Available online at www.sciencedirect.com
ScienceDirect
Journal of Invertebrate Pathology 94 (2007) 84–94
Journal of INVERTEBRATE PATHOLOGY
www.elsevier.com/locate/yjipa

Variation in susceptibility of *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae) in Australia to two *Bacillus thuringiensis* toxins

Lisa J. Bird [a,b], Raymond J. Akhurst [a,*]

[a] *CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia*
[b] *Department of Botany and Zoology, Australian National University, Canberra, ACT 2601, Australia*

Received 20 March 2006; accepted 16 August 2006
Available online 17 October 2006

Abstract

Intra-specific variation in susceptibility of *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) in Australia to the Cry1Ac and Cry2Ab δ-endotoxins from *Bacillus thuringiensis* (Berliner) (Bt) was determined to establish a baseline for monitoring changes that might occur with the use of Bt cotton. Strains of *H. armigera* and *H. punctigera* were established from populations collected primarily from commercial farms throughout the Australian cotton belts. Strains were evaluated for susceptibility using two bioassay methods (surface treatment and diet incorporation) by measuring the dose response for mortality (LC_{50}) and growth inhibition (IC_{50}). The variation in LC_{50} among *H. armigera* ($n = 17$ strains) and *H. punctigera* ($n = 12$ strains) in response to Cry1Ac was 4.6- and 3.2-fold, respectively. The variation in LC_{50} among *H. armigera* ($n = 19$ strains) and *H. punctigera* ($n = 12$ strains) to Cry2Ab was 6.6- and 3.5-fold, respectively. The range of Cry1Ac induced growth inhibition from the 3rd to 4th instar in *H. armigera* ($n = 15$ strains) was 3.6-fold and in *H. punctigera* ($n = 13$ strains) was 2.6-fold, while the range of Cry2Ab induced growth inhibition from neonate to 3rd instar in *H. armigera* ($n = 13$ strains) was 4.3-fold and in *H. punctigera* ($n = 12$ strains) was 6.1-fold. Variation in susceptibility was also evaluated for two age classes (neonates and 3rd instars) in laboratory strains of *H. armigera* and *H. punctigera*. Neonates of *H. punctigera* had the same or higher sensitivity to Bt than 3rd instars. Neonates of *H. armigera* were more sensitive to Cry2Ab than 3rd instars, while being less sensitive to Cry1Ac than 3rd instars. Differences in the two methods of bioassay used affected relative sensitivity of species to Bt toxins, highlighting the need to standardize bioassay protocols.
© 2006 Elsevier Inc. All rights reserved.

Keywords: *Helicoverpa*; *Armigera*; *Punctigera*; Cry1Ac; Cry2Ab; Toxicity

1. Introduction

Members of the *Helicoverpa* genus are key pests in Australian agriculture. Two species constitute the key pest complex in cotton: the cosmopolitan species *Helicoverpa armigera* (Hübner) and the endemic species *Helicoverpa punctigera* (Wallengren) (Common, 1953; Zalucki et al., 1986). Larvae of these species are highly polyphagous and attack a range of cultivated and uncultivated hosts (Zalucki et al., 1986). The adult stages are highly mobile and capable of migration (Farrow and Daly, 1987), allowing adaptation to a changing mosaic of hosts in ephemeral environments. Their ability to enter facultative pupal diapause enables *Helicoverpa* to maintain substantial resident populations in unstable habitats. These complex dynamics, influenced by various environmental and biological factors, has led to successful exploitation of diverse ecosystems by *H. armigera* and *H. punctigera* in Australia (Fitt, 1989).

Both *H. armigera* and *H. punctigera* are important economic targets for insecticidal products based on the soil bacterium *Bacillus thuringiensis* (Bt), that have been used

* Corresponding author. Fax: +61 2 6246 4155.
E-mail address: Ray.Akhurst@csiro.au (R.J. Akhurst).

0022-2011/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2006.08.005

commercially in Australia in the form of transgenic cotton since 1996 (Fitt and Forrester, 1998). Toxins from Bt provide good control of the lepidopteran pests. *Heliothis virescens* (F.) and *Pectinophora gossypiella* (Saunders) in the USA (Parker et al., 2000; Tabashnik et al., 2000). However, *Helicoverpa* species have naturally lower sensitivity to these toxins (Liao et al., 2002). Although there have been no problems with resistance to Bt toxins in field populations of *Helicoverpa* so far, the capacity for *H. armigera* to develop resistance to Cry1Ac has been demonstrated in several laboratories (Fan et al., 2000; Kranthi et al., 2000; Akhurst et al., 2003). Although Gunning et al. (2005) reported selection for Cry1Ac resistance in *H. armigera* from survivors of a 2001 resistance monitoring program, there is doubt about the validity of their assessment of resistance. They used an unusual bioassay technique to estimate a resistance ratio of 275-fold in their strain. However, independent testing of this strain with standard techniques by two laboratories in 2001 detected no resistance (R. Mahon and K. Olsen, pers. comm.; W. James and R. Akhurst, pers. comm.). There has been no subsequent independent testing of this strain.

Ideally, a resistance management strategy will include monitoring field populations to detect early changes in the frequency of resistance alleles so that remedial measures can be deployed to prevent the development of field level resistance. Surveys of target pest susceptibility to insecticides are necessary to establish baseline responses for monitoring possible changes in the resistance status of field populations. Studies of field populations of *Helicoverpa* from outside of Australia demonstrated considerable intraspecific variability in susceptibility to Bt toxins (Stone and Sims, 1993; Luttrell et al., 1999; Wu et al., 1999; Gujar et al., 2000). Similarly, a range of sensitivity to Bt has been demonstrated in unselected strains of *H. virescens* (Stone and Sims, 1993; Luttrell et al., 1999) and *Pectinophora xylostella* (González-Cabrera et al., 2001).

Although resistance monitoring programs have been in place since the introduction of transgenic cotton (Forrester and Bird, 1998), these programs involved the use of formulated Bt products. These formulations were not ideal for monitoring Cry1Ac resistance because they contained multiple proteins (e.g. DiPel®) or consisted of encapsulated bacteria (MVP®). Moreover, the commercial release of two-gene cotton (Bollgard II) in Australia in 2003 required that monitoring for Cry2Ab resistance be undertaken. Therefore, methods for monitoring Bt resistance in *Helicoverpa* were revised in 2002 when formulated Bt products were replaced with spore/crystal preparations of both Cry1Ac and Cry2Ab. This necessitated establishment of baseline response of *Helicoverpa* to spore/crystal preparations of Bt toxins for use in the resistance monitoring program in Australia.

The study reported here had two objectives. The first was to establish baseline levels of susceptibility to the two Bt toxins currently commercially deployed in transgenic cotton in Australia (Cry1Ac and Cry2Ab) in field derived populations of *H. armigera* and *H. punctigera*. The second was to determine whether the toxicity of these Cry proteins to *H. armigera* and *H. punctigera* is age-specific. The establishment of a realistic range of susceptibility is important for resistance monitoring because variation in susceptibility impacts on the criteria for resistance. That is, the resistance status of a field population would be determined by the unselected reference strain used. The baseline responses to these toxins across numerous populations will be useful in determining the full range of intra-specific tolerance for each species of *Helicoverpa* to allow resistance episodes to be identified with certainty.

2. Materials and methods

2.1. Insect strains

Insects of various life stages (ranging from egg to pupa) were collected between August 2001 and March 2004 from a range of cultivated and uncultivated hosts including cotton, sorghum, pigeon pea, maize, tomato and a scrophulariaceous weed host, *Verbascum virgatum*. Insect collections were primarily obtained from the major cotton growing areas in Australia, which are in New South Wales and Queensland. Two populations originated from other areas in Australia; one each from Western Australia and the Northern Territory. A minimum of 50 field collected individuals constituted any one geographically distinct strain. Each strain was reared in the laboratory on artificial diet as described in Akhurst et al. (2003) and tested within three generations of its establishment in the laboratory. In the larval stage, insect strains were maintained under a laboratory environment of 25 °C with a photoperiod of 14:10 (L:D) h. Adults were maintained in a separate facility under the same conditions of light and temperature with relative humidity maintained at 65%.

The laboratory strain ANGR was established by crossing AN02 (pyrethroid-resistant and endosulfan-susceptible strain) and GR (general laboratory strain) provided by J. Daly (CSIRO Entomology, Canberra, Australia). The ANGR strain had been cultured in the laboratory for approximately 4 years at the time of testing. The laboratory strain of *H. punctigera* was established from a population collected from uncultivated hosts from south-west Queensland provided by P. Gregg (University of New England, Armidale, Australia) in the spring of 1999.

2.2. Toxins

The Cry1Ac toxin used in mortality assays was produced from *B. thuringiensis* strain HD73, as described in Akhurst et al. (2003). The Cry1Ac used in development assays and age-specific mortality assays was produced from the HD73 strain by GeneSearch (Arundel, Queensland), and had similar potency to the Cry1Ac used in mortality bioassays.

A recombinant clone of the *cry2Ab* gene in *B. thuringiensis* (PM156) provided by L. Masson (National Research

Download English Version:

https://daneshyari.com/en/article/4558821

Download Persian Version:

https://daneshyari.com/article/4558821

Daneshyari.com