Esterase Inhibitors Restore Pyrethroid Susceptibility in Australian *Helicoverpa armigera*

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ABSTRACT

Pyrethroid resistance in Australian Helicoverpa armigera field populations is primarily a consequence of the over production of esterase isoenzymes that metabolize and possibly sequester pyrethroid insecticides. Biochemical studies show that organophosphorous compounds inhibit pyrethroid resistance associated esterases in H. armigera. Esterase inhibition by organophosphates does not occur immediately after dosage, but occurs rapidly, with maximum enzyme inhibition from 2 to 24h after dosage, depending on the inhibitor used. These enzyme inhibition studies are supported by pyrethroid bioassays using organophosphate synergists, showing excellent levels of pyrethroid synergism against resistant H. armigera. One hundred percent mortality could be achieved when H. armigera were dosed with pyrethroid, during the time of maximum organophosphate induced enzyme inhibition. Use of organophosphate synergists in the field may have the potential to restore some pyrethroid susceptibility in Australian H. armigera.

Introduction

The cotton bollworm *Helicoverpa armigera* (Hübner), is a serious pest of cotton and other summer crops in Australia, where it has a long history of insecticide resistance. Pyrethroid resistance in field populations of Australian *H. armigera* is largely due to overproduction of specific esterase isoenzymes (R_m 0.24 - 0.33), which are thought to sequester and hydrolyze pyrethroids (Gunning *et al.*, 1996). However, the degree of resistance to all pyrethroids is not identical and some compounds are more resisted than others.

Previous studies with esterase inhibitors, such as profenofos, have shown little pyrethroid synergism against resistant *H. armigera* (Forester *et al.*, 1993; Gunning *et al.*, 1991). However, these studies used only a very short organophosphate pre-treatment period and utilized only one highly resisted pyrethroid (fenvalerate) and it is possible that synergism may have been more effective with longer pre-treatment times and with other pyrethroids. Indeed, *in vivo* inhibition of esterase enzymes by organophosphates, such as profenofos in *Bemisia tabaci*, has been shown to take up to 24 hours (Byrne and Devonshire, 1991). Thus, it seemed logical to consider the possibility that esterase inhibition (and pyrethoid synergism) in resistant *H. armigera*, might also be enhanced by a longer pre-treatment period.

Materials and Methods

Insects and Bioassay

Pyrethroid resistant *H. armigera* obtained from field collections, was used in these studies. The *H. armigera* were susceptible to organophosphates. Standard 3rd instar larval bioassays (Gunning *et al.*, 1984) were used to determine pyrethroid resistance status. Pyrethroid synergism studies were carried out using a non-toxic dose of organophosphate and a standard single discriminating dose pyrethroid. Pyrethroids used were fenvalerate (which is an example of a highly resisted pyrethroid) and two isomers of cypermethrin that are much less resisted (alpha and zeta cypermethrin). Resistant *H. armigera* larvae (30 mg) were pre-treated with non-toxic doses of ethion, acephate, profenofos, chlorpyrifos and chlorpyrifos-oxon. Larvae were dosed with pyrethroid 0 - 72 h later. Mortality was assessed 48 h after pyrethroid application.

Electrophoresis and total esterase assays

Small larvae, which are the most suitable weight range for electrophoretic and biochemical analysis of esterase isoenzymes in *H. armigera* (Gunning *et al.*, 1996) were used in these experiments. Larvae (2 - 3 mg), were topically applied with 0.5 µl of acetone alone, or acetone containing non-toxic doses of ethion, profenofos, chlorpyrifos, chlorpyrifos-oxon or acephate. The larvae were provided with food and left at 25 ± 1^{o}C. At time intervals from 0.25 - 72 h. after dosage, samples of larvae (3 - 4 mg), were placed in the deep freeze and stored in at -15^{o}C until the conclusion of the experiments. Polyacrylamide gel electrophoresis preparation methods were similar to those used by Devonshire and Moores (1982). Total esterase activity of the larval homogenates was detected using 1-naphthyl acetate as a substrate, using kinetic assays, as previously described (Gunning *et al.*, 1996).

Results

Inhibition of esterase activity by organophosphates

Gel electrophoresis and assays of total esterase activity indicated that all organophosphates used in these experiments bound to *H. armigera* esterases, and in particular, those bands implicated in pyrethroid resistance (Gunning *et al.*, 1996).

Acephate rapidly bound to *H. armigera* esterases, (0.5 - 1h. after treatment), however this effect was short lived and esterase activity in larvae, was completely recovered 8h after treatment. Chlorpyrifos and its oxon also rapidly inhibited *H. armigera* esterases, however, recovery of activity was slow, with full enzyme activity not recovered until 36 h after treatment. Inhibition of esterase activity by profenofos occurred primarily between 4 and 8 h after treatment (at 8 h, some 70% of esterase activity had been inhibited). However, recovery of enzyme activity was rapid. By contrast, ethion caused almost complete inhibition of all esterase activity, between 1 to 72 h. after application.

Organophosphate /Pyrethroid Synergism

All the organophosphates used synergised pyrethroids during the time of maximum enzyme inhibition, however, the greatest effects were obtained with the less resisted pyrethroids. Pre-treatment with ethion, greatly increased pyrethroid mortality and the increased period of susceptibility persisted up to 24 hours after pre-treatment with the synergist. The synergistic effect declined over longer time intervals. Acephate also showed a strong, but short lived, synergistic effect with pyrethroids. These effects rapidly declined with time intervals greater than 3h between acephate and pyrethroid applications. Profenofos was also synergistic with pyrethroids. However, these effects were not rapid, a 4h time interval between application of profenofos and

pyrethroid was required to reach maximum mortality and the effects rapidly declined thereafter. Chlorpyrifos was also shown to be a reasonably effective pyrethroid synergist. Maximum mortality was achieved after a 4 h, pre-treatment time but this level of mortality rapidly decreased.

Discussion

These experiments showed that organophosphates most effectively inhibited esterases, which have been linked to pyrethroid resistance in Australian *H. armigera* (Gunning *et al.*, 1996). However, this did not happen immediately after application and the timing of and recovery from inhibition varied according to organophosphate. While the inhibitory effects of most organophosphates on *H. armigera* esterases were generally not long lived, the effects of ethion lasted at least 72 h.

These data, showing organophosphate inhibition of resistant *H. armigera* esterases, are supported by bioassays, in which organophosphates proved to be effective pyrethroid synergists, especially with more effective, (less resisted), pyrethroids. For each organophosphate inhibitor, pyrethroid synergism occurred at pre-treatment times that corresponded to maximum *vivo* esterase inhibition in the larvae used for biochemical studies. Ethion (which is non-toxic and therefore could be applied in larger doses) was a more effective synergist than any other and was capable of producing 100% mortality. Had it been possible to increase the synergistic doses of acephate, chlorpyrifos, and profenofos, (which can be toxic to *H. armigera*), better synergism would have been expected.

These data open up the possibility that organophosphate synergists could be used in Australia to restore some pyrethroid susceptibility in *H. armigera*. Obviously, it would be best to treat with organophosphates prior to pyrethroid spray but this approach would require two insecticide applications instead of one and would probably be rejected on economic grounds. However, the better, less resisted pyrethroids combined with organophosphate synergists, could still produce high mortality when applied as a mix with no pre-treatment time. The pyrethroids that were most easily synergized were those less resisted. The potential use of organophosphates as pyrethroid synergists would need to be carefully incorporated into a resistance management strategy because uncontrolled use of synergists designed to eliminate a major, metabolic pyrethroid resistance mechanism, may well result in the selection of other resistance mechanisms, such as Kdr.

The present results support previous findings (Gunning *et al.*, 1996) that pyrethroid resistance in field populations of Australian *H. armigera* is largely a consequence of enhanced esterase activity.

Evidence that organophosphate esterase inhibitors make excellent pyrethroid synergists has provided additional evidence that pyrethroid resistance in *H. armigera* is due to esterase detoxification and the increased production of these enzymes. These findings may have considerable practical application for *H. armigera* resistance management in Australia and may greatly assist in restoring some measure of pyrethroid susceptibility in field populations of *H. armigera*.

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