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INSECTICIDE RESISTANCE IN *HELICOVERPA ARMIGERA* IN INDIA: RECENT DEVELOPMENTS

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ABSTRACT

Seasonal changes in insecticide resistance in the cotton bollworm, *Helicoverpa armigera* (Hübner) were monitored at six widely spaced locations in India during 1993/94 using a discriminating dose bioassay technique. Insecticide / synergist combinations were used to elucidate resistance mechanisms. The status of resistance to pyrethroids, endosulfan and organophosphates is described and correlated with local farmer insecticide use strategies in the different regions and the role of migration in the spread of resistance beyond high input farming areas.

INTRODUCTION

Severe pyrethroid resistance in the cotton bollworm, *Helicoverpa armigera* (Hübner) was first recorded from cotton in eastern Andhra Pradesh, India in 1987 (Dhingra *et al.*, 1988; McCaffery *et al.*, 1989). Associated with high population pressure, field control failures and a marked decline in lint yields (from 430 to 170 kg/ha) resulted. More recently, bioassay of field collected material using discriminating doses of synthetic pyrethroids (SPs), organophosphates (OPs) and endosulfan, and various synergist combinations at monitoring centres established at six locations in India has enabled its spread, and local changes in resistance intensity and their mechanisms to be followed closely for the first time (Armes *et al.*, in press). Correlation of resistance with records of local insecticide use patterns has generated much useful information relevant to the development of management strategies, some of which are currently being tested.

MATERIALS AND METHODS

Insecticide resistance monitoring laboratories have been established in six widely spaced pulse and cotton growing locations in India, *viz.*: Andhra Pradesh Agricultural University (APAU), Guntur; Central Institute for Cotton Research (CICR), laboratories at Coimbatore, Nagpur and Sirsa; International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad; Tamil Nadu Agricultural University (TNAU) campuses at Coimbatore and Madurai, (Fig. 1).

H. armigera eggs were collected from host plants in farmers' fields over as much of the 1993/94 cropping season as possible at the six locations. Typically the range of major host plants sampled over the season comprised: wild host plants, tomato, sorghum, cotton, sunflower, pigeonpea, chickpea and groundnut. Several thousand eggs were collected weekly from each region and transferred individually to 7.5ml cells of 12-well tissue culture plates containing a chickpea artificial diet. Once the larvae reached the 30-40 mg weight range they were randomly assigned to 6 discriminating dose screens *viz.*: cypermethrin 0.1 µg/µl, fenvalerate 0.2 µg/µl, endosulfan 10.0 µg/µl, quinalphos 0.75 µg/µl (approximations to LD_{99} values for homozygous insecticide susceptible *H. armigera* strains); cypermethrin 1.0 µg/µl (introduced as a "twin" discriminating dose because of the high survival at the 0.1 µg/µl dose), cypermethrin 0.1 µg + piperonyl butoxide (Pbo) 50 µg/µl (to determine the extent of Pbo suppressible pyrethroid resistance) and at the Central region only, cypermethrin 0.1 µg + profenofos 0.1 µg/µl (to determine the extent of profenofos suppressible

pyrethroid resistance). For each location, each week at least 100 (ideally 120-250) larvae were treated topically with 1.0 µl of each discriminating dose. Rearing and insecticide assays were conducted in rooms maintained at a constant temperature of 25 ± 2 °C, under natural photoperiod (approx. 12:12 h light:dark).

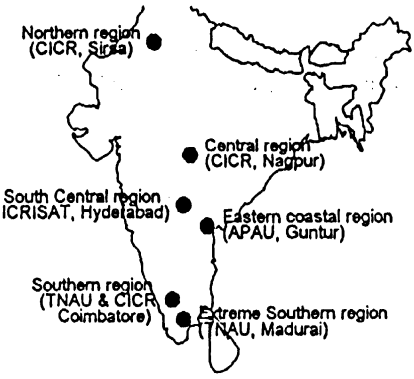

FIGURE 1. Location of *H. armigera* insecticide resistance monitoring laboratories in India.

RESULTS

In view of the large amount of data, it is not possible to present all the results in this paper. However, seasonal SP, endosulfan and OP insecticide resistance data for indicator regions: Haryana, Maharashtra, coastal Andhra Pradesh and Tamil Nadu are presented graphically in Figures 2-4. Data for the South Central region are presented elsewhere (Armes *et al.*, in press), but summarised briefly along with data for the remaining regions in Table 1.

TABLE 1. 1993/94 seasonal average *H. armigera* insecticide resistance levels at six monitoring locations in India (expressed as % survival at each discriminating dose).

Discriminating dose treatment	Northern region	Central region	S. Central Region	E. coastal region	Southern region	Extreme S. region
Cypermethrin 0.1 µg	77.1	78.2	83.2	95.6	85.2	76.3
Cyper 0.1+Pbo 50 µg	31.8	66.4	55.4	92.7	52.4	49.4
Suppression of resistance by Pbo	59.5	14.0	33.9	2.7	39.1	31.5
Cypermethrin 1.0 µg	23.9	23.9	44.5	70.8	52.0	-
Fenvalerate 0.2 µg	68.0	72.1	75.2	91.6	79.4	53.7
Endosulfan 10.0 µg	35.5	45.5	29.5	74.1	35.8	40.9
Quinalphos 0.75 µg	15.0	24.0	25.7	77.0	25.5	7.2

Pyrethroid resistance

Resistance both to cypermethrin and fenvalerate was high in all monitoring regions, with the situation being most severe (>80% survival at cypermethrin 0.1 µg) in Maharashtra, Andhra Pradesh and Tamil Nadu comprising the Central, South Central, Eastern coastal and Southern regions respectively (Figs 2 & 3).

In the Northern region the monitoring period was quite short (2.5 months), but there was a clear trend of increasing resistance with progression of the cotton growing season. SPs are still the pesticides of choice of farmers in this region for bollworm control, but in the latter half of the season these were proving to be ineffective and many farmers resorted to SP / OP mixtures. This region recorded the highest Pbo suppression of cypermethrin resistance at a seasonal average of 60% suppression.

In the Central region, SP resistance appeared to be bi-modal. Overall, both cypermethrin and fenvalerate resistance were high (averaging 78 and 72% survival respectively), with the lowest resistance levels (47 and 43% respectively) occurring mid season in October - November. For cypermethrin, the decrease in resistance correlated with a concomitant reduction in Pbo suppression which averaged 38% up to late October but only 4% for the remainder of the season. Similarly there was a marked change in profenofos suppressible SP resistance during the same period. Prior to the first week of October, profenofos suppression averaged only 6% and from November - mid March, 48%. The last collections in late March indicated significantly decreased suppression at 18%, most probably indicating a more important role for target site nerve insensitivity (*kdr*) resistance at this time.

In the Eastern coastal region the monitoring period was quite short this season for logistical reasons, but the data clearly shows that the Guntur cotton belt is still facing the most severe problem with >90% average seasonal survival at the SP discriminating doses. Even treatment at the 10x higher cypermethrin 1.0 µg dose gave an average of >70% survival. Pbo suppression was not significant at an average of only 2.7%.

The bimodal SP resistance trend observed in the Central region was also apparent in the Southern region at Coimbatore. However, here Pbo suppression remained fairly stable throughout the season, averaging 39% (range: 21-57%).

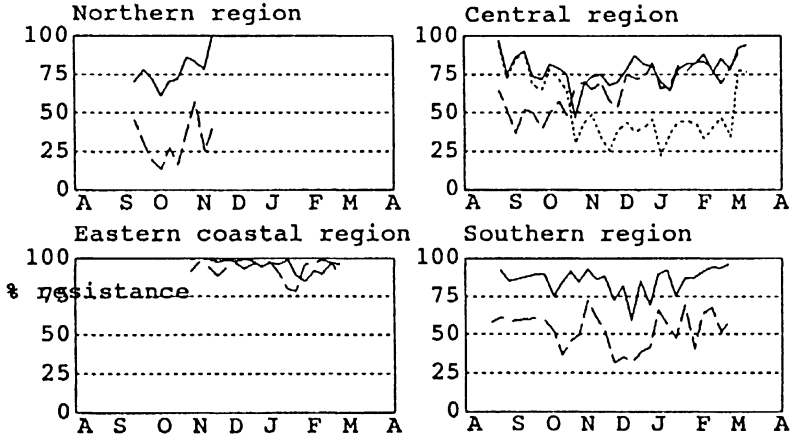

FIGURE 2. Average weekly resistance to cypermethrin (solid line) and effect of Pbo (broken line) and profenofos (dotted line), on suppression of cypermethrin resistance in *H. armigera* at four monitoring locations during the 1993/94 cropping season (based on % of 30-40 mg larvae surviving the cypermethrin 0.1 µg, cypermethrin 0.1 µg + Pbo 50 µg and cypermethrin 0.1 µg + profenofos 0.1 µg discriminating doses).

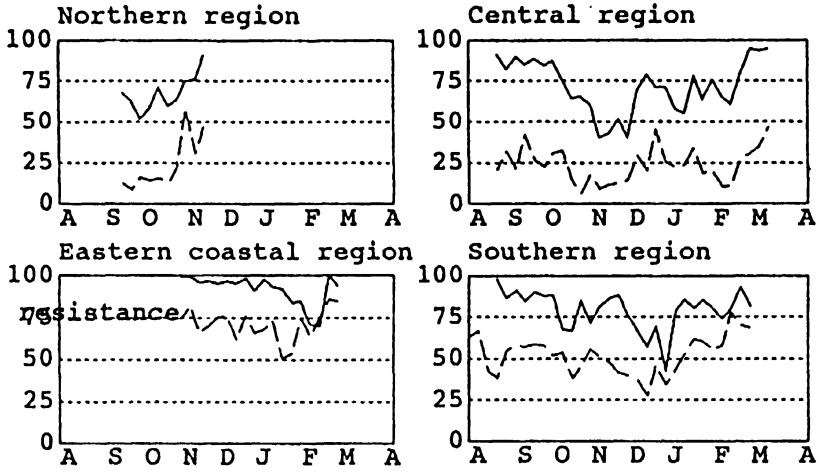

FIGURE 3. Average weekly resistance in *H. armigera* at four monitoring locations during the 1993/94 cropping season to the fenvalerate (solid line) and 10x LD_{99} cypermethrin (broken line) doses (based on % of 30-40 mg larvae surviving the fenvalerate 0.2 µg and cypermethrin 1.0 µg discriminating doses).

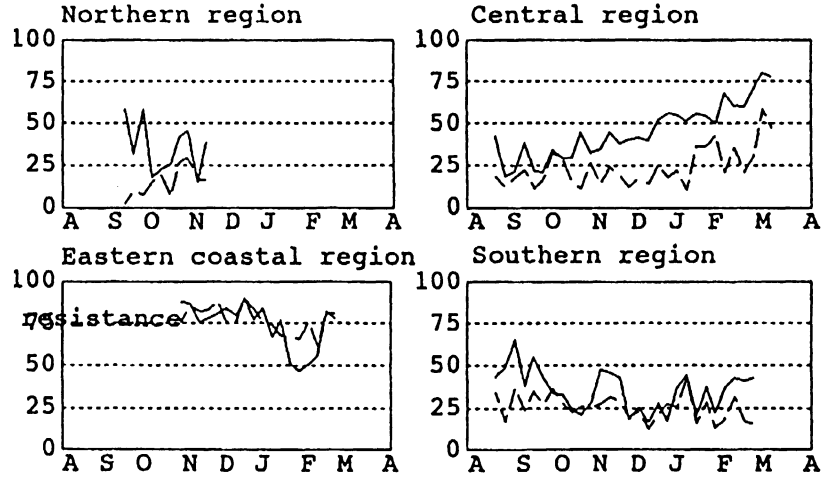

FIGURE 4. Average weekly resistance to cyclodienes (solid line) and organophosphates (broken line) in *H. armigera* at four monitoring locations during the 1993/94 cropping season (based on % of 30-40 mg larvae surviving the endosulfan 10 µg and quinalphos 0.75 µg discriminating doses).

Endosulfan resistance

Resistance to endosulfan averaged 36% in the Northern region and a seasonal decline was indicated. This probably arose because it was only widely used during the early season in Haryana, farmers preferring to use SP / OP mixtures later in the season as these were considered to be more

effective against *H. armigera*. In the Central region there was a clear trend of increasing resistance over the season (from 19% in September to 80% in March). In the Eastern coastal region, resistance was very high with a mean of over 74%. Resistance decreased from November to January and then increased again in February-March. In the Southern region, endosulfan resistance was highest early season (mean 49%), decreasing by early November to a fairly stable level (mean 32%) for the remainder of the season.

Organophosphate resistance

With the exception of the Eastern coastal region, moderate to low levels of resistance to quinalphos were recorded. Interestingly, in the Northern region, *H. armigera* was almost fully susceptible to quinalphos in September (<3% survival at quinalphos 0.75 µg), but had reached 30% resistance by mid November. Similarly in the extreme Southern region of Madurai, quinalphos resistance averaged only 7% and in several weeks 0-5% survival was observed. In the Central region, OP resistance remained fairly stable from September to January (mean 19%), but then increased markedly from then on to March (mean 37%). At Coimbatore in the Southern region, resistance remained fairly stable throughout the season (mean 26%, range: 13-36%).

DISCUSSION

The results clearly indicate that insecticide resistance in *H. armigera* is ubiquitous in India and can no longer be considered as a problem confined to the Central and Eastern coastal regions. Gene flow resulting from migration of moths from high input farming regions to areas where insecticide selection pressure is much less, is most likely to be responsible for the apparent homogeneity of resistance to SPs, cyclodienes and OPs over large areas of India (Armes *et al.*, 1992), and as reported by Daly and Gregg (1985) in Australia. Temporal differences in changes in resistance frequencies between regions are a reflection of regional variations in insecticide use and their effects on selection of resistant larval phenotypes over one or more locally breeding generations. To illustrate these points, Pbo and profenofos suppression of pyrethroid resistance provide good examples.

In the Eastern coastal region, where high insecticide inputs on cotton in particular are common (15-30 applications), Pbo synergism was not significant. The overuse of insecticides and SPs in particular appears to have resulted in strong genetic selection for nerve insensitivity (*kdr*) (West & McCaffery, 1993) as a significant mechanism, a situation which arose in Australia prior to implementation of a resistance management strategy (Gunning *et al.*, 1991). The Northern region (also an intensive cotton growing region), contrasts with the Eastern coastal situation in that insecticide inputs are in most seasons significantly lower in the north (6-12 applications), and the consequence of this appears to be high SP resistance but with a more significant role for mfo metabolism and probably (although untested) a lesser role for *kdr*.

In the Southern region, where insecticide use is highly variable (depending largely upon farmers' economic status), resistance is intermediate with moderate Pbo suppression and the likelihood of low frequencies of *kdr* resistance (West & McCaffery, 1993). It is quite feasible that 'injection' of highly resistant phenotypes by migration from the Eastern coastal region to the Central and Southern regions occurs during October-December at a time when the emergence of *H. armigera* moth populations is highest from cotton crops (Pedgley *et al.*, 1987; Armes *et al.*, 1992).

Profenofos synergism was tested only in the Central region. A 'switch' in metabolic mechanisms from significant mfo metabolism to esterase metabolism within one week implies a very rapid change in resistance genotypes. The bioassays were supported by quantitative estimates of cytochrome P450, carboxyl esterase and glutathione transferase activity indicating similar changes (Kranthi, *unpbl.*). It is difficult to account for such rapid change in terms of changes in local selection as there was no obvious shift in farmers' control tactics toward different insecticide chemistries at this time. A plausible explanation would be an influx of moths from populations subject to different

selection pressures contributing significantly to the gene pool (e.g. Daly, 1993), but we currently have no field evidence to confirm this. Levels of OP resistance were highly variable but significant across regions, probably largely as a result of different ways farmers were using these chemicals in cotton pest management. For example, in the North, endosulfan, SPs and monocrotophos (and their combinations) were the favoured chemicals for early season pest control and quinalphos resistance was low at this time. As control with these proved ineffective, there was a switch to quinalphos and chlorpyrifos in combination with SPs in the latter half of the season and this correlates with increasing quinalphos resistance at this time. Similarly, in the Central region, quinalphos resistance remained fairly stable up to January when OPs and endosulfan were the predominant choice for *H. armigera* control on chickpea by farmers.

For endosulfan, mean resistance was high across all regions and we frequently observed decreasing resistance over the early part of the cropping season. This probably arose because endosulfan is a popular early season insecticide (frequently popularised by agriculture departments as a good early season insecticide as it is considered relatively benign to natural enemies), being largely substituted later on by SPs and OPs.

Clearly the mechanisms involved and dynamics of insecticide resistance in *H. armigera* in India are complex, and for SPs at least, differs markedly from the routinely monitored situation in E. Australia where the implementation of a management strategy has moderated resistance toward a single locus mfo gene (Daly, 1993). Variable levels of selection pressure through unrestricted and inappropriate use of insecticides has resulted in selection of multiple resistance mechanisms and intractable resistance-related control problems over large areas of India. The use of agrochemicals in cotton pest management needs to be addressed urgently if *H. armigera* resistance is to be managed in the subcontinent.

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