INSECTICIDE RESISTANCE AND SYNERGISM OF PYRETHROID TOXICITY IN THE TARNISHED PLANT BUG, *LYGUS LINEOLARIS*

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Abstract

Adult tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois) were collected from a cotton growing region in Northeast Louisiana during 1995 and 1996 and tested for susceptibility to cypermethrin, acephate and oxamyl. For all three insecticides, susceptibility decreased throughout the growing season. In addition, effects of synergists on cypermethrin toxicity were evaluated using insects collected during mid-late season. Pretreatment of adults with S, S, S-tributylphosphate or triphenylphosphate decreased susceptibility to cypermethrin. Similarly, application of piperonyl butoxide or a trichloropropynyl ether also increased susceptibility to cypermethrin suggesting that enhanced metabolism is a mechanism of resistance in these insects.

Introduction

The spectrum of insect pests that attacks U. S. cotton may be changing. The tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), is emerging as an important pest of midsouth cotton (Scott *et al.*, 1986). Infestation and crop damage due to populations of TPB have increased recently both nationwide and in Louisiana. Problems with TPB and *Lygus hesperus* are well-recognized across the cotton belt with over 50% of U.S. cotton infested by these pests in 1995 and 1996 (Williams, 1996; 1997).

In recent years, TPB populations have been managed with organophosphate, carbamate and pyrethroid insecticides (Snodgrass 1996a). Resistance to these insecticides may have contributed to the increased pest status of this insect and has been documented to all three groups of insecticides in field populations of TPB (Snodgrass and Elzen, 1995; Pankey *et al.*, 1996; Snodgrass 1996a; Hollingsworth *et al.*, 1997). However, frequencies and mechanisms of resistance have not been studied. Thus, it is not clear whether current control difficulties result from large populations of predominantly susceptible insects, or smaller populations with high frequencies of resistance. An understanding of resistance mechanisms is important for the rational design of insecticide resistance countermeasures and may facilitate development of rapid assays for resistance detection and monitoring.

Synergists have been used to provide a preliminary understanding of the contribution of insecticide metabolism to resistance. Further, when conventional synergists fail to increase susceptibility, the contribution of non-metabolic mechanisms (e.g., decreased target site sensitivity) may be cautiously inferred. The objective of this research was to monitor seasonal variations in insecticide susceptibility in TPB, and to examine effects of synergists on pyrethroid toxicity.

Materials and Methods

Insects

Field strains of the TPB were collected using sweep nets during the summers of 1995 and 1996 at the Louisiana State University Agricultural Center's Northeast Research Station (Winnsboro, LA). Adults were collected from *Choreopsis* spp. during April, May and June, and from cotton (*Gossypium histurtum*. L.) during August and September. Adults from a reference-susceptible laboratory strain (USDA) were obtained from the USDA/ARS laboratory in Stoneville, MS. Insects were held overnight in wire cages and provided with washed green beans for food.

Chemicals

Technical grade acephate, oxamyl and cypermethrin were supplied by Valent (Walnut Creek, CA). Technical grade piperonyl butoxide (PBO), triphenylphosphate (TPP) and *S,S,S*-tributylphosphorotrithioate (DEF) were supplied by ChemService (West Chester, PA), Bayer Corporation (Kansas City, MO) and Sigma (St. Louis, MO), respectively. The propynyl ether, 1, 2, 4-trichloro-3-(2-propynyloxy) benzene (TCPB) was prepared and purified as described by Shan *et al.* (1997). Insecticides and synergists were dissolved in analar grade acetone. For assays with insecticides, new, 20 ml scintillation vials were treated individually as described by Plapp *et al.* (1987) and stored at 0^{o}C. Vials were used less than four months after preparation.

Insecticide Bioassays

The susceptibility of adult TPBs to insecticides was determined using a glass vial bioassay similar to that described by Snodgrass (1996b) for *Lygus* spp. Adults (2-3/vial) were exposed to a residue of either acetone (control) or insecticide in 20 ml scintillation vials. A small (<2mm) section of washed green bean was added to each vial, and vials were capped loosely to prevent escape. At least 40 adults from each strain were treated with not less than 5 concentrations of each insecticide. Vials containing insects were held upright at 27^{o}C, 50% relative humidity and a 14:10h (light: dark) photoperiod. Mortality was assessed at 24 h after treatment and defined as the inability for adults to right themselves or make coordinated movement within 15 seconds after being prodded with a pencil point. Control

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mortality never exceeded 10% and was corrected using Abbot's (1925) formula. Data were analyzed and probit regressions were estimated using a Polo Probit computer program (LeOra 1987).

Synergist Bioassays

Adult TPBs were transferred into untreated vials and treated on the dorsal surface of the thorax with 1 μl of either acetone (controls) or acetone containing synergist. In preliminary experiments, bioassays with synergists alone were used to establish maximum sublethal doses (data not shown), which were used in subsequent experiments. In bioassays with both synergists and cypermethrin, synergists were applied 30 min. prior to introduction of insects into vials containing 10 μg of cypermethrin, and mortality was evaluated as described above. Doses of synergists used were 5 μg/insect of PBO, DEF or TPP, and 0.5 μg/insect of TCPB.

Results and Discussion

Insecticide Susceptibility

Resistance to oxamyl, acephate and cypermethrin increased with time during the 1995 and 1996 growing season (Figure 1). The most rapid increase in levels of resistance was measured with cypermethrin between April and August. During this period, LC_{50} values increased by 14-fold in 1995 and more than 17-fold in 1996. In tests with oxamyl and acephate, susceptibility was generally similar between 1995 and 1996 although LC_{50} values in early season collections were lower for both insecticides in 1996 than 1995. This pattern also was seen for cypermethrin in early season collections but, in mid-late season insects, resistance to cypermethrin was higher in 1996 than 1995.

During 1996, LC_{50} values for cypermethrin were significantly higher in mid-late season field collections than that measured for insects from the USDA strain (Figure 2). Susceptibility to the discriminating concentration also decreased throughout the growing season and was correlated with LC_{50} values. Resistance to these different insecticide classes has been reported previously in field strains of TPB (Snodgrass 1996a), although it is not known whether resistance results from the expression of a single mechanism (cross-resistance) or multiple mechanisms that are specific for each chemical class (multiple resistance).

Synergism of Cypermethrin Toxicity

Pretreatment with PBO reduced survival of adults exposed to 10 μg cypermethrin by 7% in July, 23% in August and 22% in September (Figure 3). More dramatic effects were measured with DEF, which reduced survival by 33% in July, 50% in August and 54% in September (Figure 3). Toxicity of cypermethrin was also increased following pretreatment with TPP and the propynyl ether, TCPB (Figure 4). As with results with PBO and DEF, the esterase synergist (TPP) was more effective than the oxidase synergist (TCPB). These results suggest that pyrethroid metabolism, possibly mediated by esterases and monooxygenases plays a significant role in pyrethroid resistance in adult TPB. In the related pest, *L. hesperus*, organophosphate resistance has been shown to be associated with enhanced carboxylesterase activity and insensitive acetylcholinesterase (Zhu and Brindley, 1992). Finally, a proportion (5-37%) of individuals survived synergist: insecticide bioassays suggesting that additional (non-metabolic) mechanisms contribute to resistance in these insects. Further work to characterize both resistance mechanisms in these insects is needed.

In summary, this work has provided further evidence that insecticide resistance is expressed in field populations of the TPB. Implementation of new strategies for managing cotton pests (e.g., the boll weevil eradication program and transgenic cotton) may result in further shifts in the status of pests such as the TPB. An appraisal of current resistance frequencies and mechanisms in pests that have, up until now, received secondary consideration will provide baseline data for subsequent studies and provide information that information that will facilitate future efforts to mange these pests.

Acknowledgments

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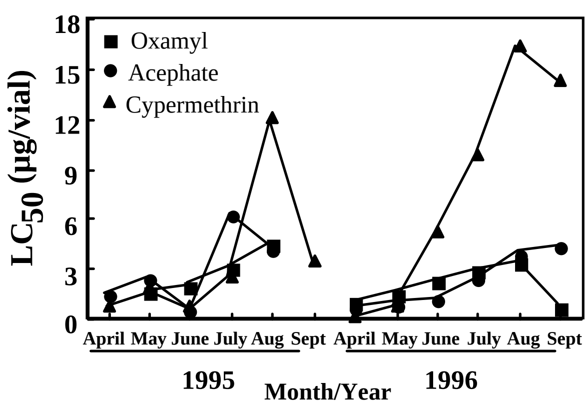

Figure 1. Susceptibility of adult *L. lineolaris* to oxamyl, acephate and cypermethrin during 1995 and 1996.

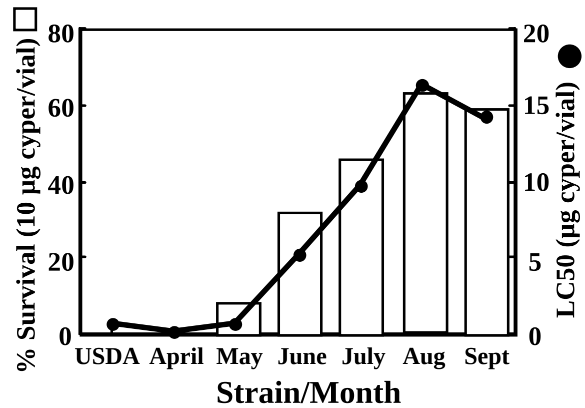

Figure 2. Frequencies and levels of resistance to cypermethrin in lab and field strains of *L. lineolaris* during 1996.

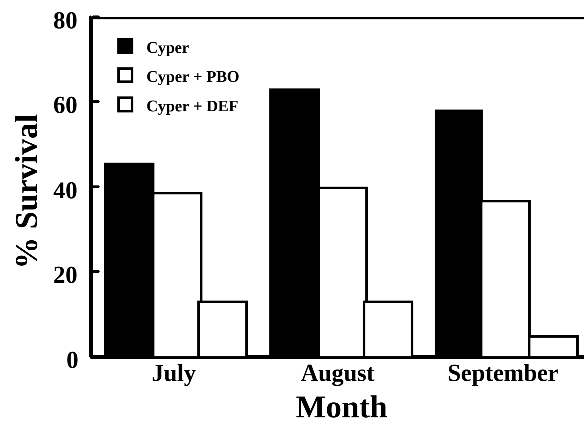

Figure 3. Synergism of cypermethrin toxicity by PBO and DEF in field-collected strains of *L. lineolaris* during 1996.

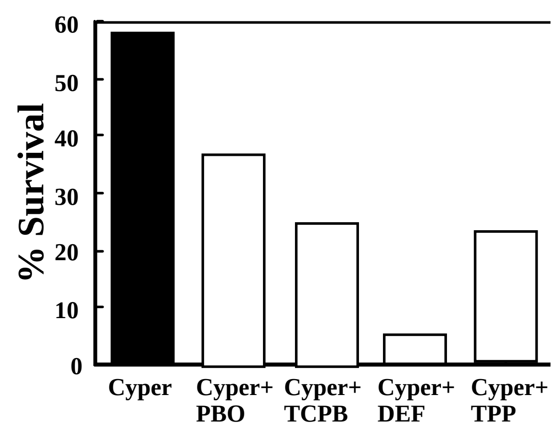

Figure 4. Synergism of cypermethrin toxicity by PBO, TCPB, DEF and TPP in *Lygus lineolaris* collected during September, 1996.