EXPRESSION AND INHERITANCE OF TARGET SITE RESISTANCE TO PYRETHROIDS IN THE TOBACCO BUDWORM, HELIOTHIS VIRESCENS

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Abstract

Pairs of adult tobacco budworms that were either target site-sensitive or -resistant to pyrethroids were mated in an attempt to develop a target site resistant strain of this insect. During the course of this study, assumptions regarding the genetic nature of this mechanism were examined; specifically, that target site resistance was monogenic and that the inheritance of this mechanism was recessive. Preliminary results from a limited number of crosses between adults of known phenotype suggest that one or both of these assumptions is invalid.

Introduction

Mechanisms of resistance to pyrethroids have been characterized in *H. virescens* and three major mechanisms are now recognized: reduced cuticular penetration of insecticides, enhanced metabolic detoxification and alteration of the primary site of action for pyrethroids in the insect nervous system (= target site resistance) (McCaffery et al. 1991; Sparks et al. 1993; Ottea et al. 1995). Target site resistance to pyrethroids is of particular concern because it confers cross resistance to all pyrethroid insecticides.

The current US insecticide resistance management (IRM) strategy for H. virescens advocates the rotation of insecticide classes. By removing selection pressure for one or more generations of the pest, the frequencies of resistance genes in a particular population can be reduced to a level where populations can be managed (Roush and McKenzie 1987). In the absence of pesticide treatment, the frequency of resistant individuals in laboratory and field populations of H. virescens decreases over time (Plapp 1981; Campanhola and Plapp 1989; Elzen et al. 1994; Plapp et al. 1990; Graves et al. 1991). In the field, this decline in resistance may be due to immigration of susceptible insects (Forrester et al. 1993, Leonard et al. 1995), or due to biological constraints associated with the expression of resistance genes. These constraints are believed to confer reduced fitness in individuals expressing resistance genes relative to susceptible individuals.

This investigation represents part of a larger study to investigate fitness costs associated with the expression of target site resistance in H. virescens. To undertake such a study, it is necessary to isolate a strain of H. virescens that is pure-breeding (i.e., genotypically homozygous) in its expression of target site resistance against an otherwise susceptible genetic background. With such a strain, measurements of life history parameters such as developmental time and overall fecundity can be measured and compared to those of a laboratory susceptible strain. However, our current knowledge about the genetics of target site resistance in the tobacco budworm is based largely on research carried out using other insects, most notably the housefly (Sawicki, 1985). Because of our lack of specific knowledge regarding expression of this mechanism in tobacco budworms, assumptions were made about the genetics of target site resistance in H. virescens. For example, expression of target site resistance was assumed to be due to a single, autosomal gene with recessive inheritance. These assumptions are of importance, as the genetic nature of a resistance mechanism a key biological factor in determining the speed at which resistance develops in response to insecticide application and which it declines in the absence of selection for resistance.

The specific objectives of this study was to use insecticide selection and single pair crosses to validate these assumptions with the long-term goal of establishing a laboratory strain that was homogenous for expression of target site resistance. Preliminary results from this study suggests that expression of this mechanism is controlled by more than one gene (or is due to multiple alterations in a singe gene) and that resistance is not inherited in simple, recessive fashion.

Materials and Methods

Insects

Field strains of *H. virescens* were collected at the Northeast Research Station (Macon Ridge) in Winnsboro, LA. in August and September 1995, and were used as a source of target site resistant insects for this study. The August strain (MRS Aug) was collected as eggs or neonate larvae from cotton and the September strain collected using pheromone traps or sweep net from adjacent stands of wild Dallas grass (*Paspalum notatum*). A reference laboratory strain (LSU) of susceptible *H. virescens* was also used. This strain has been maintained in the laboratory for 19 years without exposure to insecticides. Insects were reared in the laboratory as described previously (Leonard et al., 1988). Field strain insects were selected as adults with a discriminating dose of cypermethrin (0.23µg/insect). This dose is equivalent to 6 x LD_{80} for LSU insects. Cypermethrin was dissolved in 1µl of acetone and applied to the right eye of 1 day old adults. Survivors of selection were used in the pair mating experiments described below.

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Pair Mating

Male and female adults (3 days old) were placed pair-wise in 10 oz cups covered with cheesecloth serving as an ovipositional substrate. A piece of cellulose sponge soaked in 10% sucrose solution was placed in each cup as a food source and changed daily. Once fertile eggs had been collected from cheesecloth sheets, the parents were phenotyped for target site resistance using a neurophysiological assay (below). Progeny from each pair were reared in isolation and 30 fifth stadium larvae from each pair were phenotyped for target site resistance. Progeny from pairs expressing target site resistance were paired again in the following generation with progeny from other pairs expressing target site resistance. This cycle was repeated in order to increase the frequency of target site resistance genes in these insects.

Neurophysiological Assay

Levels of neuronal sensitivity in study insects were established using the neurophysiological assay of McCaffery et al. (1991), as modified by Ottea et al. (1995). Developmentally synchronous, fifth stadium larvae or 1-2 day old adults were dissected along the dorsal midline and pinned on a paraffin wax dish. Insects were bathed in lepidopteran saline and a peripheral nerve from the body wall was drawn by suction into an insulated recording electrode connected to a high gain, low noise amplifier (Warner Instruments, Hamden, CT). Action potentials were identified by amplitude discrimination, quantified using a MacLab 4 interface module (Analog Digital Instruments, Milford, MA) and displayed on a Macintosh computer. Immediately before use, solutions of analytical grade allethrin were prepared in saline from dilutions of stock solutions (0.01-100 mM). Numbers of action potentials during incubation of the nervous system in an allethrin-free saline/ethanol solution were recorded for 3 minutes as a control. Saline was then removed and replaced with fresh saline containing the various concentrations (0-100 µM) of allethrin. The endpoint of the assay was defined as the concentration of allethrin that causes a twofold increase in the frequency of spontaneous multiunit potentials relative to the saline/ethanol control. Frequency distributions for neurophysiological responses of LSU insects (Holloway et al. 1996) were used to establish a concentration of allethrin capable of discriminating between susceptible and resistant adults and larvae from pair mating experiments. Insects responding a concentration of 10µM allethrin or less were considered target site sensitive and insects responding or failing to respond to 100µM allethrin were considered to be target site resistant.

Results and Discussion

Information regarding the genetics of resistance mechanisms has direct impact on the management of insecticide resistant pest populations. For example, if more than one gene is responsible for the expression of target site resistance, initial frequencies of individuals possessing both genes (i.e., fully resistant insects) would be very low in populations and would increase more slowly (and decrease more rapidly) than if resistance were monogenic. Similarly, the level of dominance of a resistance allele will also contribute to the rate at which insecticide resistance develops in the field. If inheritance of resistance is recessive, insects that are heterozygous for resistance (RS) will be phenotypically susceptible and will not survive in a pesticide-treated environment. Thus, when insecticide application and selection occurs, expression of resistance genes will be limited to insects that are genotypically homozygous for resistance and overall expression of resistance genes will be lower than if resistance were dominant and heterozygotes expressed the resistance phenotype.

Based on initial assumptions about the genetics of target site resistance, the outcomes of crosses between target site-sensitive and -resistant insects were unexpected. For example, if the expression of target site resistance is due to a single gene with recessive inheritance, crosses between two resistant (homozygous = RR) parents should result in 100% resistant offspring. In actual crosses between target site-resistant parents however, only 33% of offspring expressed this mechanism (Table 1). In addition, reciprocal crosses between target site-resistant (RR) and -sensitive (RS or SS) individuals produced similarly unexpected ratios. In accordance with initial assumptions, offspring from such crosses should either be 50% resistant and 50% susceptible (for RR X RS) or 100% susceptible (for RS X RS). Actual susceptible: resistant ratios were 2:1 (in a cross with a resistant male) and 3:1 (in a cross with a susceptible male). Finally, pairing of two phenotypically sensitive individuals also resulted in an unexpected outcome. Again according to our assumptions such a cross could only result in 25% resistant offspring (if parents were RS x RS) or 100% sensitive offspring (if parents were SS x RS or SS x SS). Actual results from this cross were intermediate, with 88% susceptible and 12% resistant progeny.

Summary

Results from this study indicate that more than one gene may be responsible for the expression of target site resistance and that the gene or genes responsible are not fully recessive. Recent research into the molecular genetics of target site resistance in *H. virescens* (Park and Taylor, unpublished) in conjunction with the results from pair mating experiments in this investigation, bring into question assumptions about the inheritance and expression of target site resistance in this insect and indicate that the genetics of this important mechanism warrant further investigation.

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Table 1. Phenotypes of progeny from single pair crosses between target site-sensitive (S) or resistant (R) adults.

PARENTS			PROGENY	
			% Sensitive	% Resistant
R Male	**X**	R Female	66 %	33 %
R Male	**X**	R Female	66 %	33 %
R Male	**X**	S Female	66 %	33 %
S Male	**X**	R Female	76.7 %	23.3 %
S Male	**X**	S Female	88 %	12 %