ANALYSIS OF ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDE RESISTANCE IN THE BEET ARMYWORM, *SPODOPTERA EXIGUA* (HUBNER)

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Abstract

Initial monitoring of field populations of the beet armyworm, *Spodoptera exigua*, indicated the presence of two forms of acetylcholinesterase (AChE), the target site of organophosphorus (OP) and carbamate insecticides, using a diagnostic concentration of the carbamate, methomyl. In toxicological bioassays, the response of populations to topical applications of technical methomyl and the OP, chlorpyrifos-oxon, were related to the relative frequencies of the AChE alleles present. All populations, including a laboratory reference strain, showed a high degree of tolerance to methomyl; however, chlorpyrifos-oxon proved to be extremely potent, even against populations in which the resistant AChE variant predominated.

Introduction

Widespread tolerance to the carbamate methomyl, and the organophosphate (OP), chlorpyrifos, has been reported in Californian populations of the beet armyworm (BAW) *Spodoptera exigua* (Brewer and Trumble, 1996; Kerns *et al.*, 1998; Mascarenhas *et al.*, 1998; Sparks *et al.*, 1996). Despite substantial documentation of toxicological data, our survey of the literature provided no information on the potential involvement of biochemical mechanisms in conferring reduced susceptibility in these populations. Kim *et al.* (1997) reported increased Km values for AChEs in resistant populations of BAW from Asia, but provided no direct evidence for changes in the intrinsic sensitivity of the enzymes to the affected insecticides. Although resistance to OPs and carbamates can be due to many factors, we initiated a study to examine the impact of modified AChEs on the expression of resistance in this pest. The primary aim of the study was to develop a sensitive method for detecting AChE insensitivity in individual BAW insects, and to then use this technique to monitor the frequencies of resistant alleles in field populations and determine how these affect the toxicological response to insecticides. Our preliminary results are presented here.

Materials and Methods

Insects

The Dow AgroSciences laboratory-reared strain of BAW was used for reference purposes. Field populations were collected during 1998 from alfalfa, cotton and lettuce in San Joaquin and Imperial Valleys of California, and reared on artificial diet in the laboratory.

Insecticides

Technical grade methomyl and chlorpyrifos-oxon were gifts from DuPont Agricultural Products and Dow AgroSciences, respectively.

Toxicological Bioassays

Technical methomyl and chlorpyrifos-oxon were applied topically to the dorsum of the thorax of 3rd instar larvae (25 larvae per test) in 0.5 µl of acetone at 0.04-4.0 µg/larva and 0.001-3.0 µg/larva, respectively, and mortality determined after 48 h. Probit analyses of the concentration dependent mortality data were done using POLO-PC (Anon, 1987).

Acetylcholinesterase Insensitivity Assays

Individual 3rd instar larvae were homogenized in a final volume of 250 µl of 0.1 M phosphate buffer, pH 7.5, containing 0.1 % Triton X-100, in 96-well microtitre plates using the multi-homogenizer of ffrench-Constant and Devonshire (1987). After a 1 h solubilization period at 4 °C, three 50 µl aliquots from each homogenate were added to adjacent wells of a new microplate, and the volume adjusted to 100 µl with buffer/TX100. AChE activity was measured by the method of Ellman (1961) with modifications by Byrne and Devonshire (1993). Reactions were started by the addition of ATChI/DTNB (0.5 mM/0.005 mM final assay concentrations) and changes in absorbance at 405 nm monitored for 30 min using a SpectraMax 250 microplate reader (Molecular Devices). Activity was measured in the absence of insecticides, and in the presence of either methomyl (30 µM) or chlorpyrifos-oxon (1 µM) for each insect homogenate. AChE genotypes were determined according to Byrne and Devonshire (1993).

Results and Discussion

Two AChE variants were detected in field populations of BAW collected from sites in Imperial Valley and San Joaquin Valley using the microplate assay. Samples from these areas were typically small, and in some cases were confined to a single egg mass. However, the collections provided sufficient material with which to develop the diagnostic assay and thereby enable a preliminary survey of AChE variation. Despite the likelihood that some samples were derived from single pair matings, no populations we tested were fully homogeneous for either AChE variant, although the relative frequencies of each differed. Both variants were sensitive to the diagnostic concentration of 1 µM chlorpyrifos-oxon, but differed in their responses to methomyl. At a concentration of 30 µM methomyl, the activity of one form was unaffected during the 30 min. assay, while the second form was fully inhibited. BAW individuals heterozygous for methomyl insensitivity were also readily detected in these field populations. Because of

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the heterogeneous nature of the field populations, it has not yet been possible to make an accurate comparison of the kinetics of their AChEs with those found in the laboratory strain from Dow, which showed full sensitivity to both insecticides.

In bioassays, methomyl proved ineffective over the dose range 0.04-4.0 µg with all field populations tested. As a result, there was insufficient data for determining LD_{50}s for this compound. However, at the upper dose of 4.0 µg, 48% mortality was recorded for the Dow strain, approximating the LD_{50} for this compound reported elsewhere (Wolfenbarger *et al.*, 1997). In contrast, chlorpyrifos-oxon was extremely potent to both the laboratory and field populations. Data for two populations from San Joaquin Valley are shown in Table 1. Higher LD_{50}s were observed in the population containing the highest frequency of insects expressing the insensitive AChE.

As far as we are aware, this is the first evidence of insensitive AChE in BAW. The presence of high frequencies of the insensitive variant in the most insecticide-tolerant populations is strong evidence for a major role of target-site insensitivity in resistance to OPs and carbamates. However, the exact contribution to resistance cannot yet be fully determined until populations homogeneous for each variant are available for bioassay. This is currently underway in our laboratory. Although both AChE variants were sensitive to 1 µM chlorpyrifos-oxon in the microplate assay, the bioassay data suggest that the form showing insensitivity to methomyl is also more insensitive to the OP. This should be readily detectable using a lower diagnostic concentration of chlorpyrifos-oxon in the microplate assay, which will be more easily defined using homogeneous populations. The isolation of pure strains will also enable a more accurate assessment of the impact of each form on the survivorship of BAW in bioassays, and will further provide an opportunity to determine the underlying kinetics likely to be responsible for contributing to OP and carbamate resistance in this pest.

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Table 1. Toxicity of technical chlorpyrifos-oxon topically applied to 3rd instar BAW larvae, and the frequencies of their AChE genotypes. Dow is the laboratory susceptible strain, and SJV-1 and SJV-2 represent two field populations collected from the San Joaquin Valley during 1998.

Strain	LD_{50} µg/L_3	95% conf. limits	Slope	S.E.	AChE frequency (%)		
					SS	SR	RR
Dow	0.08	0.02 - 0.18	2.3	0.28	100	-	-
SJV-1	0.19	0.11 - 0.32	3.0	0.52	75	-	25
SJV-2	0.55	0.40 - 0.76	2.5	0.38	13	81	6