Resistance development a possibility in mirids from Australian cotton

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Abstract

With the introduction of Transgenic Bollgard® II cotton Australian populations of green mirid have required targeted insecticidal control, which may select for insecticide resistance in this pest. Unfortunately, no methods are available in Australia to detect resistance or to establish the baseline data used to confirm resistance. To achieve that a simple method is required to culture and maintain a reference strain(s) of mirids. Here we describe a method to breed and culture green mirids, which is a first step toward development of a resistance monitoring program.

Introduction

With the introduction of transgenic cotton, sucking insect pests have become more troublesome, so requiring increased targeted insecticide control. This brings with it the risk of insecticide (or miticide) resistance. Two-spotted mite has a proven ability to develop resistance if targeted with miticides and has recently developed resistance to chlorfenapyr (Intrepid®)(Herron *et al*. 2004a). Similarly, high-level organophosphate and carbamate resistance has developed in cotton aphid (Herron *et al*. 2004).

Other sporadic, but troublesome, sucking pests include green peach aphid *Myzus persicae* (Sulzer), bean spider mite *Tetranychus ludeni* Zacher, thrips (including western flower thrips) and green mirids, *Creontiades dilutus* (Stal)(Forrester and Wilson 1988). Green mirid in particular is a serious pest in Bollgard II® crops. This is due to the reduction in insecticides used against *Helicoverpa* spp., which formerly also suppressed the mirids. There is now an increase in sprays specifically targeted against green mirids, with high reliance on Regent® (fipronil), which accounts for about 70% of sprays and organophosphates (omethoate and dimethoate) which account for about 20% of sprays. Overseas data indicate that similar sucking bug pests, such as *Lygus lineolaris* in the south eastern USA, can quickly develop resistance to organophosphates and pyrethroids (Scott and Sondgrass 2000). However, Australian resistance researchers currently do not possess the capability to detect resistance in green mirids.

Pre-emptive baseline data is critical in resistance management as it establishes the natural

range in susceptibility of a particular pest to insecticide before the insecticide is used widely. The response of insects collected after the insecticide is used can then be compared back to this baseline data. Such data has been critical to management of other sucking pests, such as cotton aphid because resistance could quickly be confirmed. However, no baseline data for mirids currently exists, preventing an early confirmation of resistance and subsequent resistance management.

This is now a serious concern because mirids have increasingly required targeted control, to the extent that the use of OP's against mirids adversely affected the resistance management strategy for aphids during the 2003-2004 cotton season (Herron *et al.* 2004). This was because the use of OP's against mirids also selected for resistance to this group of insecticides in aphids. The sustainable chemical control of mirids would be greatly enhanced by the pre-emptive generation of baseline data for resistance monitoring. This would enable us to receive mirids collected from cotton crops, establish them in culture to increase numbers, then screen them for resistance to a range of insecticides. If resistance develops Australian growers could face increased control costs and/or loss of yield and delayed maturity with resultant loss of fibre quality that could easily damage Australia's reputation as a producer of quality cotton.

Here we outline a method to culture mirids so that we can produce enough insects for baseline bioassay.

Material and methods

Below we describe a method we have developed to culture green mirids. It has been a long and complicated process, with continual problems with mirids dying in transit or the cultures slowly dying out. The technique we present, though labour intensive, will reliably produce mirids in sufficient numbers for resistance testing.

Rearing cages

Eight litre plastic containers were prepared by cutting a hole and inserting a ventilation gauze into the lid. Mirids were added to the rearing cage and fed with green bean pods that were washed in 1% bleach and rinsed in distilled water to remove any contaminants or pesticides. The bean pods were kept alive by placing them into a take away food container which had 250 ml of water agar (1% w/V) in the base. After the agar was poured into container it was allowed to cool, but before it had set the bean pods were added, pushing the end into the agar. The container with beans was placed into the culture cage. Supplementary food was supplied in the form of a lump of solid brown sugar and yeast that was placed in a 35 mm petri dish and added to the ventilated cage.

Culture of Mirids

Mirids were collected from lucerne at EMAI on the 13/09/07. From this collection 9 adult mirids were stunned briefly (about 20 seconds) with carbon dioxide gas, to make handling easy and added to the cage. The cage was placed in a growth cabinet to maintain constant conditions of 27^{o}C and 10L:14D. After 7 days and subsequently twice weekly adult mirids

were removed from the bean pods and the old pods plus agar transferred to a new cage to which additional fresh pods were added. The original cage(s) with adult mirids then had fresh bean pods in agar added, thus repeating the process. The process was repeated with old pods being removed from the adult mirids to new cages with additional food. These were left until adult mirids developed that could be used for testing.

Results

Numbers of mirids increased from 9 adults to hundreds and were successfully maintained in culture without more being added until the mirids were destroyed on 01/05/08. In that time enough mirids were produced for a preliminary bioassay which involved treating leaf discs with fipronil and a water only sprayed control with the aid of a Potter spray tower to deliver a repeatable dose. The results showed that the treated mirids died and the untreated controls survived so validating our initial bioassay trial.

Discussion

Cornford and Simpson (Undated) tested field collected Australian green mirids from cotton to ascertain the relative potency of potential chemicals used for their control. Their method required that mirids be temporarily housed in plastic containers with food for two days prior to use and permanent reference colonies were not established. This has the disadvantage that reference strains cannot be re-evaluated at a later date to confirm results or test new insecticides.

Here we have demonstrated that, with perseverance, it is possible to establish and maintain a reference strain of mirids and produce enough insects for subsequent bioassay. By achieving this we have achieved the first step in the development of a method to establish baseline bioassay data for the purpose of resistance monitoring.

It was clear very early, that mirids are very fragile and do not transport readily. This has implications for future resistance monitoring as specific methods will have to be developed so that suspect resistant strains arrive in good condition. Consequently it is desirable to develop molecular based methods for resistance monitoring as soon as practical to reduce or eliminate the need for routine field strain culturing.

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