WANTED ALIVE: Large Helicoverpa larvae from Bollgard II® plants

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The possible development of resistance by *Helicoverpa* to the Bt (*Bacillus thuringiensis*) toxins in Bollgard II® is a major threat to the continued efficacy of transgenic cotton. Monitoring *Helicoverpa* field populations enables early warning of the onset of resistance and thereby presents an opportunity to refine our resistance management plan to maintain the effectiveness of Bt-cotton.

The Cotton Research and Development Corporation funds a project based at the Australian Cotton Research Institute in Narrabri, that measures resistance frequencies to the Bt toxins in Bollgard II® (Cry1Ac and Cry2Ab) in field populations *of Helicoverpa*.

Results to date suggest that in *H. armigera* and *H. punctigera*, the frequency of genes conferring resistance to Cry1Ac remains low—none of the almost 1700 genes tested scored positive for conferring resistance. Data for *H. punctigera* suggest that the baseline frequency of genes conferring resistance to Cry2Ab is at a low level—only 1 of the almost 1600 genes tested scored positive for conferring resistance.

However, data for *H. armigera* show that the baseline frequency of genes conferring resistance to Cry2Ab are unexpectedly high—7 of the almost 1700 genes tested have scored positive for resistance. Instances of this form of resistance were detected prior to the widespread deployment of Bollgard II® so this 'high' frequency probably is not due to selection by Bt-cotton. However, as this resistance is more common than desired, we must pay careful attention to any increase in frequency of this gene.

Results from screens with live larvae from Bollgard II® plants

During the 2005/06 season, we received for screening 321 live *Helicoverpa* larvae that were collected on Bollgard II® plants. All were reared in the laboratory and those that emerged as healthy moths were assigned to the F_2 screening component of the Bt resistance monitoring program.

We tested 31 lines of *H. armigera* and 27 lines of *H. punctigera* that originated from field-collected larvae on Bollgard II®, using the F_2 screen against Cry1Ac and Cry2Ab. Two of the *H. armigera* lines scored positive for an allele conferring a high level resistance to Cry2Ab and were included in the above frequency (i.e. 7 out of 1700). While the proportion of live larvae from Bollgard II® plants carrying a resistant gene to Cry2Ab is high (2 of 58) and thus of concern, it is important to carefully consider a number of factors around this finding.

1. None of the isolated Cry2Ab resistant lines, including the two from live larvae collected off Bollgard II® plants, show cross-resistance to Cry1Ac. The larvae collected off Bollgard II® were from plants that scored positive for expressing Cry1Ac and Cry2Ab using a qualitative ELISA. Therefore, it is unlikely that the larvae would have been able to survive to adulthood on Bollgard II® simply because they carried one allele conferring resistance to Cry2Ab.
2. The majority of live larvae collected off Bollgard II® plants (83 of 85) were fully susceptible to both Cry1Ac and Cry2Ab, but they managed to survive for some time on these plants.

3. For all of the lines scored as positive for resistance to Cry2Ab in the program, including the two from live larvae collected off Bollgard II® plants, we can conclude from our bioassays that only one of the larvae in each pair was carrying a resistant allele. Importantly, that individual was heterozygous (RS) rather than homozygous (RR).

We need to screen more live larvae from Bollgard II® plants!

The isolation of Cry2Ab resistant genes from larvae collected on Bollgard II® during what was a 'high pressure' year from *Helicoverpa* is consistent with the situation found previously. Nevertheless, these two cases are of concern and it is essential that we screen more larvae collected on Bollgard II® plants.

Reports from 2005/06 suggest that the incidence of live medium or large larvae on Bollgard II® plants is sporadic. Therefore we rely on alerts or collections of larvae from growers, consultants or other interested parties. If you are based in the Namoi Valley, we usually are able to respond to alerts promptly. In all other regions we request that the larvae be collected and posted to us.

We are interested in larvae of *H. armigera* and *H. punctigera* that are medium in size or larger (i.e. at least 6mm long). Ideally, the larvae would be accompanied by associated leaf samples but even if you cannot complete step 2 below, your contribution of larvae will be invaluable to the Bt resistance monitoring program. We will distribute containers and bags. Contact us now if you would like some posted to you or your group.

Step 1: Please collect the larvae and place it in a small container with air holes and some food other than Bollgard II® (e.g. conventional cotton).

Step 2: If possible, collect a leaf from the plant that the larvae was feeding on (indicated by the rectangle in Figure 1), as well as the plant in the same row that is either side of the host plant (indicated by the circles in Figure 1), and the plant in the surrounding row that is either side of the host plant (indicated by the triangles in Figure 1).

Place the leaf from each plant in a separate paper bag labelled appropriately with 'host plant' or 'surrounding plant', and the plant variety, property, collector, and field, if known, and keep them cool. *Please remove the 3rd unfurled leaf from the top of the plant as the sample.* It is critical that the leaf material is fresh.

Step 3: As soon as possible, call your IDO or arrange to have the larvae or the larvae plus associated plant parts delivered to **Trudy Staines, Australian Cotton Research Institute, Wee Waa Road, Narrabri 2390.** We can pay for the postage (i.e. tick the 'receiver pays' option on the freight form). If your property is in the Namoi valley, deliver the material to the ACRI or call Tracey Parker or Trudy on 02 6799 1500 to arrange a pick up.

We will rear the larvae in the laboratory. All larvae that emerge as healthy moths will be included in the F_2 screens for resistance as part of the Bt resistance monitoring program.

We will also examine the plant samples for the presence of Cry1Ac and Cry2Ab. The four plants surrounding the host are tested in case the grub recently moved from one of them to the current host plant. It is important to keep the leaf from each surrounding plant in a separate bag so that we can tell how many individual plants we sampled but there is no need to indicate which surrounding plant the sample is from.

For further information please contact Tracey or Trudy, ACRI, 02 6799 1500 or 0427 480 967. If you would like to be placed on the distribution list for monthly updates on Bt resistance throughout the season, email Sharon.Downes@csiro.au. Requirements for collecting eggs of *Helicoverpa* for the insecticide and Bt monitoring programs are detailed in The Australian Cottongrower Oct-Nov 2004 issue.

Caption 1, Diagram, *insert under or beside figure*

Step 2: If possible, collect a leaf from the plant that the larva was feeding on (rectangle), the plant in the same row that is either side of the host plant (circles), and the plant in the surrounding row that is either side of the host plant (triangles).