Available online at www.sciencedirect.com
ScienceDirect
Journal of Invertebrate Pathology 95 (2007) 208–213
Journal of INVERTEBRATE PATHOLOGY
www.elsevier.com/locate/yjipa

Monitoring and adaptive resistance management in Australia for Bt-cotton: Current status and future challenges ☆

Sharon Downes [a,*], Rod Mahon [b], Karen Olsen [b]

[a] CSIRO Entomology and Australian Cotton Cooperative Research Centre, Locked Bag 59, Narrabri, NSW 2390, Australia
[b] CSIRO Entomology and Australian Cotton Cooperative Research Centre, GPO Box 1700, Canberra, ACT 2601, Australia

Received 19 January 2007; accepted 13 March 2007
Available online 25 March 2007

Abstract

In the mid-1990s the Australian Cotton industry adopted an insect-resistant variety of cotton (Ingard®) which expresses the Bt toxin Cry1Ac that is specific to a group of insects including the target *Helicoverpa armigera*. A conservative resistance management plan (RMP), that restricted the area planted to Ingard®, was implemented to preserve the efficacy of Cry1Ac until two-gene transgenic cotton was available. In 2004/05 Bollgard II® replaced Ingard® as the transgenic cotton available in Australia. It improves on Ingard® by incorporating an additional insecticidal protein (Cry2Ab). If an appropriate refuge is grown, there is no restriction on the area planted to Bollgard II®. In 2004/05 and 2005/06 the Bollgard II® acreage represented approximately 80 of the total area planted to cotton in Australia. The sensitivity of field-collected populations of *H. armigera* to Bt products was assayed before and subsequent to the widespread deployment of Ingard® cotton. In 2002 screens against Cry2Ab were developed in preparation for replacement of Ingard® with Bollgard II®. There have been no reported field failures of Bollgard II® due to resistance. However, while alleles that confer resistance to *H. armigera* in the field are rare for Cry1Ac, they are surprisingly common for Cry2Ab. We present an overview of the current approach adopted in Australia to monitor and adaptively manage resistance to Bt-cotton in field populations of *H. armigera* and discuss the implications of our findings to date. We also highlight future challenges for resistance management in Australia, many of which extend to other Bt-crop and pest systems.

Keywords: *Bacillus thuringiensis*; Cotton; Resistance; *Helicoverpa armigera*; Australia

1. Introduction

The development of *Bacillus thuringiensis* (Bt) transgenic plants provide prospects for widespread control of lepidopteran pests on field crops (Shelton et al., 2000; Carrière et al., 2003), but a major risk associated with this technology is rapid adaptation of the pests targeted by the toxins (Gould, 1998). Although no failures of Bt-crops due to field resistance have been reported, field resistance to Bt toxin sprays has been detected in several *Plutella xylostella* populations and many targeted pests have been successfully selected in the laboratory for Bt resistance (Tabashnik et al., 2003; Griffitts and Aroian, 2005).

Bt-cotton that is specific to Lepidoptera has been commercially available in some parts of the world since 1996 (James, 2005). The resistance risk to this technology is considered to be especially great in Australia, China, and India because the cotton bollworm *Helicoverpa armigera* is the major pest in these countries (McGaughey and Whalon, 1992; Roush, 1997; Gould, 1998). This moth has an impressive record of rapidly evolving resistance to conventional insecticides (Forrester et al., 1993; Shen and Wu, 1995; McCaffery, 1998; Kranthi et al., 2002). In addition,

☆ Based on a spoken presentation in the Symposium "Monitoring and Managing for Bt-resistance: The challenges for the next decade" at the Society for Invertebrate Pathology 39th Annual Meeting in Wuhan, China, August 27 to September 1, 2006.

* Corresponding author.
E-mail address: Sharon.Downes@csiro.au (S. Downes).

0022-2011/$ - see front matter
doi:10.1016/j.jip.2007.03.010

transgenic cotton does not provide a high dose for *Helicoverpa* species (Luttrell et al., 1999; Liao et al., 2002).

The goal of this paper is to present an overview of the approach currently adopted in Australia to monitor and adaptively manage resistance to Bt-cotton in field populations of *H. armigera*[1]. We present up to date estimates of the frequencies of alleles conferring resistance to Bt but the detailed methodology and analysis of these data are given in another paper (Mahon et al., 2007a). We also highlight some future challenges for Bt resistance management in Australia, many of which extend to other Bt-crop and pest systems.

2. Bt-cotton in Australia

In the 1995/96 growing season transgenic cotton, known as Bollgard® in the New World and Ingard® in the Old World, became commercially available in Australia. Ingard® expressed the *cry1Ac* gene and was treated as an interim technology until a second generation of transgenic cotton was available that expressed two Bt toxins with different modes of action (Fitt, 2004). Because of the critical importance of preserving the susceptibility of *H. armigera* to Bt toxins, a conservative resistance management plan (RMP) was imposed for Ingard®, a key component of which restricted the planting of this technology to 30% of the cotton cropping area per farm (Fitt, 2000).

Early in the season Ingard® provided good control of *H. armigera* but the titer of Cry1Ac in plant material declined as plants aged which allowed larvae to survive on older cotton plants (Greenplate, 1999; Fitt and Wilson, 2000; Olsen et al., 2005). Despite the frequent need to spray crops with a Heliocide late in the season, between the 1995/96 season and 2001/02 season, the average amount of insecticide used per hectare was 44% lower on Ingard® compared to conventional cotton (Cotton Consultants Australia [CCA], unpublished data; see also Wilson et al., 2004).

In the 2004/05 growing season Ingard® cotton was completely replaced by Bollgard II®, which expresses the *cry1Ac* and *cry2Ab* genes (Fitt, 2004). It is reasonable to assume that the gene or genes conferring resistance to one of these toxins would segregate independently from those conferring resistance to the other toxin (Mani, 1985; Roush, 1989; Tabashnik, 1989; but see Tabashnik et al., 1997 for evidence that this may not be the case for the Diamondback Moth, *P. xylostella*). Due to the putative value of this dual-toxin strategy, the RMP for transgenic cotton was relaxed to allow growers to plant up to 95% of their crop to Bollgard II® (Farrell, 2006).

Bollgard II® comprised around 80% of the total area planted to cotton in Australia during the 2004/05 and 2005/06 seasons (Farrell, 2006). The expression of Cry1Ac in Bollgard II® declines as the season progresses, but the expression of Cry2Ab remains consistently high throughout the entire season (Greenplate et al., 2003). Thus, Bollgard II® plants provide good control all season. Between the 2002/03 season and 2004/05 season, the average amount of insecticide used per hectare was 85% lower on Bollgard II® compared to conventional cotton (CCA, unpublished data).

3. The current resistance management plan

The RMP for Bollgard II® was established by regulatory authorities to protect the technology. As it is difficult to precisely predict the chances of resistance developing to Bollgard II® in *H. armigera*, the management plan is a preemptive strategy that aims to significantly delay field-scale changes in resistance.

The five components of the RMP impose limitations and requirements for management on farms that grow Bollgard II®. These are: mandatory growing of refuges; control of volunteer and ratoon plants; a defined planting window; restrictions on the use of foliar Bt; and mandatory cultivation of crop residues (for further details see Farrell, 2006; Sivasupramaniam et al., 2007). The interaction of all of these elements gives researchers and regulatory authorities the confidence that the RMP will effectively slow the evolution of resistance.

4. Monitoring resistance to Bt in field populations

The Australian Cotton Research and Development Corporation (CRDC) funds a program that monitors resistance to Bt in field populations of *H. armigera*. The program has two objectives: (1) to evaluate the effectiveness of the current RMP and (2) to detect changes that might signal the onset of resistance. Insect material is collected from all of the main cotton growing regions in eastern Australia. The program currently uses a phenotypic screen in conjunction with a genic screen. It was developed using the knowledge that most previously detected cases of resistance to Bt are at least partially recessive (Andow and Ives, 2002) and considered the labour and resources available to effectively perform the work.

We term the first method 'F_0 screen'. This approach involves (1) collecting eggs of *H. armigera* from the leaves or flowers of host plants, (2) delivering them to a centralised laboratory for testing, (3) hatching the eggs in the laboratory, and (4) screening the larvae using a laboratory discriminating dose assay (Roush and Miller, 1986). Since this phenotypic screen requires significantly less labour and resources to perform than the genic screen (see below) we are able to sample repeatedly in the same area and look at trends over time within seasons and among seasons. It is a statistically inefficient method for recessive alleles (Andow and Ives, 2002) but provides a rapid check for major changes in resistance in field populations.

The second method is designed to measure precise frequencies of resistant alleles and is termed 'F_2 screen'

[1] The Australian Bt-resistance monitoring program also samples field populations of *Helicoverpa punctigera* but consideration of these data are beyond the scope of this paper.

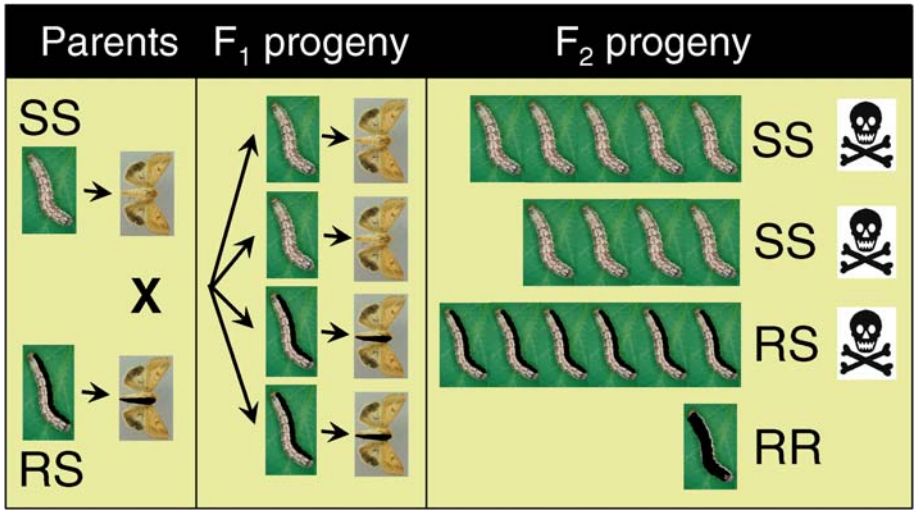

Fig. 1. F_2 screen for rare resistance alleles in *Helicoverpa armigera*. Parents are collected in the field, usually as eggs or larvae, and here one of them is indicated with one copy of the resistance allele. Their F_1 progeny are sib-mated to produce the F_2 generation. If resistance is completely recessive, in the F_2 generation only 1/16 of the larvae are expected to be homozygous for the rare resistance allele (RR), and the remaining homozygous susceptible (SS) and heterozygote progeny will be killed by the discriminating dose of toxin.

(Andow and Alstad, 1998). This approach involves following steps 1 through 3 above for the F_0 screen, (4) rearing the larvae to moths, (5) mating single pairs of moths, (6) rearing the F_1 larvae, (7) sib-mating the F_1 families, (8) collecting eggs, and (9) screening the neonates using a laboratory discriminating dose assay (Fig. 1). This method generates isofemale lines that in the second generation produce a proportion of individuals that are homozygous for each allele present in two field-derived parents (Andow and Alstad, 1998). This genic screen is labour-intensive but is particularly efficient for recessive alleles (Andow and Ives, 2002).

The Bt resistance monitoring program in Australia began in 1994 prior to the use of Ingard® by the industry, and since then F_0 screens against Cry1Ac have been used. In 2002, we developed F_0 screens against Cry2Ab, in anticipation of the widespread use of Bollgard II® in the 2004/05 season, and instigated F_2 screens for Cry1Ac and Cry2Ab resistance alleles. In the current program material is allocated to the F_2 screens until a threshold workload is reached. In practice, around 50% of the field-derived eggs are screened using each approach. Details on the development of discriminating doses, and procedures used in rearing and F_2 screening are given in Mahon et al. (2007a). Details for the discriminating doses employed in the F_0 screen are given by Bird and Akhurst (2007).

5. Findings from recent F_0 screens

We restrict our analyses of data from the F_0 screens to the 2003/04, 2004/05, and 2005/06 seasons because from 2003 we refined the protocols used for screens against Cry1Ac and commenced screens against Cry2Ab. The analyses were performed on data collected from the Namoi and Gwydir (hereafter Namoi/Gwydir) valleys in north-western New South Wales. It is only from these neighboring valleys that we have good samples of insects within and among consecutive seasons.

We summed data from farms that were sampled at the same time to reach test sizes of at least 40 individuals per period, and plotted the median value for that sample as the number of days since the first sample for that season (Fig. 2). A simple regression on the data for each season

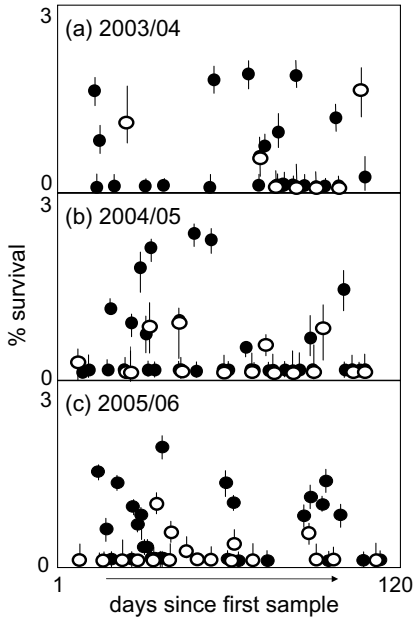

Fig. 2. The proportion (expressed as a percentage) and 95% CI of individuals that survived the F_0 screens against Cry1Ac (closed) and Cry2Ab (open) in (a) 2003/04, (b) 2004/05, and (c) 2005/06. Data are from the Namoi/Gwydir valley. The laboratory assay used Bt toxin as a contaminant of artificial diet. In all cases each data point represents at least a sample of 40 individuals.

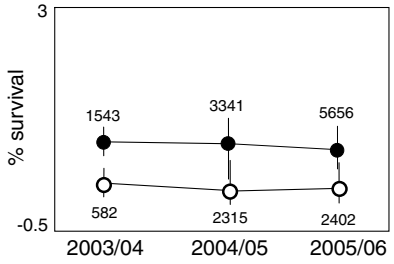

Fig. 3. The proportion (expressed as a percentage) and 95% CI of individuals that survived the F_0 screens against Cry1Ac (closed) and Cry2Ab (open) in 2003/04, 2004/05, and 2005/06. Data are from the Namoi/Gwydir valley. The laboratory assay used Bt toxin as a contaminant of artificial diet. Numbers near each data point represented the size of that sample.

showed that, for Cry1Ac and Cry2Ab F_0 screens, there is no significant shift in the proportion of surviving *H. armigera* throughout the season (2003/04, Cry1Ac, $F_{1,22} = 0.47$; 2004/05, Cry1Ac, $F_{1,19} = 0.40$, Cry2Ab, $F_{1,17} = 0.41$; 2005/06, Cry1Ac, $F_{1,28} = 1.50$, Cry2Ab, $F_{1,17} = 0.49$; in all cases $P > 0.05$). The data for Cry2Ab collected in 2003/04 were not statistically analysed due to small sample sizes but a visual inspection indicates no marked change in the proportion of survivors over time (Fig. 2a).

To examine trends among seasons we divided the total number of individuals surviving screens in a year by the total number of individuals tested in that year (Fig. 3). A Fisher's exact test showed that, for Cry1Ac and Cry2Ab F_0 screens, there is no significant difference among the three sequential years of sampling in the numbers of individuals surviving the screen relative to the number of individuals tested (Cry1Ac, $df = 2$, $\chi^2 = 0.95$, $P = 0.62$; Cry2Ab, $df = 2$, $\chi^2 = 0.10$; $P = 0.96$).

Therefore, recent data indicates that there have been no major changes in the proportions of *H. armigera* surviving F_0 screens within seasons or among seasons, and presumably no major changes in resistance in field populations.

6. Alleles conferring resistance to Cry1Ac or Cry2Ab

Since 2002/03 the F_2 method has been used on field collected *H. armigera* to screen 1680 alleles for resistance to Cry1Ac and 1684 alleles for resistance to Cry2Ab. No alleles scored positive for conferring resistance to Cry1Ac but 7 alleles scored positive for conferring resistance to Cry2Ab (Table 1). Three of the seven cases of Cry2Ab resistance alleles were detected prior to the widespread adoption of Bollgard II® by the industry in the 2004/05 season (Table 1). We followed the Baysian methods developed by Andow and Alstad (1998) to analyse F_2 screen data. The expected frequency (with lower and upper 95% credibility intervals) of resistance in Australian populations of *H. armigera* to Cry1Ac and Cry2Ab is 0.0006 (0.0000–0.0018) and 0.0049 (0.0021–0.0088), respectively. A more detailed analysis of these data, which includes additional samples from collaborators, is presented in the paper by Mahon et al. (2007a).

Table 1
The number of alleles in the F_2 screens that scored positive for conferring resistance to Cry1Ac or Cry2Ab

Year	Cry1Ac F_2 screen		Cry2Ab F_2 screen	
	Alleles tested	Scored positive	Alleles tested	Scored positive
2002/03	116	0	112	1
2003/04	244	0	248	2
2004/05	364	0	368	0
2005/06	892	0	892	4
Total	1616	0	1620	7

Data are presented separately for each growing season but have been combined for all regions and crops (i.e., conventional cotton, Bollgard II® cotton, mung bean, sunflowers, chick pea, maize, and pigeon pea). The results are presented by locality in Mahon et al. (2007a).

7. What are the implications of these data?

An Australian strain of *H. armigera*, designated BX, has been selected in the laboratory for a high level resistance to Cry1Ac (Akhurst et al., 2003). In 2002 a surviving *H. armigera* from an F_0 screen against Cry1Ac was shown to be allelic with BX (Lisa Bird, unpublished data). Therefore, alleles conferring resistance to Cry1Ac occur in field populations of Australian *H. armigera*. Our data from the F_2 screens suggests that the frequency of these alleles is very low (Table 1). We conclude that Ingard® was managed conservatively and successfully to preserve the Cry1Ac gene against *H. armigera* (see also Mahon et al., 2007a).

The first four Cry2Ab resistant isolates have been tested and are allelic (Mahon et al., 2007b). We have not yet tested the remaining three strains but it is likely that the resistance is the same form in each case (for further detail see Mahon et al., 2007b). This initial frequency of alleles conferring a high level resistance to Cry2Ab is greater than expected. The implications of this data for the evolution of resistance depend on several factors, and are covered in detail in Mahon et al. (2007b).

We have no evidence that the frequency of alleles conferring resistance to Cry2Ab is increasing but our statistical power to detect subtle shifts in incidence is weak (see below). The first isolated Cry2Ab resistance allele has been characterised as due to a single major gene that is recessive (Mahon et al., 2007c). All 7 isolated alleles that confer resistance to Cry2Ab render individuals fully susceptible to Cry1Ac (see Table 1; Mahon et al., 2007a). Costs to carrying a resistance allele are presently under investigation (R. Mahon and K. Olsen). Importantly, there may be opportunities for survival of Cry2Ab resistant larvae late in the growing season when the Cry1Ac in Bollgard II® declines to a level where only Cry2Ab is effective against *H. armigera*.

8. Challenges for the future

In Australia many users of Bt-cotton begrudge the costs of growing refuges and anecdotal evidence suggests

that not all refuge crops are well maintained (see also Dove, 2001 and references within). This situation is more prominent since Bollgard II® has become the dominant crop. The cap on the area that could be planted to Ingard®cotton made it feasible for growers to plant the maximum allowed amount of Bt-cotton and select non-Bt cotton that could be sprayed with Heliocides to serve as the refuge. Since there is no cap on the area that can be planted to Bollgard II®, if a grower chooses to plant the maximum allowed amount of Bt-cotton they must select unsprayed crops, which are highly productive for Helicoverpa but usually not profitable, as their refuge. A major challenge in Australia, and elsewhere, is to determine the cheapest way to generate sufficient moths to provide a reasonable lifetime for the technology (see also Vacher et al., 2006).

While Bollgard II® provides excellent control over *H. armigera* in Australia, there have been anecdotal reports from growers of living larvae on these plants, particularly during flowering. We have screened a sample ($n = 58$) of these larvae using the F_2 method and determined that Bt-susceptible larvae are able to survive on these plants (Downes et al., 2006). We plan to determine the prevalence of larvae, the damage they inflict to a cotton crop, and the mechanism(s) that allows them to survive on Bollgard II® plants (e.g., Gore et al., 2003; Gore and Adamczyk, 2004). This information is critical for determining a threshold level for spraying Heliocide that considers economic damage and resistance risk.

In Australia, we currently do not have a planned response action to counter resistance to Bt if it develops or a threshold increase in frequency over which such a response would be triggered. A major limitation to looking for shifts in frequencies is that the F_2 screen, although a cost-effective method for monitoring recessive resistance alleles (e.g., Andow and Ives, 2002), is labour intensive for a low throughput. In principle, DNA-based screening can be conducted with properly preserved insects of any life stage, thereby greatly reducing labour, time, and cost (Tabashnik et al., 2006). An ongoing challenge is to develop these methods for the Australian system, although it will be necessary to continue some F_2 screens to detect alleles other than those already detected (Cry1Ac = BX, Akhurst et al., 2003; Cry2Ab = SP15, Mahon et al., 2007b) and on which any DNA-based screens might be developed.

In theory there would be two main types of responses to increases in the frequencies of resistance to Bt in *H. armigera* populations (after Andow and Ives, 2002). The first would be to reduce the selection differential between resistant and susceptible individuals or reduce the survival of the resistant individuals. The second would be to modulate the relative frequencies of the resistance allele between Bt and non-Bt fields by moving adults. Although research into both of these approaches is ongoing, considerable additional investigation is required to demonstrate that they can be effectively implemented.

Acknowledgments

K. Stanford, L. Scott, K. Stewart, N. Winters, E. Cuell, R. Ison, C. Mares, L. Bird, and B. James provided valuable technical assistance and advice. In particular, K. Garsia and T. Parker performed the bulk of the technical work. We thank L. Rossiter and her team (NSW DPI) for coordinating some of the collection and delivery of eggs, and the numerous industry participants that provided alerts and/or collections of eggs for the screens. L. Rossiter provided comments that improved the clarity of this paper. The Monsanto Company provided the source of Cry2Ab and B. James (CSIRO Entomology) provided the source of Cry1Ac. The monitoring work was funded by the Australian Cotton Research and Development Corporation.

References

Akhurst, R., James, W., Bird, L., Beard, C., 2003. Resistance to the Cry1Ac—endotoxin of *Bacillus thuringiensis* in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 96, 1290–1299.

Andow, D., Alstad, D., 1998. F_2 screen for rare resistance alleles. J. Econ. Entomol. 91, 572–578.

Andow, D., Ives, A., 2002. Monitoring and adaptive resistance management. Ecol. Appl. 12, 1378–1390.

Bird, L., Akhurst, R., 2007. Variation in susceptibility of *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae) to two *Bacillus thuringiensis* toxins in Australia. J. Invert. Pathol. 94, 84–94.

Carrière, Y., Ellers-Kirk, C., Sisterson, M., Antila, L., Whitlow, M., Dennehy, T., 2003. Long term regional suppression of pink bollworm by *Bacillus thuringiensis* cotton. Proc. Nat. Acad. Sci. USA 100, 1519–1523.

Dove, A., 2001. Survey raises concerns about Bt resistance management. Nat. Biotechnol. 19, 293–294.

Downes, S., Mahon, R., Parker, T., Staines, T., 2006. Wanted alive: large Helicoverpa larvae from Bollgard II plants. The Aust. Cotton Grower 27, 8–10.

Farrell, T., 2006. Cotton Pest Management Guide 2006/07. NSW Department of Primary Industries, Orange, NSW, Australia.

Fitt, G., 2000. An Australian approach to IPM in cotton: integrating new technologies to minimise insecticide dependence. Crop Protect. 19, 793–800.

Fitt, G., 2004. Implementation and impact of transgenic Bt cottons in Australia. in: Proceedings, World Cotton Research Conference-3, Cape Town, 9–13 March 2003. Agricultural Research Council, Institute for Industrial Crops, Pretoria, Republic of South Africa.

Fitt, G., Wilson, L., 2000. Genetic engineering in IPM: Bt cotton. In: Kennedy, G., Sutton, T. (Eds.), Emerging technologies for integrated pest management: concepts, research and implementation. APS Press, USA, pp. 108–125.

Forrester, N., Cahill, M., Bird, L., Layland, J., 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. Bull. Entomol. Res. Suppl. Series, No. 1.

Gore, J., Adamczyk Jr., J., 2004. Impact of Bollworms [*Helicoverpa zea* (Boddie)] on maturity and yield of Bollgard cotton. J. Cotton Sci. 8, 223–229.

Gore, J., Leonard, B., Gable, R., 2003. Distribution of bollworm, *Helicoverpa zea* (Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var. *kurstaki* Berliner cotton. J. Econ. Entomol. 96, 699–705.

Gould, F., 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann. Rev. Entomol. 43, 701–726.

Greenplate, J., 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92, 1377–1383.

Greenplate, J.T., Mullins, J.W., Penn, S.R., Daham, A., Reich, B.J., Osborn, J.A., Rahn, P.R., Ruschke, L., Shappley, Z.W., 2003. Partial characterization of cotton plants expressing two toxin proteins from *Bacillus thuringiensis*: relative contribution, toxin interaction, and resistance management. J. Appl. Entomol. 127, 340–347.

Griffitts, J., Aroian, R., 2005. Many roads to resistance: how invertebrates adapt to Bt toxins. BioEssays 27, 614–624.

James, C., 2005. Global status of biotech/GM crops in 2005. ISAAA Briefs No. 34-2005: Executive summary. International Service for the Acquisition of Ag-biotech Applications, Ithaca, NY.

Kranthi, K., Jadhav, D., Kranthi, S., Wanjari, R., Ali, S., Russell, D., 2002. Insecticide resistance in five major insect pests of cotton in India. Crop Protect. 21, 449–460.

Liao, C., Heckel, D., Akhhurst, R., 2002. Toxicity of *Bacillus thuringiensis* insecticidal proteins for *Helicoverpa armigera* and *Helicoverpa punctigera* (Lepidoptera: Noctuidae), major pests of cotton. J. Invert. Pathol. 80, 55–63.

Luttrell, R., Wan, L., Knighten, K., 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soyabean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92, 21–32.

Mahon, R., Olsen, K., Downes, S., Addison, S. 2007a. Frequency of alleles conferring resistance to the Bt toxins Cry1Ac and Cry2Ab in Australian populations of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). in preparation.

Mahon, R., Olsen, K., Downes, S., 2007b. Isolations of Cry2Ab resistance in Australian populations of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) are allelic, in preparation.

Mahon, R., Olsen, K., Garsia, K.,Young, S., 2007c. Resistance to the Bt toxin Cry2Ab in a strain of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). J. Econ. Entomol. in press.

Mani, G., 1985. Evolution of resistance in the presence of two insecticides. Genetics 109, 761–783.

McCaffery, A., 1998. Resistance to insecticides in heliothine lepidoptera: a global view. Philos. Trans. R. Soc. Lond. Ser. B 353, 1735–1750.

McGaughey, W., Whalon, M., 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science 258, 1451–1455.

Olsen, K., Daly, J., Holt, H., Finnegan, E., 2005. Season-long variation in expression of the *cry1Ac* gene and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 98, 1007–1017.

Roush, R., 1989. Designing pesticide management programs: how can you choose? Pesticide Science 26, 423–441.

Roush, R., 1997. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pesticide Science 51, 328–334.

Roush, R., Miller, G., 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79, 293–298.

Shelton, A., Tang, J., Roush, R., Metz, T., Earle, E., 2000. Field tests on managing resistance to Bt-engineered plants. Nat. Biotechnol. 18, 339–342.

Shen, J., Wu, Y., 1995. Resistance of *Helicoverpa armigera* to insecticides and its management. China Agricultural Press, Beijing, People's Republic of China.

Sivasupramaniam, S., Head, G.P., English, L., Jin Li, Y., Vaughn, T.T., 2007. A global approach to resistance monitoring. J. Invert. Pathol. 95, 224–226.

Tabashnik, B., 1989. Influence of pesticide persistence in evolution of resistance. J. Econ. Entomol. 82, 1263–1269.

Tabashnik, B., Liu, Y.-B., Finson, N., Masson, L., Heckel, D., 1997. One gene in the diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad. Sci. USA 94, 1640–1644.

Tabashnik, B., Carrière, Y., Dennehy, T., Morin, S., Sisterson, M., Roush, R., Shelton, A., Zhao, J-Z., 2003. Insect resistance to transgenic Bt crops: lessons from the laboratory and field. J. Econ. Entomol. 96, 1031–1038.

Tabashnik, B., Fabrick, J., Henderson, S., Biggs, R., Yafuso, C., Nyboer, M., Manhardt, N., Coughlin, L., Sollome, J., Carrière, Y., Dennehy, T., Morin, S., 2006. DNA screening reveals Pink Bollworm resistance to Bt cotton remains rare after a decade of exposure. J. Econ. Entomol. 99, 1525–1530.

Vacher, C., Bourguet, D., Desquilbet, M., Lemarie, S., Ambec, S., Hochberg, M., 2006. Fees or refuges: which is better for the sustainable management of insect resistance to transgenic Bt corn? Biol. Lett. 2, 198–202.

Wilson, L., Mensah, R., Fitt, G., 2004. Integrated pest management strategies through the growing season. In: Horowitz, A., Ishaaya, I. (Eds.), Insect Pest Management. Field and Protected Crops. Springer-Verlag, Berlin, Germany, pp. 97–118.