Oviposition site selection and survival of susceptible and resistant larvae of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Bt and non-Bt cotton

T.T.A. Luong[1]*, S.J. Downes[2], B. Cribb[1,3], L.E. Perkins[1] and M.P. Zalucki[1]

[1]School of Biological Sciences, The University of Queensland, Brisbane 4072, Australia: [2]CSIRO Agriculture, Australian Cotton Research Institute, Narrabri 2390, Australia: [3]Centre for Microscopy & Microanalysis, The University of Queensland, Brisbane 4072, Australia

Abstract

In Australia Bt cotton has been planted since 1996, and has greatly improved the control of its key target *Helicoverpa armigera* (Hübner). There is no strong evidence that genetically modified cotton has been selected for significant physiological resistance to Bt toxin in field populations. There are many possible explanations for the lack of apparent selection that range from high compliance with the resistance management strategy for this technology to a lack of behavioral preference in key traits such as oviposition that could favor survival. To date most experiments that test oviposition of *H. armigera* on Bt cotton vs. conventional cotton have been done with susceptible moths. We determine the oviposition preference of a field isolated Bt resistant line of *H. armigera* and a susceptible counterpart when given a choice of non-Bt cotton and Bt-cotton with the same genetic background, and test whether there is any relationship between oviposition site selection (different plant structures) and the survival of the first instar larvae. Within cotton plants, our experiments consistently showed that both resistant and susceptible moths did not choose plants or plant parts that were less toxic in terms of Bt toxin on which to lay eggs. There was one exception in that susceptible moths were more likely to lay eggs on squares of Bt cotton plants than squares of non-Bt cotton. As expected, the mortality of susceptible *H. armigera* neonates was significantly higher on structures of Bt cotton plants than on those structures of conventional cotton, and survival was greater on flowers than on other structures of Bt cotton. This confirms opportunities for selection for resistance, and demonstrates no advantage in this respect to carrying resistance genes that might overcome the Bt toxins.

Keywords: oviposition preference, larval survival, behavioral resistance, resistant, susceptible

*Author for correspondence
Phone: +84 942 474 474
Fax: +84 573 841 214
E-mail: luong.tuyet@pyu.edu.vn

Introduction

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is an economically important polyphagous pest that causes major damage and poor yields to a range of agricultural crops (Zalucki *et al.*, 1986, 1994), particularly cotton, maize,

legumes and tomato (Sharma, 2005). To control this pest chemical pesticides have been used extensively, but they cause undesirable side effects including resistant insects (Fitt, 2003). The management of pesticide resistance is now a necessity when both chemical sprays and crops engineered to express toxins are used (Forrester et al., 1994; Tabashnik et al., 2005, 2009; Tabashnik & Carriére, 2010; Zhang et al., 2011). In Australia, cotton genetically modified to express the delta-endotoxin genes of Bacillus thuringiensis subsp. kurstaki (Bt) has been planted since the mid-1990s to control H. armigera and the native Helicoverpa punctigera (Wallengren), and has reduced insecticide usage from 30%, for the first generation single toxin varieties (Fitt, 2008), to 85% for the second generation dual toxin varieties (Wilson et al., 2013). Bt cotton was quickly adopted by growers and now comprises nearly 90% of all cotton crops (Zalucki et al., 2009). Although it has greatly improved the control of H. armigera, the threat of an increasing frequency of insects that are physiologically resistant to the toxins remains (Downes et al., 2010).

The effects of the widespread planting of Bt cotton on H. armigera biology and ecology are generally unknown and potentially large. This major crop has become an effective population sink (Rochester et al., 2002; Baker et al., 2016). Zalucki et al. (2012) showed that there was no evidence that host selection by H. armigera among tobacco, conventional cotton, and cabbage had changed since the widespread adoption of genetically modified cotton in Australia. However, Zalucki et al. (2012) carried out their experiments with susceptible moths, and did not include Bt cotton in their oviposition assays. In the USA, Torres & Ruberson (2006) suggested that Heliothis virescens (Fabricius) (Noctuidae) and Helicoverpa zea (Boddie) (Noctuidae) did not show an oviposition preference when offered non-Bt cotton and Bt cotton. They found a similar spatial distribution of eggs within plants between the two types of cotton in both pest species suggesting that moths had not changed their oviposition behavior despite variation in toxin concentration amongst structures within Bt cotton plants. Kumar & Stanley (2010) found no discrimination by H. armigera moths between Bt and non-Bt cotton in a field study in India. Nevertheless, there is evidence that H. armigera shows behavioral avoidance through oviposition site selection that effectively circumvents exposure to toxin on Bt cotton plants (Men et al., 2005; Liu et al., 2010). In China there is evidence for oviposition non-preference for Bt cotton, which would reduce selection for physiological resistance (Zhao et al., 2016). Moths preferred to oviposit on non-Bt cotton in mixed plantings of Bt and non-Bt cotton (Liu et al., 2010). In Gujarat, India, egg density was reduced with increasing Bt cotton patch size (Lodaya & Borad, 2014). The total number of eggs deposited on conventional cotton plants was about 95% greater than that on Bt cotton plants at bud–flower stage and flower–boll stage (Lodaya & Borad, 2014).

Extensive work on various aspects of oviposition behavior has been conducted on Australian populations of H. armigera (reviewed in Cunningham & Zalucki, 2014). However to date those experiments were carried out using susceptible H. armigera moths. Experiments that compare oviposition behavior of H. armigera moths that are physiologically resistant vs. susceptible to Bt cotton will clarify an important potential impact on resistance evolution: whether shifts in adult and larval behavior exist that might lead to differential survival of these genotypes on Bt cotton plants.

Here we determine the oviposition preference of H. armigera from colonies that are resistant and susceptible to Bt-toxin when given a choice of Bt and non-Bt cotton plants by asking: (1) whether females choose oviposition sites randomly or lay more eggs on plants (e.g. non-Bt cotton) that are potentially less toxic; we also ask (2) whether moths from the different strains choose less toxic parts within plants with respect to Bt expression. We base plant part toxicity on a review of published data. Finally we determine (3) whether there is any relationship between oviposition sites and the survival of the first instar larvae of the two H. armigera strains (susceptible and resistant) on different plant structures (young leaf, mature leaf, stem, square and flower) on both Bt cotton and non-Bt cotton. Survival experiments were examined using laboratory assays.

Materials and methods

Insects

The H. armigera Bt-resistant strain used in this study (SP15) was established from a single mating pair collected as eggs on corn near Griffith, NSW, in December 2002. Progeny from the pair were subjected to an F_2 screen (Andow & Alstad, 1998) and the SP15 colony was formed from F_2 offspring that survived a discriminating dose (LD 95) (1 ug cm^{-2}) of Cry2Ab (Mahon et al., 2007). The F_2 screens were performed with the specific intention of detecting resistance to Cry toxins in H. armigera. SP15 initially possessed a very restricted gene pool as it originated from a single isofemale line. Lepidopteran colonies suffer severe inbreeding depression rapidly leading to a loss of vigor that strongly influences the outcome of bioassays. Consequently, over the years since its isolation SP15 has been outcrossed to the susceptible strain, GR, numerous times, to maintain fitness and to produce a strain that is near isogenic with the susceptible strain (Mahon et al., 2007). Following each outcross, the colony was maintained without selection for one generation and then re-selected with 1–2 ug cm^{-2} Cry2Ab toxin as a diet surface treatment. Dried and ground corn (Zea mays L.) leaf material was used as a source of Cry2Ab toxin. Corn powder was provided by Monsanto (St Louis, USA) as a lyophilized leaf powder. This powder contained the transgenically expressed B. thuringiensis crystal protein Cry2Ab, at a concentration of 6 mg g^{-1} powder (Mahon et al., 2007). Toxin in the leaf was calibrated using an enzyme-linked immune-sorbent assay (ELISA) method on aliquots of leaf material after freeze-drying and homogenization. ELISA methods and protein extraction are detailed in Holt et al., (2002). All subsequent generations were selected at this dose. Moths used to establish a susceptible H. armigera colony were collected from the field from a range of crops such as chickpea, pigeon pea, cotton, etc., and bulk mated to form a colony. All colonies were maintained at the Australian Cotton Research Institute, Narrabri, New South Wales.

Resistant (SP15) and susceptible (GR) eggs of H. armigera were transferred to the laboratory of the School of Biological Science at The University of Queensland, Australia to establish colonies for experiments. Neonates were individually reared on artificial diet (modified from Teakle & Jensen, 1985) until the 3rd-instar stage in 45-well plastic trays (circular well: 23 mm in diameter, 18 mm in height; Tacca Plastics Australia Pty Ltd., Moorebank, NSW, Australia) at which time they were transferred to 32-well plastic trays (square well: 30 mm in width, 20 mm in height; Tacca Plastics Australia Pty Ltd.). The larvae remained in the 32-well trays

during pupation. Rearing trays were covered and heat-sealed with a perforated lid. Male and female pupae were separated and each sex was housed in bulk in vermiculite in an incubator at 25°C (±1) and 80% relative humidity (RH) (±1) to ensure synchronous adult emergence. Fifteen male and fifteen female moths were released together in each plastic holding container (20 cm in width × 20 cm in length × 30 cm in height) covered securely with polypropylene nappy liners as a substrate for egg laying. Moths were provided with a pot (∼30 ml) of 10% honey/sugar solution fed through a cotton wick as a food source.

The susceptible and resistant colonies were tested at least monthly for their responses to Bt toxin as part of a program monitoring populations for resistance. Only families that were confirmed to not be resistant by screening were incorporated into the susceptible colony.

Plants

A transgenic cotton cultivar containing Cry genes and a conventional cotton variety with the same genetic background as the transgenic variety but without the Cry genes were used in experiments. Conventional cotton (Sicot 71 R: hereafter 'non-Bt') and Bollgard II® cotton (Sicot 71 BR: hereafter 'Bt cotton') were used to test oviposition preference. A pool of plants was prepared as follows. Three seeds were sown in each pot (30 cm in height and 25 cm in diameter) in UC soil mix (a mixture of sand, bark and peat moss). After 2-weeks the healthiest seedling was retained and the others removed by hand. Plants were maintained in a greenhouse at The University of Queensland at 24 ± 6°C and 56 ± 10% RH. All plants were watered three times a week and supplied with a general purpose soluble fertilizer (Thrive 16: 9: 12: 2 MgO) every 4 weeks. Similar sized (50–60 cm in height) plants at the same stage of development: open flowers, squares and bolls present, were used in oviposition choice tests.

Oviposition preference

Experiments were run four times from December 2012 to April 2014 with eight cages (replicates) in total for each of two strains (table 1). There were 108 cotton plants used for each larval strain and more than 240 female moths used in total. Experiments were staged as follows: December 2012 for replicates 1 and 2, July 2013 for replicate 3, November 2013 for replicates 4, 5, and 6, and April 2014 for replicates 7 and 8. One day after eggs started to be laid in holding containers, each group of 30 moths (15 males and 15 females) were released into a large cage (180 cm × 180 cm × 180 cm), which contained cotton plants in a glasshouse. There were 12–16 plants in each cage; 6–8 Bt cotton and 6–8 non-Bt cotton plants arranged randomly. Pots with 10% sucrose-solution were placed in a plastic box with water within the cage to supply additional nutrients for the insects. Moths were acclimated to the cage environment and allowed to lay eggs for two nights. On the second day, moth survival was checked. If moths had died, more moths were added from the laboratory culture to keep a total of 30 moths (15 males and 15 females) in each cage. All eggs laid within two nights were removed from plants so that they would be clean to use in experiments on the following day. The location of plants was re-randomized daily to reduce position effects. Eggs were counted once, on the next day. The number of eggs on each plant structure: immature leaf, mature leaf, stem, square, or flower was counted for 1 day.

In the third replicate (July 2013) plants of two varieties (Bt and non-Bt cotton) were not synchronous in their development. Although plants across treatments were the same height, those that were Bt cotton had 3–4 nodes more than those that were non-Bt cotton. In this experiment, across both insect genotypes the number of eggs laid on Bt cotton (355 eggs plant^{-1}) was significantly higher than the number laid on non-Bt cotton (143 eggs plant^{-1}) (table 1). Thus, this third replicate was not used further in statistical analysis. For all other experiments plants were of similar height and at the same developmental stage.

Survival of newly hatched larvae

Experiments were performed in a temperature-controlled room set at 25 ± 1°C and with a natural light cycle. Neonates were placed in round plastic containers (120 mm diameter ×-98 mm height) with each plant structure to assess the survival of newly hatched larvae. Each container had a small pot (height 33 mm, top diameter 44 mm, base diameter 31 mm) with water to keep the plant parts fresh for 2 days. A hole was made in the lid of each small pot and the stem attached to the plant part inserted. Large containers were open at the top and covered with polypropylene nappy liners secured around their lids for air circulation. There were four treatments (young leaf, mature leaf, square and flower) for each strain (SP15 and GR) with each cotton line (Bt and non-Bt). Ten neonates were introduced into each treatment and left for 2 days. Ten replicate experiments were performed for each plant part of cotton line and larval strain. After 2 days, surviving larvae were counted and introduced to artificial diet to test their survival after a further 4 days (i.e., to 6 days of age).

Data analysis

Statistical analyses were performed using the Statgraphic Century procedure, version 15.1 (Statpoint Technologies, Inc., Washington D.C., USA). As total egg numbers varied greatly between cages (see the section Results), the data were expressed as percentage of the total egg lay on each plant. All data recorded as a percentage were arcsin-transformed to correct heterogeneity of variances prior to analysis using an analysis of variance (ANOVA) with strains and plant lines as main effects. Differences in the distribution of eggs between the two moth strains on parts within Bt vs. non-Bt cotton plants was analyzed using Wilcoxon Signed-Rank Tests. Oviposition preference and larval survival between Bt and conventional cotton of the two moth strains were analyzed using a two-way ANOVA. The mean percentage of oviposition preference on different plant structures and the mean percentages of larval survival on different positions of plant were analyzed with Tukey's Multiple Range Tests.

Results

Oviposition preference

Data for 7 out of the 8 replicates were used in the analysis of preference since in one of the replicates the plants were at different phonological stages (see the section Methods). There were no significant differences in oviposition percentages between resistant and susceptible moths (df = 1, $F = 1.89$,

Table 1. The mean (±SE) of eggs per plant ($n = 6$ or 8) laid by resistant or susceptible female moths on Bt cotton and non-Bt cotton plants in each cage ($n = 8$) from December 2012 to April 2014.

Strain	Cotton	Cage								
		1	2	3	4	5	6	7	8	Mean (±SE)
Resistant	Bt	53 ± 6a	21 ± 3a	355 ± 40a	71 ± 7a	10 ± 3a	4 ± 2a	14 ± 2a	143 ± 29a	84 ± 42a
	N-Bt	38 ± 5a	19 ± 2a	143 ± 21b	68 ± 11a	14 ± 5a	12 ± 6a	10 ± 4a	147 ± 32a	56 ± 21a
Susceptible	Bt	22 ± 12b	64 ± 10b	130 ± 11c	8 ± 2b	51 ± 14b	57 ± 16b	16 ± 3a	40 ± 11b	48 ± 13b
	N-Bt	18 ± 5b	72 ± 8b	88 ± 11d	7 ± 11b	40 ± 11b	54 ± 16b	19 ± 3a	75 ± 13b	47 ± 11b

Means within a column followed by same letter are not significantly different ($\alpha = 0.05$, Tukey's Multiple Range Test). The replicate with plants at different growth stages is highlighted.

$P = 0.17$). Both resistant and susceptible moths did not discriminate between Bt and non-Bt cotton (resistant: df = 1, $F = 0$, $P = 0.97$; susceptible: df = 1, $F = 0.29$, $P = 0.59$). The percentage of eggs that resistant moths laid on Bt cotton plants was equal to those on non-Bt cotton plants (50% on each). The results were similar with susceptible moths (48% on Bt cotton and 52% on non-Bt cotton) (fig. 1).

There was no difference in egg distribution of resistant moths with respect to parts within a plant for Bt and non-Bt cotton; young leaf ($t = 0.84$, $P = 0.40$), mature leaf ($t = 0.09$, $P = 0.92$), square ($t = -0.52$, $P = 0.60$), stem ($t = -1.34$, $P = 0.18$), and flower ($t = 1.17$, $P = 0.24$). Oviposition of susceptible moths was not different between Bt and non-Bt cotton on most structures of cotton plants including young leaves (0.84, $P = 0.40$), mature leaves ($t = -1.31$, $P = 0.19$), stems ($t = 0.18$, $P = 0.85$), and flowers ($t = -1.41$, $P = 0.16$). The exception was that susceptible moths laid significantly more eggs on squares of Bt cotton compared with non-Bt cotton plants ($t = 2.21$, $P = 0.03$; 8 and 5%, respectively).

Survival

Two-day survival

There was no effect of cotton plant line (Bt vs. non-Bt) on survival of resistant neonates amongst plant structures: young leaf (df = 1, 1 $F = 1.92$, $P = 0.18$), mature leaf (df = 1, 1, $F = 3.44$, $P = 0.08$), square (df = 1, 1, $F = 0.83$, $P = 0.37$), and flower (df = 1, 1, $F = 0.00$, $P = 1.00$) after 2 days. Overall survival of resistant larvae was 75% on Bt cotton and 81% on non-Bt cotton plants. However, cotton line did have a significant effect on the survival of susceptible neonates; 59% of larvae survived on Bt cotton for 2 days in comparison with 81% on non-Bt cotton. The survival of susceptible larvae was significantly different on young leaves (df = 1, 1 $F = 22.81$, $P = 0.0002$), mature leaves (df = 1, 1, $F = 19.55$, $P = 0.0004$), and squares (df = 1, 1, $F = 11.52$, $P = 0.004$) of the two cotton lines (fig. 2). In contrast, there was no significant difference in percentages of susceptible neonates surviving on the flowers of Bt vs. non-Bt cotton (df = 1, 1, $F = 3.38$, $P = 0.08$). Larval survival on flowers was significantly higher than on young leaves, mature leaves and squares (df = 3, $F = 3.62$, $P = 0.02$) of Bt cotton plants. In general, the survival of resistant and susceptible larvae on non-Bt cotton was similar. Resistant larvae, however, survived better on Bt cotton plants than susceptible larvae, and there was no difference amongst plant parts or cotton lines. For susceptible larvae survival was generally poorer on Bt cotton but was better on squares and flowers than other Bt cotton structures (fig. 2).

Six-day survival

The larvae alive after 2 days exposure to plant parts were moved onto artificial diet and assessed again at 6 days of age. The cotton line that larvae were initially exposed to did not affect the survival of resistant larvae after a further 4 days on diet. The percentage of resistant larvae surviving on non-Bt cotton structures appeared higher than that on Bt cotton plant parts (63 and 59%, respectively), but was not significantly different (df = 1, $F = 0.40$, $P = 0.59$). There was no significant difference between Bt and non-Bt cotton in the number of resistant larvae surviving on diet after being exposed to cotton plant structures: young leaf (df = 1, $F = 2.59$, $P = 0.25$), mature leaf (df = 1, $F = 5.49$, $P = 0.94$), square (df = 1, $F = 0.01$, $P = 0.94$), and flower (df = 1, $F = 0.54$, $P = 0.54$).

After 4 days on diet, the percentages of susceptible larvae surviving declined sharply after initial exposure to several plant structures of Bt cotton plants: young leaves (from 50 to 25%), mature leaves (from 53 to 25%) and squares (from 63 to 38%) but less so on flowers (from 70 to 47%). These differences were statistically significant among plant structures of Bt cotton plants (df = 3, 36, $F = 3.62$, $P = 0.02$) (fig. 3). There was no significant difference in survival of susceptible larvae on diet after 4 days after initial exposure to non-Bt cotton structures (df = 3, 35, $F = 0.52$, $P = 0.67$) and their average survival decreased by about 14% after 4 days, similar to resistant larvae. The number of surviving susceptible larvae after 4 days on diet was significantly different after initial exposure to Bt vs. non-Bt cotton plant parts: young leaves (df = 1, $F = 835$, $P = 0.001$), mature leaves (df = 1, $F = 65.11$, $P = 0.02$), squares (df = 1, $F = 25.14$, $P = 0.04$); the exception was flowers (df = 1, $F = 15.38$, $P = 0.06$).

As expected, after 4 days on diet susceptible larvae initially exposed to Bt cotton structures had lower survival than those initially exposed to non-Bt cotton and survival was higher on squares and flowers of Bt cotton. The average survival of susceptible larvae decreased by 25–28% on young leaves, mature leaves and squares of Bt cotton. By contrast, the overall survival of resistant larvae amongst structures on Bt and non-Bt cotton plants was similar after 2 days and 6 days.

Discussion

The oviposition experiments were run over a 2-year period in different weather condition, thus the numbers of eggs per plant were highly variable. The results of oviposition preference experiments showed that resistant and susceptible moths behaved similarly when laying eggs on Bt and non-Bt cotton. Both Bt and conventional cotton with the same genetic
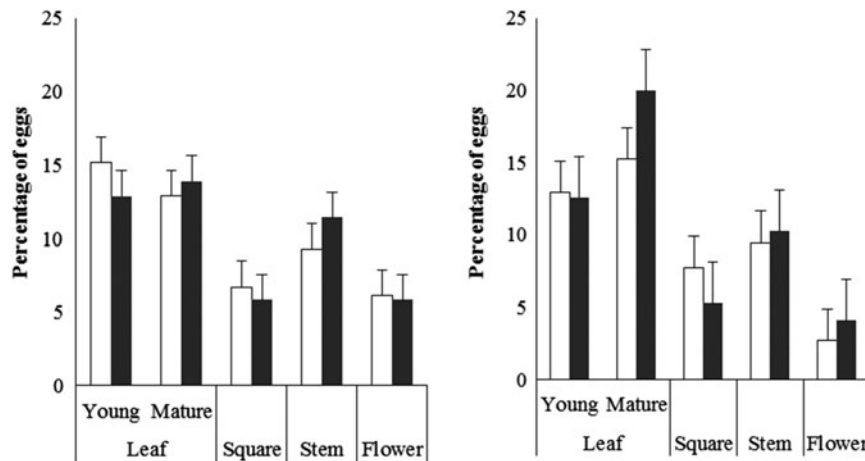
Fig. 1. Mean percentages (±SE) of eggs that resistant (left) and susceptible (right) female moths laid on different plant structures on Bt cotton (white bars) and non-Bt cotton (black bars) plants.
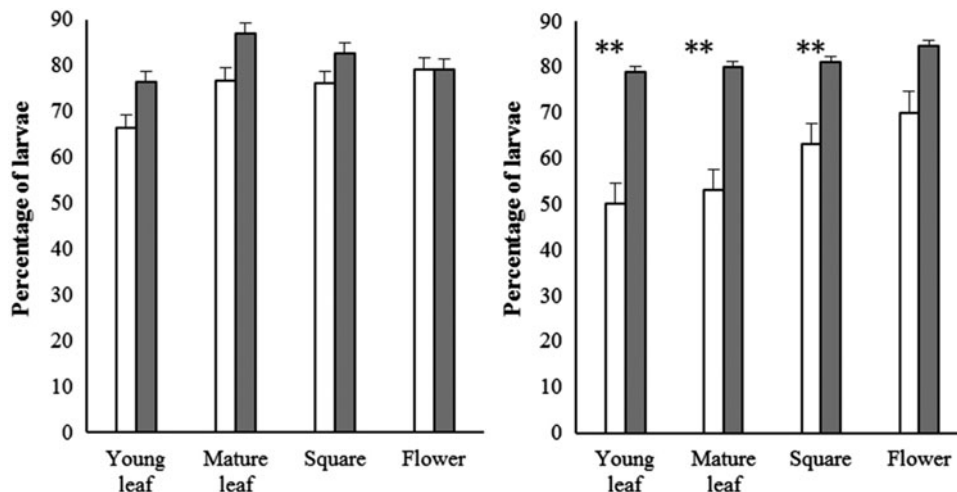

Fig. 2. Mean percentages (±SE) of resistant (left) and susceptible (right) larvae that survived on plant parts (young leaf, mature leaf, square, and flower) of Bt cotton (white) and non-Bt cotton (grey) after 2 days. Asterisks identified the significant difference in survival of susceptible neonates on flowers between Bt cotton and non-Bt cotton.

background were equally likely to receive eggs. The percentages of eggs that resistant and susceptible moths laid on Bt and non-Bt cotton were not significantly different suggesting that the two moth strains did not discriminate between Bt and non-Bt cotton plants or could distinguish them; they did not prefer one over the other. Stage of plant is important when making comparisons (Firempong & Zalucki, 1990). In one of our experiments, Bt cotton was further developed with more flower/fruiting structures, and even though both plant types were similar in height, the Bt plants received more eggs (see the section Methods). The result highlights that assays assessing host preference for Bt vs. non-Bt cotton must be carefully controlled.

Within cotton plants, we found that females consistently chose oviposition sites with little regard to the toxicity of plant parts. Most eggs were laid on leaves, especially young leaves, which are more toxic than squares on Bt cotton (Greenplate, 1999; Adamczyk *et al.*, 2001a; Gore *et al.*, 2001).

The results of these experiments corroborated previous studies (Zalucki *et al.*, 1986; Jallow *et al.*, 1999), which showed that *Helicoverpa* spp. moths preferred to lay eggs on rough or hairy surfaces (young leaves are usually the hairiest). Pink bollworm, *Pectinophora gossypiella* (Saunders) from susceptible and resistant strains also did not discriminate between Bt and non-Bt cotton bolls indicating that oviposition was independent of susceptibility to Cry1Ac (Liu *et al.*, 2002). Torres & Ruberson (2006) found that the numbers of *Helicoverpa* spp. eggs per plant were similar on both cotton types (Bt and non-Bt cotton), and on average approximately 80–95% of eggs were laid on top nodes of both types of cotton. The percentages of eggs on squares, stems and flowers were not significantly different. These results confirmed that both resistant and susceptible moths generally did not choose plant parts that were less toxic in terms of Bt toxin on which to lay eggs. There was an exception; the percentages of eggs from susceptible moths on squares were significantly different
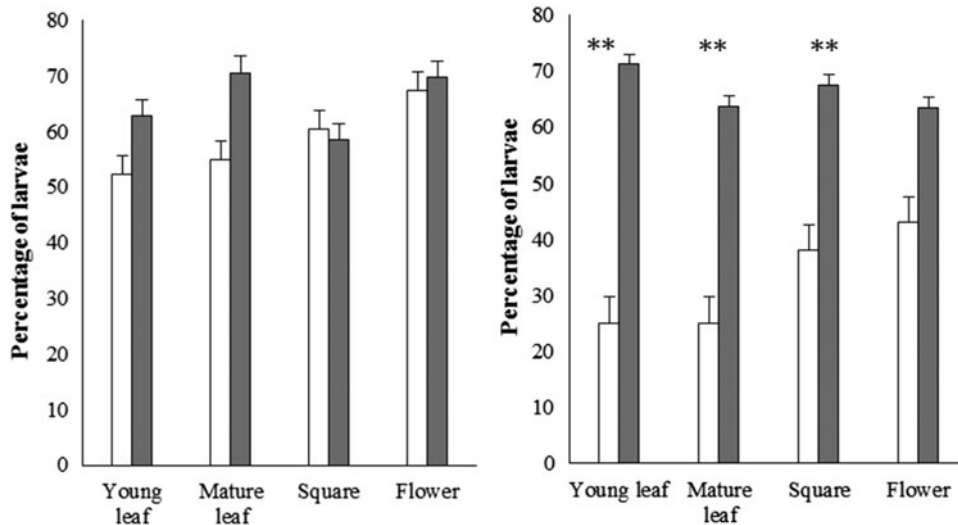
Fig. 3. Mean percentages (±SE) of resistant (left) and susceptible (right) larvae that survived on artificial diet after 6 days; 2 days on plant parts (young leaf, mature leaf, square, and flower) of Bt cotton (white) and non-Bt cotton (grey) followed by 4 days artificial diet. Asterisks identified the significant difference in survival of susceptible neonates on flowers between Bt cotton and non-Bt cotton.

between Bt and non-Bt cotton plants. Significantly more eggs were laid on squares of Bt cotton than squares of non-Bt cotton and could offer a chance for susceptible larvae to survive on Bt cotton plants since these sites become flowers that are known to express less toxin (Gore *et al.*, 2001).

As expected, data from the larval survival assay demonstrates that mortality of susceptible *H. armigera* neonates is significantly higher on different structures (young leaves, mature leaves, and squares) of Bt cotton plants compared with non-Bt cotton. These results are consistent with previous research (Gore *et al.*, 2001; Kranthi *et al.*, 2005; Arshad *et al.*, 2009; Lu, 2010). The survival of *H. zea* larvae has been found to be higher on squares and flower anthers than on other floral structures on non-Bt and Bollgard II® cotton (Gore *et al.*, 2001; Kranthi *et al.*, 2005). Arshad *et al.* (2009) showed a significantly higher mortality in neonates fed on Bt cotton leaves than those fed on Bt flower-bolls. At the very small larval stage, susceptible larvae were more likely to be found alive on flowers of Bollgard II® cotton suggesting these plant components are likely to have relatively low levels of Bt toxin (Yang *et al.*, 2008). The apparent preference for flowers by susceptible larvae may be related to the expression of Cry proteins between structures. Levels of Cry1Ac expression in Bollgard® cotton plants are typically higher on vegetative tissues compared with floral structures, such as pollen and flower petals (Greenplate, 1999; Adamczyk *et al.*, 2001*b*; Gore *et al.*, 2001). In Australian growing conditions, Lu (2010) confirmed that levels of Cry1Ac expression were highest in leaves and lower in squares and flowers; but Bt toxin was not uniform within square and flower parts; the expression of Cry 1Ac was highest in bracts followed by petals, and lowest in anthers. The fact that flowers overall expressed low levels of Bt toxin was supported by the finding in our study that there was no significant difference in percentages of susceptible neonates surviving on the flowers of Bt vs. non-Bt cotton. Low levels of expression in Bollgard II® cotton squares and flowers offer an opportunity for neonates to survive if they are able to move and find plant parts with low levels of Bt toxin. Given the oviposition pattern of moths, which would result in more eggs being laid on squares,

hatching larvae could survive better than expected at the flowering stage if they fed on such plant parts.

In general, adults of both strains did not differentiate between and within plant structures with respect to Bt-expression, except for susceptible moths laying more eggs on squares. Depositing a higher percentage of eggs on squares may lead to higher survival of larvae on flowers as they develop. Fifteen per cent of eggs were laid on Bt squares. Seventy per cent of those could survive on Bt flowers after 2 days and these larvae continued to survive at a high rate (43%) after 4 days when moved onto artificial diet with no toxin. In total, 6–7% of larvae could survive on Bt cotton if they found a non-toxic or less-toxic food at the first instar stage. Current thresholds for chemical control of Bt cotton are two small larvae (>3 mm) per meter or one medium larva (>8 mm) per meter (Fitt, 2003). If *H. armigera* female moths laid 50 eggs plant^{-1}, it is predicted that three larvae could survive on Bt cotton plants, therefore reaching the threshold for chemical spraying. Since the adoption of Bt cotton in Australia, the number of egg laid per plant has declined sharply and 50 eggs plant^{-1} is unlikely. But a combination of locally high egg load, at the right plant stage (squaring cotton), and larval movement may in part account for the 'high' level of non-resistant larvae seen occasionally. Lu *et al.* (2011) also suggested that *H. armigera* larvae movement might be an important part of the reason for survival. Their chances of survival on Bt cotton would increase if they moved away from plant parts with high toxin levels, such as leaves, to more suitable food, for example flowers (see Yang *et al.*, 2008). Larvae may establish on plants with poorer expression before moving onto higher expression areas (Lu *et al.*, 2011).

In conclusion, resistant and susceptible *H. armigera* female moths from Australia did not show differences in oviposition between Bt and non-Bt cotton plants in glasshouse experiments. Resistant and susceptible moths show broadly similar oviposition behaviour with some minor differences. In addition, the fact that some 20% of susceptible larvae survived 2 days exposure on Bt cotton plant parts after a further 4 days on artificial diet, suggests that they either avoided feeding

and could survive for a period of starvation, or fed so little that they did not receive a fatal dose. Further experimentation would be useful to determine whether *H. armigera* larvae can: (1) detect Bt toxin, (2) survive a period of starvation, and/or (3) recover from a period of starvation.

Acknowledgements

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