Pesticide Biochemistry and Physiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

A genomic approach to identify and monitor a novel pyrethroid resistance mutation in the redlegged earth mite, *Halotydeus destructor*

Owain R. Edwards[a,*], Thomas K. Walsh[b], Suzanne Metcalfe[c], Wee Tek Tay[b], Ary A. Hoffmann[d], Peter Mangano[e], Alan Lord[e], Svetlana Micic[f], Paul A. Umina[d,g]

[a] CSIRO, Underwood Ave, Floreat, WA 6014, Australia
[b] CSIRO, Clunies Ross Street, Canberra, ACT 2601, Australia
[c] CSIRO, Ecosystem Sciences Precinct, 41 Boggo Rd, Brisbane, QLD 4001, Australia
[d] School of BioSciences, Bio21 Institute, The University of Melbourne, VIC 3010, Australia
[e] Department of Agriculture and Food Western Australia, 3 Baron-Hay Ct, South Perth, WA 6151, Australia
[f] Department of Agriculture and Food Western Australia, 444 Albany Highway, Albany, WA 6330, Australia
[g] Cesar, 293 Royal Parade, Parkville, VIC 3052, Australia

ARTICLE INFO

Keywords:
Redlegged earth mite
Synthetic pyrethroid
kdr
Insecticide resistance
Genomics

ABSTRACT

Resistance mechanisms are typically uncovered by identifying sequence variation in known candidate genes, however this strategy can be problematic for species with no reference data in known relatives. Here we take a genomic approach to identify resistance to pyrethroids in the redlegged earth mite, *Halotydeus destructor*, a member of the Penthalidae family of mites that are virtually uncharacterized genetically. Based on shallow genome sequencing followed by a genome assembly, we first identified contigs of the *H. destructor* parasodium channel gene. By linking variation in this gene to known resistant phenotypes, we located a single nucleotide polymorphism in resistant mites. This polymorphism results in a leucine (L) to phenylalanine (F) amino acid substitution in the II6 region (predicted) of the gene (L1024F). This novel mutation has not previously been linked to pyrethroid resistance, although other polymorphisms have been identified in the two-spotted spider mite, *Tetranychus urticae* at the same locus (L1024V). The sequencing approach was successful in generating a candidate polymorphism that was then validated using laboratory bioassays and field surveys. A high throughput Illumina-based sequencing diagnostic was developed to rapidly assess resistance allele frequencies in pools of mites sourced from hundreds of populations across Australia. Resistance was confirmed to be widespread in the southern wheatbelt region of Western Australia. Two different resistance mutations were identified in field populations, both resulting in the same amino acid substitution. The frequency and distribution of resistance amplicon haplotypes suggests at least two, and probably more independent origins of resistance.

1. Introduction

The redlegged earth mite (RLEM), *Halotydeus destructor*, is a soil mite specialized to Mediterranean climates, originating from southern Africa [1,2]. It was introduced into Australia around the beginning of the 20th century, and has since spread across the southern agricultural regions of the Australian continent and continues to spread inland [3]. *H. destructor* causes economically-important feeding damage on several pasture and crop species, especially early season during crop establishment [1]. *H. destructor* thrives in legume-dominated pastures, while also using many broad-leaf weed species for both food and shelter. At high population densities, mites can also cause substantial damage to germinating and establishing canola, cereal and pulse crops [4]. *H. destructor* enters a summer diapause in late spring, triggered by environmental cues, primarily day length; diapause eggs are retained in the cadaver of parental females, until development is triggered the following autumn by a combination of temperature and rainfall cues [1,5].

Growers in southern Australia have become increasingly reliant on chemical controls to assist with management of *H. destructor* [5]. Seed treatments, bare-earth insecticide treatments with organophosphate or synthetic pyrethroid insecticides help protect germinating crops from *H. destructor* populations, and post emergence sprays are also applied [6]. A late season insecticide application with organophosphate insecticides targeting mites entering diapause (Timerite™) has been highly successful in suppressing mite populations in the following

* Corresponding author.
E-mail address: owain.edwards@csiro.au (O.R. Edwards).

https://doi.org/10.1016/j.pestbp.2017.12.002
Received 6 June 2017; Received in revised form 3 December 2017; Accepted 8 December 2017

autumn, especially in Western Australia (WA) [7], although less so in eastern states [8,9]. Grazing management of pastures in spring is also an important cultural control to minimising RLEM population carry-over between seasons [10].

Mites spend the majority of their time on the soil surface so *H. destructor* is inadvertently exposed to many insecticide applications targeting other crop pests [6]. This includes neonicotinoid seed treatments and foliar applications of organophosphate and synthetic pyrethroids, with up to 4–5 applications of the latter often occurring in some crops within high rainfall regions in high risk years [11].

Localized resistance to synthetic pyrethroid insecticides was first detected in *H. destructor* within WA in 2007 [12], and has since been reported in > 25 properties scattered throughout the southern half of the WA wheatbelt [13]. This resistance confers an extremely high level of protection, exceeding 200,000-fold resistance against some synthetic pyrethroids [12]. The resistance phenotype is also very discrete, with resistant individuals readily differentiated from susceptible individuals using a discriminating dose. Based on these characteristics, we hypothesized that the mechanism of resistance was likely to be an altered target site, and based on known resistance mechanisms in insects [14] and other acarines [15,16], we hypothesized that the likely mechanism (s) would be knockdown resistance (*kdr*), conferred by a mutation in the parasodium channel gene.

Because *H. destructor* are not easily handled and cultured, traditional resistance bioassays are conducted using field collections (suction samples) of mites. This is cumbersome and requires tests with large numbers of individuals and adequate controls [13,17]. While such assays are an important part of routine monitoring, studies on the detection and spread of resistance would be greatly facilitated by the development of a rapid assay of field collected mites, especially post application of insecticides, that can monitor resistance. Such assays are already available for mites that show pyrethroid resistance due to sodium channel gene targets [18,19]. However, the development of such an assay for *H. destructor* is challenging because of the poor genetic resources available for this species that do not extend much beyond a few allozyme, microsatellite and mitochondrial markers [20,21,22].

The first aim of this study was to develop genomic resources for eventual identification and sequencing of the *H. destructor* parasodium channel gene. This involved sequencing and assembling the *H. destructor* genome, which provides additional resources for other studies on this mite including the location of SNP markers (Hoffmann and Rasic, unpublished). The second aim was to compare gene sequences between known resistant and susceptible populations to identify candidate mutations putatively responsible for resistance. We then validated candidate mutations using individuals from additional known resistant and susceptible populations, as well as using survivors and non-survivors of dose-response chemical bioassays. The final aim was to develop a molecular diagnostic for the resistance mechanism, and to apply this diagnostic to field populations of *H. destructor* to better understand the evolutionary history, distribution, and dynamics of pyrethroid resistance in this pest.

2. Methods

2.1. Mite sampling

H. destructor were sampled from various crop, pasture and broad leafed plants using suction sampling as described in Hoffmann et al. [17]. In 2013, two resistant populations were collected from locations in WA (Cranbrook, Boyup Brook) where resistance had previously been shown to be at high frequency by laboratory bioassays. Susceptible mites were collected near Arthur River, WA, at which no survivorship was observed at diagnostic doses of bifenthrin (0.01 g AI/L) determined previously to be indicative of pyrethroid resistance [13].

In 2014 and 2015, mites were collected from > 100 sites in each of WA and south-eastern Australia. In WA, where resistance is known to exist, sampling was concentrated on farms on which reported spray failures had occurred, as well as in paddocks on and around areas with documented resistance. In south-eastern Australia, mites were collected from farms with varying histories of insecticide use. This sampling regime was necessary to adequately survey for resistance in *H. destructor*, because this species does not disperse rapidly [2], and hence resistance can remain localized for several years [13]. The date, time and GPS were recorded for each sample. Live mites were stored in plastic containers with paper towel and leaf material until returned to the laboratory, where they were held at 4 °C until used for bioassays and/or transferred to 95% ethanol for molecular analysis.

2.2. Identification and sequencing of the parasodium channel gene

Rather than use a traditional approach of degenerate PCR to characterise the parasodium channel gene, we decided to take a whole genome sequencing approach. A low coverage genome sequencing approach was predicted to be cost-effective, with knowledge that the spider mite (*Tetranychus urticae*) genome was small (estimated at 90 Mb) and with low complexity [23]. A number of sequencing libraries were constructed using the Nextera® XT (Illumina, San Diego, CA, USA) library construction kit, optimised for small genomes < 100 MB. Sequencing libraries were made using 1 or 5 individual mites from resistant and susceptible populations as determined through laboratory bioassays (see below). The libraries were barcoded and sequenced on an Illumina MiSeq (150 bp PE) at the CSIRO Black Mountain Laboratories. All sequences were trimmed and assembled using CLC genomics workbench. The sequence reads from all libraries were used to generate an initial assembly and then each library was assembled separately. Reads from each library were then mapped back to the assembled genomes. Contigs containing sequence for the sodium potassium channel were identified by tblastn [24]. Consensus sequence for individuals was compared with the GenBank database by Blast and examined for polymorphisms. The full parasodium channel gene sequences of all individual sequencing libraries were aligned and compared between the source populations to identify candidate resistance mutations present at high frequency in the resistant population and near-absent from the susceptible population.

To determine the genotype of mites at the putative resistance locus, PCR primers (Table S1) were designed to amplify a 313 bp region that included the target mutation. The PCR protocol was performed using Platinum Taq (Invitrogen, Carlsbad, CA, USA) and the following reaction mix: 2.5 μL of 10 × Buffer, 0.5 μL of 0.2 mM each dNTPs, 0.75 μL MgCl2 (50 mM), 0.5 μL of each 10 μM primer, 2 μL of template DNA and water to 25 μL. The PCR conditions were: 1 cycle at 94 °C for 2 min; 35 cycles at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 45 s; and 1 cycle at 72 °C for 5 min. The PCR products were directly Sanger sequenced (Biological Resources Facility, John Curtin School of Medicine, Australian National University, Australia). The nucleotide(s) at the putative resistance locus were recorded for each individual, including the identification of heterozygotes. All polymorphic loci within the amplified sequence were also noted, and the nucleotide(s) for each individual recorded.

DNA was then extracted from 10 to 100 pooled mites (depending on the life stage distribution in the sample) using the QIAGEN (Aarhus, Denmark) DNeasy ® Blood & Tissue kit or the Zymo ZR (Irvine, CA, USA) Tissue & Insect DNA MiniPrep™ extraction kit according to the manufacturer's instructions. DNA was then quantified and 2 μL (or 10 ng DNA) was used in subsequent PCRs (PCR protocol as described above). Three different methods of amplicon sequencing were used: (1) Nextera® XT libraries were created following the manufacturer's instructions from each amplicon and sequenced (Illumina MiSeq 150 bp PE); (2) direct amplicon sequencing was performed with appropriate amplicon specific primers (Table S1) modified from the Illumina 16S amplicon sequencing protocol and following the protocol for barcoding and clean up as described for 16S sequencing using a 150 bp PE