1603 Mating of Helicoverpa armigera (Lepidoptera: Noctuidae) moths in relation to their plant hosts as larvae within Australia cotton farming systems

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Transgenic (Bt) cotton, based on the inclusions of genes for Cry1Ac and Cry2Ab toxins, predominates in Australian cotton production systems. Such cotton is grown to reduce feeding damage by lepidopteran pests, especially *Helicoverpa armigera* and *H. punctigera*. The major threat to this farming strategy is that *H. armigera*, which has previously developed resistance to several pesticides, might also become resistant to Bt. Refuge crops (e.g. pigeon pea, maize, sorghum and conventional cotton), which can produce large numbers of Bt susceptible *H. armigera*, are grown in association with Bt cotton to reduce the chances of such resistance developing. A key assumption in this refuge crop strategy is that mating between moths of different crop origin occurs at random. The work reported here used analyses of carbon isotope signatures within moths captured whilst mating within 5 Bt cotton crops and one conventional cotton crop, to identify their host plant origins (i.e. where they fed as larvae) and test if indeed mating appeared to be random according to origin. The results supported the notion of random mating. However, the work was restricted to distinguishing origins from C3 plants (e.g. cotton and pigeon pea) from C4 plants (e.g. sorghum and maize). Different methods will be required to further specify particular plants within these groupings.

Keywords: *Bt resistance, Helicoverpa, host plant origins, random mating*

Introduction

Substantial economic and environmental advantages (e.g. reduced insecticidal sprays) have resulted from the growing of transgenic (Bt) cotton (*Gossypium hirsutum* L.) in Australia (Fitt et al. 1994; Fitt, 2000, 2004), which reduces feeding damage from key pests, *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera : Noctuidae). These transgenic cotton varieties are currently based on Bollgard II®, which incorporates the genes for two insecticidal toxins, Cry1Ac and Cry2Ab. Bollgard II® replaced Ingard® cotton in 2004, which only included the gene for Cry1Ac. Approximately 80% of the national crop is now Bt cotton. However, development of resistance to Bt in *Helicoverpa* moths is the most important entomological risk facing the industry (Fitt, 2000). *H. armigera* has already proven capable of developing resistance to several other insecticides in the field (Forrester, et al. 1993; Fitt, 1994) and to Bt in laboratory cultures (Akhurst, et al. 2003). Recent research has shown that there are unexpectedly high baseline levels of resistance to Cry2Ab in field populations of *H. armigera* (Mahon et al. 2007; Downes et al. 2007). Consequently, a Resistance Management Plan (RMP) has been implemented to prevent or delay field-scale resistance to Bt (Roush, et al. 1998; Farrell, 2006). Part of this plan requires growers of Bt cotton to provide suitable refuge crops (no Bt exposure for the insects) as reliable sources of large numbers of non-selected and thus hopefully Bt susceptible moths that will mate with potentially resistant moths coming from the Bt crops, thus reducing the likelihood of resistance emerging.

The relative production of *Helicoverpa* moths from the various refuge crop options has been identified (Tann, et al. 2002, 2005; G. Baker, C. Tann and G. Fitt, unpublished data), and is accompanied by extensive knowledge of various aspects of the ecology of *Helicoverpa* spp.

in Australian agricultural landscapes (Zalucki et al. 1986, 1994; Fitt, 1989; Fitt and Cotter, 2004; Zalucki and Furlong 2005). However, we need to be sure that sufficient moth movement is occurring on the landscape, and that moths, from different crop sources, are indeed inter-mating. A primary assumption of the RMP is that mating is random amongst moths originating from separate plant hosts.

The use of stable carbon isotopes as natural markers of the host plant upon which larvae of Lepidoptera have fed has proven very useful in tracing the origins of captured moths (Gould et al. 2002). C3 and C4 plants possess 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 (*Sorghum bicolor* (L.)) and maize (*Zea mays* L.). Both of these latter plants are accepted as refuge crop options for use with Bt cotton (and almost exclusively support *H. armigera*, not *H. punctigera*). There is evidence that some Chenopodeaceae and many herbaceous weed species may also be C4. C3 plants include legumes such as pigeon pea (*Cajanus cajan* (L.)), as well as cotton and sunflower (*Helianthus annuus* (L.)). Pigeon pea is a particularly popular choice as a refuge option for use with Bt cotton in Australia, because of its high production of *Helicoverpa* (and hence less area required under the RMP per unit of Bt cotton, compared with other refuge options). In the present study, we analysed *H. armigera* moths, especially pairs of mating moths, captured in cotton fields in northern New South Wales (NSW) and southern Queensland (Qld), Australia, for carbon isotope signatures to identify their likely plant host origins and to determine the mating patterns among moths from different plant host sources.

Materials and Methods

To verify the carbon isotope signatures of *H. armigera* moths that have fed as larvae on different key plant types (cotton and common refuge crops used in Australia), pupae were collected from soil beneath two C3 crops (conventional cotton [n = 69 pupae] and pigeon pea [22]) and two C4 crops (sorghum [25] and maize [57]) in the vicinity of Narrabri, northern NSW. The pupae were reared to moths in the laboratory, dried before feeding, and then analysed individually (4-5 mg of head and a small part of the thorax) for carbon isotopes using mass spectrometry (Carlo Erba M.S.) at the University of New England, NSW. In preliminary work (data not shown here), we found no difference between carbon isotope signatures of the head and the remainder of the body of *H. armigera* moths.

Over 4 consecutive cotton growing seasons, we manually collected *H. armigera* moths at night, both mating and non-mating, within 6 cotton fields using head torches : at Keytah (Gwydir Valley) in 2002-03, Drayton (Upper Namoi Valley) in 2003-04, Shangri-la (Upper Namoi Valley) and South Callandoon (Macintyre Valley) in 2004-05, and Redcamp and Taratan (both Lower Namoi Valley) in 2005-06. Each collection occurred over 1 or 2 nights. These moths were analysed for carbon isotopes as described above.

Statistical tests were applied using Statistix®.

Results

Carbon delta results (mean ± S.E.) varied significantly amongst moths reared from the pupae collected in soil beneath the four crops (C3 plants, conventional cotton = -28.77 ± 0.11 and pigeon pea = -26.82 ± 0.24; C4 plants, sorghum = -13.33 ± 0.09 and maize = -11.72 ± 0.08; One way ANOVA, $F = 5,962.1$, $p < 0.001$). The carbon delta means differed between all field sources (LSD test, $p < 0.05$).

Table 1 provides the frequencies of moths collected at night at each of the 6 sites, in relation to the carbon delta values recorded from carbon isotope analyses. Amongst the 131 moths collected at Keytah, there were 27 mating pairs. Likewise for Drayton there were 199 moths with 30 mating pairs, Shangri-la 130 and 22, South Callandoon 77 and 17, Redcamp 370 and 130, and Taratan 288 and 126. Clear distinctions were apparent in the data sets at most sites between moths bearing carbon delta values similar to those of C3 and C4 plants, such as cotton and pigeon pea and sorghum and maize respectively (a notable exception occurred at Drayton where two peaks in the data were not obvious).

Based on the assumption that a carbon delta value of < -20 is indicative of a moth arising from a C3 plant host and a carbon delta equal to or > -20 is indicative of a moth resulting from a C4 plant host, Table 2 indicates the observed frequencies of matings between moths 1) where both were of C3 origin, 2) where both were of C4 origin, and 3) where one moth was of C3 origin and the other of C4 origin, for each of the 6 cotton fields. Expected occurrences of such matings were generated from the total captures (mating and single moths) in each field, again taking a carbon delta of -20 as the discriminating value. The results of Multinomial Tests are included in Table 2. In all cases the frequencies of observed mating classes were not significantly different from what would have been expected if mating occurred at random, although the results for Redcamp were very nearly so.

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. 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 likely to be at random, with the possible exception at Redcamp where results were marginal. However, Li et al. (2005) have suggested differential mating, according to plant host origin, can occur in *H. armigera,* based on laboratory studies. Whether or not the production and / or fitness of *H. armigera* offspring might vary according to the plant host origins of their parent moths, in particular where mixed origin matings occur, is however unknown.

The precise locations of the mating moths we collected in the Bt crops were not recorded. The mating moths were collected during random walks throughout the cotton fields. In future studies we intend to gather more spatially explicit data 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 had carbon delta values which varied between sites. Sometimes we collected moths with carbon deltas which resembled those we found in moths reared from pupae dug up from under particular crops (e.g. there was a peak in carbon delta frequency in the Redcamp moths which closely matched what was found for "sorghum" moths (i.e. carbon deltas of approximately -13), but rarely were moths collected

that matched those from cotton crops (i.e. mean of -28). Whether or not such variation reflected true rarity of moths from some host plant origins, the inclusion of some moths from plant hosts other than those we collected pupae beneath which then "blurred" profiles in the data sets, or "contamination" of carbon delta signatures through moth feeding following emergence is unknown.

Whilst the carbon delta data from moths of known plant host origin suggested capacity to discriminate cotton from pigeon pea origins, in practice we saw no opportunity to discern such origins in the moths we captured over Bollgard II® crops. We are exploring different methods to discern such origins. This is particularly important given the widespread use of pigeon pea crops as refuges.

Redcamp and Taratan are approximately 15 km apart in the Lower Namoi Valley in northern NSW. It seems highly unlikely that the moths caught at these sites, with C3 carbon isotope signatures (< -20), originated from the local Bollgard II® crops (*Helicoverpa* larvae were rarely seen on these crops; C. Tann unpublished data). On the other hand, the Redcamp site had crops of sorghum, maize, sunflowers and unsprayed conventional cotton nearby which did have large numbers of *Helicoverpa* larvae on them in advance of the moth collections. These crops could have provided the moths of both C3 and C4 origin caught over the Bollgard II® crop at Redcamp. In contrast, the Taratan site only had pigeon pea refuge crops in the near vicinity, and these did not appear to be producing many *Helicoverpa* larvae. A maize crop, approximately 1 km from the Bollgard II® crop at Taratan did however have large numbers of *Helicoverpa* larvae on it. The captured moths at Taratan, with a bias of C4 carbon isotope signatures, could perhaps have been from that maize crop, 1 km away.

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. Research on the latter is currently underway.

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Table 1. Frequency of *Helicoverpa armigera* moths with different carbon delta values, collected at night over cotton fields (Conventional, Ingard® or Bollgard II®) at six sites in northern NSW and southern Qld.

Carbon Delta	Keytah 2002-03 [Ingard®]	Drayton 2003-04 [Conventional®]	Shangri-la 2004-05 [Bollgard II®]	South Callandoon 2004-05 [Bollgard II®]	Redcamp 2005-06 [Bollgard II®]	Taratan 2005-06 [Bollgard II®]
-8		1	1	3		
-9		1		5		
-10	5	4	1	6	4	
-11	7	5	7	17	11	18
-12	15	4	18	16	32	16
-13	8	12	10	4	38	38
-14	6	20	12	4	31	41
-15	3	15	7	3	19	50
-16	3	17	11	3	9	25
-17	3	3	6		2	15
-18	3	15	3	2	5	6
-19	3	11	3		2	1
-20	4	9	3		1	
-21	8	17	7			1
-22	4	9	4		4	1
-23	16	7	1	4	20	4
-24	25	10	8	3	26	10
-25	16	9	16	4	58	15
-26	2	7	9	1	42	22
-27		6	3	2	37	13
-28		4			21	11
-29		1			6	
-30		5			2	1
-31		2				
-32		2				
-33		2				
-34		1				
-35						

Table 2. Observed incidence of pairs of mating *Helicoverpa armigera* moths with different carbon delta values, collected at night over cotton fields (Conventional, Ingard® or Bollgard II®) at six sites in northern NSW and southern Qld. Multinomial test results are included. Expected values (based on frequencies of moths with carbon deltas < or > -20) are included in parentheses.

Site	Carbon Delta		
	Both Moths < -20 C3 x C3	Both Moths > -20 C4 x C4	Mix of < and > -20 C3 x C4
Keytah (2002-03) $\chi^2 = 2.74$, P > 0.05	11 (8.9)	7 (4.9)	9 (13.2)
Drayton (2002-03) $\chi^2 = 0.52$, P > 0.05	7 (6.3)	10 (8.8)	13 (14.9)
Shangri-la (2004-05) $\chi^2 = 2.30$, P > 0.05	2 (3.4)	6 (8.1)	14 (10.5)
South Callandoon (2004-05) $\chi^2 = 0.59$, P > 0.05	0 (0.6)	12 (11.4)	5 (5.0)
Redcamp (2005-06) $\chi^2 = 5.68$, P = 0.059	57 (44.7)	22 (22.2)	51 (63.1)
Taratan (2005-06) $\chi^2 = 0.64$, P > 0.05	7 (9.2)	67 (67.0)	52 (49.8)