SUMMARY OF LABORATORY STUDIES

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Many aspects of insecticide resistance, by their nature, are most suited for laboratory experimentation. The body of work reported here examines pyrethroid resistance in the tobacco budworm using traditional laboratory studies as well as new approaches. Abundant testing of the response of field and laboratory tobacco budworm populations to pyrethroids relative to nonselected populations have been conducted with topical assays and foliar assays. The adult vial test (AVT) has also been used to test the response of adult males to the pyrethroids. Additionally, enzyme and neurophysiological assays have been used to examine the predominant mechanisms by which pyrethroid resistance is manifested by certain populations of the tobacco budworm. Bioassay procedures, in conjunction with classical genetic crossing studies, elucidate some aspects of the inheritance of pyrethroid resistance in this insect.

The topical assay of larvae and the AVT are the basis for all long term monitoring results reported here. Topical assay studies have been conducted for many years and results were available for some tobacco budworm populations even before pyrethroids were commercially available (Luttrell et al., Staetz et al., and Watson and Kelly). AVT results are now available for tobacco budworm populations in many areas for four years. It is the long term use of these assays which has provided valuable information on changes in susceptibility from year to year and within geographic locations. Susceptibility of the tobacco budworm to pyrethroids was relatively stable prior to 1986. Increases in values obtained from topical assays were observed in 1986 and 1987 in various locations. The lowest levels of susceptibility generated with cypermethrin for any Mississippi population occurred in 1986 (Luttrell et al.) and in Texas, Arizona and several Mexico sites, in 1988 (Martinez-Carrillo, Staetz et al., Watson and Kelly).

AVT studies were initiated on a broad scale in 1987. Use of this procedure has continued in many cotton growing areas since that time. This procedure is based on the premise that one of the key mechanisms of resistance to the pyrethroids in the tobacco budworm, kdr (knockdown resistance), is expressed in both the adult and larvae. This procedure would not be an effective monitoring tool if other key mechanisms (metabolic,

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reduced penetration) were predominant, nor has it been shown to be particularly effective in predicting control failures in the field. However, it's relative ease of use compared to topical larval assay is a strong asset. This has allowed more intense monitoring of tobacco budworm populations within a cotton growing season and thus, an opportunity to observe susceptibility changes at more frequent intervals than allowed by the topical assay. In general, AVT results indicate that survival levels to pyrethroids within a given year are lower early and increase during the cotton growing season (Graves et al., Staetz et al., Luttrell et al.). Across all rates tested, the highest levels of survival in Mississippi were reported in the delta area as compared to the eastern hill counties in 1987 and 1988. Survival in the hill counties increased in 1989 (Luttrell et al.). In Louisiana, survival at the 10 ug/vial rate was generally highest in areas of extensive cotton production and lowest in areas with little or no commercial cotton production (Graves et al.). Adult survival was higher in Brazos County, Texas than in any other area tested in Texas, California and Arizona in 1987, 1988 and 1989 (Staetz et al.). Apparently, high adult survival at 10 ug/vial cannot be used as a discriminating dosage because field information does not confirm that survival is related to the lack of control of larval populations with field sprays of pyrethroids.

The foliar spray bioassay has been used in limited degree but results from Mississippi studies using field populations of tobacco budworm showed decreased mortality in 3rd instar larvae as compared to a susceptible strain (Elzen and Luttrell et al.). This technique has been demonstrated to have some utility because it closely represents field conditions.

Each of the methods used in monitoring susceptibility levels of tobacco budworm has particular advantages and disadvantages. All appear to have a place in monitoring for resistance in the tobacco budworm and should be maintained in future monitoring programs. Further examination and refinement of these studies such as that presented by Firko and Hayes and Wolfenbarger et al. will allow research to obtain more precise results with these procedures and ultimately draw better conclusions. Luttrell et al. suggests that monitoring procedures should be developed which provide precision to the level that resistance in 1 in 100 larvae could be detected. Methods other than those currently used would be necessary to attain this goal.

The mechanisms of resistance in strains of the tobacco budworm to pyrethroids are examined in the work by McCafferty et al. Although other mechanisms of resistance such as reduced penetration and behavioral resistance have been reported elsewhere for the tobacco budworm (McCafferty 1989, Sparks), McCafferty et al. focus on the mechanism of nerve insensitivity and increased monooxygenase activity in this report. Nerve insensitivity is thought to be a result of changes at the nerve membrane active site of pyrethroids and is responsible for the type of resistance most commonly called knockdown resistance (kdr). Monooxygenase enzymes are involved in metabolic breakdown of the pyrethroids. In highly resistant, laboratory selected strains of tobacco budworm, McCafferty et al. describe nerve insensitivity and increased

monooxygenase activity as major mechanisms. However, a large variation in expression was reported among the strains and even within a given strain.

For example, neurophysiological assays demonstrated that the nervous system of 13 of 14 susceptible strain (BRC) individuals responded to the lowest cypermethrin concentration tested while in a highly resistant strain (PEG87), 33% responded similar to the susceptible strain and 22% remained unaffected by the highest concentration tested. Others responded to concentrations between these. Another highly resistant strain (DuPont) responded much more uniformly in this assay with 77% failing to respond at all to the highest concentration of cypermethrin. Two Texas field strains (Hearne, Snook) and one Mississippi field strain (Itta) also demonstrated a wide range of response in this assay. Additionally, bioassay results using piperonyl butoxide indicated that the PEG87 and DuPont Strains possessed a resistance mechanism based on enhanced monooxygenase activity that was particularly strong in the PEG87 strain. Results showed this mechanism to be absent in the Hearne, Snook and Itta field strains. McCafferty et al. suggest that there is significance in the fact that highly selected strains such as PEG87 and DuPont possess both mechanisms while the field strains possess only nerve insensitivity. The possibility that continued selection may enhance the monooxygenase mechanisms in strains already possessing the nerve insensitivity mechanism is proposed. The issue of the potential for a shift in the expression of major resistance mechanisms should be explored for the tobacco budworm based on events which have occurred in Australia with *Helicoverpa armegera*. Additionally, it is clear that the mechanisms involved in pyrethroid resistance are not all expressed to the same degree in all populations of the tobacco budworm. Thus, it is essential that efficient neurophysiological and enzyme assays be developed to examine tobacco budworm populations on a routine basis to provide information on those specific resistant mechanisms.

In the work reported by Watson and Kelly, a laboratory selected permethrin resistant strain of tobacco budworm was crossed with a susceptible strain to determine the nature of inheritance of the resistance. It was found that the resistance was autosomal and incompletely dominant. Backcrosses indicated either more than one gene is responsible for the resistance or that the strain was not completely homozygous for resistance. However, this population was shown to be 26100X times more tolerant to permethrin than a susceptible laboratory colony. These studies also indicate the genes responsible for resistance are not sex linked and that it is possible that there is one major loci and a few minor loci which control resistance in this insect. It is clear that many issues remain with regard to the overall understanding of the genetic basis of pyrethroid resistance in the tobacco budworm. Further work on understanding the number of alleles of a gene or genes involved in each of the known mechanisms of pyrethroid resistance in the tobacco budworm is needed. Additionally, work estimating the frequency within a population of the genes responsible for resistance is essential to the efforts of resistance management.

The formanidines have been of interest with regard to pyrethroid resistance since chlordimeform was shown to synergize permethrin against resistant tobacco budworms (Plapp and Campanhola 1986). Laboratory work reported here supports their results in that resistant tobacco budworm larvae treated with the combination of permethrin and chlordimeform resulted in similar mortality to that of susceptible larvae treated with permethrin alone (Watson et al.). The combination also produced high mortality in larvae resulting from crosses of resistant and susceptible tobacco budworm. Mosuysi and Terry report synergism with permethrin plus chlordimeform in a resistant tobacco budworm strain, a slight synergism in a field strain and no synergism in a susceptible strain. Chlordimeform and SN-49844, an amitraz metabolite, showed higher degrees of synergism than amitraz in the resistant tobacco budworm.

Selection of larvae with permethrin alone, permethrin plus SN-49844 and permethrin plus amitraz for four generations resulted in a strong tolerance to permethrin regardless of agents used in the selection. This indicates that these two formanidines may not have the potential to delay resistance as was demonstrated with chlordimeform by Crowder et al. (1984). Additionally, Leonard et al. found SN-49844 and amitraz to be less active on eggs of susceptible, pyrethroid resistant and field strains of tobacco budworm. Sparks et al. demonstrated that chlordimeform was more consistent in enhancing tobacco budworm larval movement and permethrin uptake than amitraz or SN-49844. Results with binary mixtures of pyrethroids and acephate against eggs and larvae of a resistant strain did not result in synergism to any degree. Using third instar larvae, mortality was additive with this mixture (Hoskins et al.). The use of mixtures in contrast to using sequential treatments of two or more insecticide classes remains a key unsolved issue in current resistance management theory. Future work is essential to provide a better understanding of when and where these strategies are most effective in the prevention or delay of insecticide resistance.

LITERATURE CITED

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