERPK
AY378700.1        KILVT------LSVVVTVAVLND-----KYRKPVTHRMSPWVKLLFIGGLPLILLMRGPE
                  ::        :::*:  *       :    **:* **.          ::::. :

AY378699.1(R)     KDDDEEDEA-----SGNPDGEG-VHLDSETPPDVDLGLGGGGGGGGGLSLGGSVGGWSSK
AY378702(R)       AGGSLPKVLLMVPLRDLN---GKAFSKTLIMDEMQSG-------S------SPHSL----
AJ251838.1(S)     KTRLRWM-MEMPGMSGPPHPHHTSPSDLPAPAPPSSA---------TPS---------K-
AY378698.1(R)     GDDAGDE-E----DEAPKGT----LLDTPPDVDMNLGLGGGGGGGGGLSLGGVGGVGGK-
AY378700.1        QDDKDLA-S----KRGHHHNHGATKLSAAAAAAMAAAA----S---STAKSNPDSVRLH-
                                 .       .

AY378699.1(R)     TASSADYEDDKYELPLALPADDSAFGDQGLPPLPSSLPA---AADDDLFGAANSKCPAAA
AY378702(R)       -RRM--------QGDKVGAGGCNGLHVTTATNR---------------FMSEAG------
AJ251838.1(S)     -HKMEA----MELADLHHP-NCK-------------------------------INRK
AY378698.1(R)     -RNDADYEL-----PLALPAGCNGLGSQGLPPLPLPLPLHLAAADKDLFGGAAACPAAAA
AY378700.1        -HLHQHLHLHLQLKHLKHDKGCNGMKAASHNRF---------GASAGAFGGLPSHHLGDG
                            .

AY378699.1(R)     AAAAALHPHDVSPSFDSNKPSHEMEKTIEDGNNSDHHLFIAQHVKNKDKFESQQHNNDES
AY378702(R)       ----LPVLSDVAAR--KKYPEIELEKSIDN---IHDVMFISSHLHVQRD---QDK--FNA
AJ251838.1(S)     ASAERRES--ESSD--SLILSPEASK---A---TEAVEFIAEHLRNEDQ--------YIQ
AY378698.1(R)     AAAAALHPHDVPSS--FDKPSHEMEK---T---IFDARFIAQHVKNKDK--------FES
AY378700.1        ------SLSDVYVV--RKPYPFEMEK---A---IHDKGFIQNHVAEQDEFGGLDE--FDQ
                            * .*          ** .*: : .

AY378699.1(R)     VEEDWGQYVAMDQQLRLFLWIFTIACWMGTFFQWSIILQAGHNNDSSLYDTTSPVDSSQS
```

```
AY378702(R)       EDEDWK-YVAMV-MDRLFLWLFTIALLTSLVGQLSILGEAP-----SLYDDTKSHHLLDI
AJ251838.1(S)     IREDWK-YVAMV-IDRLQLYLFFFVT---TAGTLGILMDAP-----HIFETVDQDKIIEI
AY378698.1(R)     VEEDWK-YVAMV-LDRLFLWIFTIAC---VMGTAIILLQAP-----SLYDTTIDIKYSKI
AY378700.1        DDQDWG-FVAMV-LDRLFLWIFTIAG---IVGTFKILSEAP-----GLYDKTKLIDMATK
                  :**  :***    ** *::* :.         *: :*       ::: .   .

AY378699.1(R)     DIRPIDIKFQKIAIAKKKFMLLLGTSSEPHHHLPEEG-
AY378702(R)       D-----VQLSDIA----NIKQIFN---------LTEN-
AJ251838.1(S)     Y-----GG---------K--------------------
AY378698.1(R)     A-----K-----K----KYMLMMG---------PEEG-
AY378700.1        G-----IDMAASG----VALLQFL---------PDIGN
```

Indoxocarb resistance:

>KM027335

MSEDLDSISEEEPSLFRPFTRESLAAIEARIADELARQKENEKKRAEGEVRYEDEDEDEGPQPDATLEQGLPVPVRMQGSFPPELASTPLEDIDPY
YHNQKTFVVVSRGRDIFRFSATDALWMLDPFNPIRRVAIYILVHPLFSFFIITTILVNCILMIMPSTPTVESTEVIFTGIYTFESAVKVMARGFILQPFT
YLRDVWNWLDFVVIALAYVTMGIDLGNLAALRTFRVLRALKTVAIIPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKCVKSF
PEDGSWGNLTDENWERFCQNETNWYFENGAYPLCGNSSGAGQCEPGYVCLQGYGPNPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQL
VLRSAGSWHVLFFVVIIFLGSFYLVNLILAIVAMSYDELQKKAEEEEQAEEEALREAEQKAAARADKQEAREAHAREVAAAAEAAAYAEAHPAKS
PSFSCQSYELFVNQERGNQDDNTRERMSLRSDPFQDSVSTQPAHKPDPHSEARRQRKVSMASLSLPGSPFNLRRGSRGSHQMALRPNGRPR
YPPGADRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIIPVYYANLGSRHSSYTSHQSRLSYTSHGDLLGGLGKAQTKEAKLRNRSASRN
HSVTSQPHAYPLPRQDSSLASRPLREYEMSTAECTDDAGKVLKPSNDNPFIESSEQPNVVDMRDVMVLNEIIEQAGRQSRTSDQNVSVYYFPT
AEDDEDGPTMKEKLLECLMKGIDIFCVWDCCWLWLEFQKYVALLVFDPFVELFITLCIVVNTLFMALDHHDMDRDMEKALKSGNYFFTATFAIE
ATLKLIAMSPKYYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFG
KNYVDHVDRFPDGDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCILVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSTPTADQET
NKIAEAFNRISRFNEWVKKSIADFLKILKNKLTNQIAIHAPGLKAALCGRCVSSDRVDNELELGTDLEDTVLYKDKKLKDQVEVAIGDGMEFTIPVN
GIISGDNKYKKGKILMNNINAITDNHRDNRLECELNHHGYPLQDDDTISQKSYGSHKIRSFKDESHKGSADTIDGEEKKDASKEELGLEEEEIEEEED
KLEGLKDIIVAADEDVVDDSPAECCPDKCYKQFPFLAGDDESPFWQGWGMLRLKTFRLIENTYFETAVITMILLSSLALALEDVHLPHRPILQDILY

YMDRIFTVIFFLEMLIKWLALGFQKYFTNAWCWLDFIIVMVSLINFVAALCGAGGIQAFKTMRTLRALRPLRAMSRMQGMRVVVNALVQAIPSIF
NVLLVCLIFWLIFAIMGVQLFAGKYFKCVDLNHTTLSHEIIPDRNACILENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSREVGR
QPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYYNAMKKMGSKKPLKAIPRPKWRPQAIVFEIVTDKKFDMII
MLFIGLNMLTMTLDHYQQAESFSVVLDYLNMIFIVIFSSECMSKIFALRYHYFVDPWNWFDFVVVMFSILSLVLSDIIEKYFVSPTLLRVVRVAKVG
RVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFGMSFFMHVKNKGGLDDVYNFKTFVQSMILLFQMSTSAGWDGVLDGIINEEECD
LPDNERGYPGNCGSATIGITYLLSYLVIS**F**LI**V**INMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQRFDPDGTQYIRYDQLSDFLDVLEPPLQI
HKPNKYKIISMDIPICRGDMMFCVDILDALTKDFFARK

>EF535530.1 (Can’t find point mutation-but figure shows sequence)
MHTCRPRGVHSIALVLALAIAWLPHADHAAGAGGGGMFGDVNISAILDSLSVSYDKRVRPNYGGPPVDVGATMHVLSISSLSEVKMDFTLDFY
FRQFWTDPRLAYKKRTGVETLSVGSEFIRNIWVPDTFFVNEKQSYFHIATTSNEFIRIHHSGSITRSIRLTITASCPMDPQYFPMDRQLCNIEIESFG
YTMRDIRYKWNEGPNSVGVPSEVSLPQFKVLGHRQRAMEISLTTGNYSRLACEIQFVRSMGYYLIQIYIPSGLIVIISWVSFWLNRNATPARVSLG
VTTVLTMTTLMSSTNAALPKISYVKSIDVYLGTCFVMVFASLLEYATVGYMAKRIQMRKQRFTAVQKMAAEKKMQIDGPPGTSEPLPPPRTSTLT
RPPPPSRSSEVRFKVHDPKAYSKGGTLENTINGSRGPAPAPVPAAPQPDEEAGPPPHLIHASKGINKLLGTTPSDIDKYSRIVFPVCFVCFNLMY
WIIYLHVSDVVADDLVLLGEEN

BIFENTHRIN RESISTANCE: not completed!

```
FJ906807.1(bifenthrinR)     ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906804(bifenthrinR)       ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906805.1(bifenthrinR)     ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906806.1(bifenthrinR)     ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906811.1(bifenthrinR)     ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906808                    ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906809(bifenthrinR)       ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
FJ906810.1(bifenthrinR)     ---------TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSKKVYLFPN
AJ131759(BifenthrinS)       ------SWPTLNLLISIMGRTIGALGNLTFVLCIIIFIFAVMGMQLFGKNYTEKMYMFKD
AJ131760(BifenthrinR)       ------SWPTLNLLISIMGRTIGALGNLTFVLCIIIFIFAVMGMQLFGKNYTEKMYMFKD
U38813(bifenthrinS)         VFKLAKSWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYIDHKDRFKD
AJ440727.1(bifenthrinS)     ----AKSWPTLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYTDNVDRFPG
AY094601.1(bifenthrinR)     ----AKSWPTLNLLISIMGRTVGAIGNLTFVLCIIIFIFAVMGMQLFGKNYT--------
                            ******.***.*.* .******* **.********** **

FJ906807.1(bifenthrinR)     AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906804(bifenthrinR)       AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906805.1(bifenthrinR)     AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906806.1(bifenthrinR)     AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906811.1(bifenthrinR)     AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906808                    AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906809(bifenthrinR)       AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
FJ906810.1(bifenthrinR)     AEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVIIGHLVMLNLF
AJ131759(BifenthrinS)       HELPRWNFTDFLHSFMIVFRVLCGEWIESMWDCLHVGEPTCIPFFLATVVIGNLVVLNLF
AJ131760(BifenthrinR)       HELPRWNFTDFLHSFMIVFRVLCGEWIESMWDCLHVGEPTCIPFFLATVVIGNFVVLNLF
U38813(bifenthrinS)         HELPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMYVGDVSCIPFFLATVVIGNLVVLNLF
AJ440727.1(bifenthrinS)     GELPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMHVGDVSCIPFFLATVVIGYLVVLNLF
AY094601.1(bifenthrinR)     ------------------------------------------------------------
```

```
FJ906807.1(bifenthrinR)     LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWVKNRILTGLKQLRSKTRNQIR
FJ906804(bifenthrinR)       LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLKQLRSKTRNQIR
FJ906805.1(bifenthrinR)     LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLKQLRSKTRNQIR
FJ906806.1(bifenthrinR)     LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLKQLRSKTRNQIR
FJ906811.1(bifenthrinR)     LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWVKNRILTGLKQLRSKTRNQIR
FJ906808                LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLKQLRSKTRNQIR
FJ906809(bifenthrinR)       LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLKQLRSKTRNQIR
FJ906810.1(bifenthrinR)     LALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLKQLRSKTRNQIR
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         LALLLSNFGSSSLSAPTAD-NDTNKIAEAFNRIARFKNWVKRNIADCFKLIRNKLTNQIS
AJ440727.1(bifenthrinS)     LALLLSNFGSSSLSAPTAD-NETNK-----------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------


FJ906807.1(bifenthrinR)     DTAQWPSRGGGSSGGMVDRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906804(bifenthrinR)       DTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906805.1(bifenthrinR)     DTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906806.1(bifenthrinR)     DTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906811.1(bifenthrinR)     DTAQWPSRGGGSSGGMVDRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906808                DTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906809(bifenthrinR)       DTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
FJ906810.1(bifenthrinR)     DTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIRDKKLLAAAAYGET
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         DQPS---EHGD-------NELELGHDE-------IMGDGLIKKGMKGETQLEV-------
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
```

```
FJ906807.1(bifenthrinR)     VVGPDGLEYSLTESGKVTLKSTAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906804(bifenthrinR)       VVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906805.1(bifenthrinR)     VVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906806.1(bifenthrinR)     VVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906811.1(bifenthrinR)     VVGPDGLEYSLTESGKVTLKSTAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906808                  VVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906809(bifenthrinR)       VVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
FJ906810.1(bifenthrinR)     VVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLILTESALIEKLSPSVT
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         -AIGDGMEFTIHGDMKNNKPKKS----------K-FMNNTTM---------
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------


FJ906807.1(bifenthrinR)     NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906804(bifenthrinR)       NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906805.1(bifenthrinR)     NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906806.1(bifenthrinR)     NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906811.1(bifenthrinR)     NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906808                  NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906809(bifenthrinR)       NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
FJ906810.1(bifenthrinR)     NHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLLVSASTINNNNVNKVKPS
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         ----IGN----------SINHQD-----NRLEHELNHRGLS----------
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906804(bifenthrinR)       LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906805.1(bifenthrinR)     LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906806.1(bifenthrinR)     LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906811.1(bifenthrinR)     LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906808                LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906809(bifenthrinR)       LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
FJ906810.1(bifenthrinR)     LHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSSESQQHLVNNNVSNNNDSSA
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         IQDDDT-------------------------ASINSYGSHKNRPF--------------
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**D**PSPL
FJ906804(bifenthrinR)       QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**A**PSPL
FJ906805.1(bifenthrinR)     QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**A**PSPL
FJ906806.1(bifenthrinR)     QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**D**PSPL
FJ906811.1(bifenthrinR)     QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**D**PSPL
FJ906808                QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**A**PSPL
FJ906809(bifenthrinR)       QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**A**PSPL
FJ906810.1(bifenthrinR)     QQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGGPVPISFQHSYSSLNRL**D**PSPL
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         ----------KDESHKGSAETIEGEEKRDVSKE-------DLGLD------EELDE---
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906804(bifenthrinR)       NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906805.1(bifenthrinR)     NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906806.1(bifenthrinR)     NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906811.1(bifenthrinR)    NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906808                NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906809(bifenthrinR)       NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
FJ906810.1(bifenthrinR)     NHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCLPEYWYHRFPCCL--EETAFWIKW
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         -EAEGDEGQLDG----DIIIHAQNDDEIIDDYPADCFPDSYYKKFPILAGDEDSPFWQGW
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------


FJ906807.1(bifenthrinR)     REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906804(bifenthrinR)       REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906805.1(bifenthrinR)     REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906806.1(bifenthrinR)     REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906811.1(bifenthrinR)    REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906808                REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906809(bifenthrinR)       REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
FJ906810.1(bifenthrinR)     REVRSKCYKLVEDKYFETLVITLILISSMTLALEDVNLKERPWLEYSLKYIDQFFTIIFT
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         GNLRLKTFQLIENKYFETAVITMILMSSLALALEDVHLPDRPVMQDILYYMDRIFTVIFF
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSALNLGAEFAGLAKIQAFKTMRTLRAFRPL
FJ906804(bifenthrinR)       CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSALNLGAEFAGLAKIQAFKTMRTLRAFRPL
FJ906805.1(bifenthrinR)     CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSALNLGAEFAGLAKIQAFKTMRTLRAFRPL
FJ906806.1(bifenthrinR)     CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSALNLGAEFAGLAKIQAFKTMRTLRAFRPL
FJ906811.1(bifenthrinR)     CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSLINFTVGQLGFSNIPAFKTMRTLRALRPL
FJ906808                CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSLINFTVGQLGFSNIPAFKTMRTLRALRPL
FJ906809(bifenthrinR)       CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSLINFTVGQLGFSNIPAFKTMRTLRALRPL
FJ906810.1(bifenthrinR)     CEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSLINFTVGQLGFSNIPAFKTMRTLRALRPL
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         LEMLIKWLALGFKVYFTNAWCWLDFVIVMLSLINLVAVWSGLNDIAVFRSMRTLRALRPL
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------


FJ906807.1(bifenthrinR)     RAMSRSKGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906804(bifenthrinR)       RAMSRSKGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906805.1(bifenthrinR)     RAMSRSKGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906806.1(bifenthrinR)     RAMSRSKGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906811.1(bifenthrinR)     RAMSRLEGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906808                RAMSRLEGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906809(bifenthrinR)       RAMSRLEGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
FJ906810.1(bifenthrinR)     RAMSRLEGMRVVVNALIQAIPAIFNVLLVCLIFWLIFAIMGVQLFAGKFSYCRDRNTEEK
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         RAVSRWEGMKVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCKDGNDTVL
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906804(bifenthrinR)       SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906805.1(bifenthrinR)     SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906806.1(bifenthrinR)     SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906811.1(bifenthrinR)     SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906808                 SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906809(bifenthrinR)       SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
FJ906810.1(bifenthrinR)     SDPNEIENKTICDQHNETLEWYTPMVNFDNVFNGYLSLFQVATFKGWTIIMDHAIDSREV
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         SH-EIIPNRNACK--SENYTWENSAMNFDHVGNAYLCLFQVATFKGWIQIMNDAIDSREV
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     HQQPVYENSILMYLYFVFFIIFGSFITLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906804(bifenthrinR)       HQQPVYENSILMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906805.1(bifenthrinR)     HQQPVYENSILMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906806.1(bifenthrinR)     HQQPVYENSILMYLYFVFFIIFGSFITLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906811.1(bifenthrinR)     HQQPVYENSILMYLYFVFFIIFGSFITLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906808                 HQQPVYENSILMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906809(bifenthrinR)       HQQPVYENSILMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
FJ906810.1(bifenthrinR)     HQQPVYENSILMYLYFVFFIIFGSFITLNLFIGVIIDNFNEQKKKGGGSREMLMTEDQKK
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         DKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKK
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906804(bifenthrinR)       YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906805.1(bifenthrinR)     YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906806.1(bifenthrinR)     YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906811.1(bifenthrinR)    YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906808                YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906809(bifenthrinR)       YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
FJ906810.1(bifenthrinR)    YLNAMKKMGSKKPMKAIPRPRFKLQAIIFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQAS
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         YYNAMKKMGSKKPLKAIPRPRWRPQAIVFEIVTDKKFDIIIMLFIGLNMFTMTLDRYDAS
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------


FJ906807.1(bifenthrinR)     AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906804(bifenthrinR)       AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906805.1(bifenthrinR)     AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906806.1(bifenthrinR)     AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906811.1(bifenthrinR)    AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906808                AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906809(bifenthrinR)       AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
FJ906810.1(bifenthrinR)    AFMEHILEMCNLFFIAVFTAECMLKIFALRFHYFREPWNVFDFVIVILSIASSALKDFVE
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         EAYNNVLDKLNGIFVVIFSGECLLKIFALRYHYFKEPWNLFDVVVVILSILGLVLSDIIE
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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FJ906807.1(bifenthrinR)     NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906804(bifenthrinR)       NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906805.1(bifenthrinR)     NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906806.1(bifenthrinR)     NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906811.1(bifenthrinR)    NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906808                NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906809(bifenthrinR)       NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
FJ906810.1(bifenthrinR)     NYLISPTLLRVVRVVKIGRVLRLVKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAI
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         KYFVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAI
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------


FJ906807.1(bifenthrinR)     FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906804(bifenthrinR)       FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906805.1(bifenthrinR)     FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906806.1(bifenthrinR)     FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906811.1(bifenthrinR)    FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906808                FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906809(bifenthrinR)       FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
FJ906810.1(bifenthrinR)     FGMSFFMNVKQRYGLDETFNFGTFFRSFILLFQMCTSAGWDGVLAAIMDESKCEKDSEV-
AJ131759(BifenthrinS)       ------------------------------------------------------------
AJ131760(BifenthrinR)       ------------------------------------------------------------
U38813(bifenthrinS)         FGMSFFMHVKEKSGINAVYNFKTFGQSMILLFQMSTSAGWDGVLDAIINEEDCDPPDNDK
AJ440727.1(bifenthrinS)     ------------------------------------------------------------
AY094601.1(bifenthrinR)     ------------------------------------------------------------
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>AJ440727.1
AKSWPTLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFG
KNYTDNVDRFPGGELPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMHVGDVSCIPFFL
ATVVIGYLVVLNLFLALLLSNFGSSSLSAPTADNETNK

>AY094601.1
AKSWPTLNLLISIMGRTVGAIGNLTFVLCIIIFIFAVMGMQLFG
KNYT

>AJ131759
SWPTLNLLISIMGRTIGALGNLTFVLCIIIFIFAVMGMQLFGKN
YTEKMYMFKDHELPRWNFTDFLHSFMIVFRVLCGEWIESMWDCLHVGEPTCIPFFLAT
VVIGNLVVLNLF

>AJ131760
SWPTLNLLISIMGRTIGALGNLTFVLCIIIFIFAVMGMQLFGKN
YTEKMYMFKDHELPRWNFTDFLHSFMIVFRVLCGEWIESMWDCLHVGEPTCIPFFLAT
VVIGNFVVLNLF

>FJ906804
TLNLLITIMGKTLGDLGNLTFVLAIIVFIFAVMGMQLFGANYSK
KVYLFPNAEIPRWNFKDFMHSFMIVFRVLCGEWIESMWSCMLVCGFVCVPFFLATVII
GHLVMLNLFLALLLSSFGASNLSSPTSESADTKKLQEAFDRFGRAHKWLKNRILTGLK
QLRSKTRNQIRDTAQWPGRGGASSGGMVGRSMMLGSESVLDEGDIIMMDGLNASGLIR
DKKLLAAAAYGETVVGPDGLEYSLTESGKVTLKSSAQTVLNSVKLSQAVKDLSSDKLI
LTESALIEKLSPSVTNHSDEGNRLHGDVSTVNNNNVNDRSTSLNNTNFNNSLNSNNLL
VSASTINNNNVNKVKPSLHDANGCSPFPLDDNHHHHHLVQQQPHGGDYHGESGYYGSS
ESQQHLVNNNVSNNNDSSAQQSSSLSVTPSHHLNHPSSHPTAHHYNASQLSKVHPAGG

PVPISFQHSYSSLNRLAPSPLNHFNGPGEDVMGEEMNANKMVTVTADVNINDHPDDCL
PEYWYHRFPCCLEETAFWIKWREVRSKCYKLVEDKYFETLVITLILISSMTLALEDVN
LKERPWLEYSLKYIDQFFTIIFTCEMLLKWFAYGFKSYFSNAWCWLDFIIVMVSALNL
GAEFAGLAKIQAFKTMRTLRAFRPLRAMSRSKGMRVVVNALIQAIPAIFNVLLVCLIF
WLIFAIMGVQLFAGKFSYCRDRNTEEKSDPNEIENKTICDQHNETLEWYTPMVNFDNV
FNGYLSLFQVATFKGWTIIMDHAIDSREVHQQPVYENSILMYLYFVFFIIFGSFFTLN
LFIGVIIDNFNEQKKKGGGSREMLMTEDQKKYLNAMKKMGSKKPMKAIPRPRFKLQAI
IFDIVTNKKFDMLIMLFIMLNMFVMSLDHYQASAFMEHILEMCNLFFIAVFTAECMLK
IFALRFHYFREPWNVFDFVIVILSIASSALKDFVENYLISPTLLRVVRVVKIGRVLRL
VKGARGIRTLLFALAMSLPALFNICLLLFLVMFIYAIFGMSFFMNVKQRYGLDETFNF
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>U38813
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The following table was taken from Pesticide Biochemistry and Physiology, Volume 121, June 2015, Pages 97–101, Toxicodynamic mechanisms and monitoring of acaricide resistance in the two-spotted spider mite, Deok Ho Kwona, , , J. Marshall Clarkb, Si Hyeock Leea, c, http://dx.doi.org/10.1016/j.pestbp.2014.12.011

Acaricides	Location	References
Organophosphate/carbamate	Oxyanion hole	[15] and [17]
"	Near catalytic serine	[16]
"	Peripheral anionic site	[16] and [17]
"	–	[16]
"	Anionic subsite	[15], [16] and [17]
Pyrethroid	6th transmembrane in domain II	[18]
"	Intracellular loop between domain II and III	[18] and [19]
"	6th transmembrane in domain III	[19]
Abamectin	3rd transmembrane	[20] and [21]
"	3rd transmembrane	[21]
Etoxazole	5th transmembrane in CTR region	[22]
Bifenazate	Q_o pocket	[23] and [24]
"	"	"
"	"	"
"	"	"
"	"	"

Table 1.
The point mutation associated acaricide resistance in *T. urticae*.

Genes	Accession number[a]	Mutation	Amino acid position: Model species[c]	Amino acid position: *T. urticae*	Acaricides
Acetylcholinesterase (Tuace)	tetur19g00850	G228→S/A	119	228	Organophosphate/carbar
		A309→S	201	309	"
		A391→T	280	391	"
		G436→A	328	436	"
		F439→W/C/Y	331	439	"
Voltage-sensitive sodium channel (Tuvssc)	tetur34g00970	L→V	1024	1014 (1022)[d]	Pyrethroid
		A→D	1222	1366 (1376)[d]	"
		F→I	1538	1694 (1704)[d]	"
Type-1 glutamate-gated chloride channel (TuGluCl1)	tetur02g04080	G→D	234	314 (323)[d]	Abamectin
Type-3 glutamate-gated chloride channel (TuGluCl3)	tetur10g03090	G→E	234	326	"
Chitin synthase (TuChS1)	tetur03g08510	I→F	1094	1017	Etoxazole
Cytochrome b (TuCytb)	YP_001795379.1[b]	G→S	137	126	Bifenazate
		I→T	147	136	"
		S→F	152	141	"
		D→G	172	161	"
		P→T	271	262	"

a Accession ID of *Tetranychus urticae* in Bogas (http://bioinformatics.psb.ugent.be/orcae/overview/Tetur).

b Accession ID in GenBank.

c Amino acid positions numbered by following model species: *Tuace*, *Torpedo californica* (1E3QA); *Tuvssc*, *Musca domestica* (NP_001273814.1); *TuGluCl1* and *TuGluCl3*, *Drosophila melanogaster* (#A