Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **03UA002**

Project Title: Inducible tolerance to Bt-toxin: Significance, mechanism and new management strategies.

Project Commencement Date: 1.1. 2008 **Project Completion Date:** 31.12. 2010

CRDC Program: **Farming Systems (Crop Protection)**

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Part 3 – Final Report Guide (due 31 October 2008)

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Reports on *Bacillus thuringiensis* (Bt)-resistance in the cotton bollworm, *Helicoverpa armigera,* in field populations pose a great threat to transgenic cotton's long-term sustainability. Although the frequency of resistance alleles in Australian field populations of *H. armigera* in Bollgard II cotton plantations has dropped significantly, surviving larvae are found occasionally, particularly in late season or on low toxin-expressing plant tissues. Bt-resistance is thus a continuing threat. Therefore, the selection pressure imposed on insect populations in transgenic crops expressing *B. thuringiensis* crystal toxins requires continuous monitoring to prevent or delay insect populations evolving resistance and/or tolerance in the field.

Our understanding of the diverse biological pathways leading to insect resistance against Bt-toxins outside mutations in major Bt-receptor genes (type I resistance) is still limited. Further, one of the unintended outcomes of intensive selection pressures has been the emergence of new Bt-resistance mechanisms in pest insects. For example, in addition to genetic resistance based on target site mutations (which produces individuals resistant to high toxin concentrations), we have shown that exposure of insect larvae to low to medium levels of Bt crystal toxins causes the induction of immune and metabolic responses, resulting low-level resistance (which we refer to here as inducible tolerance) in insect population that can be transmitted to offspring by epigenetic inheritance mechanisms (caused by gene and protein regulatory mechanisms) (Rahman et al., 2004). For several years, in collaboration with Ray Akhurst's group (CSIRO Entomology), we have been actively engaged in understanding the relative contribution of recessive resistance and also inducible tolerance mechanisms in insects that has been selected with Bt toxins in the laboratory as model systems for Bt-resistance (Akhurst et al., 2003; Rahman et al., 2004, 2007; Ma et al., 2005).

The most effective resistance management strategy for transgenic cotton is the refuge strategy involving an integrated set of practices to avoid or delay the evolution of genetic resistance in insect pests. It is based on the assumption that any mutants emerging as heterozygotes in transgenic cotton plantations are less likely to mate with each other if surrounded by susceptible insects, thus avoiding emergence of homozygous insects, which would become difficult to control even with transgenic plants expressing high levels of the toxin (Bates et al., 2005). Since the refuge strategy relies on resistance levels of heterozygotes being well below the levels of toxin expression in transgenic crops, it is important to understand whether other resistance mechanisms interact with incompletely recessive type I resistance to contribute to the overall resistance in the field.

To determine the cause of *H. armigera* survival in Bolgard II cotton, we proposed to monitor survivors for relative contribution of inducible tolerance mechanisms to overall resistance. This provides a basis for developing improved resistance management strategies to cope with the evolution of new threats to the use of Bt-toxin in transgenic cotton. The task was to test the genetic and epigenetic potential of inducible tolerance to Bt-toxins in *H. armigera* populations. This involved establishing laboratory Cry1Ac and Cry2Ab tolerant cultures with an incremental increase of respective toxin exposure.

The key questions in this project were: Do inducible tolerance mechanisms exist in field and laboratory populations of *H. armigera*? If these do, are they transmitted to offspring by a maternal effect? If transmitted by a maternal effect can the level of tolerance be increased

over generations under continuous selection pressure? And finally: what are molecular mechanisms of inducible immune and metabolic tolerance in field-derived laboratory populations of *H. armigera?*

We suspect that resistance traits caused by recessive mutations and inducible mechanisms may coexist in field insect populations under continuous selection pressure. Given that many type I resistance mechanisms are incompletely recessive, the main question was whether additive effects of the two mechanisms in heterozygotic pest insects can overcome toxin levels in transgenic crops, or survive when toxin expression declines in crops. The outcomes from this project will have direct impacts on the management of Bt resistance in cotton cropping systems.

Objectives

2. List the project objectives and the extent to which these have been achieved.

Objective 1: Assessment of inducible tolerance mechanisms in the field populations in relation to target site resistance

Laboratory cultures of susceptible ANGR and transgenic cotton field-collected *H. armigera* populations were established at Adelaide University, Waite Campus. A composite of susceptible ANGR and field derived insects (Waite Susceptible strain) was exposed to incremental increases of the Cry1Ac and Cry2Ab toxins to elevate the levels of tolerance to respective toxin. Both Cry1Ac and Cry2Ab tolerant strains are currently at generation 24 (F_{24}) in the course of incremental increased of selections and are maintained on 0.1mg/ml and 3µg/ml of Cry1Ac (crude bacterial suspension; note that the concentration refers to total protein within the crude preparation) and Cry2Ab (transgenic maize extract containing Cry2Ab toxin), respectively. **These cultures were a central experimental resource for the project.**

Field collected populations were examined first for genetic resistance and being identified as non-resistant in diagnostic genetic crosses. The inducible tolerance mechanisms were investigated in the offspring of susceptible, field and laboratory selected populations for Cry1Ac and Cry2Ab. We have observed that tolerance in *H. armigera* is induced by gut-derived toxins (both Cry1Ac and Cry2Ab) through differential regulation of immune and metabolic activities (larval induction) transmitted to offspring by an epigenetic mechanism showing a maternal effect (embryonic induction). Results for Cry1Ac were published in a referred international journal [Rahman et al. (2011a), "Developmental penalties associated with inducible tolerance in *Helicoverpa armigera* to insecticidal toxins from *Bacillus thuringiensis*", *Applied and Environmental Microbiology* 77:1443-1448], and a manuscript [Rahman et. al. (2011b), "Inducible tolerance is associated with differential regulation of receptor, immune and metabolic genes in laboratory selected Cry2Ab tolerant *Helicoverpa armigera* larvae]" is in preparation for *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*.

Objective 2: Molecular and genetic characterization of inducible mechanisms in field-collected insects

We have investigated the nature and degree of differential expression of immune and metabolic responses in Bt (Cry1Ac and Cry2Ab)- tolerant and susceptible insect larvae that are highly relevant to understand inducible tolerance. We have established that the inducible tolerance is associated with differential regulation of immune and metabolic activities in field-derived but laboratory-selected Bt tolerant larvae. By performing reciprocal genetic crosses between field-derived WS and tolerant strains, and analysing tolerance levels in the offspring we have established that the mode of acquisition of tolerance under continuous

incremental selection pressure is largely caused by inducible immune and metabolic response with an epigenetic transmission (Rahman et al., 2011a, 2011b).

In collaboration with Professor David Heckel (Max Planck Institute, Jena, Germany) we have analysed transcription of ca 6000 defined cDNA tags in laboratory tolerant and susceptible *H. armigera* insects using microarray technology. We have investigated neonate larvae, midgut tissues and carcasses of 4th instar larvae from susceptible, induced and tolerant insects for gene expression patterns in the respective treatment groups. When we have analysed gene expression patterns of neonate larvae, we have observed that a significant number of genes, including key receptor, immune and catalytic genes are significantly differentially transcribed in Cry2Ab tolerant neonate larvae (Cry2Ab, 0.3µg/ml, F_8, RR at LC_{50}, 5.4) compared to susceptible control (Figure 3, Table 4), which may have practical implication for Bt resistance management strategies. Although Gene Ontology (GO) data illustrated that some of the differentially expressed genes are involved in binding, immunity , catalytic activity, metabolic and cellular processes (Table 4a); some of the highly up/down regulated genes (Table 4b) remain to be investigated. We have also performed several hybridizations of midgut tissues and carcasses from different groups of Cry2Ab induced and non-induced susceptible larvae. The preliminary data from selection experiments with Cry2Ab seem to support a multi-gene tolerance effect. These experiments are ongoing and we anticipate would continue pending further funding.

In conjunction with an ARC Discovery project (DP0881071), the molecular mechanism of inducible tolerance was established [Ma et al. (2011), "Insect tolerance to the crystal toxins Cry1Ac and Cry2Ab is mediated by the binding of monomeric toxin to lipophorin glycolipids causing oligomerization and sequestration reactions", *Journal of Biological Chemistry (under review)*]. We have indentified interactions of mature Bt toxin with lipid particles in the gut lumen of Bt-tolerant insects. We have investigated the lectin activity of Cry toxin domain II and observed that it is similar in different Cry toxins. The observed aggregation and sequestration of toxins by lipid particles is based on the interaction with the same glycolipids [Ma et al. (2011), under review]. This begs the question as to whether the observed aggregation and sequestration of toxins by lipid particles creates conditions in insect populations favourable for cross-tolerance that are potential threats for long-term use of Bt toxins. Our full dose response cross-tolerance bioassays data for laboratory selected Cry1Ac and Cry2Ab populations suggests a low but significant cross-tolerance.

Objective 3: Identify relevant characters of inducible Bt-tolerance and develop diagnostic tools to detect inducible Bt-tolerance in the field.

Because the sequestration reaction is expected to remove lipid particles from involvement in lipid metabolism [Rahman et al. (under review) *Journal of Insect Physiology*; Ma et al. (under review) *Journal of Biological Chemistry*], the observed increase in tolerance to the toxin after immune induction (Rahman et al., 2004) is expected to be associated with high fitness costs. Therefore, we have investigated genetic and physiological traits such as, inbreeding depression and developmental penalties in laboratory selected populations, where we observed that the developmental penalties are associated with inducible tolerance in field-derived laboratory selected Bt tolerant insects, as evidenced by lowered larval weights and increased developmental times. However, once the populations were kept at constant toxin levels, the developmental penalties slowly diminished over subsequent generations. This could indicate possible genotypic selection of allelic combinations of multi-gene functions that reduce developmental penalties. This may in long term provide tolerant populations with the adaptive potential to acquire resistance mechanisms that are genetically transmitted and involve target site mutations in important resistance genes. Developmental penalties alone or

in combination with differential gene regulation can be used as potential markers to identify effects of transient and/or inducible mechanisms in the field populations (Rahman et al., 2011a, 2011b). These are important findings that may allow predictions on low level tolerance in the field and have practical significance for adapting pest management protocols to counter inducible tolerance. The implications for cotton IPM strategies are profound; and highlight the importance of refuges and preventing resistance from ever getting well-established at modest frequencies even at low resistance magnitudes (that is, on the order of 5 fold mechanisms at frequencies of 10-20% or higher, that could select for multi-gene families). As discussed further below, it is especially important for susceptible females to reach sites harbouring potentially resistant populations.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

A. Bacillus thuringiensis (Bt) crystal toxins

The toxins used were *B. thuringiensis* crystal toxins Cry1Ac and Cry2Ab. Cry1Ac was a bulk, crude, commercial bacterial suspension of *B.thuringiensis* strain HD73 containing Cry1Ac toxin, kindly supplied by Dr. John L. Reichelt, Bacterial Fermentation Pty. Ltd., Arundel, Queensland, Australia. Cry2Ab was from Monsanto Australia Pty. Ltd. (transgenic maize powder containing Cry2Ab). A crude bacterial suspension was synthesised from a coding sequence gi|40311|emb|X55416.1| *B. thuringiensis ssp. kurstaki* HD-1 plasmid gene for the crystal protein CryIIAb 1902 bp and cloned into an *E. coli* expression vector pQE30 (Qiagen). Our ***E. coli* expression Cry2Ab toxin could potentially be used by other research groups.**

B. Insect Populations

Susceptible ANGR strain: An established Bt toxin-susceptible *H. armigera* laboratory strain (ANGR) was maintained at 25±0.5°C (14/10h, L/D photoperiod) on artificial diet (modified from Teakle and Jensen, 1985) in the constant temperature room, Waite Campus, University of Adelaide, South Australia, without exposure to any insecticides. Freshly prepared artificial diet (2ml) was dispensed in 45-well plastic trays (45×12) and left to dry in a fume hood for about 15~20 minute. Neonate larvae were placed individually in each well and heat sealed with Mylar (heat-sealable polyester film). A total of 20 to 25 fine holes were then punched into the film to allow air exchange. Trays were placed in constant temperature room under standard culture conditions (see above). After seven days, about 432 (36×12) late 3rd to early 4th instar larvae were transferred individually to comparatively larger 36-well plastic trays containing freshly prepared artificial diet for growing larvae, heat sealed with 11x6" 3MIL/10MP DBL-PNCHD/PERF lids (Oliver-Tolas Healthcare Packaging, Grand Rapids, MI 49504 USA) and placed in constant temperature room. About 300-350 pupae were surface sterilized with 0.1% bleach for 2~3 minutes and rinsed well under cold running water. Once dry, the pupae were set in rectangular (50×30cm) cage for emerging adults. Sterile 0.02% honey solution containing ascorbic acid (2g/L) was supplied in honey pots for the adults. Once mated, about 40~50 moths were then moved to an egg-laying bucket (2.5L, surface painted with black paint) covered with nappy liner for egg laying. Eggs were collected from the nappy liner on a daily basis and sterilized in 0.1% bleach solution before being set for larval emergence. The population was maintained on a weekly basis for a constant supply of egg and/or neonate larvae for experimental purposes. This ANGR Bt

susceptible strain was used as the reference strain for comparison with field-collected populations.

Field collected populations: Initially, three batches of *H. armigera* eggs (constituting F_2 offspring from surviving larvae in transgenic cotton that were cultured in the laboratory in the absence of toxins) were obtained from Dr Sharon Downes and her colleagues, CSIRO, Narrabri. A further batch of eggs was collected from transgenic maize and cotton fields across the Narrabri cotton growing district. The laboratory colony was started from about 900 eggs and maintained in freshly prepared artificial diet under standard culture conditions (see above) without exposure to any insecticides.

C. Base-line tolerance bioassays and establishment of Waite Susceptible(WS) strain

After establishment of the laboratory colonies, neonate larvae were tested for base line tolerance to Cry1Ac (a bulk, crude, commercial bacterial suspension of *B.thuringiensis* strain HD73 containing Cry1Ac toxin, kindly supplied by John L. Reichelt, Bacterial Fermentation Pty., Ltd., Arundel, Queensland, Australia). Bioassays were performed with a broad range of toxin concentrations mixed with freshly prepared artificial diet for neonate larvae. Larval mortality was recorded on day 1, 5, 10, 15 and 20.

Although the susceptible ANGR strain was kept in the laboratory without any overt inbreeding depression, the ANGR population was combined with susceptible field populations that tested negative for target site mutation (resistant alleles) and inducible tolerance. The combined susceptible strain [**Waite Susceptible (WS) strain]** has been reared at the Waite Campus for the last three years without exposure to any toxin. The WS strain provides a resource for genetic diversity to avoid inbreeding depression, if there is any, and potentially overcome fitness costs under incremental selection pressure in the laboratory. WS strain was tested for base line tolerance to Cry2Ab (transgenic maize powder obtained from Monsanto) following the methodology mention above.

D. Laboratory selection of Waite Susceptible (WS) strain to Cry1Ac and Cry2Ab toxins

Given that field survivors do not show any resistance due to target site mutations (Dr. Sharon Downes, Bt resistance monitoring report 2007/2008), the possibility exists that inducible tolerance is assisting larvae to survive on certain parts of the plants or late in the season when toxin expression is low. Therefore, we initiated laboratory selection of field derived WS strain using low to medium level of Cry1Ac and Cry2Ab toxins.

About 450 neonate larvae were individually selected on artificial diet initially containing sub-lethal concentrations of Cry1Ac crystal preparation (0.005mg/ml, note that the concentration refers to total protein within the crude preparation) or Cry2Ab (0.03µg/ml Cry2Ab, transgenic maize extract containing Cry2Ab toxin) in 45-well plastic trays (45×10) to establish whether continuous exposure to Bt crystal toxins increases tolerance to the corresponding toxin together with an elevated immune and metabolic responses. In contrast to other lepidopteran insect species, such as diamondback moth *Plutella xylostella* and flour moth *Ephestia kuehniella*, where high inducible immune and metabolic tolerance have been achieved by incremental increases, *H. armigera* posed some technical problems. Firstly, *H. armigera* larvae are cannibalistic and must be kept in isolation. Secondly, the level of selection in the artificial food proved to be a critical precondition for successfully culturing tolerant *H. armigera* when maintaining broad genetic diversity under continuous incremental selection pressure. Thirdly, developmental penalties associated with incremental selection in *H. armigera* larvae proved to be critical for the establishment of tolerant strains.

Given the high numbers of individual larvae required for bioassays and selected lines, careful operational and logistic preparations went into the implementation of the selection

process, including the physical separation of each insect culture in different rooms to prevent any larval escapees from contaminating any of the other strains. After trial and error, we established Cry1Ac and Cry2Ab tolerant strains with incremental increase of toxin selections. The toxin concentration was maintained at the same level for at least ten generations (except for the neonate using in bioassays), and the relative tolerance against WS strain was assessed using full-dose response bioassays.

E. Full-dose response bioassays of WS, Cry1Ac and Cry2Ab tolerant strains

To estimate acquired tolerance under incremental toxin selections, full-dose response bioassays were performed using an artificial diet overlaid with respective crude toxin suspension. After the first batch of full dose response bioassays using Cry1Ac (from John L. Reichelt) and Cry2Ab (transgenic maize extract), we primed our own source of *E. coli* expression Cry2Ab toxin to optimise experimental errors in mortality bioassays and to meet expected shortfall of transgenic maize extract for laboratory selection (see above).

Bioassays were performed using an artificial diet overlaid with a crude bacterial suspension containing Cry1Ac or Cry2Ab respectively. Because the exact concentration of toxin in crude bacterial extracts was not known, preliminary assays were conducted using a broad range of dose concentrations of crude extracts to determine the appropriate doses for the formal assay. Full dose response bioassays were then conducted with 10 concentrations (plus a Milli-Q water control) and at least 45 neonate larvae for each concentration. Toxin-containing stock solutions were diluted in Milli-Q water to specific concentrations, and 50 and 200µl aliquots were spread evenly on top of the artificial diet in each well for Cry1Ac and Cry2Ab respectively. Trays were left in a fume hood until completely dry. One neonate larva was placed in each well, and trays were then placed in a ventilated room under standard culture conditions (see above). The efficacy (larval mortality) of the treatments was assessed on day 7. Full-dose line bioassays of neonate larvae from WS strain were also performed for Cry1Ac and Cry2Ab. The tolerant strains were compared with the WS strain to estimate the relative tolerance levels in the two strains against the respective toxin.

F. Analysis of transcription of ca 6000 defined cDNA tags in laboratory selected Bt tolerant, induced and non-induced WS H. armigera larvae

In collaboration with the Max Planck Institute, Jena, Germany (Professor David Heckel's group), we analysed transcription of ca 6000 defined cDNA tags of laboratory Bt tolerant and susceptible *H. armigera* larvae using microarray technology. RNAs were extracted from larvae that were either induced in earlier stages with the sub-lethal dose of the toxin, or exposed to toxin for seven generations. RNAs from tolerant (Cry2Ab, 0.3µg/ml, F_8, RR at LC_{50}, 5.4) neonate larvae, gut tissue and carcass of induced larvae were compared with RNAs of the same tissues from WS control larvae. The RNAs were sent to Heckel's group, where the RNAs were separated into mRNA, labelled and incubated with microarray chips from Agilent.

The first part of the experiment was to investigate the differential gene regulation in Cry2Ab tolerant larvae compared to WS control. Therefore, RNAs were extracted from four-day old Cry2Ab tolerant (Cry2Ab, 0.3µg/ml, F_8, RR at LC_{50}, 5.4) and WS control larvae reared on toxin-free diet. The second part of the experiment was to investigate the differential gene regulation in Cry2Ab exposed WS larvae compared to non-exposed WS control. Briefly, three to four day-old WS larvae were either exposed to sub-lethal concentration of Cry2Ab (0.03µg/ml mixed with artificial diet) or Milli-Q water (non-exposed control). Larval mortalities for toxin exposed and control were recorded after seven days exposure. Although larval mortality did show non-significant difference (11.8% mortality, n=220 for toxin

exposed compared to 6.7%, n=225 for non-exposed control), we observed a significant fitness costs in some of the larvae that survived Cry2Ab exposure compared to non-exposed WS control. Therefore, we sorted the exposed larvae in three groups, namely Induced Group I, Induced Group II and Induced Group III, based on larval size and body mass. Susceptible larvae reared in toxin free conditions (from routine cultures) that were identical size (or body mass) to those of Induced Groups were used as reference. Control Group I and Control Group II were 7-8 days and 4-5 days younger than their counterparts Induced Group I and Induced Group II, respectively, but non-significant difference in age between groups III. We extracted RNA from whole larvae for Group I, but from mid-gut and carcases for Groups II and III.

G. Reciprocal genetic crosses

To establish whether the acquisition of tolerance under continuous incremental selection pressure is caused by differential immune and metabolic responses with an epigenetic transmission, or by resistance alleles that pre-existed in the field populations in low frequencies and which were increased in selected populations, we performed reciprocal genetic crosses between WS and tolerant strains, and analysed tolerance levels and fitness costs in the offspring.

About 150 pupae each from WS (S♀xS♂), Cry1Ac and Cry2Ab tolerant strains (T♀xT♂) were sexed following Nacrajan et al. (1979). For T♀xS♂, the tolerant female pupae (T♀) were crossed with susceptible male pupae (S♂), and for S♀xT♂, susceptible female pupae (S♀) were placed with tolerant male pupae (T♂). Full-dose response bioassays were performed following the methodology above with F_1 neonate offspring from S♀xS♂, S♀xT♂, T♀xS♂, and T♀xT♂ genetic crosses.

H. Experimental design to explore epigenetic and genetic contributions to inducible tolerance in laboratory selected populations

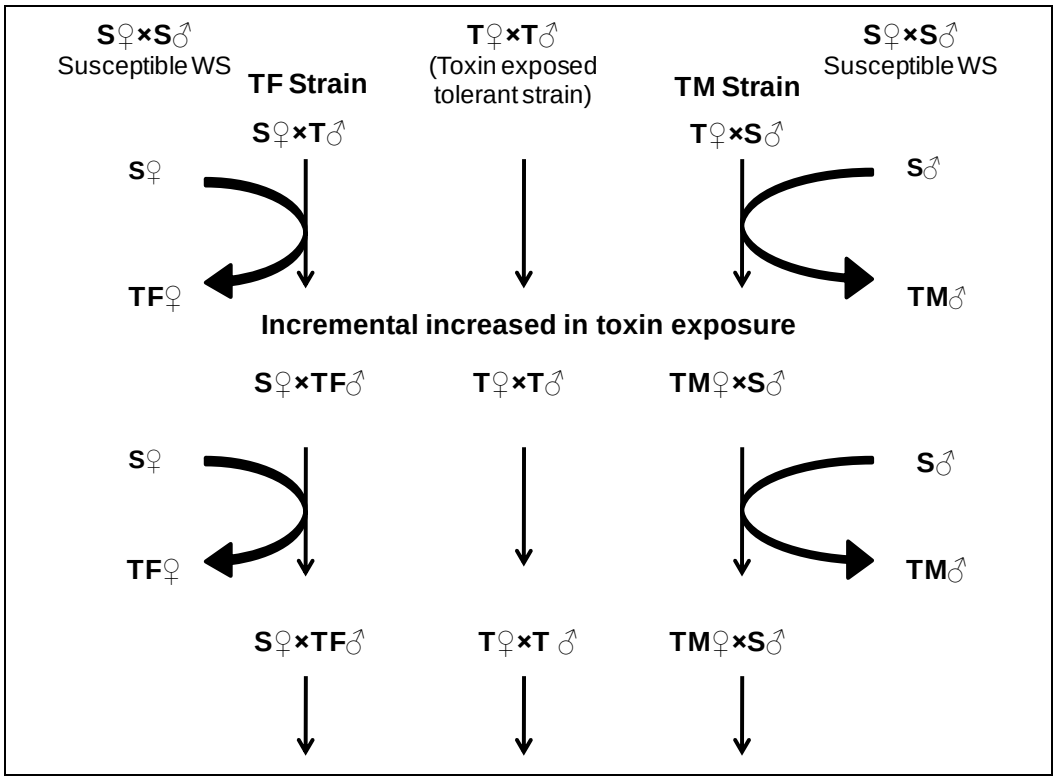
In contrast to resistance caused by target site mutations, the inducible tolerance requires continuous selection pressure to be noticeable. As indicated above, the insects were cultured in the laboratory under incremental selection pressure and assessed for acquired tolerance to the toxin. One of the emphases of the project was to establish a genetic design that allows exposing laboratory cultures to the toxin and assessing tolerance in subsequent generations after continuous and increasing selection pressure. From trial and error we established an experimental design that allowed us to control and measure epigenetic and genetic contributions to tolerance separately.

This involves maintenance of multiple *H. armigera* strains in the laboratory with distinct genetic backgrounds. The first strain is the Waite susceptible (WS) culture, which is a combined population of susceptible ANGR and field-collected insects that test negative for target site mutation (resistant alleles) and inducible tolerance. The susceptible strain provides a resource for genetic diversity to avoid inbreeding depression in this and the other strains. Immediately after establishment of a viable susceptible *H. armigera* culture at the Waite Campus, two sup-populations of the WS strain were exposed to low levels of Cry1Ac and Cry2Ab in artificial food. After establishment of tolerant strain (T♀×T♂) under incremental exposure to the toxin, we started three additional strains namely, TF and TM strains for Cry2Ab. After initial reciprocal crosses, the female pupae from S♀×T♂ offspring were replaced by susceptible female pupae (S♀) in TF-strain, but in the TM-strain, the male pupae from T♀×S♂ offspring were replaced by susceptible male pupae (S♂). The exchange of females and males in the two strains respectively, were repeated in every generation. While the Waite Susceptible (WS) strain was reared without toxin, the tolerant (T♀×T♂), TF and

TM strains were kept under continuous selection pressure. The acquired tolerance for T♀×T♂, TF and TM strains were assessed in full dose response bioassays and compared with WS strain.

An important feature of this design is the repeated out-crossing of exposed strains with susceptible females, which should prevent incremental increases in maternally inherited inducible tolerance, whereas out-crossing with susceptible males provides a measure of potential tolerance levels achieved by inducible mechanisms.

The principle of the design is schematically outlined below:



This design has the following advantages:

- It makes experimental assessment of inducible tolerance possible under continuous selection pressure in controlled environments.
- It provides evidence for transmission of tolerance to subsequent generations.
- It allows incremental increases in toxin exposure and measurement of corresponding tolerance levels.
- Out-crossing with susceptible males in the TM-strain provides evidence for transmission of tolerance to subsequent generations.
- Out-crossing with susceptible females in the TF-strain interrupts maternally inherited inducible tolerance. Any increase in tolerance is due to genetic effects (genotype selection).
- Out-crossing in the TF- and TM-strains provides genetic diversity in the two selected strains and prevent inbreeding depression.
- The tolerant and TM-strains provide an indication of the potential of inducible tolerance in the field and generate data on the fitness costs associated with tolerance.
- The TF-strain provides an estimate of epigenetic contributions to the tolerance levels achieved and provides experimental evidence for the importance of uninterrupted female transmission of tolerance.

- As foreshadowed above, this design tests the foundation of possible management strategies to prevent inducible tolerance mechanisms becoming a problem in the field.

I. Molecular mechanisms of inducible tolerance in field-derived laboratory selected Cry1Ac and Cry2Ab tolerant insects

Field-collected insect survivors were analysed for immune- or metabolic up-regulation. However, direct measurements from field-collected larvae were not possible, since collected insects have to be kept for at least one generation to monitor for any parasitism. By that time any transient induction process may have dissipated. We therefore decided to use our WS and tolerant laboratory cultures to study molecular mechanisms of inducible sequestration of toxins. A manuscript entitled "Insect tolerance to the crystal toxins Cry1Ac and Cry2Ab is mediated by the binding of monomeric toxin to lipophorin glycolipids causing oligomerization and sequestration reactions" is attached herewith for further information. This aspect of the project was investigated in conjunction with an ARC Discovery project (DP0881071).

Since our observations suggest a generalised aggregation and sequestration of Bt crystal toxins by lipid particles, we suspected that this may create a condition where cross-tolerance is possible in laboratory-selected insects. Therefore, during the course of incremental selection, we determined tolerance and cross-tolerance amongst Cry1Ac and Cry2Ab tolerant populations compared with the WS strain. Full-dose response bioassays were performed using an artificial diet overlaid with crude toxin(s) suspension following the methodology above. Neonate larvae from WS, tolerant Cry1Ac and Cry2Ab strains were used in full-dose response bioassays for Cry1Ac and Cry2Ab. LC_{50}, LC_{90} and LC_{99} values of tolerant strains for each toxin were compared with corresponding LC_{50}, LC_{90} and LC_{99} values of WS strain.

J. Measurement of genetic and physiological trails in Waite Susceptible, and Cry1Ac and Cry2Ab tolerant populations

During the course of incremental selection, we investigated genetic and physiological trails such as, inbreeding depression and developmental penalties in the laboratory selected Cry1Ac and Cry2Ab tolerant strains, and compared those with WS strain. We measured developmental penalties in term of reduced body weight and extended larval development time. Developmental penalties were measured in single or multi dose toxin bioassays following the methodology described above for full dose response bioassays with the modification that toxin suspension (or buffer only) was mixed through the diet prior to pouring into wells. This was performed to ensure constant toxin exposure over the assay period. About 300~500 neonate larvae were individually reared on freshly prepared artificial diet mixed with each dose concentration in 45-well plastic trays. The larval survivors and their body weight were recorded at 10^{th} day exposure. Larval developmental period (days) from neonate to pupa was recorded for each larva. We investigated several genetic and physiological traits such as larval developmental time, larval body mass, pupal weight, sex ratio, fecundity, etc., in WS and tolerant insects reared on toxin or toxin free diet for any sign of inbreeding depression that may have correlation with the acquisition of tolerance in laboratory selected insects.

K. Melanization assays

Since inducible tolerance is associated with the relative amounts and activities of immune and metabolic components at the gut lining, we assumed that the phenoloxidase mediated immune response discriminates between susceptible, tolerant and/or resistant insects. Therefore, we assessed the phenoloxidase mediated immune response in susceptible, field

and laboratory selected tolerant populations by melanization assays of cell-free plasma from 3rd instar larvae.

Eight to ten third-instar larvae were chilled on ice for 5 min, washed with ice-cold 70% ethanol, and then washed with ice-cold phosphate-buffered saline (PBS). Hemolymph was extracted by cutting a foreleg and bleeding larvae directly into 1.5 ml of ice-cold PBS. The suspension was centrifuged for 5 min at 3,000×*g*, and the supernatant was transferred to a quartz cuvette. The optical absorbance was measured (Varian DMS 100S spectrophotometer) at *A*280nm to determine the relative protein concentration, and then the degree of melanization (i.e., the *A*490) was measured every minute for 30 min. At least five replicates for each sample were used.

L. Statistical analysis

Mortality data were analyzed (POLO-PC software; LeOra Software, Berkeley, CA) to estimate the lethal dose concentrations (LCs). For each probit analysis, the mortality was corrected using Abbott's formula. Differences in susceptibility were considered significant when the 95% confidence intervals did not overlap at LC_{50} values. The resistance ratio (RR) was expressed as the ratio of the LC_{50} value of the relevant sample to that of the Waite susceptible insects. Developmental penalties and melanisation were analysed using Student *t* test paired analyses (SigmaPlot 10; Systat Software, Inc.). Microarray data were analysed with Welch's t test (1947) and Benjamini-Hochberg Method (1995).

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

To investigate the reasons why some *H. armigera* larvae survive on Bollgard II cotton, field-collected larvae were examined first for genetic resistance and after being identified as non-resistant in diagnostic genetic crosses, eggs from these insects were collected from CSIRO, Narrabri (kindly provided by Sharon Downes) to establish our own colony. The field-derived populations were compared with the susceptible ANGR strain in terms of their relative tolerance to Cry1Ac, and respective ability to be induced by exposure to low to medium level of toxin. Bioassays of F_2-offspring from surviving larvae of the 2007/2008 growing season showed no significant difference to susceptible ANGR insects (Dr. Sharon Downes, Bt resistance monitoring report 2007/2008). No survivors from field collected populations were found with tolerance to Cry1Ac (Figure 1). Similarly, the field derived laboratory population (WS strain) had no resistance to Cry2Ab.

Laboratory assessment did not reveal any signs of differential immune and/or metabolic activities in field derived laboratory insects compared to the ANGR susceptible (Figure 2a, 2c & 4). Between field-collection and melanisation assays in our laboratory, insects have been kept on toxin-free diet for more than three generations. Given that the differential immune and/or metabolic activities are for transient regulatory process, we suspected that the effects generated in the field could have faded away by the time it was examined. We therefore concluded that if tolerance in the field was due to differential regulation of immune and/or metabolic activities, it was indeed transient and in the absence of the toxin and elicitors, the tolerance would disappear within a few generations.

To test this assumption, we embarked on a long-term exposure of sub-populations of WS insects to Cry1Ac and Cry2Ab to examine the potential for inducible immune and metabolic tolerance to reach measurable levels high enough to allow larvae to survive on plant or plant tissues with low expression of the toxin(s). We investigated the acquired tolerance under

incremental laboratory selections, differential regulation of immune and/or metabolic activities, differential gene expression and associated fitness costs.

When we compared sub-populations of WS insects kept under incremental exposure to low to medium concentrations of Cry1Ac and Cry2Ab for up to 18 generations, we made the following observations:

A. Given that *H. armigera* can suffer from inbreeding depression, merging of ANGR and susceptible field populations introduce field-related genotypes and increase genetic diversity in the susceptible experimental insects (Waite Susceptible strain). In none of the populations maintained for the past three years we detected any reduction of reproductive success due to inbreeding depression.

B. When we analysed gene expression patterns in neonate larvae of Cry2Ab tolerant (Cry2Ab, 0.3µg/ml, F_8, RR at LC_{50}, 5.4) and WS control, we have observed that a significant number of genes (~1800) are significantly differentially regulated in tolerant larvae (Cry2Ab, 0.3µg/ml, F_8, RR at LC_{50}, 5.4) compared to susceptible control (Figure 3, Table 4). Gene Ontology (GO) data illustrated that some of the differentially expressed (up to 40 fold up/down regulated) genes are key Bt receptors and the others are involved in immunity, catalytic activity, metabolic and cellular processes (Table 4a). Importantly, some of the highly up/down regulated (up to 194 fold) genes are unknown in function (Table 4b) and remained to be investigated. We have performed several hybridizations of midgut tissues and carcasses from different groups of Cry2Ab induced and non-induced susceptible larvae. The preliminary data from selection experiments with Cry2Ab seem to support a multi-gene tolerance effect. It would also be interesting to see the gene expression patterns in Cry1Ac tolerant insects. By understanding the highly altered unknown genes, it is likely we will gain better understanding of the inducible tolerance mechanisms. More importantly, significant differential expression of key receptor genes opened up avenues for further research that may lead to establish monitoring tool to detect inducible tolerance and/or resistance in the field and able to develop management options to specially counter tolerance build-up.

C. The level of acquired tolerance had increased significantly after eleven rounds of Cry1Ac selection (Table 1, T♀×T♂/ S♀×S♂ at L_{C50}, RR: 29.8). Transmission of tolerance was likely due to an epigenetic mechanism showing a strong maternal effect (Table 1, compare RR of larvae from T♀×S♂ to those from S♀×T♂; and Figure 5).

D. Although the selection for Cry2Ab was increased 100 fold compared to 20 fold for Cry1Ac, a low but significant tolerance (RR=15.3 at LC_{50}) was acquired when we compared with non selected WS strain at seven and eighteen generation (Table 2, RR=5.39 and 15.3 respectively at LC_{50}). Like Cry1Ac, the transmission of the tolerance was likely due to an epigenetic mechanism showing a maternal effect (Table 2, compare RRs of T♀×S♂ to those of S♀×T♂).

E. Further, for the Cry2Ab exposure, we divided the starting population into two sub-populations. Every generation, we replaced females (in one population, TF strain) and males (in the other population, TM strain) with Waite susceptible insects. This ensures genetic diversity and if the transmission of the elevated immune and metabolic status is achieved by epigenetic mechanisms with a maternal effect, one of the two populations (the one where males are replaced, TM strain) would be expected to show transmission and possible increase in tolerance, while the other (TF strain) would not. Full-dose response bioassays for TM and TF strains revealed a low but significant increase in

tolerance to Cry2Ab in TM (Table 2b, RR at LC_{50} value 16.8 with non-overlapping confidence limit with TF) but not in TF strain.

F. When tolerant females were crossed with susceptible males (T♀×S♂), the offspring were more similar to the tolerant than the susceptible strain, and tolerant males crossed with susceptible females (S♀×T♂), the offspring were more similar to the susceptible strain (Table 1 & 2). Because the females are the heterogametic sex in Lepidoptera, this effect is in the opposite direction of what could be attributed to sex linkage. If sex linkage were the case, the crosses with resistant males would be expected to contribute two resistance alleles to the offspring, with the females being hemizgygotes and very resistant, while the male offspring would be heterozygotes. In the crosses using susceptible males, only the male offspring would carry resistance. The fact that tolerance was largely transmitted by the females and not the males suggests that a large fraction of the tolerance levels observed in laboratory-selected lines was not genetically controlled, but transmitted by putative epigenetic effect. The mechanism of this epigenetic transmission is not known. While we cannot rule out DNA modifications of male or female genes, we suspect that immune proteins circulating in female hemolymph may be incorporated into growing oocytes and mediate embryonic induction processes.

G. Exposure to the toxin preparation in their diet had physiological implications for larvae, which exhibited significant differential regulation of putative receptor, immune and metabolic genes. Because growth and developmental effects are often a result of differential activation of immune and physiological functions imposed by the gut-derived toxin, we sought to determine how these changes are correlated with the presence of toxin in offspring of susceptible and tolerant insects. When susceptible larvae encountered each of the two toxins in the diet, the larvae showed dramatic changes in physiology, resulting in significant delays in growth and development (Figure 2a-g). We assume that exposure to toxins shut down food digestion and induced defence reactions through up and/or down regulation putative metabolic and immune genes. Since the delays in growth and development were correlated with the toxin concentrations, we investigated developmental penalties in the laboratory selected Cry1Ac and Cry2Ab strains under incremental increased in respective toxin exposure starting with non-lethal dose concentration.

a) We first examined developmental penalties in susceptible larvae exposed to toxin for the first time. Prior to forming our experimental susceptible population from the two susceptible (ANGR and field) subpopulations, we examined developmental penalties in the two subpopulations separately to rule out any inherent differences. After exposure of neonates to a sublethal concentration of Cry1Ac preparation (0.01mg/ml), the time required to reach pupation was significantly longer compared to insects kept on toxin-free diet (T =32.55, degrees of freedom (df) =140, and P< 0.0001 for ANGR and T =20.62, df =179, and P < 0.0001 for the field population) without any overt differences between the susceptible ANGR and field-derived populations (T =0.05, df =218, and P =0.961 for control and T =2.40, df =101, and P =0.019 for Cry1Ac exposed; see Figure 2c). Likewise, when larval body mass was compared after 10 days of sub-lethal exposure (0.01mg/ml), it was significantly reduced compared to control larvae (T =36.48, df =351, and P <0.0001 for ANGR and T =35.57, df =275, and P < 0.0001 for the field population) without any overt differences between the two strains (T = 1.58, df =287, and P =0.115 for control and T =2.66, df =339, and P =0.008 for Cry1Ac exposed, see Figure 2a)

b) After merging the two susceptible populations, one larval cohort was exposed to toxin suspension, and a control cohort was kept on toxin-free diet. In the first generation after toxin application, the toxin-exposed population responded with developmental penalties similar to the two separate populations, i.e., with developmental delays (data not shown) and reduced body weight (T=0.71, df = 426, and P =0.476 for control and T =0.04, df =510, and P=0.967 for Cry1Ac exposed; Figure 2a). These measures were significantly different from the population kept on toxin-free diet (T =35.49, df =314, and P <0.0001). However, after five generations of continuous exposure to this level of toxin, the mean larval weight increased such that they were significantly different (T =23.72, df =295, and P <0.0001) to those in the first generation after exposure (Figure 2a, compare F_1-Cry1Ac with F_5-Cry1Ac). In contrast, mean body mass of the control and toxin-exposed populations were not significantly different in the fifth generation (T =1.19, df =140, and P =0.236), although the tolerant population showed more variation (Figure 2a, F_5-Cry1Ac). This suggests that exposure to the toxin causes significant developmental penalties, which are at least partly reversed after continuous exposure at the same concentration.

c) We looked at how developmental penalties in the tolerant population respond to an incremental increase in dietary toxin exposure. At generation 6 (F_6) we increased the Cry1Ac toxin concentration to 0.02 mg/ml and maintained at this level until generation 12. We compared the tolerant and susceptible populations for developmental penalties in generations 6 and 12, with larvae being either not exposed to toxin or exposed to increased concentrations of toxin. Developmental penalties (reduced body weight) in the control larvae (never exposed to the toxin) were similar to that of tolerant insects that were not exposed (T =2.66, df =116, and P =0.009 at generation 6 and T =1.49, df =278, and P =0.137 at generation 12; Figure 2b). The developmental penalties of tolerant insects were significantly reduced in subsequent generations when the toxin level was maintained (T =15.67, df =146, and P <0.0001 at generation 6 for a 0.01mg/ml exposure and T =13.88, df =328, and P<0.0001 at generation 12 for a 0.02 mg/ml exposure), but the increase in toxin concentrations imposed additional developmental penalties, which were again reduced in subsequent generations (Figure 2b). Similarly, delays in the development of surviving susceptible and tolerant (F_{12}, 0.02 mg/ml) larvae exposed to increasing concentrations of Cry1Ac were significantly reduced in the tolerant strain (Figure 2c).

d) Developmental penalties in Waite susceptible and 2Ab tolerant strains under incremental exposure to Cry2Ab toxin indicated similar physiological implications for larvae, resulting in significant delays in growth and development (Figure 2d-g) as well.

H. Given the relatively high developmental penalties imposed on *H. armigera* larvae exposed to Cry1Ac and Cry2Ab toxins in the laboratory, we asked how inducible tolerance, stability of the elevated status and developmental penalties are correlated. We made three important observations related to our understanding of tolerance and its increased levels under continuous exposure to toxin. Here we consider that the term developmental penalties is different from the term "fitness costs," which is generally used in the context of mutational alterations in resistance genes (Gahan et al., 2005) causing pleiotropic effects due to absence of resistance gene products or changes in the function of mutant gene products.

a) Developmental penalties in the presence of constant levels of the toxin decreased significantly over subsequent generations but increased considerably under incremental increases in toxin exposure. The question is, what are the reasons for this reduction in fitness costs in subsequent generations.

b) Since it is unlikely that this reduction in developmental penalties is due to genetic adaptation, it is important to understand how immune and metabolic activities in induced larvae influence the developmental penalties as well as tolerance to the toxin. As mentioned above, significant number of genes (~1800) are significantly differently regulated in tolerant larvae compared to susceptible control (Figure 3, Table 4), where some of the genes involved in immunity, catalytic activity, metabolic and cellular processes and others are important Bt receptor genes (Table 4a). Again, some of the highly up/down regulated (up to 194 fold) genes are unknown in function (Table 4b). Therefore we precisely asked whether the immune and metabolic activities in larvae that are exposed to toxin in the diet (larval induction) differ from the induction that occurs in offspring from induced insects (embryonic induction). Our data suggest that the developmental penalties imposed by two induction processes (larval induction and embryonic induction) differ considerably. Where larvae exposed to toxin preparation in their gut have significantly delayed growth and development, the transmission of the elevated immune status to offspring is much less costly, affecting growth and development less than in their toxin-containing parents, while elevated immune and tolerance levels are maintained. Further, the observed developmental penalties are influenced by toxin concentration as well as the presence or absence of toxin in the gut.

c) This raises the important question as to whether the reduction in developmental penalties on subsequent generations under selection pressure is the result of a metabolic adaptation process regulated by differential expression of metabolic and immune genes or due to changes in the relative contributions of the two induction processes. As discussed, the phenoloxidase mediated melanisation was dramatically different in plasma from larvae induced by the two mechanisms (Figure 4). Further, when susceptible larvae were exposed to the toxin for the first time, developmental delays and reduced body size/mass were direct indicators of the developmental penalties accrued by the larval induction of immune activities. These and other observations suggest that the larval induction process is a highly damaging process, where the physiology of the larva is disrupted and switches from food digestion to defence reactions. This involves damage to the gut lining and exposure of hemolymph with gut content. One of the reasons why plasma melanisation is inhibited under these conditions may be the release of soluble protease inhibitors to prevent the systemic melanisation and coagulation in the hemocoel. While the elevated immune status was maintained, we noticed overt signs of decreased developmental penalties in subsequent generations in the presence of toxin and even faster in the absence of the toxin. This suggests that the induction is reversible, probably to prevent the excessive expenditure of internal resources if toxin is no longer present.

The reduction in developmental penalties may be due to changes in the relative contributions of larval and embryonic induction process. As mentioned above when larvae are exposed for the first time all changes caused by the exposure to the toxin are due to larval induction processes. If the toxin levels are maintained over subsequent generations, the embryonic induction causes some tolerance to the toxin which reduces the need for the insect to stimulate the immune and metabolic activities by larval induction mechanisms. The more the embryonic induction predominates, the

more the developmental penalties will be reduced. Any increase in toxin will enlarge the larval induction process and thus the overall developmental penalties. If the toxin is kept at the same levels in subsequent generations, the developmental penalties will be reduced again, while tolerance to the toxin is acquired at a higher level. While not much is known about the embryonic induction process, growth and development is less affected in larvae from induced parents kept on toxin-free diet even though the level of tolerance is maintained. In contrast to larval induction, embryonic induction is part of a developmental process and may be less damaging and costly to the insect.

I. Although prophenoloxidase (PPO) is not directly involved in the tolerance to Bt toxins (Rahman et al, 2007, Gassmann et al, 2009), we sought to determine whether, and under what conditions, PPO-dependent melanization in cell-free hemolymph (plasma) can be used as a quick and reliable measurement of insect immune status. Since Cry1Ac and Cry2Ab tolerant insects are immune induced, and the acquired tolerance is transmitted to offspring by epigenetic processes, we first examined whether larval immune induction by gut-derived elicitors (larval induction) differs from transmissible induction passed on to offspring (embryonic induction). Because a comparison of the rate of melanization in plasma was difficult in tolerant (induced) and susceptible (non-induced) larvae due to variable growth and development, we compared similar aged or similar sized larval offspring from insects maintained for 10 generations on toxin-containing diet. The rate of melanization in tolerant larvae (which at the time of measurement were kept on toxin-free diet) was significantly higher than in susceptible larvae both for Cry1Ac and Cry2Ab (Figure 4) a direct reflection of the elevated immune status. Because tolerant larvae showed a significant reduction in developmental penalties even in the presence of the toxin (Figure 2), we could compare similar sized/aged larvae even if exposed to toxin at the time of measurement. Unexpectedly, when tolerant larvae were reared on a toxin-containing diet at the time of measurement, melanization reactions were inhibited almost to the level of susceptible larvae (Figure 4).

Our observations suggest that insect larvae respond to gut-derived Bt toxins with an elevated immune response in the hemolymph (larval induction), which can be measured by the rate of melanization in cell-free plasma, only if larvae were kept on a toxin-free diet prior to plasma isolation. This confirms that toxin exposure causes an elevation of cell-free immune components, such as PPO in hemolymph which is transmitted to offspring and persists for at least one generation in the absence of toxin. In contrast, hemolymph derived from toxin-containing larvae contains a multitude of factors that among other effects inhibit melanization, while amounts of PPO and other immune/metabolic components were increased (data not shown). The simplest explanation for this observation is that immune and associated metabolic components are induced in the hemolymph by the presence of toxin in the gut. Although the molecular bases of the Bt toxin-mediated induction are not known, possible causes may involve signalling functions of Bt toxin or toxin-mediated damage to gut lining, and contact of gut-derived elicitors with hemolymph, including enterobacteria, which enhance the induction and toxicity but are not obligatory to toxicity. Whatever the mechanism, gut-derived toxin has multiple effects in hemolymph, such as the reduction of hemolymph proteins and an increase in PPO and the number of circulating hemocytes, as well as a reduction in hemocyte phagocytosis and an increase in PO-inhibiting proteins. Our data suggest that induction of the immune defense by gut-derived elicitors generates a wide range of metabolic responses that includes defense reactions against an intruding toxin or pathogen and also down regulation of the insect's own defense responses, such as coagulation and melanization, that are potentially damaging if allowed to spread within

the hemocoel. In particular, an increase in protease inhibitors may explain the observed inhibition of melanization reactions in induced larval plasma. Such inhibition may have other effects; for example, toxin-exposed susceptible larvae negatively affect predators, with some protease inhibitors having insecticidal activities. In addition to this larval induction process, the elevated immune status is transmitted mainly by induced tolerant mothers to offspring, apparently by epigenetic means (embryonic induction). Whatever the reasons for the difference in melanization reactions, our data clearly show that induction of the larval immune system by gut-derived toxins differs from the embryonic induction responsible for the transmission of the elevated immune status to offspring.

J. In conjunction with an ARC Discovery project the molecular mechanism of toxin binding to lipid particles (pro-coagulant in insects) was established [Ma et al. (2011). *Journal of Biological Chemistry, under review*). Our data shows that monomers of Cry1Ac and Cry2Ab bind to the same neutral glycolipid moiety found in membranes and in lipid particles. Both toxins and a recombinant peptide representing the domain II of Cry1Ac form tetramers after interaction with lipid particles, which cross-link particles to form large aggregates. We assume that the toxin forms a pre-pore complex in the presence of high concentrations of lipid particles. Since immune induction is associated with increased secretion of lipid particles into the gut lumen, the toxin is likely to cross-link particles forming aggregates, which sequester the toxin into coagulation products that are excreted. This could indicate that glycolipids on lipid particles are a possible target for toxin monomers, which upon binding to the glycolipids form tetramers that can form aggregates and thereby sequester the toxin inside the gut lumen before it can interact with receptors on the brush border membrane. Further, the sugar determinant of insect glycolipids resembles glycolipids from nematodes, which cause resistance when altered by mutant galactosyltransferases. Our observations suggest a mode of toxin sequestration by cell-free coagulation reactions, where the gut-derived monomeric toxin proteins become adhesive lectin complexes after interaction with lipid particles in the gut lumen. This suggests that while Cry1Ac and Cry2Ab bind to different glycoprotein receptors on the BBM causing toxicity by different pathways, their binding to the same glycolipid moiety suggest that sequestration of the toxins, resulting in tolerance, may be based on the same mechanism.

K. Although Cry1Ac and Cry2Ab bind to different glycoprotein receptors on the BBM causing toxicity by different pathways, their binding to the same glycolipid moiety suggest a similar sequestration of the two toxins resulting in tolerance. Therefore during the course of incremental selection we investigated the potential for cross tolerance between Cry1Ac and Cry2Ab tolerant populations, and compared with WS strain. Full-dose response bioassays of neonate larvae from WS, tolerant Cry1Ac and Cry2Ab populations revealed a low but significant cross-tolerance between Cry1Ac and Cry2Ab populations (Table 3). However, whether this cross-resistance will threaten management of resistance in the field will depend on the correlation between developmental penalties and/or fitness costs, mechanism of acquired tolerance and/or resistance, and extent of cross-resistance under extreme selections in the laboratory.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

The development of preventative resistance management strategies is a crucial objective of Program 4 (Crop Protection), given the emphasis on cotton plant resistance against insect pests. The immediate threat to transgenic cotton is the emergence of type I resistance in *H. armigera* populations, which is now managed with the introduction of Bollgard II, a transgenic cotton pyramiding two toxins using separate receptors. Nevertheless, other less known resistance mechanisms (i.e., dominant resistance traits, Gunning et al., 2005; inducible tolerance, Rahman et al., 2004, 2010, 2011) alone or in combination with type I resistance have the potential to create problems particularly when pest populations are continuously exposed to the toxin over many generations.

We have several novel outcomes that may lead to establish monitoring tool to detect inducible tolerance and/or resistance in the field and able to develop new or modified IPM strategies to specially counter insect tolerance build-up in Bollgard II.

Firstly, we have observed that a significant number of genes are significantly differently regulated in Cry2Ab tolerant larvae compared to susceptible control, where some of the genes involved in immunity, catalytic activity, metabolic and cellular processes. Importantly, some of the highly up/down regulated (up to 40 fold) genes are key Bt receptor genes and others are unknown in function ((up to 194 fold). Although, our data suggests a multi-gene tolerance mechanisms in Cry2Ab tolerant larvae, by understanding the highly altered unknown genes in Cry2Ab tolerant insects, and gene transcripts of Cry1Ac tolerant insects it is likely we will gain better understanding of the inducible tolerance mechanisms. More importantly, significant differential expression of key receptor genes opened up avenues for further research that may lead to establish monitoring tool and/or management strategies to prevent or delay development of tolerance in field populations.

Secondly, we have observed that inducible tolerance contributes significantly to the overall tolerance in field derived laboratory selected insects. Our observations suggest that exposure to gut-derived toxins (both Cry1Ac and Cry2Ab) allows insects to acquire tolerance through differential regulation of immune and metabolic activities (larval induction) that is transmitted to offspring by putative epigenetic mechanism showing a maternal effect (embryonic induction). The observed developmental penalties that are associated with inducible tolerance under incremental exposure to the toxins may have potential impacts on our thinking of how we perform long term IPM strategies.

At this stage the developmental penalties of tolerant insects may be prohibitive for field populations to acquire high levels of tolerance and/or resistance. However, studies in other insect species indicate that prolonged exposure to the toxin over time may decrease developmental penalties [Rahman et al. (2011), Epigenetic transmission of inducible immune and metabolic tolerance mechanisms to Bt-toxins in Australian field populations of diamondback moth *Plutella xylostella, in preparation for PNAS*]. It remains to be seen whether *H. armigera* shows reduction in developmental penalties after prolonged exposure to lethal toxin concentration.

Thirdly, we have observed that the induction process differs if the toxin is eaten by larvae through diet compared to the embryonic induction process through transmission of the elevated immune status to offspring. Melanization assays in plasma from offspring of

induced larvae that were kept on toxin-free diet (embryonic induction), show a direct correlation with the immune status (and tolerance levels), while melanisation reactions in plasma from larvae that were kept on toxin-containing diet (larval induction) were inhibited. This may enable development of easy and quick bioassay to measure immune induction and tolerance.

Fourthly, our observations suggest that incremental increases in tolerance to Bt toxins in *H. armigera* are achievable under laboratory conditions. Therefore, the potential for emergence of high levels of tolerance and/or resistance exists in the field. One way to prevent this from occurring is to interrupt the linage of induced females. i.e., it is possible to prevent high levels of tolerance emerging in the field by ensuring out-crossing selected populations with susceptible females. One outcome of the project is therefore to enhance strategies for susceptible females to reach sites harbouring potentially resistant populations, where encouraging sons from induced females to mate with susceptible females may interrupt the transmission of tolerance. This is consistent with the refuge strategy used in the field, where the out-crossing of spontaneous mutants with susceptible insects can delay resistance by maintaining heterozygocity in mutant field populations.

Finally, we observed that Bt cry toxins (Cry1Ac and Cry2Ab) have similar glycolipid binding properties although bind to different glycoprotein receptors (Ma et al., under review). Our data suggests that while Cry1Ac and Cry2Ab bind to different glycoprotein receptors on the BBM causing toxicity by different pathways (Tabashnik et al., 2009), their binding to the same glycolipid moiety suggest that sequestration of the toxins resulting in tolerance, may be based on the same mechanism (Rahman et al., under review; Ma et al., under review). Again, our observations that in Cry2Ab induced larvae significant number of genes are highly up or down regulated, and that these are key Bt receptor genes and genes of unknown function. These open up avenues for further research on molecular and genetic basis of inducible tolerance under extended selections of laboratory and field populations that may allow predictions of Bt tolerance in the field. Overall, the outcomes may provide fundamental basis for the design of new or modified resistance management strategies to effectively control *H. armigera* for long-term sustainability of transgenic cotton.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

None, other than the implications for the resistance management strategy as outlined above.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

The *E. coli*-expressed Cry2Ab developed in our laboratory, but could potentially be used by other research groups.

c) required changes to the Intellectual Property register.

Possible IP could be claimed for the two tolerant strains. If this possibility arises material transfer agreements can be designed.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

This project has contributed much to our understanding of low level of Bt resistance acquired by novel inducible immune and metabolic tolerance in field derived *H. armigera* strain. We haven't detected inducible tolerance and type I resistance in field derived insects, but our laboratory selections suggest that the potential exist for inducible tolerance mechanism that may contribute to overall resistance in the field, with the potential to cause ecological and economic problems in cotton production. We have shown that exposure to gut-derived toxins (both Cry1Ac and Cry2Ab) allows insects to acquire tolerance through differential regulation of immune and metabolic activities (larval induction) that is transmitted to offspring by an epigenetic mechanism showing a maternal effect (embryonic induction). We observed significant developmental penalties associated with inducible tolerance that may have potential impacts on long term cotton IPM strategies. We have identified a molecular mechanism of inducible tolerance of Cry1Ac and Cry2Ab that is based on similar glycolipid binding properties rather than mutation in glycoprotein receptors. The implication for Bt resistance management is that while pyramiding different toxins may delay resistance based on target site mutations in glycoprotein receptor genes, it may not protect against development of inducible and broad cross-tolerance based on glycolipid-mediated toxin sequestration. Importantly, we observed significant differential expression of key receptor, immune and metabolic genes in Cry2Ab tolerant insects that opened up avenues for further research. This may lead to establish monitoring tool and/or management strategies to prevent or delay development of field insect tolerance to cotton Bollgard II. Most importantly, we have highlighted the importance of susceptible females in blocking a form of resistance that can be maternally transmitted, and the need to make sure susceptible females move from refuges to sites where resistant insects may concentrate.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

Differential gene regulation in tolerant larvae can be used as marker for field monitoring of inducible tolerance.

(b) for the future presentation and dissemination of the project outcomes.

Dr. Mahbub Rahman intends to present some of the results in World Cotton Research Conference -5 (WCRC-5) that will be held in Mumbai, India, November 7-11, 2011. A number of manuscripts are either in preparation or under review for prestigious international journals.

(c) for future research.

In the light of our observations that tolerance to Cry1Ac and Cry2Ab in field derived laboratory *H. armigera* populations can be induced by exposing low to medium levels of toxin, and that there is potential for inducible tolerance in conjunction with receptor mutations to reach levels high enough to allow larvae to survive on plant or plant tissues with low expression of the toxin(s), our differential gene expression data

seems opened up avenues for further research on molecular and genetic basis of inducible tolerance and identifying monitoring tools for it. Therefore, we intend to continue the current project pending further funding from CRDC.

8. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

List of publications-

Rahman, M. M., R. Glatz, R. Roush, and Schmidt, O. (2011a). Developmental penalties associated with inducible tolerance in *Helicoverpa armigera* to insecticidal toxins from *Bacillus thuringiensis*. *Applied and Environmental Microbiology* **77(4)**: 1443-1448.

Rahman, M. M**.,** Roush, R., Heckel, D. G., Vogel, H. and Schmidt, O. (2011b). Inducible tolerance is associated with differential regulation of receptor, immune and metabolic genes in laboratory selected Cry2Ab tolerant *Helicoverpa armigera* larvae (in preparation for *Proceedings of the National Academy of Sciences of the United States of America).*

Ma, G., Rahman, M. M., Grant, W., and Schmidt, O. (2011). Insect tolerance to the crystal toxins Cry1Ac and Cry2Ab is mediated by the binding of monomeric toxin to lipophorin glycolipids causing oligomerization and sequestration reactions (*under review in Journal of Biological Chemistry*).

B. Have you developed any online resources and what is the website address?

No

Part 4 – Final Report Executive Summary

Insects can respond to selection pressure by mobilising new defence mechanisms. In contrast to recessive resistance mechanisms based on rare target site mutations in receptor proteins, leading to extreme resistance to high *B. thuringiensis* (Bt) toxin concentrations, we observed a "hidden" inducible immune and metabolic tolerance in field derived laboratory populations of *H. armigera* to Cry1Ac and Cry2Ab toxins, with potential to cause ecological and economic problems in cotton production. Given that offspring from insects surviving in the field are tolerant of Bt but do not exhibit resistance to Cry1Ac and Cry2Ab due to target site mutations, it is likely that other mechanism(s) is assisting larvae to survive on certain parts of the plants or late in the season when toxin expression is low. Importantly for cotton bollworm management, we have the following three main outcomes:

1) Tolerance in *H. armigera* larvae is induced by gut-derived toxins (both Cry1Ac and Cry2Ab) and creates sub-populations of insects that show significant levels of tolerance without displaying mutational changes in a major resistance gene locus. Instead, the tolerant phenotype is caused by differential regulation of immune and metabolic activities (larval induction), and is transmitted to offspring by an epigenetic mechanism showing a maternal effect (embryonic induction). While the epigenetic contribution to incremental increases in tolerance is prominent under low to medium selection pressure, other resistance mechanisms that are transmitted genetically may predominate over time with incremental increase in toxin exposure.

2) Testing gene expression in tolerant and susceptible neonate larvae indicates that a range of genes are expressed significantly differently (both higher and lower expression) in tolerant insects compared to susceptible control. Although some of these differentially expressed genes are important key receptor, putative immune and catalytic genes, most of the differentially regulated genes (up to 190 fold) are unknown in function. By understanding these highly altered unknown genes, it is likely we will gain an understanding of the tolerance mechanism and able to develop management options to specially counter tolerance buildup. Further, significant differential regulation of key receptor genes opens up avenues for further research that may lead to establish monitoring tool to detect inducible tolerance and/or resistance in the field.

3) There appears to be significant developmental penalties under laboratory selection, as evidenced by lowered larval weights and increased developmental times. However, once the populations were kept at constant toxin levels, the developmental penalties slowly diminished over subsequent generations. This could indicate possible genotype selection of allelic combinations of multi-gene functions that reduce developmental penalties. This may in long term provide tolerant populations with the adaptive potential to acquire resistance mechanisms that are genetically transmitted and involve target site mutations in important resistance genes. Our findings have practical significance for adapting pest management protocols to counter inducible tolerance, particularly the importance of susceptible females in blocking this form of resistance.

Lastly, laboratory selected *H. armigera* (25 generations selected with Cry1Ac, 63 fold resistance) are able to complete their larval development on transgenic cotton expressing Cry1Ac and produced fertile adults (Akhurst et al., 2003). Therefore, whether incremental increases in tolerance can support selection for target site mutations in key receptors allowing insect larvae to survive on Bt-plants is another key issue that requires investigation.

For further details please contact Dr. Mahbub Rahman at School of Agriculture, Food and Wine, University of Adelaide, Australia:(mahbub.rahman@adelaide.edu.au; rahman.drmahbub@yahoo.com.au)

FIGURES:

Figure 1. Base-line susceptibility of field derived laboratory population of *H. armigera* larvae to crude bacterial suspension containing Cry1Ac toxin. Neonate larvae from field derived populations were either exposed to freshly prepare artificial diet mixed with different concentrations of crude bacterial suspension containing Cry1Ac (0.01, 0.1 and 1 mg/ml) or MilliQ water (toxin free control). The larval mortality was recorded at day 1, 5, 10, 15 and 20. A long established Bt susceptible ANGR strain was used as a reference strain to compared field-derived strain. N=96 for each concentration in each of the strains. Note that the concentration refers to total protein within the crude preparation.

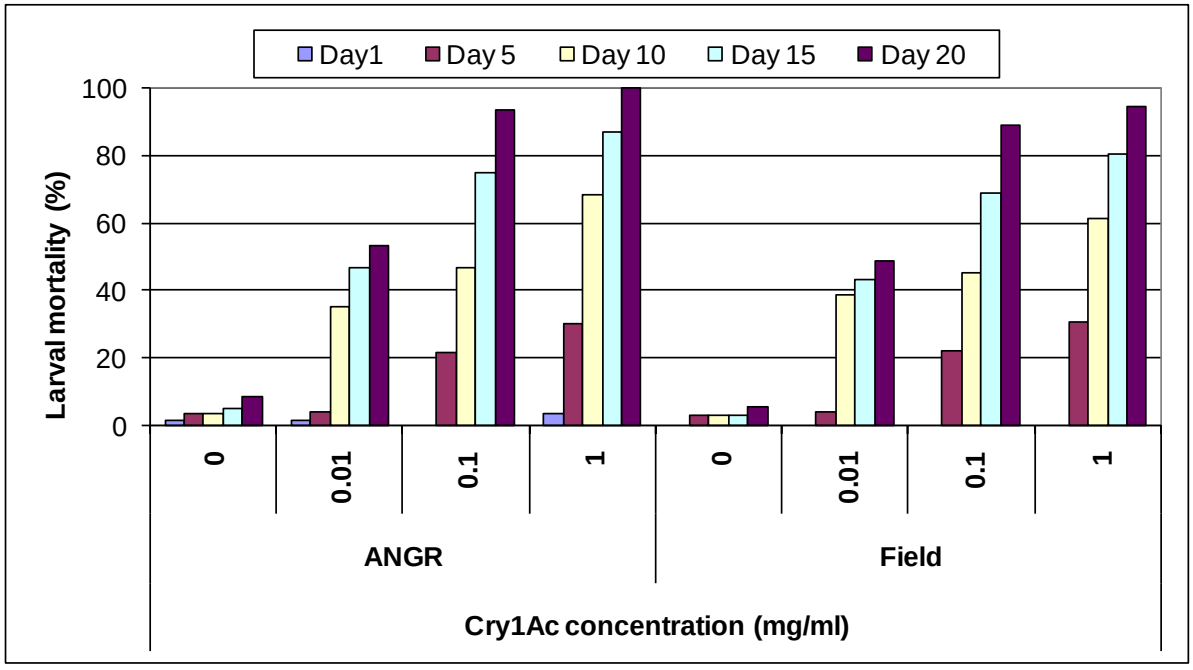
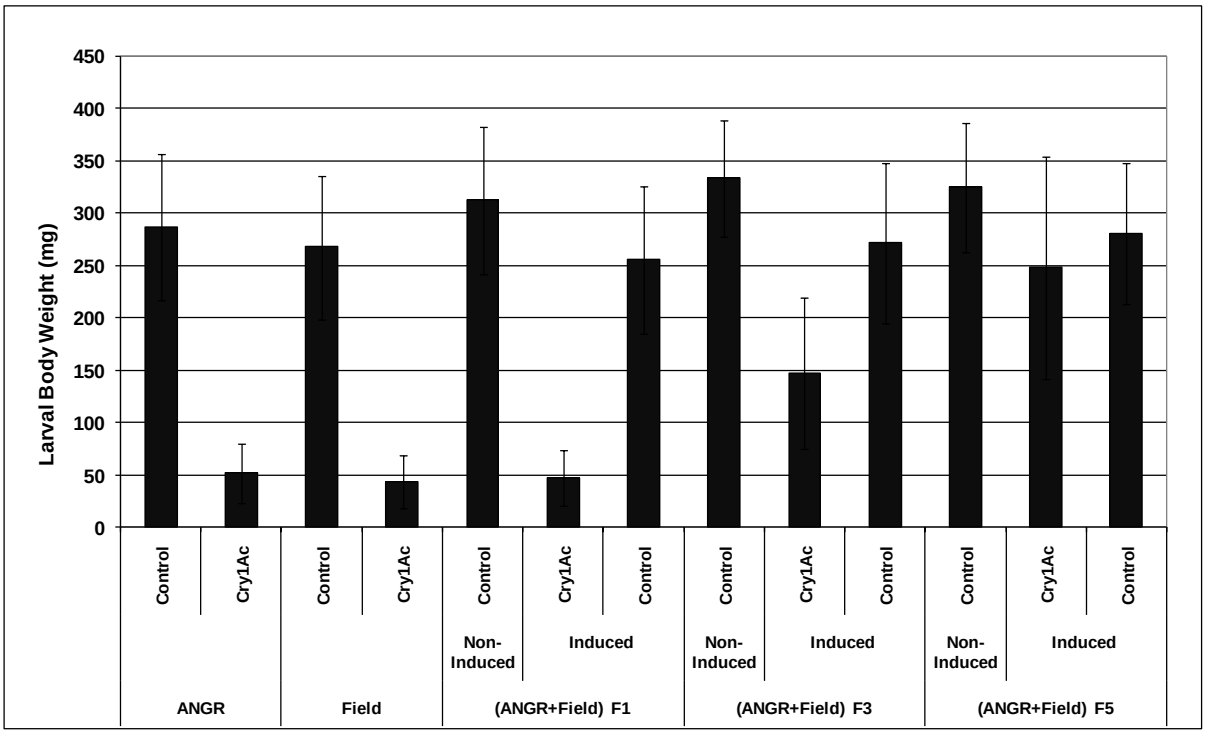
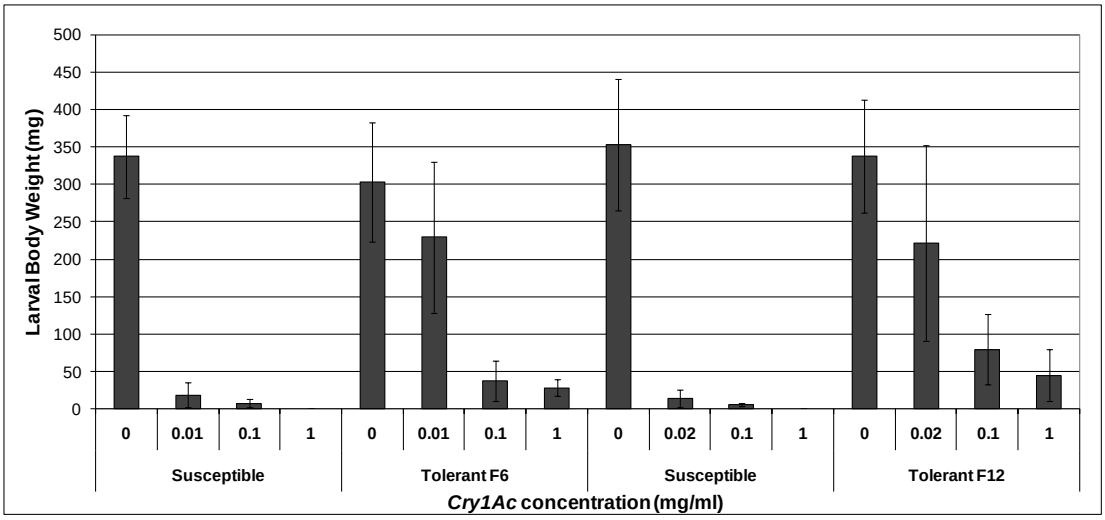


Figure 2. Laboratory exposure to low to medium level of Cry1Ac and Cry2Ab imposed significant developmental penalties in larval insects.

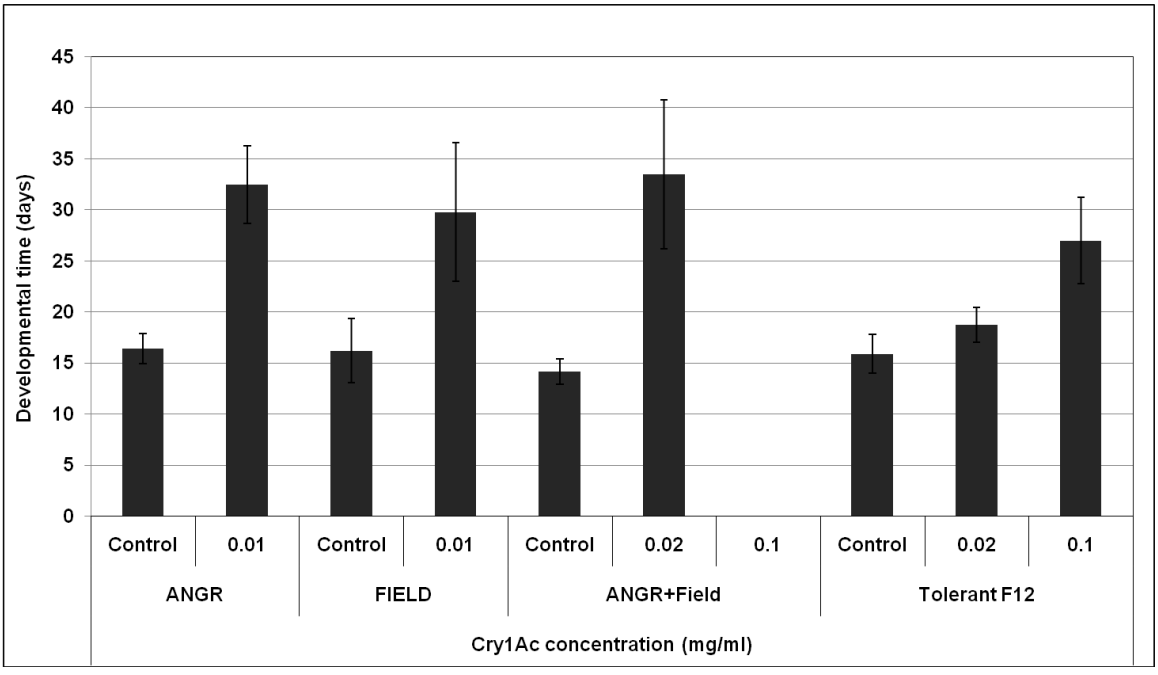
a) Reduced body weight in 10-day-old larvae when neonates were exposed to 0.01 mg/ml concentration of Cry1Ac suspension (note that the concentration refers to total protein within the crude preparation) and compared to larvae not exposed to the toxin (control). ANGR and field-collected larvae were equally susceptible to the toxin and combined to form Waite susceptible (ANGR×Field) population that was continuously exposed to same level of Cry1AC suspension over five generations. Results are shown for the newly induced population (ANGR×Field)F_1, in the third generation (ANGR×Field)F_3, and in the fifth generation (ANGR×Field)F_5. Each bar represents measurements from 300 to 500 individual larvae.



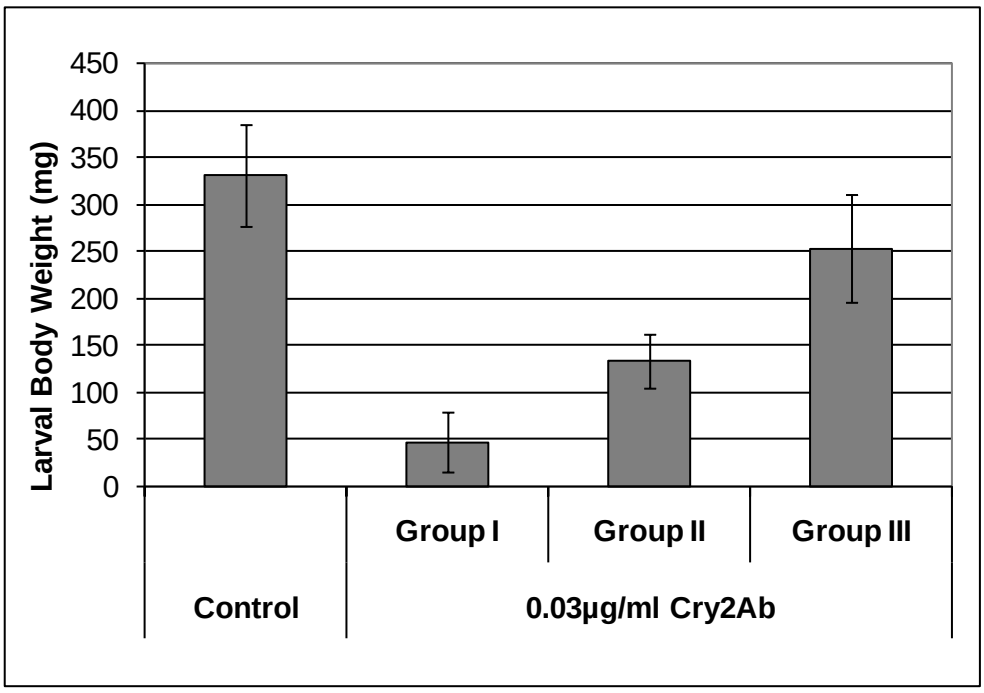
b) Reduced body weight in 10-day-old susceptible and tolerant larvae after neonate larvae were exposed to increasing Cry1Ac concentrations. Tolerant larvae were exposed to a 0.01-mg/ml crude Cry1Ac suspension for five generations (F_5) and to a 0.02-mg/ml crude Cry1Ac suspension in subsequent generations (F_{12}). Each bar represents measurements from 300 to 500 individual larvae. Note that the concentration refers to total protein within the crude preparation.



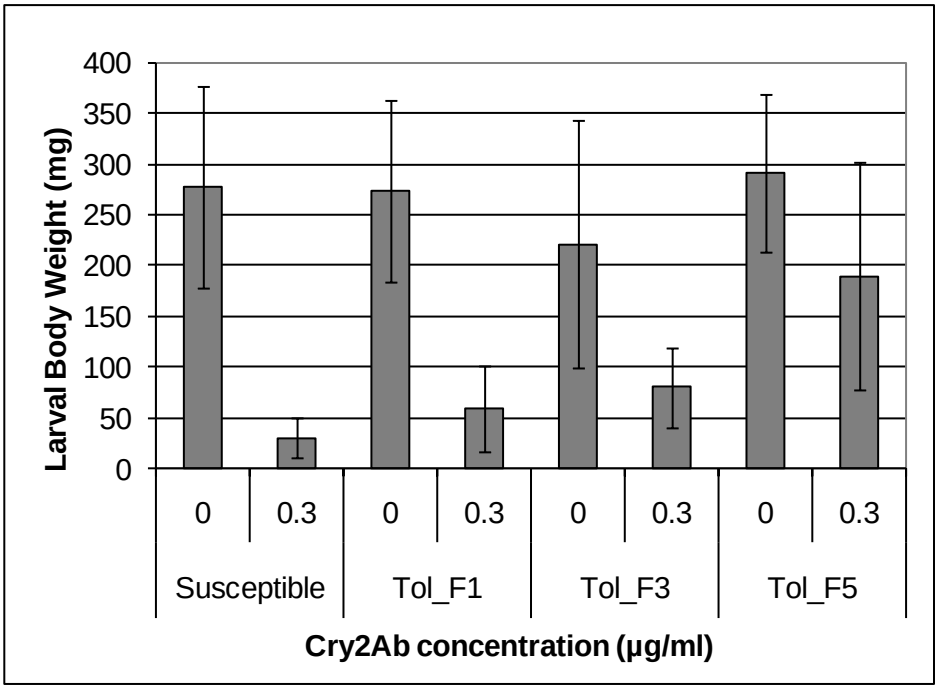
c) Delay in larval development (from neonate to onset of pupation) of surviving susceptible and tolerant larvae (F_{12},0.02-mg/ml Cry1Ac suspension) exposed to increasing concentrations of Cry1Ac. No bars indicate that all insects died during larval stages. Note that the concentration refers to total protein within the crude preparation.



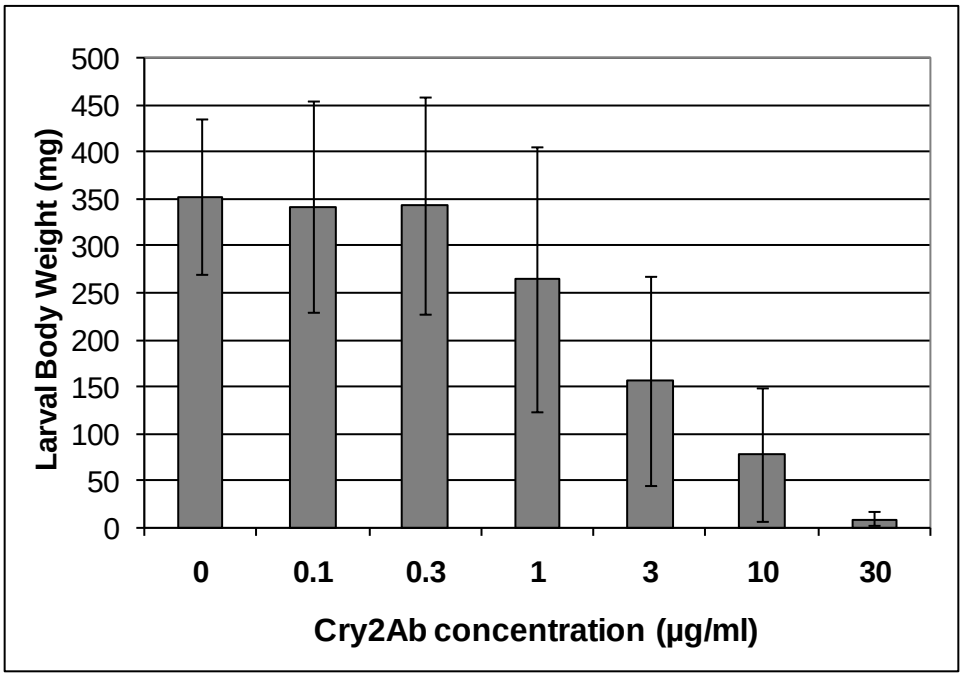
d) Reduced body weight in Cry2Ab exposed WS larvae. Four day-old Waite susceptible larvae were exposed to 0.03µg/ml Cry2Ab (transgenic maize extracts) mixed with freshly prepared artificial diet. After seven days exposure larval mortality (11.8%, n 220 in treatment compared to 6.7%, n 225 in control) and larval body weight were recorded. A significant fitness costs were recorded in some of the larvae that survived in Cry2Ab exposure. To further laboratory exposure and analysis of transcription of ca 6000 defined cDNA tags in exposed and non-exposed larvae, the exposed larvae were then sort in three groups, namely Induced Group I, Induced Group II and Induced Group III depending on their size. WS larvae reared in toxin free diet that were identical size to those of Induced Groups were used as control. Larvae in control groups were significantly younger in age compared to treatment.



e) The developmental penalty (reduced body weight) in exposed larvae was significantly reduced in subsequent generations when the toxin concentration was maintained at a constant level.



f) Larval body weight under incremental increase of Cry2Ab exposure in10 day old Cry2Ab tolerant **(**tolerant $F_{7,}$ 0.3µg/ml**)** larvae



DF=805, F=168 and P-value<0.0001

g) Larval developmental period (from neonate to onset of pupation) under incremental increase of Cry2Ab exposure in Cry2Ab tolerant **(**tolerant $F_{7,}$ 0.3µg/ml**)** larvae

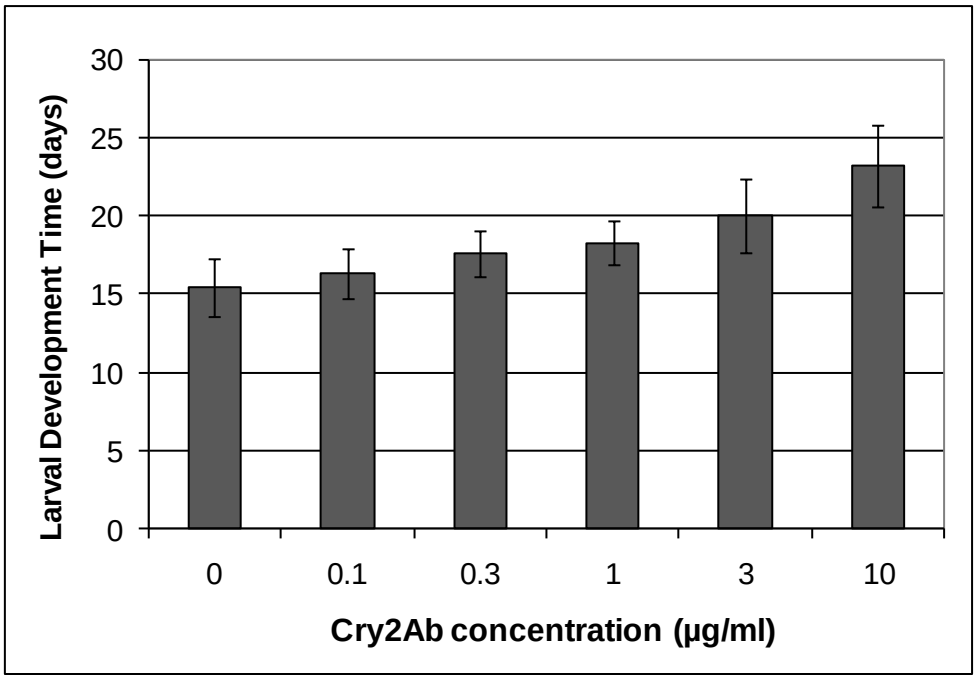


Figure 3. Microarray analysis of transcription of ca 6000 defined cDNA tags in susceptible (non-induced control) and laboratory Cry2Ab tolerant (induced) *H. armigera* neonate larvae.

a. Differentially expressed 1801genes in Cry2Ab tolerant neonate larvae. Data showing as Log2 ratio of susceptible/tolerant (Group1/Group2) 4 day-old neonate larvae raring on toxin-free artificial diet. Statistical analysis was performed by Welch's t test and Benjamini-Hochberg Method (1995).

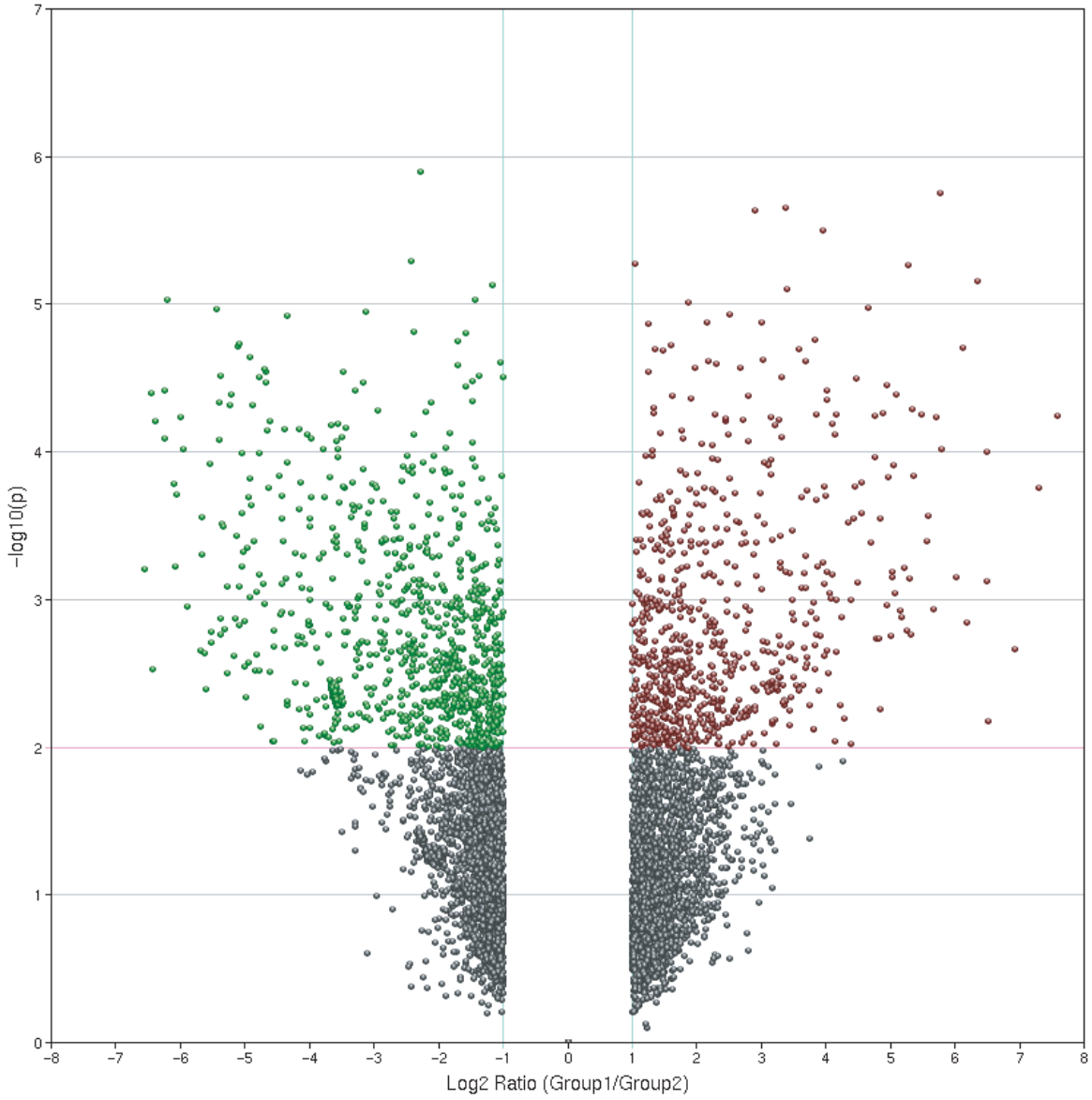


Figure 4. The immune status of Waite Susceptible (WS) and tolerant larvae reared on toxin free and toxin contaminated artificial diet from egg hatch neonate. The absorbance of cell-free plasma was first measured at 280nm to determine the relative protein concentration, and then the absorbance at 490nm was recorded every minute for 30 minute on a 100s spectrophotometer (Varian). The average of five separate measurements is shown. Bars represent SD.

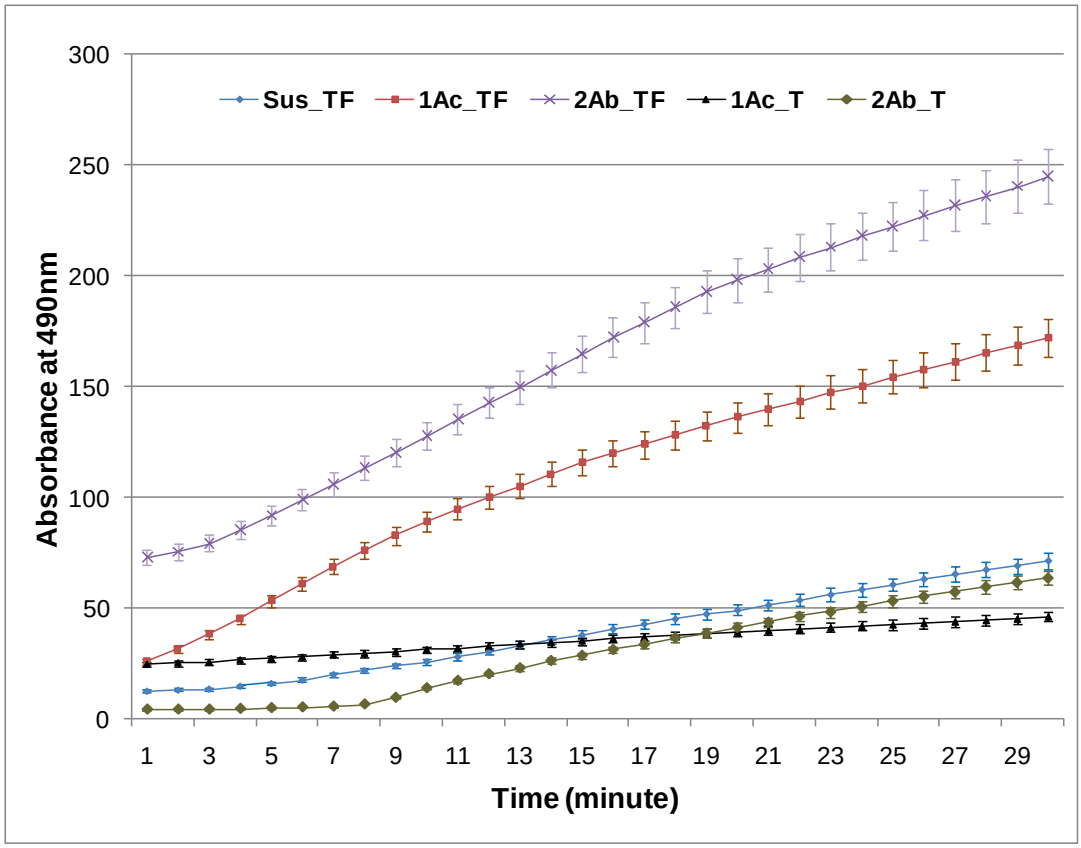
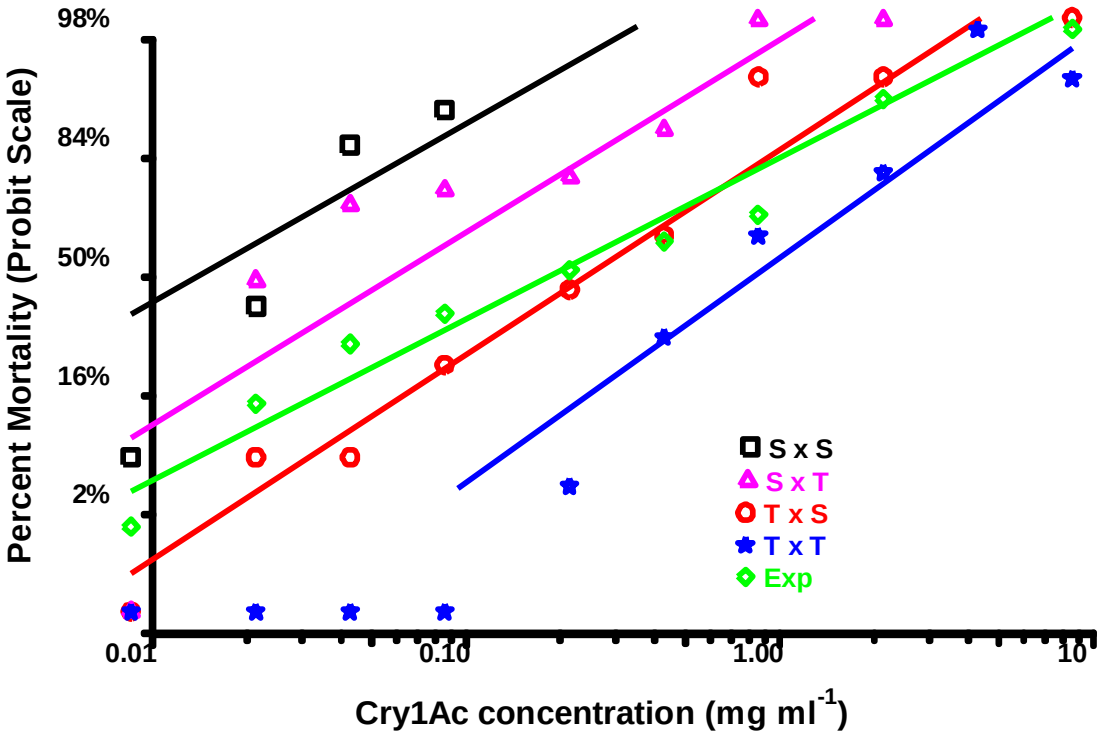


Figure 5. Log dose versus percentage of mortality (probit scale) of the unselected Waite susceptible (S×S), a sub population of Waite susceptible exposed to sub-lethal concentration of bacterial suspension containing Cry1Ac toxin for twelve generation (T×T) and their reciprocal genetic crosses T♀×S♂ (T×S) and S♀×T♂ (S×T) together with the values predicted for a single gene transmission (EXP)



TABLES:

Table 1. Differential Cry1Ac toxicities to neonate larvae derived from Waite susceptible (S×S), Cyr1Ac tolerant (T×T), and their reciprocal genetic crosses (S×T and T×S). Note that concentrations refer to total protein in the crude bacterial suspension. 95% CI, 95% confidence intervals; RR, resistance ratio.

Insect strain	LC_{50} (mg/ml)	95%CL	RR	LC_{90} (mg/ml)	95%CL	RR	LC_{99} (mg/ml)	95%CL	RR	Mean slope +/-SE
S♀×S♂	0.04	0.02-0.06	1	0.1	0.06-0.33	1	0.23	0.11-1.75	1	2.88+/-0.23
S♀×T♂	0.06	0.05-0.08	1.57	0.46	0.32-0.76	4.69	2.65	1.46-6.20	11.65	1.38+/-0.14
T♀×S♂	0.32	0.24-0.43	9.26	2.29	1.54 -3.86	23.1	11.23	6.19-25.67	48.48	1.51+/-0.12
(T♀×T♂) F_{12}	1.04	0.84-1.29	29.8	4.33	3.25-6.30	43.78	13.84	9.03-24.83	60.98	2.07+/-0.18

Table 2. Differential Cry2Ab toxicities to neonate larvae derived from Waite susceptible (S×S), Cyr2Ab tolerant (T×T,), and their reciprocal genetic crosses.

2a. Bioassays were performed with crude transgenic maize extract containing Cry2Ab

Insect Strain	LC_{50} (mg/ml)	95%CL	RR	LC_{90} (mg/ml)	95%CL	RR	LC_{99} (mg/ml)	95%CL	RR	Mean slope+/-SE
S♀×S♂	0.018	0.015-0.021	1	0.11	0.09-0.17	1	0.51	0.31-1.08	1	1.60+/-0.14
S♀×T♂	0.022	0.017-0.029	1.22	0.14	0.09-0.30	1.3	0.65	0.31-2.42	1.28	1.59+/-0.20
T♀×S♂	0.034	0.024-0.049	1.89	0.50	0.26-1.69	4.56	4.48	1.41-38.39	8.79	1.10+/-0.11
(T♀×T♂) F_8	0.097	0.05-0.31	5.39	1.14	0.35-10.79	10.11	8.54	1.62-201.53	16.66	1.12+/-0.08

2b. Bioassays were performed with crude bacterial (*B. thuringiensis ssp. kurstaki* HD-1 plasmid gene for the crystal protein Cry2Ab into an *E. coli* expression vector pQE30) suspension containing Cry2Ab. Note that concentrations refer to total protein in the crude bacterial suspension.

Insect Strain	LC_{50} (mg/ml)	95%CL	RR	LC_{90} (mg/ml)	95%CL	RR	LC_{99} (mg/ml)	95%CL	RR	Mean slope+/-SE
S♀×S♂	0.71	0.54-0.94	1	4.44	3.07-7.27	1	19.76	11.28-43.34	1	1.61+/-0.13
S♀×T♂	2.79	1.71-4.93	3.91	18.6	9.3-63.13	4.19	87.12	31.51-594	4.41	1.56+/-0.13
T♀×S♂	4.21	2.67-7.37	5.9	54.07	24.51-194.52	12.18	433.48	132.5-3163	21.94	1.16+/-0.11
(T♀×T♂) F_{18}	10.89	6.85-22.18	15.27	129.91	48.68-1228	29.26	980.3	210-37120	49.61	1.19+/-0.20
TF-F_{10}	0.46	0.33-0.61	1	5.374	3.58-9.16	1	40.14	20.82-99.44	1	1.20+/-0.10
TM-F_{10}	7.73	6.12-9.88	16.8	37.822	26.53-61.81	7.04	137.96	80.61-299.93	3.44	1.86+/-0.17

Table 3. Differential cross tolerance to Cry1Ac and Cry2Ab between Cry1Ac and Cry2Ab tolerant populations. Note that concentrations refer to total protein in the crude bacterial suspensions.

Insect Strain	Toxin	LC_{50} (mg/ml)	95%CL	RR	LC_{90} (mg/ml)	95%CL	RR	LC_{99} (mg/ml)	95%CL	RR	Mean slope+/-SE
Cry2AB											
WS	**Cry2Ab**	0.713	0.541-0.935	**1**	4.44	3.07-7.27	**1**	19.76	11.28-43.34	**1**	1.61+/-0.13
Tol_2Ab	**Cry2Ab**	10.89	6.85-22.18	**15.27**	129.91	48.68-1228	**29.26**	980.3	210-37120	**49.61**	1.19+/-0.20
Tol_1Ac	**Cry2Ab**	8.878	6.88-12.057	**12.45**	49.827	31.55-97.38	**11.22**	203.33	102.9-567.5	**10.29**	1.71+/-0.19
Cry1AC											
WS	**Cry1Ac**	0.04	0.02-0.06	**1**	0.1	0.06-0.33	**1**	0.23	0.11-1.75	**1**	2.9+/-0.23
Tol_1Ac	**Cry1Ac**	1.04	0.84-1.29	**29.8**	4.33	3.25-6.30	**43.78**	13.84	9.03-24.83	**60.98**	2.07+/-0.18
Tol_2Ab	**Cry1Ac**	0.529	0.355-0.742	**13.2**	2.288	1.502-4.602	**22.9**	7.551	3.93-25.28	**32.83**	2.02+/-0.24

Table 4. Differential gene expression in Cry2Ab tolerant larvae.

Table 4a. A list of *H. armigera* receptor, immune, developmental and metabolism-related genes that were differentially transcribed (2< fold) in Cry2Ab tolerant neonate larvae.

Gene identifier	Seq. length	Fold change	Seq. name	Sequence description	P-value	E-value
Har_00172514	665	-85.81	Har_454S-C22794	AGAP007875-PA [Anopheles gambiae str. PEST]	0.00294527	0.07
Har_00022747	778	-76.03	Har_454S-C13373	isoform e	8.14E-05	4.47E-36
Har_00012039	875	-74.15	Har_454S-C11395	endonuclease-reverse transcriptase	9.31E-06	2.30E-18
Har_00010326	891	-51.86	Har_454S-C11072	f-box lrr	0.00218917	2.62E-49
Har_00010093	893	-49.4	Har_454S-C11018	tropomyosin 2	0.0022814	5.17E-05
Har_00095721	1351	-41.89	Har_454S-C7701	enolase-phosphatase e-1	8.19E-05	2.30E-06
Har_00028990	715	-41.82	Har_454S-C14543	tmf1-like protein	0.00172754	0.01
Har_00016699	835	-39.76	Har_454S-C12240	cadherin	0.00157886	4.00E-32
Har_00070515	1129	-38.82	Har_454S-C4200	low molecular lipoprotein 30k precursor	0.00316357	3.29E-85
Har_00110725	939	-38.52	Har_454S-C9841	isoform c	0.00081332	1.55E-87
Har_00030709	695	-37	Har_454S-C14862	isoform b	4.09E-05	1.76E-19
Har_00044415	540	-36.07	Har_454S-C17228	2-oxoglutarate dehydrogenase	0.00241243	1.66E-35
Har_00005806	922	-32.94	Har_454S-C10257	viral a-type inclusion protein	0.00025828	0
Har_00031895	683	-31.57	Har_454S-C15073	isoform a	0.00462428	2.00E-44
Har_00118897	711	-31.3	Har_454S-C3923	loc100135188 protein	0.00045019	2.88E-28
Har_00011725	878	-30.44	Har_454S-C11331	equilibrative nucleoside transporter	0.00096892	4.97E-37
Har_00078924	1063	-29.69	Har_454S-C5414	anarchy 1	4.83E-05	8.35E-27
Har_00039391	610	-28.78	Har_454S-C16286	cuticular protein 4 from low complexity family (agap003334-pa)	0.00303489	9.80E-07
Har_00121318	3437	-28.3	Har_454S-C6293	proclotting enzyme	0.00237224	8.05E-151
Har_00042577	569	-27.64	Har_454S-C16861	isoform a	0.00068115	7.00E-14

Har_00037313	630	-27.41	Har_454S-C15949	isoform d	3.12E-05	1.95E-16
Har_00001241	1116	-24.46	Hvi_Timeless_HeliobaseV1 Contig995	circadian clock protein	0.00309902	4.21E-116
Har_00019071	811	-24.4	Har_454S-C12699	cg33964 cg33964-pa	6.17E-05	9.10E-19
Har_00057586	977	-23.31	Har_454S-C2455	vacuolar protein sorting 33a	0.00162907	1.46E-68
Har_00050945	243	-22.17	Har_454S-C19047	embryonic development factor	0.00014581	2.85E-13
Har_00108398	953	-21.64	Har_454S-C9512	penicillin-binding1a family	0.00121121	3.52E-15
Har_00072663	1541	-21.4	Har_454S-C4515	domain containing 24	0.00019943	5.31E-34
Har_00110095	942	-21.32	Har_454S-C9755	protein	0.00040206	3.20E-24
Har_00107616	959	-20.56	Har_454S-C9410	major facilitator superfamily domain containing 9	0.00072135	2.35E-30
Har_00075715	943	-19.48	Har_454S-C494	fibroin p25	0.00123488	2.16E-04
Har_00078421	1078	-18.7	Har_454S-C5339	membrane-associated lps-inducible tnf alpha factorprotein	0.00361845	2.75E-25
Har_00117621	221	-18.11	Har_454S-C26783	kiaa2010 protein	0.00199165	2.48E-12
Har_00050036	343	-18.02	Har_454S-C18630	tubulin beta	0.00176007	2.82E-13
Har_00121229	238	-17.78	Har_454S-C27406	deoxyribonuclease ii beta	0.00068166	2.36E-07
Har_00006942	912	-17.7	Har_454S-C10443	short-chain dehydrogenase	0.00015965	5.29E-77
Har_00058680	834	-16.89	Har_454S-C2564	tpa:cuticle protein	0.0091445	2.06E-45
Har_00092815	1555	-16.86	Har_454S-C7316	protein geranylgeranyltransferase typebeta subunit	0.00199372	2.89E-112
Har_00119693	275	-16.27	Har_454S-C20670	family protein	0.00139699	4.14E-04
Har_00097580	1253	-16.22	Har_454S-C7976	sugar transporter	0.00149116	3.60E-67
Har_00075971	539	-16.05	Har_454S-C497	zinc finger protein 514	0.00602088	1.39E-10
Har_00100723	1128	-15.85	Har_454S-C8414	torso-like protein	0.00644365	1.94E-42
Har_00034048	661	-15.62	Har_454S-C15415	molybdopterin synthase small subunit mocs2a	0.00020053	1.19E-18
Har_00117960	468	-14.76	Har_454S-C27142	glutathione s-transferase 4	0.00477463	3.07E-47
Har_00104817	997	-14.45	Har_454S-C9005	tyrosine-protein kinase pr2	0.00052214	3.03E-84
Har_00042115	575	-14.33	Har_454S-C16771	ef hand domainmember b	0.00836813	5.24E-17
Har_00036088	642	-14.15	Har_454S-C15748	protein	0.00267799	2.23E-07
Har_00018801	814	-13.89	Har_454S-C12648	guanine nucleotide-binding protein subunit beta 1	9.48E-05	3.15E-19
Har_00003227	1406	-13.58	gi|30230468|gb|AY253870.1|	pheromone binding protein precursor	0.00461043	4.59E-28
Har_00110735	939	-12.57	Har_454S-C9843	larval cuticle protein 14	0.00394906	1.26E-04
Har_00084348	1018	-12.51	Har_454S-C6181	lysozyme	0.00756908	4.97E-74
Har_00121033	819	-12.5	Har_454S-C21043	chromosome 5 open reading frame 28	0.00071811	1.34E-17
Har_00011322	882	-12.4	Har_454S-C11255	gustatory receptor 60	0.00923224	9.56E-12
Har_00052865	851	-12.15	Har_454S-C2058	carboxylesterase-8 variant 1	0.00502415	1.67E-42
Har_00047380	791	-12.11	Har_454S-C1783	reverse transcriptase	0.0068941	1.81E-08
Har_00105488	984	-11.94	Har_454S-C9107	rad25 xp-b dna repair helicase	0.00036594	1.42E-86
Har_00116995	185	-11.92	Har_454S-C26176	cg16798 cg16798-pa	0.00044416	1.28E-06
Har_00087037	2032	-11.92	Har_454S-C6529	cytochrome p450	0.00040669	2.16E-173
Har_00121864	262	-11.89	Har_454S-C26921	bcp inhibitor	0.00010914	0.01
Har_00027213	734	-11.85	Har_454S-C14205	isoform a	0.00645243	4.34E-51
Har_00049570	382	-11.56	Har_454S-C18448	cytochrome c	0.00914034	9.49E-37
Har_00004401	932	-11.28	Har_454S-C10019	u3 small nucleolar ribonucleoprotein protein mpp10	0.00518063	4.99E-14
Har_00004913	928	-11.18	Har_454S-C10108	carboxylesterase	2.86E-05	6.97E-58
Har_00017633	827	-11.14	Har_454S-C12416	uncharacterized protein flj10769	0.00017173	3.22E-29
Har_00112496	1266	-11.05	Har_454S-C20058	isoform a	0.00203982	1.19E-33
Har_00017469	828	-10.61	Har_454S-C12385	pig-a protein	0.00049447	1.59E-90

Har_00005721	923	-10.61	Har_454S-C10244	ring finger protein 8	0.00164715	1.07E-08
Har_00020986	793	-10.33	Har_454S-C13057	glucose dehydrogenase	0.00110925	9.26E-37
Har_00120395	252	-9.78	Har_454S-C25467	eukaryotic translation initiation factor 4e	0.0032505	6.55E-05
Har_00024378	763	-9.64	Har_454S-C13666	maltose phosphorylase	0.00213196	1.30E-24
Har_00014755	854	-9.45	Har_454S-C11875	protein kinase	0.00291254	0.02
Har_00091830	1653	-9.44	Har_454S-C7186	lipid storage droplet-isoform c	0.00584814	5.85E-117
Har_00092948	1540	-9.33	Har_454S-C7335	juvenile hormone epoxide hydrolase	0.0023001	1.56E-118
Har_00068689	984	-9.15	Har_454S-C3943	molybdopterin cofactor sulfurase	0.00055426	2.30E-93
Har_00069422	1172	-9.02	Har_454S-C4055	carboxypeptidase inhibitor	0.00046228	0
Har_00114623	312	-8.83	Har_454S-C23290	apoptosis antagonizing transcription factor	0.00629999	1.08E-04
Har_00103848	1020	-8.83	Har_454S-C8871	kallikrein 7 (stratum corneum)isoform cra_b	0.00030805	1.06E-07
Har_00118058	256	-8.7	Har_454S-C27299	metaxin 1	0.00511824	2.92E-13
Har_00087075	1760	-8.66	Har_454S-C6537	beta lactamase domain	0.00533824	2.44E-94
Har_00026311	1094	-8.61	Har_454S-C1403	cathepsin b	0.00397771	1.35E-128
Har_00118615	188	-8.57	Har_454S-C27884	pyridoxal-dependent decarboxylase	0.00194384	3.69E-16
Har_00013567	863	-8.52	Har_454S-C11681	sugar transporter	0.00025901	3.66E-69
Har_00011324	882	-8.35	Har_454S-C11256	dna adenine methyltransferase	0.00234644	2.20E-08
Har_00007052	914	-8.29	Har_454S-C10465	thap4 protein	0.00021601	2.05E-34
Har_00031101	690	-8.03	Har_454S-C14932	cuticular protein	0.00393461	1.52E-31
Har_00110289	941	-7.96	Har_454S-C9781	cg6459	0.00134172	2.83E-81
Har_00121917	185	-7.71	Har_454S-C27987	reverse transcriptase	0.0053176	5.54E-12
Har_00003056	229	-7.6	gi|46358270|emb|AJ555185.1|	allatotropin	0.00108714	1.84E-38
Har_00034094	661	-7.46	Har_454S-C15422	5-nucleotidase domain protein	0.00166651	1.26E-16
Har_00050953	240	-7.45	Har_454S-C19057	nad-dependent deacetylase sirtuin4	0.00078072	3.27E-09
Har_00044872	532	-7.32	Har_454S-C17301	troponin c	0.00041145	1.98E-30
Har_00023435	771	-7.31	Har_454S-C13493	glutamateionotropic kainate3	0.0004031	2.43E-10
Har_00063356	822	-7.23	Har_454S-C3138	chemosensory protein 2	0.00021639	8.73E-33
Har_00061002	959	-7.2	Har_454S-C2828	multidrug resistance-associated protein	0.00195357	2.50E-48
Har_00068761	887	-7.17	Har_454S-C3952	novel krab box and zincc2h2 type domain containing protein	0.0003862	3.11E-10
Har_00166020	450	-7.08	Har_454S-C18022	regulatory protein cii	0.00137616	0
Har_00060883	949	-7.01	Har_454S-C2811	ankyrin unc44	0.00039028	1.78E-06
Har_00011323	882	-6.98	Har_454S-C11255	gustatory receptor 60	0.00593202	9.56E-12
Har_00050052	341	-6.97	Har_454S-C18636	atp-dependent rna helicase dbp9	0.00588332	5.90E-19
Har_00094251	1446	-6.94	Har_454S-C7501	amp dependent ligase	0.00251123	0
Har_00139023	399	-6.94	Har_454S-C18356	fibropellin partial	0.00354638	0.05
Har_00050228	322	-6.92	Har_454S-C18713	autophagy-specific gene 12	0.00048101	3.91E-18
Har_00029453	710	-6.86	Har_454S-C14635	chromaffin granule amine transporter	0.0077968	1.05E-59
Har_00035258	650	-6.85	Har_454S-C15619	neuroserpin precursor (serpin i1) (protease inhibitor 12)	0.00346091	9.51E-38
Har_00004081	1202	-6.81	gi|169260701|gb|EU497670.1|	odorant receptor or83b	0.00082209	5.70E-05
Har_00072763	1035	-6.79	Har_454S-C4529	required for meiotic nuclear division 5 homolog a	0.00299737	2.05E-06
Har_00112587	278	-6.62	Har_454S-C20116	kynureninase	0.0096002	2.10E-12
Har_00114790	223	-6.59	Har_454S-C23553	cg7120 cg7120-pa	0.00103823	2.64E-14
Har_00082495	573	-6.53	Har_454S-C5917	24-dehydrocholesterol reductase	0.00746361	4.51E-69
Har_00152629	1325	-6.3	Har_454S-C6525	hypothetical protein [Plasmodium chabaudi chabaudi]	0.00842435	0.04
Har_00031045	691	-6.14	Har_454S-C14919	isoform c	0.00102902	1.42E-45

Har_00018672	815	-6.14	Har_454S-C12621	ectodermal cg6611-pa	0.00219949	3.25E-32
Har_00030160	701	-6.11	Har_454S-C14764	phospholipase a1	0.00165618	1.25E-12
Har_00118691	256	-6.03	Har_454S-C28002	valacyclovir hydrolase	0.00038695	5.69E-17
Har_00093428	1499	-5.96	Har_454S-C7395	fetal alzheimerfalz	0.0027176	1.79E-79
Har_00118690	256	-5.93	Har_454S-C28002	valacyclovir hydrolase	0.00122669	5.69E-17
Har_00054708	1109	-5.92	Har_454S-C22081	carbonyl reductase 1	0.00523539	1.82E-64
Har_00000232	1669	-5.85	Har_CYP450_Contig181	cytochrome family subfamily polypeptide 2	0.00853676	1.44E-63
Har_00028161	1171	-5.78	Har_454S-C1438	salivary gland-expressedisoform a	0.0020893	1.70E-23
Har_00070566	1716	-5.76	Har_454S-C4210	sugar transporter	0.00050792	9.07E-54
Har_00045013	1231	-5.69	Har_454S-C1733	cg31997 cg31997-pa	0.00670744	1.43E-52
Har_00040100	603	-5.69	Har_454S-C16402	glutathione s-transferase	0.00125665	8.07E-70
Har_00093537	805	-5.64	Har_454S-C741	adenylate cyclase	0.00010577	6.77E-67
Har_00077887	879	-5.63	Har_454S-C5252	repressor of rna polymerase iii transcription maf1	0.00443483	2.04E-30
Har_00040498	598	-5.52	Har_454S-C16474	isoform h	0.00840134	7.73E-09
Har_00035256	650	-5.52	Har_454S-C15619	neuroserpin precursor (serpin i1) (protease inhibitor 12)	0.00170526	9.51E-38
Har_00064288	807	-5.51	Har_454S-C3269	lipopolysaccharide binding protein	0.0007999	4.64E-23
Har_00024151	873	-5.38	Har_454S-C1362	isoform b	0.00338481	6.93E-57
Har_00112531	615	-5.34	Har_454S-C20077	cg9117 cg9117-pa	0.00202328	1.49E-34
Har_00007277	913	-5.33	Har_454S-C10503	isoform b	0.00300692	1.87E-90
Har_00013761	862	-5.3	Har_454S-C11709	glutathione s-transferase	0.00031729	9.89E-99
Har_00103029	1042	-5.29	Har_454S-C8756	pupal cuticle protein 78e	0.00224712	4.37E-73
Har_00089418	2371	-5.28	Har_454S-C6850	moderately methionine rich storage protein	0.00280176	0
Har_00030523	696	-5.26	Har_454S-C14831	pin2-interacting protein 1	0.00190344	2.61E-45
Har_00001022	1657	-5.23	Har_IMPI_HA2-GN-G-15_A18	scavenger receptor cysteine-rich protein	0.0018707	1.18E-49
Har_00001022	1657	-5.23	Har_IMPI_HA2-GN-G-15_A18	scavenger receptor cysteine-rich protein	0.0018707	1.18E-49
Har_00032987	672	-5.17	Har_454S-C15251	tan cg12120-pa	0.0010968	1.15E-36
Har_00076295	835	-5.14	Har_454S-C5012	nicotinic acetylcholine receptor alpha9 subunit	0.00522032	2.39E-26
Har_00099357	1177	-5.14	Har_454S-C8219	uncharacterized protein kiaa1370 homolog	0.00218132	5.18E-12
Har_00015279	849	-5.09	Har_454S-C11967	isoform b	0.00019941	2.68E-16
Har_00016657	835	-5.08	Har_454S-C12234	cg10914 cg10914-pa	0.00369189	7.39E-11
Har_00091826	1653	-5.07	Har_454S-C7185	isoform a	0.00299293	2.42E-88
Har_00057622	731	-5.02	Har_454S-C2458	tissue factor pathway inhibitor	0.00750437	1.73E-23
Har_00105323	986	-5.02	Har_454S-C9087	zincmym domain containing 1	0.00800871	1.04E-04
Har_00007517	911	-4.93	Har_454S-C10545	hexamerin 2 beta	0.0010602	1.55E-20
Har_00078181	897	-4.91	Har_454S-C5296	transcription factor iiia	0.00079651	3.18E-10
Har_00110876	938	-4.91	Har_454S-C9863	voltage and ligand gated potassium	0.00074008	0.02
Har_00006001	921	-4.88	Har_454S-C10286	lipopolysaccharide binding protein	0.0009355	8.67E-43
Har_00001663	190	-4.87	gi|116833144|gb|DQ875243.1|	immune inducible protein	0.00074062	1.16E-32
Har_00120501	515	-4.83	Har_454S-C26430	tenascin c	0.000967	2.33E-46
Har_00013555	863	-4.82	Har_454S-C11680	lipopolysaccharide binding protein	0.001217	2.09E-40
Har_00017564	827	-4.82	Har_454S-C12401	briggsae cbr-npp-21 protein	0.00713669	5.92E-13
Har_00070248	1899	-4.78	Har_454S-C4167	mitochondrial ribosomal protein l33	0.0028316	5.69E-20
Har_00015572	842	-4.75	Har_454S-C12027	cecropin-like protein	0.00011682	1.15E-19
Har_00117314	191	-4.75	Har_454S-C26464	glutathione s-transferase 1	0.00403743	3.44E-22
Har_00083564	1274	-4.64	Har_454S-C6079	amino acid transporter	0.00140935	3.38E-84

Har_00065420	3279	-4.64	Har_454S-C3428	zinc transporter	0.0023743	1.38E-75
Har_00117231	294	-4.63	Har_454S-C26414	replication factor c (activator 1) 5	0.00483957	1.52E-46
Har_00119970	317	-4.62	Har_454S-C22774	folliculin isoform 1	0.00634796	1.84E-07
Har_00080997	1186	-4.61	Har_454S-C5701	elegans proteinconfirmed by transcript evidence	0.00047916	6.16E-29
Har_00003027	591	-4.6	gi|47027881|gb|AY588581.1|	cytochrome p450	0.00041355	4.45E-69
Har_00114917	635	-4.57	Har_454S-C23760	domain containing 3	5.38E-05	8.32E-07
Har_00068371	1458	-4.56	Har_454S-C3885	nicotinamide riboside kinase 1	0.00079894	3.24E-09
Har_00055151	1594	-4.51	Har_454S-C2244	cytochrome p450	0.00045369	6.55E-168
Har_00001662	190	-4.46	gi|116833144|gb|DQ875243.1|	immune inducible protein	0.00026438	1.16E-32
Har_00048585	430	-4.43	Har_454S-C18149	cytochrome p450	0.00021984	5.40E-47
Har_00023189	773	-4.4	Har_454S-C13451	novel proteinvertebrate ferm domain containing 4a	0.00530262	0
Har_00073024	853	-4.35	Har_454S-C4562	novel protein	0.00121508	1.39E-12
Har_00110557	940	-4.32	Har_454S-C9813	domain containing 3	4.59E-05	1.29E-25
Har_00055152	1594	-4.3	Har_454S-C2244	cytochrome p450	0.00083855	6.55E-168
Har_00049832	360	-4.3	Har_454S-C18544	dehydrogenase reductase (sdr family) member 13	0.00051855	1.68E-13
Har_00087038	2032	-4.28	Har_454S-C6529	cytochrome p450	0.00255837	2.16E-173
Har_00007493	912	-4.23	Har_454S-C10541	membrane-associated phospholipase a1 beta	0.00353567	5.02E-27
Har_00097417	1260	-4.23	Har_454S-C7954	nad dehydrogenase	0.00486755	4.15E-168
Har_00053669	1031	-4.21	Har_454S-C21162	tetraspanin 29fb	0.00010642	5.47E-09
Har_00000860	1530	-4.14	Har_CYP450_HAH003329_1	cytochrome p450	0.00285732	1.75E-169
Har_00000073	1475	-4.12	Har_CYP450_Contig158	cytochrome p450	0.00067216	2.94E-158
Har_00098447	1219	-4.12	Har_454S-C8095	nicotinic acetylcholine receptor alpha9 subunit	0.00219734	5.93E-43
Har_00038660	617	-4.11	Har_454S-C16161	isoform cra_a	0.00957891	2.89E-46
Har_00080379	1261	-4.06	Har_454S-C5616	ankyrin repeat-containing	0.00105425	2.46E-31
Har_00046351	502	-4.04	Har_454S-C17600	longevity assurance factor 1	0.00130613	1.18E-12
Har_00120503	515	-4.01	Har_454S-C26430	tenascin c	0.00399118	2.33E-46
Har_00051903	690	-3.98	Har_454S-C19926	nicotinic acetylcholine receptor alpha 9-ii subunit	0.00177126	4.77E-09
Har_00061179	893	-3.97	Har_454S-C2850	imp dehydrogenase gmp reductase	0.00286254	0
Har_00052968	752	-3.96	Har_454S-C2066	c10 protein	0.0015365	4.61E-27
Har_00056636	797	-3.96	Har_454S-C2372	retinol-binding protein	0.00478796	2.85E-94
Har_00092040	1626	-3.93	Har_454S-C7212	cg3106 cg3106-pa	0.00880558	1.11E-53
Har_00029022	714	-3.91	Har_454S-C14548	tenascin c	0.00489349	7.84E-58
Har_00122032	153	-3.9	Har_454S-C27599	nima-related kinase 8	0.0080424	0
Har_00073118	1444	-3.84	Har_454S-C4577	dihydrouridine synthase domain containing protein	0.00999375	4.76E-13
Har_00022622	779	-3.76	Har_454S-C13344	carboxylesterase	0.00776809	4.89E-75
Har_00116823	191	-3.76	Har_454S-C25942	short-chain dehydrogenase reductase sdr	0.00017318	2.16E-16
Har_00035905	644	-3.73	Har_454S-C15719	reverse transcriptase	0.00013093	0.01
Har_00082020	780	-3.73	Har_454S-C5844	sugar transporter	0.00483075	1.27E-30
Har_00046280	503	-3.73	Har_454S-C17587	valacyclovir hydrolase	0.00085816	1.48E-23
Har_00021323	791	-3.72	Har_454S-C13119	phosphoglucomutase	0.00877358	7.97E-65
Har_00078088	909	-3.7	Har_454S-C5279	modifier ofisoform a	0.00147218	0.01
Har_00037838	625	-3.69	Har_454S-C16030	elongation factor tu gtp binding domain containing 1	0.00109576	1.19E-34
Har_00074702	754	-3.68	Har_454S-C4796	heat shock protein	0.00013855	7.24E-09
Har_00019061	811	-3.67	Har_454S-C12697	activin receptor type ii	0.00464457	1.12E-101
Har_00048586	430	-3.67	Har_454S-C18149	cytochrome p450	0.00125834	5.40E-47

Har_00051416	871	-3.67	Har_454S-C1959	glutathione s-transferase	0.00826067	2.56E-102
Har_00113628	403	-3.66	Har_454S-C21657	abcatp-binding protein	0.00638787	0.07
Har_00116945	234	-3.66	Har_454S-C26105	lots wife cg33968-pa	0.00563657	8.66E-18
Har_00022634	779	-3.66	Har_454S-C13346	protein phosphatase 2c	0.00261683	1.47E-71
Har_00002283	5421	-3.63	gi|163866851|gb|EU327674.1|	cytochrome p450	0.00324742	1.57E-33
Har_00015702	844	-3.56	Har_454S-C12053	pol polyprotein	7.48E-05	6.68E-84
Har_00082953	445	-3.54	Har_454S-C5988	thioredoxin family trp26	0.00013484	3.66E-06
Har_00010936	885	-3.51	Har_454S-C11189	phosphomevalonate kinase	0.00440697	6.00E-30
Har_00024267	764	-3.49	Har_454S-C13641	copia-type polyprotein	0.00607267	2.89E-24
Har_00096018	1329	-3.49	Har_454S-C7750	nonclathrin coat protein gamma1-cop	0.00599416	2.77E-129
Har_00117326	170	-3.48	Har_454S-C26475	dead (asp-glu-ala-asp) box polypeptide 52	0.00109277	8.08E-08
Har_00120142	677	-3.45	Har_454S-C24180	dihydrouridine synthase domain containing protein	0.0055301	2.79E-14
Har_00074419	543	-3.43	Har_454S-C4756	synapse defectiverhohomolog 2	0.00105078	9.76E-04
Har_00032392	678	-3.42	Har_454S-C15163	cuticle protein	0.00270157	5.14E-55
Har_00100755	1124	-3.41	Har_454S-C8419	alpha gamma epsilon	0.00534691	4.15E-135
Har_00113073	897	-3.41	Har_454S-C20857	trithorax protein ash2	0.00355819	1.21E-17
Har_00000960	1479	-3.36	Har_CYP450_HARM_Contig3	cytochrome p450	0.00259011	4.12E-168
Har_00120137	677	-3.36	Har_454S-C24180	dihydrouridine synthase domain containing protein	0.00473443	2.79E-14
Har_00044046	546	-3.36	Har_454S-C17155	three prime repair exonuclease 1	0.00363225	0.02
Har_00047125	1223	-3.35	Har_454S-C1778	peptidoglycan recognition protein	0.00692408	3.12E-84
Har_00024486	762	-3.35	Har_454S-C13689	organic cation transporter	0.00691481	5.28E-18
Har_00000138	1936	-3.34	Har_CYP450_Contig167	cytochrome p450	0.00214018	2.88E-167
Har_00076079	1638	-3.33	Har_454S-C4984	hat family dimerisation domain containing protein	0.00277965	1.43E-08
Har_00041576	582	-3.31	Har_454S-C16670	af453824_1antifungal peptide gallerimycin	0.00095296	0
Har_00023359	772	-3.27	Har_454S-C13480	chemosensory protein 11	0.00164611	6.55E-46
Har_00119900	648	-3.24	Har_454S-C22269	coiled-coil-helix-coiled-coil-helix domain containing 7	0.00903918	3.52E-16
Har_00081817	1165	-3.23	Har_454S-C5815	sugar transporter	0.0021081	3.30E-19
Har_00001614	417	-3.22	gi|116833176|gb|DQ875259.1|	cobatoxin-like protein	0.00925358	2.10E-58
Har_00004221	2176	-3.22	gi|27151863|gb|AF482905.2|	delta-9 desaturase 1	0.00055729	0
Har_00069092	1994	-3.21	Har_454S-C4000	serine proteaseserpin	0.00879817	9.62E-34
Har_00087264	2013	-3.21	Har_454S-C6563	methylenetetrahydrofolate dehydrogenase (nadp dependent) 2-like	0.00294664	5.26E-88
Har_00115482	251	-3.2	Har_454S-C24525	aldo-keto reductase familymember d1	0.0003252	4.08E-20
Har_00114175	367	-3.19	Har_454S-C22603	asteroid cg4426-pa	0.00729128	2.10E-08
Har_00010705	887	-3.19	Har_454S-C11142	morn repeat protein	0.00109461	6.04E-06
Har_00119933	376	-3.19	Har_454S-C22469	three prime repair exonuclease 2	0.00030595	9.66E-09
Har_00115292	330	-3.18	Har_454S-C24241	nonclathrin coat protein gamma1-cop	0.0005411	9.84E-22
Har_00097593	1252	-3.17	Har_454S-C7978	cg11905 cg11905-pi	0.00660453	9.62E-12
Har_00108723	861	-3.17	Har_454S-C957	switch-associated protein 70 (swap-70)	0.00572793	2.19E-05
Har_00121824	266	-3.16	Har_454S-C25924	gallerimycin-like protein	0.00062506	5.26E-23
Har_00041575	582	-3.15	Har_454S-C16670	af453824_1antifungal peptide gallerimycin	0.00070043	0
Har_00048397	437	-3.13	Har_454S-C18093	gallerimycin-like protein	0.00402092	8.84E-23
Har_00117021	227	-3.13	Har_454S-C26205	smad nuclear interacting protein	0.00016524	4.35E-09
Har_00102636	1054	-3.12	Har_454S-C8701	short-chain dehydrogenase	0.00460615	4.80E-51
Har_00120432	186	-3.11	Har_454S-C25880	abl interactor 2	0.00119898	
Har_00091233	1735	-3.11	Har_454S-C7105	pitchoune cg6375-pb	0.00213991	0

Har_00045983	510	-3.09	Har_454S-C17511	aliphatic nitrilase	0.0029033	3.57E-41
Har_00072456	385	-3.07	Har_454S-C4487	trithorax protein ash2	0.00401181	1.41E-52
Har_00083153	499	-3.03	Har_454S-C6020	immune inducible protein	0.00348301	9.63E-52
Har_00030138	1414	-3.03	Har_454S-C1476	ectodermal cg6611-pa	0.00530523	1.43E-30
Har_00107646	958	-2.87	Har_454S-C9415	prophenoloxidase activating factor	0.00371249	1.18E-05
Har_00117674	525	-2.82	Har_454S-C26864	immunoglobulin-binding protein 1	0.00309332	2.19E-38
Har_00074703	754	-2.8	Har_454S-C4796	heat shock protein	0.00384801	7.24E-09
Har_00085437	2212	-2.7	Har_454S-C6325	prophenoloxidase activating factor	0.00489381	1.48E-123
Har_00121911	201	-2.67	Har_454S-C27960	heat shock transcription factor	0.00332439	5.79E-06
Har_00075909	1029	-2.52	Har_454S-C4962	serine protease-like protein	0.00204889	5.21E-95
Har_00091969	1633	-2.15	Har_454S-C7204	mature-parasite-infected erythrocyte surface antigen	0.00569133	5.24E-11
Har_00057175	756	-1.9	Har_454S-C2422	beta--glucan recognition protein 3	0.00597199	1.25E-49
Har_00119291	2864	1.84	Har_454S-C6807	chaperonin containingsubunit 7	0.004833	8.30E-162
Har_00050622	280	1.85	Har_454S-C18894	aubergine protein	0.0046653	1.04E-39
Har_00097609	1252	1.9	Har_454S-C7981	serine protease	0.0011637	2.18E-64
Har_00015983	842	1.99	Har_454S-C12107	cytochrome p450	0.0091582	1.92E-107
Har_00080657	1054	2.05	Har_454S-C5655	serine hydrolase-like	0.0013649	1.15E-28
Har_00108436	953	2.08	Har_454S-C9518	chymotrypsin-like protease	0.000397	5.67E-69
Har_00018910	812	2.1	Har_454S-C12670	chymotrypsin-like protease	0.0004759	4.61E-63
Har_00167855	417	2.16	Har_454S-C24745	abc transporter atp-binding protein	0.008159	0.09
Har_00080583	1703	2.17	Har_454S-C5644	heat shock cognate 70 protein	0.0059163	0
Har_00115527	199	2.23	Har_454S-C24671	beta--glucan recognition protein 2	0.0096713	1.93E-17
Har_00024778	760	2.26	Har_454S-C13735	heat shock cognate 70 protein	0.0038444	1.52E-33
Har_00000247	704	2.33	Har_CYP450_Contig183	cytochrome p450	0.0002548	3.08E-34
Har_00000886	756	2.38	Har_CYP450_HAH005732_1	cytochrome p450	0.0088111	1.15E-40
Har_00020830	795	2.54	Har_454S-C13032	chymotrypsin-like protease	2.01E-05	2.28E-67
Har_00041442	584	2.56	Har_454S-C16644	cytochrome p450	0.0045844	5.60E-62
Har_00015137	850	2.57	Har_454S-C11940	serine hydrolase-like	0.0004589	1.98E-27
Har_00094078	1456	2.6	Har_454S-C7480	serine protease	0.0046302	6.15E-93
Har_00095523	1363	2.71	Har_454S-C7674	antichymotrypsin precursor	0.0011688	3.62E-128
Har_00000464	492	2.81	Har_CYP450_Contig218	cytochrome p450	0.0080721	3.18E-67
Har_00048745	426	2.9	Har_454S-C18190	alkaline phosphatase	0.0011197	2.34E-47
Har_00069700	588	2.92	Har_454S-C4094	serine protease	0.0003671	2.88E-37
Har_00007145	2463	2.93	Har_454S-C1048	cytochrome p450	0.0097127	0
Har_00059630	1393	2.96	Har_454S-C268	lipopolysaccharide binding protein	0.0030349	0
Har_00080982	2035	3	Har_454S-C570	serine protease	0.0027886	2.62E-119
Har_00092078	1620	3.01	Har_454S-C7218	dopa decarboxylase protein	0.0001851	0
Har_00067065	1308	3.01	Har_454S-C3687	riken cdnaisoform cra_a	1.90E-05	8.79E-39
Har_00053383	518	3.02	Har_454S-C20932	galactosesoluble 9	0.0038451	9.44E-12
Har_00088757	1781	3.04	Har_454S-C6754	serine protease	0.0078448	1.14E-43
Har_00098353	1222	3.04	Har_454S-C8084	lipase	0.0029482	0
Har_00089288	861	3.04	Har_454S-C683	mitochondrial 28s ribosomal protein s25	0.0022633	1.94E-78
Har_00103817	1023	3.05	Har_454S-C8867	mitochondrial 28s ribosomal protein s25	0.0093859	2.33E-79
Har_00113275	893	3.07	Har_454S-C21057	cg13886 cg13886-pa	4.14E-05	1.36E-13
Har_00119050	1785	3.07	Har_454S-C4948	niemann-pick type c-	0.0040965	2.44E-41

Har_00011683	878	3.07	Har_454S-C11323	peroxisomal biogenesis factor 19	0.0002453	4.96E-45
Har_00080254	565	3.08	Har_454S-C5598	amp dependent coa ligase	0.0018859	2.97E-26
Har_00054778	718	3.08	Har_454S-C22129	mitochondrial ribosomal	0.0002377	2.89E-63
Har_00111615	934	3.09	Har_454S-C9969	laminin gamma-1	0.0066448	0.01
Har_00044225	543	3.09	Har_454S-C17192	sugar transporter	0.0043671	2.65E-09
Har_00079001	991	3.11	Har_454S-C5421	mgc108042 protein	0.0077163	2.40E-49
Har_00066516	966	3.12	Har_454S-C3605	cutc copper transporter homolog	0.0067208	1.67E-41
Har_00117415	270	3.13	Har_454S-C26564	kynureninase	0.0002697	4.41E-30
Har_00098127	1229	3.14	Har_454S-C8056	gtp binding protein	0.0075569	0
Har_00038964	614	3.14	Har_454S-C16211	type iv collagen	0.0080811	8.64E-27
Har_00098277	1225	3.15	Har_454S-C8075	solute carrier family 41	0.0090953	1.13E-33
Har_00003476	1936	3.16	gi|1498590|gb|U64800.1|HAU64800	cytochrome p450	0.0029479	0
Har_00107013	965	3.17	Har_454S-C9326	prefoldin subunit 6	0.0007407	1.03E-41
Har_00065050	979	3.21	Har_454S-C3372	aaa atpase containing von willebrand factor type aomain	0.0021785	2.22E-07
Har_00046213	505	3.21	Har_454S-C17565	galactosesoluble 9	0.0027843	1.92E-26
Har_00048743	426	3.22	Har_454S-C18190	alkaline phosphatase	0.0066228	2.34E-47
Har_00015600	845	3.22	Har_454S-C12034	aldo-keto reductase	0.0017128	3.64E-106
Har_00101408	1099	3.25	Har_454S-C8517	rrna processing protein ebp2	0.0061413	1.47E-114
Har_00101159	1107	3.26	Har_454S-C8477	adult-type muscle actin 2	0.0006397	1.91E-21
Har_00087186	2502	3.26	Har_454S-C6550	juvenile hormone epoxide hydrolase	0.000215	1.21E-96
Har_00064112	847	3.27	Har_454S-C3243	arystal structure of epiphyas postvittana takeout 1	0.0078216	9.24E-25
Har_00008664	980	3.28	Har_454S-C1075	isoleucyl trna synthetase	0.0033709	4.45E-16
Har_00099302	1178	3.28	Har_454S-C8213	transaldolase 1	0.0051223	2.80E-120
Har_00098355	1222	3.3	Har_454S-C8084	lipase	0.0009101	0
Har_00116103	517	3.31	Har_454S-C25271	duf-like 1	0.0036539	8.27E-15
Har_00003478	1936	3.33	gi|1498590|gb|U64800.1|HAU64800	cytochrome p450	0.0038548	0
Har_00116689	291	3.33	Har_454S-C25826	isoform a	0.0034006	2.15E-16
Har_00002119	1230	3.33	gi|194024961|gb|EU818704.1|	odorant receptor	0.0010211	0
Har_00087491	1128	3.34	Har_454S-C6590	trypsin-like protease	0.0065696	1.57E-111
Har_00087551	2100	3.34	Har_454S-C6598	carboxylesterase	0.0066602	0
Har_00109377	946	3.35	Har_454S-C9661	dna mismatch repair protein mlh1	0.0011579	8.30E-50
Har_00104562	1001	3.35	Har_454S-C8976	kiaa0564 protein	0.0001333	1.63E-61
Har_00082219	940	3.35	Har_454S-C5877	lipase	0.0047098	1.65E-97
Har_00023969	767	3.36	Har_454S-C13585	trypsin-like protease	0.0069471	6.76E-114
Har_00092077	1620	3.36	Har_454S-C7218	dopa decarboxylase protein	0.0003993	0
Har_00044548	538	3.36	Har_454S-C17248	ethylmalonic encephalopathy 1	0.0038016	1.52E-41
Har_00087188	2502	3.36	Har_454S-C6550	juvenile hormone epoxide hydrolase	0.0013152	1.21E-96
Har_00030062	702	3.37	Har_454S-C14749	reverse transcriptase-like protein	0.0018852	7.41E-05
Har_00106752	968	3.37	Har_454S-C9281	short-chain dehydrogenase	0.0062596	2.90E-52
Har_00056787	1368	3.39	Har_454S-C2385	lectin galactoside-binding soluble 4	0.0006135	7.23E-40
Har_00049273	401	3.39	Har_454S-C18346	cuticular protein 67fb	7.09E-05	2.30E-10
Har_00100157	1148	3.41	Har_454S-C8330	gag-pol polyprotein	0.0039242	2.24E-04
Har_00076041	793	3.42	Har_454S-C4979	ubiquitin-like 7	0.005206	2.75E-14
Har_00021524	789	3.44	Har_454S-C13150	prophenoloxidase activating factor	0.0093919	6.33E-54
Har_00045485	520	3.44	Har_454S-C17415	promoting protein	0.0075935	1.70E-36

Har_00078170	1243	3.47	Har_454S-C5294	proline synthetase co-transcribed homolog	0.0028283	1.09E-108
Har_00092072	1621	3.48	Har_454S-C7217	isoform b	0.0011234	2.55E-117
Har_00070206	876	3.49	Har_454S-C4162	vitellogenin	0.0017567	4.32E-73
Har_00048262	445	3.51	Har_454S-C18057	3-hydroxybutyrate dehydrogenase type 2	0.0004882	1.70E-13
Har_00036126	642	3.53	Har_454S-C15753	epidermal protein	0.0047283	1.75E-44
Har_00065959	1623	3.54	Har_454S-C3510	cytochrome p450	0.0089935	1.17E-114
Har_00053380	518	3.54	Har_454S-C20932	galactosesoluble 9	0.0032209	9.44E-12
Har_00095878	1336	3.56	Har_454S-C7726	apolipoprotein d	0.0097808	6.28E-89
Har_00089896	2086	3.58	Har_454S-C6921	cg9246 cg9246-pa	0.0022579	2.49E-149
Har_00071785	550	3.58	Har_454S-C439	cuticle protein 1	0.0041406	2.82E-38
Har_00035930	644	3.6	Har_454S-C15721	lipase	0.0086917	5.99E-53
Har_00001857	1512	3.61	gi|171740898|gb|EU325554.1|	lipase	0.0029632	0
Har_00052842	2395	3.61	Har_454S-C2056	sugar transporter	0.0020054	3.07E-106
Har_00066155	903	3.63	Har_454S-C3542	hit zinc finger family protein	0.0030904	2.59E-44
Har_00107305	1130	3.63	Har_454S-C937	tho complex 7	0.0051979	1.22E-55
Har_00010663	887	3.65	Har_454S-C11137	peptidase d	0.0062068	8.90E-50
Har_00097122	1277	3.67	Har_454S-C7913	juvenile hormone epoxide hydrolase	0.0099909	5.47E-103
Har_00116983	220	3.68	Har_454S-C26146	mgc79752 protein	0.0025162	6.96E-23
Har_00082968	1263	3.68	Har_454S-C599	rna binding motif protein 8a	0.0012211	2.36E-74
Har_00081227	763	3.71	Har_454S-C5733	methylenetetrahydrofolate dehydrogenase	0.0019887	7.92E-115
Har_00086176	1048	3.72	Har_454S-C6423	cg14903 cg14903-pa	0.0022272	9.93E-41
Har_00032777	674	3.74	Har_454S-C15217	cg1134 cg1134-pa	0.00913	1.96E-44
Har_00082598	1179	3.74	Har_454S-C5931	cg6443 cg6443-pa	4.31E-05	7.35E-83
Har_00082220	940	3.75	Har_454S-C5877	lipase	0.0067771	1.65E-97
Har_00012569	870	3.76	Har_454S-C11495	aconitase 1	0.0004049	3.17E-44
Har_00079615	1339	3.76	Har_454S-C5507	juvenile hormone-inducible	0.0004337	8.36E-33
Har_00067603	811	3.8	Har_454S-C3764	pickpocket	0.001361	1.00E-25
Har_00010193	892	3.81	Har_454S-C11046	tpa:cuticle protein	0.0074088	2.72E-30
Har_00082868	1219	3.83	Har_454S-C5970	elegans proteinpartially confirmed by transcript evidence	0.004637	0.01
Har_00108354	952	3.83	Har_454S-C9509	mitochondrial ribosomal	0.0045263	1.65E-68
Har_00044617	537	3.84	Har_454S-C17259	promoting protein	0.0028917	1.13E-36
Har_00019783	804	3.88	Har_454S-C12839	s-adenosylmethionine synthetase	0.0039827	1.94E-13
Har_00102972	1039	3.9	Har_454S-C8747	vitellin-degrading protease precursor	0.0069311	1.02E-122
Har_00023518	771	3.94	Har_454S-C13508	cral trio domain-containing protein	0.0080305	4.02E-05
Har_00012277	1409	3.94	Har_454S-C1144	vacuolar atp synthase subunit d	2.70E-05	8.40E-108
Har_00044208	543	3.98	Har_454S-C17190	low-mr gtp-binding protein	0.00019	1.29E-13
Har_00004054	687	3.99	gi|194718833|gb|EU769006.1|	dopa decarboxylase	0.0087851	2.21E-98
Har_00087860	1979	4	Har_454S-C6633	c20orf24 homolog	0.0002226	3.50E-36
Har_00120708	389	4.02	Har_454S-C27730	pinball wizard	0.0041753	0
Har_00105274	1270	4.05	Har_454S-C908	glutathionetheta class (agap000761-pa)	0.0056789	2.99E-85
Har_00040395	599	4.07	Har_454S-C16459	juvenile hormone-inducible	0.0020147	2.10E-14
Har_00118376	470	4.07	Har_454S-C27595	insect-derived growth factor-a-like protein	0.0090354	1.22E-11
Har_00033683	665	4.16	Har_454S-C15358	ribonuclease h1	0.0060031	5.70E-41
Har_00050993	232	4.18	Har_454S-C19081	glucose dehydrogenase	0.0005731	1.34E-26
Har_00043561	554	4.18	Har_454S-C17064	inositol 2-dehydrogenase	0.0052895	9.23E-37

Har_00010922	885	4.2	Har_454S-C11185	mitochondrial solute carrier	0.003558	1.66E-115
Har_00091601	1678	4.26	Har_454S-C7157	cyclin-dependent kinase 7	0.0069199	7.24E-149
Har_00062189	1038	4.28	Har_454S-C2975	loc549181 protein	0.004246	4.59E-98
Har_00025830	749	4.29	Har_454S-C13937	cg13350	0.0010342	8.17E-93
Har_00035303	650	4.3	Har_454S-C15625	rbd protein	0.0002292	6.54E-63
Har_00010474	869	4.35	Har_454S-C111	transaldolase 1	0.0006923	5.17E-63
Har_00117445	153	4.37	Har_454S-C26597	glycoside hydrolases	0.0004791	9.69E-09
Har_00015515	846	4.37	Har_454S-C12014	jumping translocation breakpoint protein	0.00128	6.37E-42
Har_00015720	844	4.41	Har_454S-C12058	estrogen sulfotransferase	0.000413	5.22E-84
Har_00002257	2083	4.43	gi|182894535|gb|EF547544.2|	carboxylesterase	0.0016557	0
Har_00094209	1447	4.46	Har_454S-C7498	sugar transporter	0.0045118	3.08E-28
Har_00119117	1740	4.5	Har_454S-C5689	valyl-trna synthetase	0.0065626	1.22E-05
Har_00053000	962	4.54	Har_454S-C20672	mitochondrial oxodicarboxylate carrier	0.0085662	8.73E-17
Har_00096246	1315	4.55	Har_454S-C7782	membrane-associated lps-inducible tnf alpha factorprotein	2.45E-05	1.35E-19
Har_00106754	968	4.57	Har_454S-C9281	short-chain dehydrogenase	0.0077723	2.90E-52
Har_00023684	769	4.61	Har_454S-C13536	jumping translocation breakpoint protein	0.0006631	3.14E-34
Har_00102982	1043	4.62	Har_454S-C8750	carboxylesterase	0.001425	2.39E-87
Har_00002095	2284	4.63	gi|194295557|gb|EU729323.1|	alkaline phosphatase	0.006034	0
Har_00064086	1001	4.63	Har_454S-C3237	jumping translocation breakpoint protein	0.0027748	2.44E-41
Har_00063353	822	4.65	Har_454S-C3138	chemosensory protein 2	0.0049443	8.73E-33
Har_00101338	1102	4.73	Har_454S-C8507	isoform a	0.0028101	1.62E-12
Har_00079108	875	4.73	Har_454S-C5435	rad51 homolog c	0.006535	3.32E-33
Har_00116586	641	4.75	Har_454S-C25751	apoptosis antagonizing transcription factor	0.0020846	8.35E-27
Har_00120959	1262	4.76	Har_454S-C7950	cortical granule protein with ldl-receptor-like repeats	0.0080376	4.67E-14
Har_00038960	614	4.77	Har_454S-C16210	galactosesoluble 9	0.001525	1.90E-20
Har_00070741	1081	4.77	Har_454S-C4235	lipase	0.003207	1.91E-117
Har_00119411	1328	4.8	Har_454S-C7748	ftsk spoiiie family protein	0.0019143	2.94E-22
Har_00101780	1079	4.81	Har_454S-C8571	lipase	0.0034688	1.00E-89
Har_00082028	1583	4.82	Har_454S-C5845	synaptic vesicle protein	0.0046414	1.83E-45
Har_00105039	991	4.83	Har_454S-C9041	nonsense-mediated mrna decay protein	0.0039205	5.41E-118
Har_00035927	644	4.84	Har_454S-C15721	lipase	0.0014336	5.99E-53
Har_00121094	695	4.84	Har_454S-C22030	tbrg1 protein	0.0004249	8.22E-09
Har_00095105	1386	4.85	Har_454S-C7613	transcription factor protein	0.0016943	2.92E-04
Har_00060920	1033	4.89	Har_454S-C2816	methoprene-tolerant protein	5.57E-05	3.10E-63
Har_00064088	1001	4.89	Har_454S-C3237	jumping translocation breakpoint protein	0.0001846	2.44E-41
Har_00089099	2874	4.98	Har_454S-C6806	sarcosine dehydrogenase	0.0052708	0
Har_00096217	1317	5.02	Har_454S-C7776	bhlhzip transcription factor bigmax	0.0001121	1.53E-63
Har_00099392	1176	5.03	Har_454S-C8223	glycerol-3-phosphate dehydrogenase	0.0073886	4.08E-33
Har_00000729	654	5.05	Har_CYP450_Contig271	cytochrome p450	0.0049125	7.64E-58
Har_00015918	843	5.06	Har_454S-C12092	glutathione s-transferase	0.0084989	7.68E-64
Har_00067599	811	5.06	Har_454S-C3764	pickpocket	0.0094739	1.00E-25
Har_00069320	389	5.08	Har_454S-C4040	split hand foot malformationtype 1	0.009498	1.85E-12
Har_00115771	609	5.11	Har_454S-C24926	amp dependent coa ligase	0.0020544	2.55E-43
Har_00020691	796	5.12	Har_454S-C13005	equilibrative nucleoside transporter	0.0023262	7.44E-10
Har_00011196	882	5.2	Har_454S-C11235	pancreatic triacylglycerol lipase	0.0046473	3.54E-99

Har_00028667	719	5.21	Har_454S-C14480	g patch domain containing 4	0.004915	1.41E-22
Har_00084648	805	5.26	Har_454S-C6218	serine protease	0.0020909	5.59E-37
Har_00027287	994	5.26	Har_454S-C1422	membrane-associated lps-inducible tnf alpha factorprotein	0.005639	2.27E-23
Har_00111426	935	5.3	Har_454S-C9945	riken cdnaisoform cra_a	0.0002045	2.39E-39
Har_00033685	665	5.33	Har_454S-C15358	ribonuclease h1	0.0069904	5.70E-41
Har_00070205	876	5.34	Har_454S-C4162	vitellogenin	0.0019267	4.32E-73
Har_00059345	1460	5.38	Har_454S-C2645	transcription factormitochondrial	0.0040336	2.94E-97
Har_00024223	765	5.44	Har_454S-C13633	26-kda lectin	0.0030672	3.55E-30
Har_00067062	1308	5.46	Har_454S-C3687	riken cdnaisoform cra_a	5.88E-05	8.79E-39
Har_00087142	1989	5.48	Har_454S-C6545	glucose dehydrogenase	0.0025839	6.57E-75
Har_00054466	1258	5.49	Har_454S-C21921	rrna processing protein ebp2	0.0002666	6.74E-114
Har_00094767	1410	5.5	Har_454S-C7566	cytochrome p450	0.0012052	1.03E-75
Har_00061663	555	5.5	Har_454S-C2910	tpa_inf: hdc19504	0.0067433	4.24E-13
Har_00031005	691	5.59	Har_454S-C14912	imp dehydrogenase gmp reductase	0.0062224	6.03E-04
Har_00064074	918	5.61	Har_454S-C3236	jumping translocation breakpoint protein	7.60E-05	7.32E-42
Har_00052900	800	5.64	Har_454S-C20614	imp dehydrogenase gmp reductase	0.0099694	8.03E-04
Har_00082135	703	5.66	Har_454S-C5861	isoform a	1.18E-05	9.88E-50
Har_00104238	1010	5.67	Har_454S-C8925	juvenile hormone esterase	0.0001519	1.89E-134
Har_00069688	1032	5.75	Har_454S-C4093	phosphatidylinositol glycan anchorclass h	0.0065373	2.11E-11
Har_00049815	362	5.79	Har_454S-C18534	tissue factor pathway inhibitor	0.0028875	2.76E-16
Har_00099187	1180	5.82	Har_454S-C8198	reverse transcriptase	0.0092139	1.45E-54
Har_00064077	918	5.85	Har_454S-C3236	jumping translocation breakpoint protein	0.0030149	7.32E-42
Har_00015936	842	5.91	Har_454S-C12099	carboxylesterase esterase fe4	0.0057051	3.68E-50
Har_00015516	846	5.94	Har_454S-C12014	jumping translocation breakpoint protein	0.0023532	6.37E-42
Har_00002682	1536	5.95	gi|62913890|gb|AY950637.1|	cytochrome p450	0.0028205	0
Har_00064865	972	5.97	Har_454S-C3345	jumping translocation breakpoint protein	0.0055867	8.01E-42
Har_00115212	567	6.04	Har_454S-C24159	coiled-coil domain containing 12-like protein	0.0055177	1.15E-24
Har_00113426	522	6.04	Har_454S-C21263	aldehyde oxidase	0.0005911	1.88E-06
Har_00000646	538	6.06	Har_CYP450_Contig259	cytochrome p450	0.0026818	3.06E-09
Har_00012566	870	6.1	Har_454S-C11495	aconitase 1	0.001285	3.17E-44
Har_00048834	1210	6.12	Har_454S-C1822	growth-blocking peptide	0.0052449	1.16E-30
Har_00089613	2252	6.2	Har_454S-C6876	kynurenine aminotransferase	0.0007774	5.31E-169
Har_00054250	862	6.25	Har_454S-C2174	coiled-coil domain containing 53	0.0024424	4.10E-28
Har_00098973	1191	6.27	Har_454S-C8171	serine protease	0.000303	2.56E-91
Har_00083952	1217	6.29	Har_454S-C6127	sarcosine dehydrogenase	0.0063821	5.27E-92
Har_00078849	1034	6.3	Har_454S-C5403	nuclear protein e3-3	0.0047542	3.68E-40
Har_00002368	1944	6.48	gi|81248545|gb|DQ256407.1|	cytochrome p450	0.0092944	0
Har_00114247	967	6.51	Har_454S-C22674	beta lactamase domain	0.0004177	1.45E-68
Har_00091528	1692	6.53	Har_454S-C7143	prophenoloxidase activating factor	0.0028783	2.15E-124
Har_00089940	2068	6.63	Har_454S-C6928	phenylalanine hydroxylase	0.0085235	0
Har_00082611	841	6.65	Har_454S-C5933	serine protease	0.0003532	1.27E-42
Har_00101686	1089	6.67	Har_454S-C8559	ribosomal protein l19e	0.0062495	1.94E-18
Har_00116686	291	6.77	Har_454S-C25826	isoform a	0.0056945	2.15E-16
Har_00047792	463	6.8	Har_454S-C17929	retinoid- and fatty acid-binding glycoprotein	0.0016233	1.75E-63
Har_00067843	1885	6.85	Har_454S-C38	glucose dehydrogenase	0.0023067	6.97E-54

Har_00005455	925	6.92	Har_454S-C10199	sodium-dependent phosphate transporter	0.0054044	6.37E-54
Har_00044979	531	6.97	Har_454S-C17320	pancreatic triacylglycerol lipase	8.42E-05	2.58E-30
Har_00093340	1509	7.07	Har_454S-C7382	transmembrane protein 165	0.0079531	3.71E-32
Har_00003810	1801	7.31	gi|22085152|gb|AF285831.1|	cytochrome p450	0.0039877	2.82E-177
Har_00026255	745	7.35	Har_454S-C14018	equilibrative nucleoside transporter	0.0004908	3.12E-44
Har_00006411	919	7.4	Har_454S-C10354	multi-binding protein	0.0095661	4.14E-37
Har_00071441	948	7.41	Har_454S-C4345	glycoside hydrolases	0.0027252	1.69E-73
Har_00120146	493	7.49	Har_454S-C24250	periplasmic copper-binding	2.32E-06	5.08E-05
Har_00026781	739	7.52	Har_454S-C14126	juvenile hormone binding protein	0.0056031	1.01E-79
Har_00013713	862	7.57	Har_454S-C11702	isoform a	0.0041528	2.21E-82
Har_00117121	225	7.57	Har_454S-C26296	aldo-keto reductase	0.0004061	1.49E-25
Har_00012886	868	7.57	Har_454S-C11551	amp dependent coa ligase	0.0028787	6.35E-45
Har_00096086	1326	7.57	Har_454S-C7758	arystal structure of drosophila ance	0.0006875	0
Har_00005950	922	7.69	Har_454S-C10278	amp dependent coa ligase	0.0002684	1.13E-34
Har_00039889	605	7.9	Har_454S-C16363	proteasome maturation protein	0.0001886	2.81E-30
Har_00101396	1099	8.09	Har_454S-C8515	microfibrillar-associated protein 1	0.0037568	1.82E-56
Har_00036675	636	8.1	Har_454S-C15837	sarcosine dehydrogenase	0.0019259	1.56E-61
Har_00105979	976	8.23	Har_454S-C9179	mitochondrial ribosomal	0.0001184	3.37E-56
Har_00008857	903	8.24	Har_454S-C10787	cg12253 cg12253-pa	0.0003063	6.45E-19
Har_00080253	565	8.62	Har_454S-C5598	amp dependent coa ligase	0.000123	2.97E-26
Har_00009830	895	8.65	Har_454S-C10974	fumarylacetoacetate hydrolase	0.0041839	1.34E-85
Har_00060838	1002	8.71	Har_454S-C2807	-methenyltetrahydrofolate synthetase (5-formyltetrahydrofolate cyclo-ligase)	0.0041197	1.35E-39
Har_00119816	255	8.83	Har_454S-C21810	rna binding motif protein 19	5.81E-05	6.86E-19
Har_00067167	1734	8.86	Har_454S-C3704	cg9471 cg9471-pb	0.0001423	3.99E-57
Har_00121154	279	8.87	Har_454S-C24650	methylated-dna--protein-cysteine methyltransferase	0.0001123	3.83E-18
Har_00054061	924	8.89	Har_454S-C2152	spermatogenesis associated factor spaf	0.0040987	5.40E-61
Har_00107124	964	8.95	Har_454S-C9343	zinc carboxypeptidase	0.0030701	1.02E-65
Har_00057317	1816	9.05	Har_454S-C2432	reverse transcriptase	0.0065983	1.59E-104
Har_00058900	711	9.11	Har_454S-C2588	cysteinyl-trna synthetase	0.0030586	3.42E-13
Har_00006392	919	9.23	Har_454S-C10352	phenylalanine hydroxylase	0.0065154	5.23E-40
Har_00044082	545	9.4	Har_454S-C17161	loc407663 protein	0.0037478	1.55E-17
Har_00118447	531	9.43	Har_454S-C27655	rad50 homolog	0.0094963	1.29E-13
Har_00096757	1289	9.53	Har_454S-C7854	deoxyhypusine hydroxylase monooxygenase	0.0041385	8.45E-144
Har_00006039	921	9.74	Har_454S-C10291	low-expression lectin 1	0.0005594	2.90E-22
Har_00096242	1315	9.76	Har_454S-C7782	membrane-associated lps-inducible tnf alpha factorprotein	0.0020256	1.35E-19
Har_00116271	501	9.78	Har_454S-C25425	phospholipase a2 receptor 1	0.0006077	2.63E-12
Har_00059272	1428	9.86	Har_454S-C2636	4-hydroxyphenylpyruvate dioxygenase	0.0003678	4.82E-151
Har_00067726	1382	9.96	Har_454S-C3784	replication protein a 70 kda dna-binding subunit	7.86E-05	6.77E-102
Har_00099078	1187	10	Har_454S-C8183	structural maintenance of chromosomes smc3	0.0026606	4.36E-75
Har_00216101	862	10.1	Har_454S-C5081	hypothetical protein [Plasmodium berghei strain ANKA]	0.0037815	0.05
Har_00050604	1381	10.12	Har_454S-C1889	apolipoprotein d	0.0049914	2.91E-84
Har_00035422	649	10.35	Har_454S-C15647	spermatogenesis associated 5	2.20E-06	8.35E-26
Har_00025967	748	10.37	Har_454S-C13967	gamma-glutamyl cyclotransferase	0.0049137	5.94E-43
Har_00109617	944	10.51	Har_454S-C9692	transposase	7.84E-06	2.36E-43
Har_00012551	870	10.71	Har_454S-C11492	mki67 (fha domain) interacting nucleolar phosphoprotein	0.0020924	3.06E-55

Har_00114677	325	10.76	Har_454S-C23359	sugar transporter	0.007025	4.94E-05
Har_00008101	830	10.87	Har_454S-C1065	protein takeout	0.0006645	1.18E-53
Har_00001116	903	10.9	Har_HA1-FN-M-09_A17_LPSBP	low-expression lectin 1	0.0056896	1.03E-23
Har_00048450	436	10.9	Har_454S-C18104	thioredoxin domain containing 15	0.0024567	4.85E-21
Har_00006038	921	11.16	Har_454S-C10291	low-expression lectin 1	0.0010048	2.90E-22
Har_00083294	3090	11.29	Har_454S-C6041	phosphoglucomutase 2	0.0033797	8.29E-127
Har_00089366	2426	11.65	Har_454S-C6843	cg11905 cg11905-pi	0.0037955	1.10E-18
Har_00016676	835	11.89	Har_454S-C12237	transposase	2.02E-05	3.27E-29
Har_00089745	2174	12	Har_454S-C6891	polyprotein	0.0041094	9.38E-14
Har_00074677	933	12.39	Har_454S-C4793	trypsin-like serine protease	0.0002011	5.92E-148
Har_00005949	922	12.48	Har_454S-C10278	amp dependent coa ligase	0.0037804	1.13E-34
Har_00102097	1073	12.6	Har_454S-C8615	cytochrome c oxidase copper chaperone	0.002126	2.16E-14
Har_00069659	1047	12.65	Har_454S-C4088	low-expression lectin 1	0.0006571	3.55E-22
Har_00036678	636	12.84	Har_454S-C15837	sarcosine dehydrogenase	0.0005092	1.56E-61
Har_00117746	232	12.88	Har_454S-C26913	cral trio domain-containing protein	0.0008354	9.92E-22
Har_00045549	1287	12.93	Har_454S-C1743	polycomb protein l	0.0014538	6.17E-78
Har_00013888	861	12.94	Har_454S-C11727	transposase	2.43E-05	1.08E-36
Har_00025987	898	12.94	Har_454S-C1397	zincfyve domain containing 19	0.0028884	3.07E-21
Har_00094778	1409	12.98	Har_454S-C7568	dihydroxyacetone kinase 2 homolog	0.0001812	2.73E-82
Har_00034046	661	12.99	Har_454S-C15415	molybdopterin synthase small subunit mocs2a	0.0027216	1.19E-18
Har_00026041	1717	13.71	Har_454S-C1398	ribosome biogenesis regulatory protein homolog	0.0012187	5.72E-88
Har_00067949	1373	14	Har_454S-C3820	death-associated protein	0.0076078	1.79E-38
Har_00115201	769	14.02	Har_454S-C24158	gag-pol polyprotein	0.0010446	4.10E-34
Har_00013887	861	14.17	Har_454S-C11727	transposase	1.74E-05	1.08E-36
Har_00077976	1333	14.35	Har_454S-C5263	juvenile hormone-inducible	5.53E-05	1.33E-14
Har_00069658	1047	14.46	Har_454S-C4088	low-expression lectin 1	0.0002096	3.55E-22
Har_00090573	1873	14.61	Har_454S-C7013	sugar transporter	0.0005941	7.48E-49
Har_00004892	928	14.78	Har_454S-C10103	dead (asp-glu-ala-asp) box polypeptide 18	0.0041096	9.20E-69
Har_00088529	1666	15.45	Har_454S-C672	mitochondrial carnitine acylcarnitine carrier protein	3.16E-06	2.54E-154
Har_00095055	1392	15.66	Har_454S-C7605	monocarboxylate transporter	0.0007867	2.35E-86
Har_00002596	764	15.66	gi|86279143|gb|DQ236224.1|	piggybac transposase	0.0001728	4.14E-13
Har_00002597	764	16.06	gi|86279143|gb|DQ236224.1|	piggybac transposase	0.0001999	4.14E-13
Har_00106652	969	16.07	Har_454S-C9270	endonuclease reverse transcriptase	0.0021974	1.60E-42
Har_00001551	263	16.07	gi|116833216|gb|DQ875279.1|	mitochondrial ribosomal	3.81E-05	2.94E-28
Har_00002096	2284	16.1	gi|194295557|gb|EU729323.1|	alkaline phosphatase	0.0006548	0
Har_00106651	969	16.21	Har_454S-C9270	endonuclease reverse transcriptase	4.47E-05	1.60E-42
Har_00090772	1832	16.3	Har_454S-C7039	5 nucleotidase	0.0031388	2.39E-128
Har_00088443	1085	16.68	Har_454S-C6706	metallothionein b	0.0008957	4.16E-05
Har_00058832	747	17.84	Har_454S-C25789	aldolase	0.0010077	3.47E-86
Har_00083673	1214	17.86	Har_454S-C6093	proline synthetase co-transcribed homolog	0.0022458	2.38E-90
Har_00114772	1020	18.84	Har_454S-C23495	serine protease htra2	0.001326	1.61E-72
Har_00213108	433	20.89	Har_454S-C18121	similar to conserved hypothetical protein [Hydra magnipapillata]	0.0095572	0
Har_00016364	838	21.55	Har_454S-C12179	aldolase	0.000284	8.04E-90
Har_00118904	1382	21.79	Har_454S-C3947	rna binding motif protein 34	0.0001699	2.20E-19
Har_00095383	1371	23.45	Har_454S-C7652	lysosomal thiol reductase ip30 isoform 2	0.0001596	2.40E-59

Har_00065295	1179	23.56	Har_454S-C3408	upf0420 protein c16orf58 homolog	0.0002604	1.28E-18
Har_00103052	1041	25.29	Har_454S-C8759	cg1443 cg1443-pa	1.05E-05	3.20E-34
Har_00014257	858	25.38	Har_454S-C11793	riken cdna 2410131k14 gene	7.07E-05	1.26E-53
Har_00076903	1488	26.99	Har_454S-C5107	dolichyl-phosphate annosyltransferase polypeptidecatalytic subunit	0.0001094	4.05E-116
Har_00015856	843	28.59	Har_454S-C12083	acetyl-coa synthetase	0.0002798	7.81E-08
Har_00121919	177	28.78	Har_454S-C28084	triacylglycerol lipase	0.0054662	9.15E-04
Har_00049994	346	29.31	Har_454S-C18616	trypsin-like serine protease	5.44E-05	1.34E-16
Har_00006807	1841	30.63	Har_454S-C1042	cg4797	0.001098	5.11E-143
Har_00052174	767	32.53	Har_454S-C2002	gamma-glutamyl cyclotransferase	0.0006982	1.06E-42
Har_00058998	3403	32.79	Har_454S-C2599	serine protease inhibitor	0.000647	4.83E-148
Har_00020876	1103	33	Har_454S-C1304	cytochrome c oxidase copper chaperone	0.0001222	3.86E-14
Har_00035725	646	33.38	Har_454S-C15690	cg1443 cg1443-pa	0.000896	3.94E-44
Har_00120030	172	36.23	Har_454S-C23081	splicing factor 3b subunit 5	0.0013215	
Har_00012119	2276	37.03	Har_454S-C1141	fumarylacetoacetate hydrolase domain containing 2a	0.0006131	9.97E-99
Har_00083575	921	38.31	Har_454S-C608	isoform a	0.0016271	6.25E-33
Har_00064914	946	38.53	Har_454S-C3353	coiled-coil-helix-coiled-coil-helix domain containing 7	5.39E-06	5.49E-24
Har_00036894	634	40.06	Har_454S-C15871	isoform a	0.0017165	1.04E-33
Har_00110932	938	40.27	Har_454S-C9872	ryanodine receptor homologue	0.00031795	7.74E-102
Har_00078337	1503	40.53	Har_454S-C5322	interferon gamma inducible protein 30	5.16E-05	1.03E-58
Har_00052176	767	41.04	Har_454S-C2002	gamma-glutamyl cyclotransferase	0.0001453	1.06E-42
Har_00113664	257	44.88	Har_454S-C21759	aldehyde oxidase	5.57E-05	2.65E-06
Har_00085213	979	48.2	Har_454S-C6296	viral a-type inclusion protein repeat containing protein	0.0002728	1.49E-11
Har_00033238	669	52.59	Har_454S-C15292	transmembrane protein 93	5.81E-05	2.61E-41
Har_00113819	1134	55.41	Har_454S-C22137	glucose dehydrogenase	9.66E-05	1.12E-52
Har_00085767	1149	64.65	Har_454S-C6372	transmembrane protein 39b	0.0007015	2.79E-07
Har_00105801	980	72.21	Har_454S-C9150	af250284_109 amv109	0.001443	1.03E-20
Har_00076922	2373	89.99	Har_454S-C5111	cathepsin l	0.0001002	0
Har_00083574	921	90.2	Har_454S-C608	isoform a	0.0007436	6.25E-33

Table 4b. A list of *H. armigera* genes with unknown sequence description that were differentially transcribed (5< fold) in Cry2Ab tolerant neonate larvae.

Gene Identifier	Sequence length	Fold change	Sequence name	Sequence description	P-value
Har_00206109	285	-93.99	Har_454S-C21682	NA	0.00061837
Har_00020470	797	-87.03	Har_454S-C12968	NA	3.99E-05
Har_00198526	858	-84.36	Har_454S-C11795	NA	6.18E-05
Har_00187637	158	-76.14	Har_454S-C23518	NA	3.86E-05
Har_00135635	156	-69.09	Har_454S-C26929	NA	0.00016581
Har_00174028	708	-68.06	Har_454S-C14654	NA	0.00060072
Har_00187999	306	-66.64	Har_454S-C18789	NA	0.00019581
Har_00134874	887	-64.12	Har_454S-C11112	NA	5.77E-05
Har_00167811	184	-62.35	Har_454S-C27327	NA	9.60E-05
Har_00121023	214	-59.41	Har_454S-C20527	NA	0.0011192
Har_00127813	1193	-51.04	Har_454S-C443	NA	0.00049915
Har_00170002	1106	-50.52	Har_454S-C8485	NA	0.00027472
Har_00166831	818	-46.86	Har_454S-C12575	NA	0.00012046
Har_00153597	1360	-46.22	Har_454S-C7683	NA	0.00195451

Har_00170764	462	-46.18	Har_454S-C17935	NA	0.001665
Har_00137839	193	-42.22	Har_454S-C22351	NA	4.65E-05
Har_00212432	285	-41.57	Har_454S-C21651	NA	3.06E-05
Har_00158759	313	-41.52	Har_454S-C20808	NA	0.00135243
Har_00124707	198	-40.84	Har_454S-C24295	NA	0.0003037
Har_00175324	278	-37.88	Har_454S-C24034	NA	4.80E-05
Har_00233245	868	-35.52	Har_454S-C11554	NA	0.00135506
Har_00178305	282	-35.09	Har_454S-C21266	NA	0.00036966
Har_00124409	898	-34.33	Har_454S-C4450	NA	1.94E-05
Har_00226625	748	-34.11	Har_454S-C13952	NA	0.00081107
Har_00165996	391	-34.04	Har_454S-C18398	NA	1.85E-05
Har_00232179	190	-33.95	Har_454S-C27981	NA	0.00148899
Har_00129155	171	-32.98	Har_454S-C23164	NA	0.00010135
Har_00182563	188	-32.91	Har_454S-C25601	NA	0.00059042
Har_00156605	1151	-32.69	Har_454S-C2025	NA	0.00047093
Har_00196622	397	-32.32	Har_454S-C20834	NA	0.00286169
Har_00165086	873	-32.26	Har_454S-C11441	NA	0.00140871
Har_00198073	228	-30.75	Har_454S-C23472	NA	0.00264679
Har_00163465	703	-30.53	Har_454S-C14738	NA	0.00015087
Har_00205562	525	-30.47	Har_454S-C20860	NA	2.27E-05
Har_00216963	288	-29.24	Har_454S-C24095	NA	0.00040373
Har_00226305	1219	-28.19	Har_454S-C1996	NA	0.00088454
Har_00140205	1054	-27.61	Har_454S-C3358	NA	0.0030147
Har_00209295	653	-27.12	Har_454S-C15578	NA	0.00730628
Har_00031624	686	-26.11	Har_454S-C15022	NA	2.76E-05
Har_00070084	661	-25.89	Har_454S-C4143	NA	0.00106688
Har_00179577	565	-25.67	Har_454S-C20520	NA	2.88E-05
Har_00164167	1069	-25.39	Har_454S-C6042	NA	3.38E-05
Har_00138543	545	-24.89	Har_454S-C4622	NA	0.00017385
Har_00183199	157	-23.65	Har_454S-C24763	NA	0.0090999
Har_00125440	190	-23.61	Har_454S-C23593	NA	0.00205919
Har_00182995	221	-23.47	Har_454S-C26347	NA	0.00908283
Har_00225026	1003	-21.82	Har_454S-C3080	NA	0.00126385
Har_00220631	188	-21.57	Har_454S-C20884	NA	0.00077845
Har_00218421	827	-21.55	Har_454S-C12399	NA	0.0002845
Har_00173923	1116	-20.9	Har_454S-C3398	NA	0.00213425
Har_00191710	252	-20.8	Har_454S-C25505	NA	7.06E-05
Har_00205012	1113	-20.48	Har_454S-C824	NA	0.00011827
Har_00171069	226	-20.31	Har_454S-C27904	NA	0.0048323
Har_00172364	792	-20.26	Har_454S-C5840	NA	0.00514583
Har_00139366	500	-20.22	Har_454S-C22667	NA	1.19E-05
Har_00173466	692	-18.58	Har_454S-C14901	NA	0.00048721
Har_00190812	204	-17.95	Har_454S-C20258	NA	7.00E-05
Har_00203961	686	-17.81	Har_454S-C15016	NA	0.00024562
Har_00164846	243	-17.76	Har_454S-C25568	NA	0.00550213
Har_00205124	153	-17.42	Har_454S-C26197	NA	0.00082797
Har_00153809	781	-17.36	Har_454S-C13311	NA	0.00177725
Har_00224317	872	-17.16	Har_454S-C11465	NA	0.0074462
Har_00211653	350	-16.52	Har_454S-C24723	NA	0.0037134
Har_00141188	196	-16.5	Har_454S-C27121	NA	7.56E-05
Har_00184232	407	-16.32	Har_454S-C25339	NA	0.00537849
Har_00194519	546	-16.05	Har_454S-C17150	NA	0.0009946
Har_00199948	281	-16.01	Har_454S-C23389	NA	0.00735086

Har_00180135	237	-15.96	Har_454S-C20648	NA	0.000321
Har_00192927	226	-15.92	Har_454S-C27913	NA	0.00028344
Har_00128440	486	-15.9	Har_454S-C17744	NA	0.00085143
Har_00151719	871	-15.85	Har_454S-C11477	NA	0.00149873
Har_00154818	885	-15.5	Har_454S-C11188	NA	0.00166256
Har_00014841	853	-14.93	Har_454S-C11891	NA	0.00213203
Har_00161545	404	-13.74	Har_454S-C18327	NA	0.00048821
Har_00172338	977	-13.65	Har_454S-C9176	NA	0.00821387
Har_00167777	1903	-13.63	Har_454S-C6996	NA	0.00020224
Har_00045921	903	-13.49	Har_454S-C1750	NA	0.00106084
Har_00073864	881	-13.38	Har_454S-C4680	NA	0.00832725
Har_00209219	345	-13.34	Har_454S-C26949	NA	0.00531071
Har_00123501	403	-13.03	Har_454S-C18331	NA	0.00173938
Har_00187814	610	-12.78	Har_454S-C21009	NA	0.00014918
Har_00194151	649	-12.57	Har_454S-C24160	NA	0.00032523
Har_00160732	155	-12.28	Har_454S-C25434	NA	0.00040446
Har_00171578	764	-12.15	Har_454S-C20930	NA	0.00645009
Har_00229581	636	-12.09	Har_454S-C15848	NA	0.00474814
Har_00124052	219	-11.97	Har_454S-C21171	NA	8.42E-05
Har_00156031	173	-11.73	Har_454S-C20102	NA	6.51E-05
Har_00192429	608	-11.72	Har_454S-C23998	NA	9.65E-05
Har_00218812	489	-11.71	Har_454S-C21990	NA	0.00347547
Har_00128816	819	-11.59	Har_454S-C12548	NA	0.00022095
Har_00211924	211	-11.16	Har_454S-C20854	NA	0.00499697
Har_00236950	184	-11.07	Har_454S-C26806	NA	0.00133352
Har_00228161	186	-11.03	Har_454S-C22313	NA	0.00165483
Har_00125590	850	-10.93	Har_454S-C11936	NA	0.00017491
Har_00137887	674	-10.84	Har_454S-C15228	NA	6.83E-05
Har_00177221	1106	-10.83	Har_454S-C5105	NA	0.00742419
Har_00213299	737	-10.73	Har_454S-C14153	NA	0.00106704
Har_00139556	217	-10.55	Har_454S-C27732	NA	0.00111998
Har_00126269	799	-10.51	Har_454S-C2232	NA	0.0011035
Har_00136527	867	-10.21	Har_454S-C11577	NA	0.00596044
Har_00198737	180	-10.2	Har_454S-C22912	NA	0.00027436
Har_00134488	545	-10.11	Har_454S-C17159	NA	0.00016104
Har_00155827	972	-10.11	Har_454S-C2185	NA	0.00753361
Har_00149253	397	-10.07	Har_454S-C24595	NA	0.00022894
Har_00132254	635	-9.93	Har_454S-C15855	NA	0.00024949
Har_00217792	602	-9.84	Har_454S-C16421	NA	3.85E-05
Har_00234200	177	-9.84	Har_454S-C20594	NA	0.00120492
Har_00209778	786	-9.75	Har_454S-C13222	NA	0.0013358
Har_00132610	210	-9.65	Har_454S-C20703	NA	0.00719206
Har_00186092	266	-9.64	Har_454S-C21460	NA	0.00257058
Har_00127581	1349	-9.56	Har_454S-C21005	NA	0.00111595
Har_00184181	825	-9.46	Har_454S-C21695	NA	0.00039895
Har_00021482	789	-9.44	Har_454S-C13144	NA	0.00023557
Har_00158601	155	-9.44	Har_454S-C22734	NA	0.00093818
Har_00143081	703	-9.43	Har_454S-C3540	NA	0.00197793
Har_00163611	788	-9.33	Har_454S-C13174	NA	0.00043895
Har_00193625	615	-9.3	Har_454S-C24823	NA	0.00290702
Har_00188080	278	-9.11	Har_454S-C18905	NA	0.00191286
Har_00152317	759	-9.01	Har_454S-C13742	NA	0.00013169
Har_00173642	821	-8.95	Har_454S-C12520	NA	3.38E-05

Har_00193006	724	-8.86	Har_454S-C14385	NA	0.00032324
Har_00236511	1143	-8.75	Har_454S-C20760	NA	1.13E-05
Har_00184212	368	-8.61	Har_454S-C1110	NA	0.00619796
Har_00236854	918	-8.57	Har_454S-C10385	NA	0.00075901
Har_00225968	1481	-8.36	Har_454S-C71	NA	0.00651817
Har_00135368	820	-8.34	Har_454S-C12530	NA	0.002787
Har_00196845	798	-8.24	Har_454S-C12963	NA	0.00439697
Har_00115881	194	-8.19	Har_454S-C25070	NA	0.00016381
Har_00193720	232	-7.84	Har_454S-C21247	NA	0.00017459
Har_00227024	283	-7.77	Har_454S-C18880	NA	0.00235592
Har_00227785	546	-7.71	Har_454S-C20241	NA	5.19E-05
Har_00159295	969	-7.56	Har_454S-C9254	NA	0.0055612
Har_00136032	903	-7.54	Har_454S-C10779	NA	0.00089415
Har_00220584	809	-7.5	Har_454S-C2670	NA	0.00091663
Har_00216310	184	-7.42	Har_454S-C24114	NA	0.00196048
Har_00165275	198	-7.29	Har_454S-C27508	NA	0.00216831
Har_00136175	1223	-7.27	Har_454S-C2094	NA	0.0064064
Har_00233329	222	-7.2	Har_454S-C27710	NA	0.00190741
Har_00198883	634	-7.17	Har_454S-C15881	NA	0.00173886
Har_00167786	159	-7.14	Har_454S-C21868	NA	0.00494257
Har_00145680	181	-7.04	Har_454S-C25304	NA	0.00374425
Har_00197288	327	-6.87	Har_454S-C18689	NA	0.00069487
Har_00211532	1336	-6.72	Har_454S-C3750	NA	0.00457225
Har_00175119	639	-6.68	Har_454S-C15795	NA	0.00224146
Har_00211590	601	-6.66	Har_454S-C21841	NA	0.0002558
Har_00196377	266	-6.54	Har_454S-C24708	NA	0.00575535
Har_00178506	288	-6.5	Har_454S-C18858	NA	0.00974342
Har_00159427	487	-6.47	Har_454S-C21662	NA	0.00039512
Har_00139317	457	-6.46	Har_454S-C17972	NA	0.00028096
Har_00175358	205	-6.39	Har_454S-C28045	NA	0.00048441
Har_00185725	210	-6.35	Har_454S-C23591	NA	0.00048804
Har_00007918	909	-6.32	Har_454S-C10618	NA	0.0002555
Har_00172426	414	-6.32	Har_454S-C23687	NA	0.00110043
Har_00067829	1171	-6.31	Har_454S-C3797	NA	0.00062801
Har_00236623	358	-6.29	Har_454S-C22615	NA	0.00032254
Har_00156146	569	-6.09	Har_454S-C16858	NA	0.00038645
Har_00138381	461	-6.07	Har_454S-C27814	NA	0.00605797
Har_00204724	359	-6.06	Har_454S-C24228	NA	0.00224405
Har_00222357	817	-6.03	Har_454S-C20154	NA	0.00453693
Har_00223185	887	-5.95	Har_454S-C11158	NA	0.00409305
Har_00161635	842	-5.95	Har_454S-C2473	NA	0.00100343
Har_00222166	162	-5.92	Har_454S-C27465	NA	0.00672123
Har_00160484	320	-5.91	Har_454S-C24202	NA	0.0001589
Har_00163632	419	-5.88	Har_454S-C23585	NA	0.00061015
Har_00140077	347	-5.86	Har_454S-C24810	NA	0.00051829
Har_00163028	589	-5.76	Har_454S-C22429	NA	0.00085925
Har_00122858	225	-5.76	Har_454S-C25413	NA	0.00263217
Har_00113015	812	-5.69	Har_454S-C20754	NA	0.00247132
Har_00166321	157	-5.68	Har_454S-C24044	NA	0.00614428
Har_00124986	832	-5.64	Har_454S-C569	NA	0.00749332
Har_00221551	216	-5.6	Har_454S-C21169	NA	0.00083443
Har_00196754	828	-5.56	Har_454S-C12382	NA	0.00644061
Har_00081310	894	-5.53	Har_454S-C5743	NA	0.00013285

Har_00003552	316	-5.52	gi|124481989|gb|EF152217.1	NA	0.00117666
Har_00149885	1046	-5.52	Har_454S-C8740	NA	0.00198897
Har_00184314	378	-5.51	Har_454S-C23993	NA	0.00309431
Har_00147400	614	-5.47	Har_454S-C16221	NA	0.00045114
Har_00216328	201	-5.46	Har_454S-C24113	NA	0.002216
Har_00164750	959	-5.43	Har_454S-C1912	NA	0.00043173
Har_00239910	783	-5.41	Har_454S-C13268	NA	5.07E-06
Har_00166712	869	-5.39	Har_454S-C11535	NA	0.00059035
Har_00212539	222	-5.39	Har_454S-C25372	NA	0.00708658
Har_00130030	160	-5.3	Har_454S-C20845	NA	0.00236122
Har_00138488	918	-5.3	Har_454S-C21324	NA	0.00384884
Har_00225815	622	-5.29	Har_454S-C16086	NA	0.00014021
Har_00216406	238	-5.27	Har_454S-C20319	NA	0.00585278
Har_00199447	651	-5.26	Har_454S-C15600	NA	0.00012642
Har_00229921	187	-5.23	Har_454S-C22620	NA	7.62E-05
Har_00182041	419	-5.21	Har_454S-C23757	NA	1.54E-05
Har_00059427	1024	-5.2	Har_454S-C2654	NA	0.00087703
Har_00177073	796	-5.19	Har_454S-C12997	NA	0.00073633
Har_00166699	1075	-5.19	Har_454S-C79	NA	0.00253841
Har_00229481	385	-5.14	Har_454S-C25735	NA	0.00209916
Har_00211352	602	5	Har_454S-C16415	NA	0.0094012
Har_00197698	902	5.09	Har_454S-C229	NA	0.0019888
Har_00143762	285	5.11	Har_454S-C25382	NA	0.0004133
Har_00223731	526	5.14	Har_454S-C24370	NA	0.0036137
Har_00239517	904	5.16	Har_454S-C10757	NA	0.0016654
Har_00180898	781	5.17	Har_454S-C1052	NA	0.000762
Har_00205694	208	5.39	Har_454S-C24415	NA	0.0023518
Har_00239323	1092	5.45	Har_454S-C8546	NA	6.18E-05
Har_00239322	1092	5.51	Har_454S-C8546	NA	0.0003311
Har_00194317	568	5.55	Har_454S-C20322	NA	0.0005582
Har_00207850	1146	5.56	Har_454S-C23611	NA	0.0010096
Har_00162677	162	5.69	Har_454S-C20968	NA	0.0068329
Har_00219080	188	5.75	Har_454S-C20217	NA	0.0027424
Har_00121406	834	5.77	Har_454S-C21400	NA	0.0032881
Har_00228626	221	5.99	Har_454S-C25101	NA	0.0001915
Har_00213636	881	6.13	Har_454S-C11260	NA	0.0002921
Har_00233867	758	6.34	Har_454S-C13775	NA	0.0062639
Har_00225348	2045	6.36	Har_454S-C4288	NA	2.68E-05
Har_00130333	241	6.47	Har_454S-C19054	NA	0.0059158
Har_00092453	1582	6.54	Har_454S-C7270	NA	6.11E-05
Har_00124817	280	6.75	Har_454S-C22436	NA	0.0004098
Har_00074513	470	6.78	Har_454S-C4775	NA	0.0011183
Har_00011591	982	6.86	Har_454S-C1131	NA	0.0061716
Har_00146306	317	6.9	Har_454S-C23270	NA	0.0014436
Har_00122478	227	6.95	Har_454S-C24215	NA	4.14E-05
Har_00216327	198	6.97	Har_454S-C24112	NA	0.0057613
Har_00176921	275	7.04	Har_454S-C24185	NA	0.0011764
Har_00106242	973	7.45	Har_454S-C9216	NA	0.0052416
Har_00203852	424	7.97	Har_454S-C22564	NA	0.0008533
Har_00239021	215	7.98	Har_454S-C27194	NA	1.34E-05
Har_00237569	915	8.1	Har_454S-C10461	NA	2.35E-05
Har_00208313	347	8.13	Har_454S-C21458	NA	0.0021881
Har_00159578	188	8.23	Har_454S-C20217	NA	0.0081016

Har_00031054	691	8.51	Har_454S-C14920	NA	0.003939
Har_00178701	200	8.54	Har_454S-C26214	NA	0.0019604
Har_00022059	784	8.57	Har_454S-C13244	NA	0.0022417
Har_00216533	693	8.79	Har_454S-C14888	NA	0.0037915
Har_00190788	178	8.8	Har_454S-C24946	NA	0.0036621
Har_00132389	928	8.9	Har_454S-C2434	NA	0.0037527
Har_00147732	207	8.94	Har_454S-C22138	NA	0.0035933
Har_00235587	224	8.96	Har_454S-C27358	NA	0.0003965
Har_00207543	563	9.02	Har_454S-C5388	NA	0.0069647
Har_00120717	158	9.2	Har_454S-C27851	NA	6.54E-05
Har_00139184	424	9.31	Har_454S-C21903	NA	0.0022971
Har_00161193	786	9.36	Har_454S-C20888	NA	0.0029951
Har_00223017	788	9.42	Har_454S-C13173	NA	0.0039715
Har_00152097	226	9.6	Har_454S-C27662	NA	0.0033726
Har_00186575	467	9.65	Har_454S-C20916	NA	6.10E-05
Har_00184759	637	9.68	Har_454S-C2855	NA	0.0017769
Har_00194714	385	9.76	Har_454S-C25549	NA	0.0033247
Har_00231730	178	9.87	Har_454S-C22245	NA	3.10E-05
Har_00217124	878	10.19	Har_454S-C11316	NA	0.0048134
Har_00227682	225	10.23	Har_454S-C23262	NA	0.0051549
Har_00123329	542	10.44	Har_454S-C17202	NA	0.0006434
Har_00225561	516	10.98	Har_454S-C20542	NA	0.0019997
Har_00189083	240	11.04	Har_454S-C27270	NA	0.004548
Har_00144382	150	11.11	Har_454S-C25632	NA	0.0003373
Har_00064046	601	11.19	Har_454S-C3232	NA	0.0012239
Har_00226349	1506	11.27	Har_454S-C4749	NA	0.0013813
Har_00197264	252	11.5	Har_454S-C25350	NA	0.0025743
Har_00140567	1398	12.29	Har_454S-C7597	NA	0.0008403
Har_00221822	172	13.14	Har_454S-C19287	NA	0.0024457
Har_00191235	858	13.39	Har_454S-C2113	NA	0.0051425
Har_00144838	783	13.94	Har_454S-C24224	NA	0.0075658
Har_00216919	178	14.15	Har_454S-C20538	NA	0.0020464
Har_00192805	272	14.23	Har_454S-C22631	NA	0.0029325
Har_00189225	194	14.43	Har_454S-C27426	NA	0.0017317
Har_00169076	214	14.49	Har_454S-C25926	NA	0.0013425
Har_00184640	171	15.04	Har_454S-C19292	NA	0.0017739
Har_00159185	210	15.48	Har_454S-C27811	NA	0.0005583
Har_00151890	192	15.68	Har_454S-C21844	NA	0.0023874
Har_00066404	1043	16.89	Har_454S-C3591	NA	0.0009882
Har_00210576	219	17.17	Har_454S-C23714	NA	6.44E-05
Har_00158627	591	17.22	Har_454S-C20394	NA	0.0006803
Har_00071843	2338	17.57	Har_454S-C4397	NA	7.61E-05
Har_00148513	225	17.68	Har_454S-C21180	NA	0.0090566
Har_00212886	728	17.87	Har_454S-C14314	NA	5.58E-05
Har_00042406	571	18.61	Har_454S-C16828	NA	0.0051737
Har_00139443	217	19.6	Har_454S-C21158	NA	0.006448
Har_00238203	162	20.41	Har_454S-C19319	NA	0.0003016
Har_00235572	215	20.89	Har_454S-C27683	NA	0.0010001
Har_00190705	241	22.29	Har_454S-C23256	NA	3.15E-05
Har_00188169	523	22.62	Har_454S-C4296	NA	0.0007686
Har_00240133	285	26.05	Har_454S-C23891	NA	0.0004062
Har_00157331	168	27.18	Har_454S-C23045	NA	5.70E-05
Har_00198798	791	27.34	Har_454S-C13108	NA	0.0018418

Har_00042404	571	28.25	Har_454S-C16828	NA	0.0018424
Har_00194226	185	30.82	Har_454S-C20098	NA	3.56E-05
Har_00194160	230	31.36	Har_454S-C24166	NA	0.0001477
Har_00193085	212	32.31	Har_454S-C22826	NA	0.0017459
Har_00133658	699	34.18	Har_454S-C21241	NA	4.11E-05
Har_00121915	179	35.74	Har_454S-C27968	NA	0.0011774
Har_00188489	401	39.43	Har_454S-C20579	NA	0.0007138
Har_00203345	667	47.45	Har_454S-C2945	NA	0.0004011
Har_00218677	1053	50.72	Har_454S-C8697	NA	0.0011535
Har_00237544	224	54.62	Har_454S-C19108	NA	1.75E-06
Har_00227185	801	69.35	Har_454S-C23838	NA	1.95E-05
Har_00125490	179	81.11	Har_454S-C23629	NA	6.92E-06
Har_00122822	602	91.31	Har_454S-C20072	NA	0.0067002
Har_00227476	406	120.9	Har_454S-C18310	NA	0.0021713
Har_00185697	233	158.4	Har_454S-C24482	NA	0.0001741
Har_00234354	202	193.5	Har_454S-C25341	NA	5.66E-05