MECHANISMS OF RESISTANCE TO PYRETHROIDS IN THE BOLLWORM, HELICOVERPA ZEA

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Abstract

Neuronal sensitivity to the pyrethroid insecticide, allethrin was measured in larvae of the bollworm, Helicoverpa zea (Boddie). An established neurophysiological assay was used to record and compare sensitivities in laboratory-susceptible and field strain larvae collected from a wild host, corn, conventional and transgenic (NuCOTN33) cotton in Louisiana during 1995 and 1996. Laboratory and field-collected larvae were equally target sensitive during 1995, but a low frequency of insensitive individuals was detected in conventional and transgenic cotton collections during 1996.

Introduction

The bollworm, Helicoverpa zea (Boddie), together with the tobacco budworm Heliothis virescens (F.) are well established as two of the most important cotton pests in the US (Sparks 1981, Luttrell 1994). Pyrethroid insecticides remain effective for the control of H. zea, even at low application rates, in contrast to declining efficacy against the related cotton pest, H. virescens (Sparks et al. 1993). Although both species share a history of insecticide resistance and potential as serious pests (Wolfenbarger et al. 1981, Sparks 1981, Leonard et al. 1988), in recent years H. virescens has predominated as a cotton pest and has been difficult to control with conventional insecticides largely due to problems associated with the development of resistance (Elzen et al. 1992, Sparks et al. 1993).

Pyrethroid resistance has been shown to be multifactorial in H. virescens and is due to reduced penetration, increased metabolism and altered target-site mechanisms (McCaffery et al. 1991, Ottea et al. 1995). Reduced target sensitivity to type I pyrethroids (allethrin) and type II pyrethroids (cypermethrin) has been reported previously to be a major mechanism of pyrethroid resistance in H. virescens (Ottea et al. 1995, McCaffery et al. 1995). This mechanism confers cross-resistance across the pyrethroid class of insecticides and contributes to protection from pyrethroid poisoning in both the larval and adult life stages (Holloway and McCaffery 1994, 1996). Detection and monitoring of the frequency of target site resistance in field strains of H. virescens provides useful information for incorporation into pyrethroid insecticide resistance management strategies.

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Several studies have reported recent changes in pyrethroid susceptibility in field populations of H. zea, in Central and South America (Ernst and Dittrict 1992), and in the US (Abd-Elghafar et al. 1993, Kanga et al. 1996). However, little information exists on possible mechanisms of pyrethroid resistance in this insect. Early studies showed that laboratory strain H. zea larvae were capable of metabolizing [^{14}C]permethrin (Bigley and Plapp 1978). Both metabolic and non-metabolic mechanisms have been proposed to reduce pyrethroid susceptibility in this species (Ernst and Dittrict 1992, Abd-Elghafar et al. 1993, Kanga et al. 1996, Abd-Elghafar and Knowles 1996). No neurophysiological studies have been undertaken to determine neuronal sensitivity to pyrethroids in H. zea.

The aim of the current study was to establish a baseline for neuronal sensitivity to pyrethroids in H. zea and to determine what, if any, frequency of expression of reduced pyrethroid target-site sensitivity was expressed in field strains of this species.

Materials and Methods

Insects

Laboratory strain (USDA-LAB) H. zea were obtained as pupae from the United States Department of Agriculture laboratory in Stoneville, MS. Field strains of H. zea were collected at the Louisiana State University Agricultural Center's Northeast Research Station, Winnsboro, LA. In 1995, insects were collected as eggs from transgenic cotton (NuCOTN33) during September (95Bt) and as adults (using sweep nets) from a wild host (Dallas grass, Paspalum notatum) during November (95SN). In 1996, larvae were collected from field corn in June (96JC), conventional cotton in July (96JL) and Bt cotton (NuCOTN33) in August (96ABt). Insects were reared in the laboratory as described previously (Leonard et al. 1988) without exposure to insecticides. Adults were mass-reared in 3.8 l cardboard cartons covered with cotton gauze, at 27°C, 50% RH and a 14:10 (light: dark) photoperiod, and were fed a 10% sucrose : water diet. Field corn silks were placed above the cartons to stimulate moth oviposition. Larvae were reared individually in 30 ml plastic cups on a pinto bean diet. Developmentally synchronous 5th stadium larvae weighing 181- 352 mg from the parental (P1) or first filial (F1) generation were used for assays.

Chemicals

Technical grade allethrin (94.7%) and cis/trans (60:40) cypermethrin (94%) were supplied by Roussel Uclaf (Paris, France) and FMC (Princeton, NJ), respectively. Technical grade piperonyl butoxide (PBO) and *S*,*S*,*S*-tributyl phosphorotrithioate (DEF) were supplied by ChemService (West Chester, PA) and Bayer Corporation (Kansas City, MO). For neurophysiological assays, stock solutions of allethrin were dissolved in absolute ethanol and suspended in lepidopteran saline (Weevers 1966) at final concentrations of 0.0001- 100μM. For larval bioassays,

cis/trans cypermethrin was dissolved in Analar grade acetone at final concentrations of 0.005 - 2.5μg/μl.

Insecticide Bioassays

The susceptibility of 5th stadium larvae to cypermethrin was determined by topical bioassay similar to the standard Entomological Society of America method for Heliothis spp (Anonymous 1970). Larvae were treated on the dorsal surface of the mesothorax with a 1 μl drop of acetone (controls) or acetone containing cypermethrin, using a Hamilton glass micro-syringe fitted to a repeating ratchet dispenser. At least 40 larvae from each strain were treated as controls and at each dose of cypermethrin. Not less than 5 doses of cypermethrin were chosen from preliminary bioassays giving mortality between 0-99%. Treated larvae were held at 27°C, 50% RH and a 14:10 (light: dark) photoperiod and mortality assessed after 72h. The criterion for mortality was inability for larvae to make coordinated movement 15 seconds after prodding with a pencil point. Control mortality never exceeded 5% and was corrected using Abbot's (1925) formula. Data were analyzed and probit regressions were estimated using a Polo Probit computer program. Due to low numbers of field stain larvae cypermethrin toxicity was not evaluated with the 95Bt field strain. Measurements of toxicity in 1996 were limited to diagnostic (single) dose assays.

Diagnostic dose and synergism assays

Field-collected larvae in 1996 were topically treated on the dorsal side of the mesothorax with a single diagnostic dose of 0.79 μg cypermethrin as described above. This diagnostic dose is the LD_{90} for cypermethrin in the USDA-LAB strain. Larvae killed by this dose after 72h were considered to be susceptible. Synergists were used to gain a preliminary understanding of the contribution of pyrethroid metabolism in 1996 field strains. Subsets of 40 larvae from each strain were topically pretreated on the dorsal side of the abdomen with 20 μg of either the monooxygenase synergist piperonyl butoxide (PBO), or the esterase synergist *S,S,S*-tributyl phosphorotrithioate (DEF) 30 min. prior to application of the diagnostic dose of cypermethrin or acetone (controls) to the mesothorax. No control mortality was recorded in insects pretreated with 20 μg of either synergist.

Neurophysiological assays

A rapid neurophysiological assay designed for detecting neuronal sensitivity to pyrethroids in H. virescens (Gladwell et al. 1990, Ottea et al. 1995) was modified for use with larval H. zea. Larvae were decapitated, opened dorsomedially and pinned onto a disposable paraffin wax dish. The inner surface of the body wall and its associated nervous tissues were exposed by dissection and bathed in saline. Extracellular recordings of spontaneous nervous activity were made using a blunted 27 gauge syringe needle modified as a disposable suction recording electrode (coated externally with an insulating layer of polyurethane) and a stainless steel #3 entomological pin as a reference electrode. Electrodes were connected to a high gain low, noise amplifier (Warner Model DP301, Hamden, CT) where nerve signals were amplified and filtered before relay to a MacLab-2e analog recording and analysis instrument (Analog Digital Instruments, Milford, MA) interfaced with a portable Macintosh Powerbook 180 computer. The number of action potentials discriminated above a preset threshold was recorded in successive 3 minute periods, first with the preparation bathed in saline alone, then with a 3 minute saline/ethanol control period. Nerve preparations were then exposed to a range of allethrin concentrations using a cumulative dose-response assay described by Ottea et al. (1995). H. zea nerve preparations were exposed to a range of allethrin concentrations (0.0001 - 100μM). The endpoint of the assay was defined as the lowest concentration of allethrin evoking a 2-fold increase in rate of nerve firing compared with the firing rate during the saline-ethanol control period. Thirty to thirty-five larval neurophysiological recordings were made for each strain.

Results

Insecticide bioassays

In 1995, dose-response assays were conducted with the USDA-LAB strain and the 95SN field strain. The LD50 for the 95SN strain was 1.7 fold greater than that of the susceptible USDA-LAB strain (0.24 and 0.41, respectively); however, based on overlap of 95% fiducial limits (FL) for LD_{50} and LD_{90} values for these two strains, this difference was not statistically significant (Table 1).

Diagnostic dose and synergism assays

Survival of larvae following treatment with 0.79 μg cypermethrin increased through the 1996 season from a low of 17% in June to a high of 51% in August (Table 1). This increase may have reflected sustained pyrethroid selection pressure in this region during the production season, an inference that was corroborated by observations of H. zea survivors in commercial cotton fields adjacent to the Macon Ridge site receiving synthetic pyrethroid applications.

Low levels of synergism were observed following pretreatments with PBO and DEF throughout the 1996 season (Table 2). Pretreatment with PBO reduced survival of larvae treated with the diagnostic dose of cypermethrin by 4% in June, 5% in July and 4% in August. Pretreatment with DEF increased survival of larvae treated with the cypermethrin diagnostic dose by 3% in June. This result probably reflects variability in the bioassay data rather than true negative synergism, particularly since DEF reduced survival of cypermethrin treated larvae in July and August (10% and 8%, respectively).

Neurophysiological assays

The majority of larval nerve preparations from each of the strains responded to low (< 1 μM) concentrations of allethrin with a characteristic increase in nerve firing rate. All USDA-LAB strain larvae responded at < 1μM allethrin

and 1μM was chosen as a diagnostic concentration to discriminate between target site- susceptible and -resistant individuals (shown as vertical dashed line in Figs. 1 and 2). Based on this criterion all individuals from both the 95SN and 95Bt field collections were target-site susceptible (Fig. 1) Neurophysiological profiles for the 1996 field strains are shown in Fig. 2. The profiles of the June field strain is similar to those of the 95Bt and 95SN strains. However, both the July and August field collected strains contain a low frequency of target site- resistant individuals (10% and 13%, respectively) that failed to respond to allethrin concentrations >1 μM.

Discussion

Insecticide resistance management strategies for H. virescens currently advise restriction of early season pyrethroid use to preserve efficacy later in the growing season, with increasing emphasis on the use of transgenic Bt cotton for this species (Plapp et al. 1990, Bagwell 1996). Bt cotton has been show to be less effective in controlling H. zea than H. virescens (Macintosh et al. 1990) and H. zea infestations of Bt cotton were effectively controlled with pyrethroids in the midsouth during the 1996 cotton season. Preservation of the efficacy of the pyrethroids for the cost effective control of H. zea is an important component of cotton insecticide resistance management strategy. Recent registration of synthetic pyrethroids for use in field corn and grain sorghum against winter pests may further intensify selection pressure for resistance in H. zea.

Results from diagnostic dose bioassays indicate that pyrethroid-resistant individuals were present in field populations of H. zea collected in Louisiana in 1996, albeit at low frequencies at the beginning of the growing season. This agrees with earlier work describing low frequencies of pyrethroid resistant bollworms in 1988-1993 in Texas (Kanga et al. 1996) and with reports of pyrethroid resistance in field populations of bollworm in Arkansas and Illinois (Abd-Elghafar et al. 1993). In the current study, survival of bollworm larvae treated with the diagnostic dose increased from June to August, which may reflect sustained pyrethroid selection pressure in conventional and Bt cotton in Northeast Louisiana during the 1996 growing season. Results from adult vial tests (Plapp et al. 1986) of male moths collected from the same site during 1995 and 1996 revealed general susceptibility of H. zea to pyrethroids in Louisiana; however, seasonal increases in survival at a diagnostic dose of 5 μg cypermethrin was noted (Bagwell et al. 1996 and this volume). In contrast, in Central and South America pyrethroid use patterns exert intensive selection pressure on populations of H. zea and resistance to deltamethrin, cyfluthrin, and cyhalothrin have reached prohibitive levels in cotton growing areas (Ernst and Dittrich 1992).

Previous studies have suggested that the development of pyrethroid resistance in US populations of H. zea is unlikely (Kanga et al. 1996), in contrast to the situation of widespread pyrethroid resistance in H. virescens. The wider host range and increased mobility of H. zea relative to H. virescens may have contributed to a delay of resistance and a decade of highly effective pyrethroid control of the former species (Leonard et al. 1988, Plapp et al. 1990). The potential for H. zea to develop pyrethroid resistance by expressing mechanisms found in the more closely related species, Helicoverpa armigera (Hubner) has been suggested (Kanga et al. 1996) and close phylogenetic similarities exist between the two species (Cho et al. 1995). Today, pyrethroid resistance in Australian H. armigera has been attributed to increased metabolism rather than reduced neuronal sensitivity (Gunning et al. 1991), although the latter mechanism was reported at the onset of pyrethroid resistance (Gunning et al. 1984). Previous studies have reported PBO synergism of cypermethrin toxicity in H. zea (Kanga et al. 1996), and, although synergist efficacy was poor in the current study, enhanced metabolism may be a component of reduced pyrethroid susceptibility. However, the possible contribution of unsynergisable and/or target-site resistance mechanisms in field populations of this species cannot be eliminated.

The history of DDT use and resistance in H. zea suggests that pre-selection for target-site resistance to pyrethroids may have occurred in this species as it is thought to have in H. virescens (Sparks 1981, Sparks et al. 1993). Thus, it is possible that a low frequency of target site- resistant individuals were present in field populations of H. zea but were not detected in 1995 because of small sample sizes. In 1996 however, target site resistance was detected in field populations of H. zea at measurable frequencies.

In contrast to H. virescens, the expression of target-site resistance appears to remain at low frequencies in H. zea at the present time. The frequency of expression of reduced neuronal sensitivity in field populations of H. zea currently lags behind those in H. virescens, probably reflecting differences in the intensity of selection pressure for pyrethroid resistance in the two species. The potential for an increase in the expression of this resistance mechanism in H. zea requires further monitoring and may provide insight for incorporation into the pyrethroid insecticide resistance management strategy.

This work has provided an opportunity to establish baselines of target site sensitivity to pyrethroids in laboratory and field strains of H. zea. Monitoring the frequency of expression of reduced neuronal sensitivity in H. zea is relevant to the preservation of pyrethroid efficacy for control of this species on cotton as well as other crops. It may also offer an opportunity to study the expression of this resistance mechanism in this pest species before it becomes well established in the field.

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Table 1. Susceptibility of 5th stadium H. zea measured 72h after topical application of technical grade cypermethrin.

Strain	n	LD_{50} (μg/larva) [95% FL]	LD_{90} (μg/larva) [95% FL]	Slope ± SE
USDA	262	0.24 [0.16- 0.37]	0.79 [0.48- 2.47]	2.48 ± 0.31
95SN	190	0.41 [0.29- 0.62]	2.62 [1.41- 8.20]	1.59 ± 0.26

Table 2. Susceptibility of 5th stadium H. zea measured 72h after topical application of technical grade cypermethrin ± pretreatment with PBO or DEF

	Treatment/% Survival		
Strain	cyper	cyper+ PBO	cyper+ DEF
USDA	0	0	0
96 JC	17	13	20
96JL	30	25	20
96ABt	51	47	43

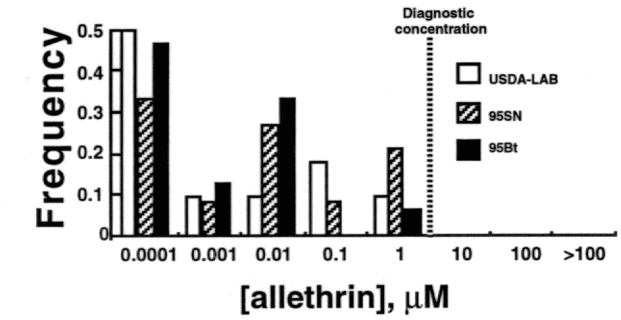

Figure 1. Neuronal sensitivity to allethrin in larval H. zea collected in Louisiana during 1995.

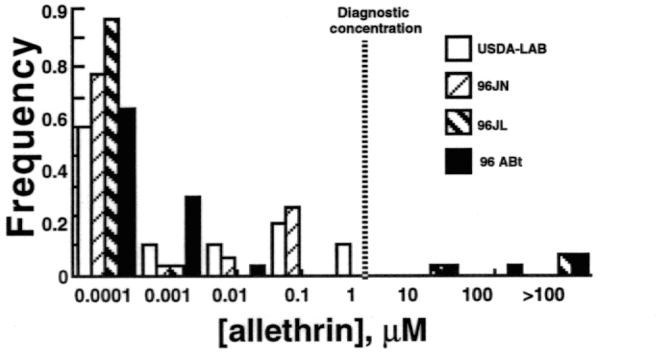

Figure 2. Neuronal sensitivity to allethrin in larval H. zea collected in Louisiana during 1996.