Mating of *Helicoverpa armigera* moths in Bollgard II® cotton

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Introduction

Transgenic (Bt) cotton varieties (now based on Bollgard II®, which incorporates the genes for two insecticidal toxins, Cry1Ac and Cry2Ab) currently form the basis of cotton production within Australia, with approximately 80% of the national crop planted last season to Bt cotton. Substantial economic and environmental advantages have resulted from growing Bt cotton, and this is well recognised within the industry (Fitt 2000, 2004). However, development of resistance to Bt in *Helicoverpa* moths, especially *H. armigera* which has already proven capable of developing resistance to several other insecticides, is the most important entomological risk facing the industry as it relies more heavily on Bt technology. The present Resistance Management Plan (RMP) is designed to prevent or delay field-scale resistance (Farrell and Johnson 2005). Mandatory requirements are placed on growers of Bt cotton to provide suitable and attractive refuge crops (no Bt exposure for the insects) as reliable sources of large numbers of Bt susceptible moths that will mate with potentially resistant moths coming from the Bt crops, thus reducing the likelihood of resistance emerging. This approach will assist greatly in delaying resistance provided susceptible moths are consistently produced throughout the cotton season.

Much of CSIRO’s recent research has been directed (and continues to be) towards evaluating the efficacy of refuge crop options (Tann et al 2002, 2005). This research compliments other extensive knowledge of the ecology of *Helicoverpa* spp. that has already been compiled. We need to be sure that refuges are productive sources of *Helicoverpa*, sufficient moth movement is occurring on the landscape, and that moths from different crop sources are inter-mating. Indeed, a primary assumption of the RMP is that mating is random amongst moths originating from separate plant hosts. Bt resistant strains of *H. armigera* have been generated in the laboratory Akhurst et al 2003; Mahon et al 2006) and research conducted by S. Downes, R. Mahon and K. Olsen (see paper this volume) has shown that there are unexpectedly high baseline levels of resistance to Cry2Ab in field populations of *H. punctigera* and *H. armigera*. This potentially puts substantial pressure on Cry1Ac as the remaining toxin that we know is expressed poorly at the end of the season.

Not all refuges will attract significant populations of *Helicoverpa* in all seasons. But refuges do need to be well maintained to provide an opportunity for them to do so. As long as refuges are growing well within a local area, then we anticipate there should be sufficient moth movement to attract the required susceptible moth population to some of them and they then will, we assume, provide large numbers of susceptible moths more broadly across the landscape. This spatial scale of production and movements of moths on the landscape is the focus of our research which is ultimately aimed at recommending strategies to produce effective refuges at minimal cost for growers. In the mean time, growers need to be diligent by ensuring that their designated refuge is well managed and has the potential to consistently produce susceptible moths.

Over recent years, we have used a range of methods in our research on understanding moth movement within, and surrounding, refuge and Bt cotton crops. For example, source crops for moths can be sprayed with a marker such as strontium (a naturally occurring rare metal), by targeting a large established population of small larvae. The larvae ingest the strontium and carry it as a marker in their tissues into adulthood. Distribution of the resultant, emerging marked moths from sprayed crops can then be monitored by making extensive collections across the landscape, both during, and following, the optimal emergence period. The collected moths are analysed for abnormally high levels of strontium, which are indicative of their origins. However, the exact timing of the marker application is crucial and needs to be targeted to a large, single cohort of larvae. Re-capture rates are often poor. In recent years, a greater than previously experienced presence of mixed populations of *Helicoverpa* species has complicated the methodology (in particular, the ease of identifying high populations of *H. armigera*, the species of most interest for resistance development). We have thus sought alternative marking methods to measure landscape movements of *Helicoverpa*.

The use of carbon isotopes as a “natural marker” of *Helicoverpa* has proven very useful in this regard (see below). This short paper presents some preliminary results of carbon isotope analyses that we have conducted using both *H. armigera* pupae collected within a range of different crops and reared to adulthood, and mating moths collected within Bt crops. The research is a follow on from a preliminary study by G. Fitt and P. Gregg (unpublished data) who first recognised the potential for using carbon isotope signatures for identifying the plant host origins of *Helicoverpa* moths in Australia. Others (e.g. Gould et al 2002) have used the technique to identify plant host origins for other moths overseas.

Methods

C3 and C4 plants are so designated because of their different photosynthetic pathways. Such plant groups differ in the relative abundance of naturally occurring carbon isotopes, and they pass such differences on to insect herbivores which feed on them (Smith and Epstein 1971; O’Leary 1988; Ambika et al 2005). In general, C4 plants are tropical plants, or summer growing annuals in temperate regions. The main group of C4 plants are grasses, including sorghum and corn crops. Both of these latter plants are accepted as refuge options for use in concert with Bt cotton (and only support *H. armigera*). There is evidence that some Chenopodeaceae and many herbaceous weed species may also be C4. C3 plants include legumes such as pigeon pea, as well as cotton, sunflower, canola, and safflower. Pigeon pea is a particularly popular choice as a refuge option for use within the RMP for Bt cotton.

Firstly, to verify the carbon isotope designation of different plant types, we collected pupae of *H. armigera* from beneath two C3 (unsprayed cotton and pigeon pea) and two C4 (sorghum and corn) crops in the vicinity of ACRI, Narrabri, northern N.S.W, during the 2005-06 cotton season. We reared these pupae to moths in the laboratory, dried them before they fed, and analysed them individually (4-5 mg of head and a small part of the thorax) for their carbon isotope signatures using specialised mass spectrometry at UNE. We present here data for the first 10 moths analysed in this way from the four crops.

Secondly, at the optimum emergence time as indicated by regular surveys of large populations of *Helicoverpa* larvae in corn and sorghum refuge crops in the vicinity of Wee Waa, we manually collected mating *H. armigera* moths in three Bt cotton crops (Bollgard II®) associated with such refuges, at night using head torches during the 2005-06 cotton season. These moths were analysed for carbon isotope signatures, as above. We present here data thus far compiled for 265 mating pairs of moths.

Results

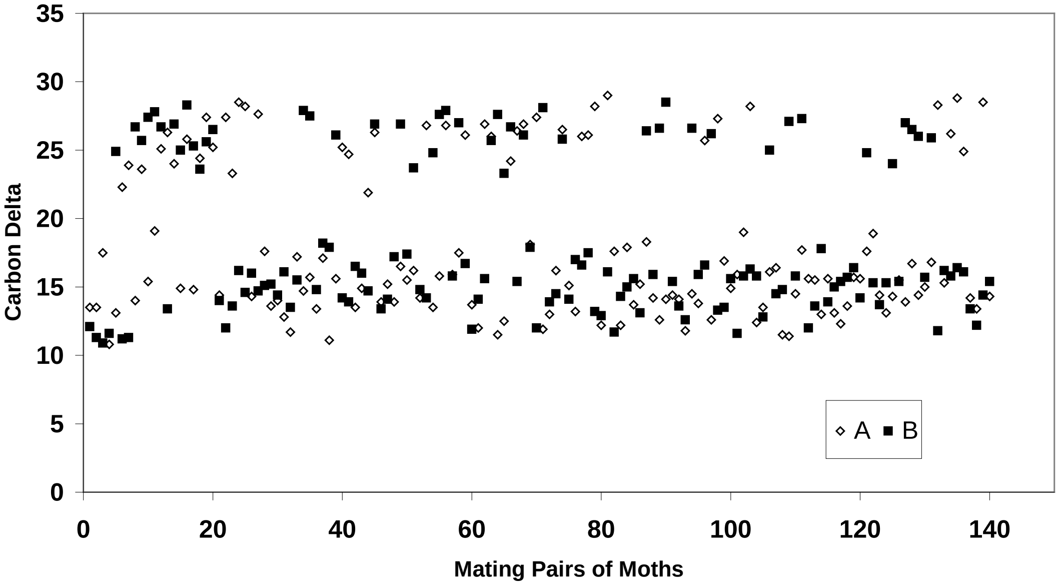
Carbon delta results (mean ± S.E.) varied significantly amongst moths reared from the pupae collected in soil beneath the four crops (unsprayed cotton = -28.79 ± 0.33, pigeon pea = -26.38 ±

0.34, sorghum = -13.24 ± 0.20, corn = -11.45 ± 0.09; One way ANOVA, F = 1153.1, $p < 0.001$). The carbon delta means differed between all field sources (LSD test).

Figure 1 below illustrates carbon delta data for the first 140 mating pairs of *H. armigera* collected and analysed in the three Bollgard II® cotton crops near Wee Waa (i.e. a subset of the total collection). Data are arranged with the two members of each mating pair randomly assigned as 'A' or 'B' and vertically aligned. Clearly, the moths varied in their carbon isotope signatures. Within some mating pairs, carbon delta data were similar for individual moths; within other mating pairs, carbon delta data differed markedly.

If we accept that a carbon delta value < - 20 is indicative of a C3 plant host and a carbon delta value of > - 20 is indicative of a C4 plant host (see results given above for moths reared from pupae collected beneath such plant hosts), then overall we observed 63 matings between moths from C3 sources only, 96 matings between moths from C4 sources only, and 106 matings where moths came from different plant hosts (i.e. C3 x C4 matings), across the 265 mating pairs collected near Wee Waa. If we assume that the mating moths reflect a representative sample of those present in the Bt cotton crops, then we can calculate if the observed matings between moths of C3 and C4 plant origins differ from what we might expect to occur at random (given the relative abundance of such moth types in our catches). From our catches we would expect by chance (i.e. random mating) to have found 50.8 C3 x C3 matings, 130.4 C3 x C4 matings and 83.8 C4 x C4 matings. Chi square analysis suggests that our findings differ significantly from what we would expect through random mating ($\chi^2 = 9.28$, $p < 0.05$). That is, we observed more matings between moths from the same plant origins, and fewer matings between moths from different plant origins than we would have expected by chance.

Figure 1. Carbon isotope analyses of mating pairs of *Helicoverpa armigera* caught over Bollgard II® cotton crops in the vicinity of Wee Waa, NSW, where the associated refuge crop was a C4 plant. A and B represent different individuals within each of 140 mating pairs of moths.



Discussion

A core assumption of the RMP for Bt cotton is that mating between *H. armigera* individuals is random, i.e. irrespective of their plant host origin. In particular, moths generated from refuge crops and Bt cotton should inter-mate freely. However, whilst the work reported here demonstrates that a substantial degree of mating does occur between moths from separate plant sources (and within Bt cotton crops), it also suggests that such mating is not strictly random. It would seem that moths from similar plant host sources are more likely to mate with each other than those from different sources. Mechanisms driving such outcomes are not yet properly understood, but some recently published data (Li et al 2005) from overseas laboratory studies has also suggested such differential mating can occur in *H. armigera*. Whether or not the production and / or fitness of *H. armigera* vary according to the plant host origins of their parent moths, in particular where mixed origin matings occur, is unknown to us.

In other work of our own, we have observed similar trends in our data for mating *H. armigera* moths and their plant host origins, but have not obtained significant differences from what we would expect through random mating. [Our collections of mating moths in these other studies were much smaller than those reported here, and on those occasions we relied on catches of non-mating moths to estimate our expected values for random mating]. For example, at one property in the Gwydir Valley, we collected mating *H. armigera* moths from an Ingard® cotton crop, associated with a sorghum refuge crop, and at another property in the Upper Namoi Valley we collected similarly from a sprayed, conventional crop adjacent to a sorghum crop. In both cases, there was a <u>tendency</u> for enhanced mating of moths from similar plant hosts to occur and reduced mating of moths from different plant hosts than would be expected by chance.

The precise locations of the mating moths we collected in the Bt crops near Wee Waa were not recorded. The mating moths were collected during random walks throughout the cotton fields, up to 1 Km from the dedicated refuge we had earlier been monitoring. In future studies we intend to gather more spatially explicit data (perhaps via GPS) on the incidence of inter-mating of moths from different plant hosts within Bt cotton fields. This should better indicate the efficacy of such mating.

The mating moths we collected near Wee Waa mostly had carbon delta values of either -25 to -28 or -11 to -17. Whilst the former match quite closely with carbon delta data observed for moths emerging from pupae collected under cotton and pigeon pea crops, the latter data was not as closely matched with the carbon delta values collected from moths emerging from pupae collected under sorghum and corn. Whether or not this variation reflects that these field collected moths originated from other C4 plant hosts on the landscape, or some “contamination” through feeding since emergence is unknown.

One of the intriguing results from our work near Wee Waa was the abundance of the mating moths that we collected in the Bollgard II® crops that bore C3 carbon isotope signatures (i.e. 44 % of the mating moths). It seems highly unlikely that these moths originated from the Bollgard II® cotton crops (larvae were rarely seen on these crops). They may have come from other cotton crops in the region, from pigeon pea, or some other unknown source. We intend to analyse the remains of the C3 – signatured moths we have collected (e.g. for gossypol which is a characteristic for cotton), to determine if their origins were cotton or another plant.

Helicoverpa moths are known to mate several times. *H. armigera* moths mate first at about 3 days old and peak in oviposition at 7 days, but the timings of the extra matings, their relationships with the crop origins of the moths and their relevance to egg production are poorly understood. Sperm precedence is known to occur in Lepidoptera (Bissoondath and Wiklund 1997; Higginson et al 2005), but which mating (first, last, neither) takes precedence is variable between species. No information seems to be available for *H. armigera* on this. Against the backdrop of managing Bt resistance and ensuring that matings between moths from refuges and Bt crops are effective, it is desirable to understand how frequently moths are mating in particular habitats and if sperm precedence is occurring. With regard to the latter, we are currently completing experiments in collaboration with S. Downes to determine the existence of sperm precedence in *H. armigera*.

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