Abstract

This study has investigated the role of esterases in conferring pyrethroid resistance in Australian populations of the cotton bollworm, *Helicoverpa armigera*. To achieve this, studies were performed at the population, individual, biochemical and genetic levels. Using native polyacrylamide gel electrophoresis (native PAGE) esterase patterns and levels were assessed in resistant field and laboratory strains and in a susceptible laboratory strain. Esterases were assigned the standard nomenclature of regions A to F, based on the relative mobilities of groups of esterases on native PAGs. The esterases previously associated with pyrethroid resistance and further potential esterases were identified by native PAGE and through comparisons of larvae surviving and those killed by pyrethroid exposure. Resistant field strains showed a general increase in esterase expression and a higher frequency of individuals with additional esterases with relative mobilities between 0.24 and 0.3. Populations from eastern Australia were characterised by native PAGE for esterase expression and were also measured for frequencies of pyrethroid resistance. Frequencies of pyrethroid resistance among populations were not significantly different. However, frequencies of pyrethroid resistance in adult females in some populations were significantly higher than in adult males. Other populations showed more equal frequencies of resistance between the sexes. In collaborative work, levels of cytochrome P450, CYP6B7, mRNA expression in resistant and susceptible larvae were measured by northern blot analysis. Esterase and CYP6B7 expression were not correlated with pyrethroid resistance.

Native PAGE and microplate esterase assays showed that esterases in *H. armigera* larvae were not induced by pyrethroids, the synergist piperonyl butoxide (PBO) or acetone. Similarly, esterase expression in larvae was not significantly influenced by the diet on which larvae were reared. Esterases were found to be expressed throughout the *H. armigera* lifecycle, particularly in the larval stages. Native PAGE of dissected tissues from fourth-instar larvae showed esterase expression in each of seven tissues examined, particularly in the midgut. Esterase expression in adults from three strains was also compared by native PAGE. The resistant strain showed greater esterase expression and a larger range of esterases than resistant and susceptible laboratory populations. Adult and larval esterases were compared using esterase inhibitors and by native PAGE.

The esterases of fourth-instar larvae were further characterised biochemically. Differences in substrate specificities of esterases were detected using native PAGE followed by staining with various naphthyl and methylumbelliferyl ester substrates. Twenty-two potential esterase inhibitors were also used to characterise *H. armigera* larval esterases. The organophosphate (OP) insecticides were potent esterase inhibitors, showing inhibition based on chemical structure. The α-cyano pyrethroids were also effective esterase inhibitors, allowing identification of esterases potentially involved in pyrethroid resistance. The non-cyano pyrethroids were poor esterase inhibitors. Other inhibitors such as eserine and a juvenile hormone transition state analogue showed selective inhibition of *H. armigera* esterases. The inhibitors allowed identification of possible carboxylesterases, cholinesterases and juvenile hormone esterases. Two previously developed partition assays were optimised for the detection of ^{14}C-labelled pyrethroid metabolism in larval enzyme preparations. Pyrethroid esterase activity, using partition assays and thin layer chromatography, was demonstrated in fourth-instar larvae. One major product of metabolism was detected, the production of which was abolished by the addition of an organophosphate insecticide. This indicated esterase-mediated pyrethroid detoxification. A resistant field strain showed higher rates and levels of pyrethroid metabolism than laboratory resistant and susceptible strains. Larval esterases more readily hydrolysed cypermethrin than permethrin and showed a clear preference for hydrolysis of the *trans*-isomers of both pyrethroids. Little or no hydrolysis of the *cis*-isomer(s) of either pyrethroid was detected. A correlation between levels of α-naphthyl acetate esterase and permethrin esterase activities was detected.

Single pair crosses between resistant field and laboratory susceptible strains and native PAGE of the progeny over two generations allowed a study of the genetics of *H. armigera* esterases. The region C esterase, or a factor controlling its expression, was shown to probably be autosomally inherited. Sex-linkage of the region D esterases, or a factor controlling their expression, was indicated by native PAGE and tested using linkage analysis with a gene known to be sex-linked in *Heliothis virescens* (collaborative work). A 701 b.p. fragment of a putative *H. armigera* esterase gene was isolated and sequenced.