# MONITORING PYRETHROID RESISTANCE IN THE POD BORER, *HELICOVERPA ARMIGERA* IN ANDHRA PRADESH, INDIA

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## ABSTRACT

**Data for dose/response monitoring of pyrethroid resistance in *Helicoverpa armigera* (Hübner) at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad and the coastal cotton growing region of Andhra Pradesh during the period 1986-92 are summarized and discussed. The use of a discriminating dose technique for monitoring pyrethroid resistance in India was evaluated using a provisional dose of 1 g cypermethrin applied topically to 3rd - 4th instar larvae in the weight range 30-50 mg. At ICRISAT Center, data for the 1991-92 cropping season showed that pyrethroid resistance was high at the beginning of the rainy season in June, but declined markedly in August probably as a result of dilution of residual resistant populations by populations emerging from unsprayed wild hosts and early season unsprayed crops. Resistance levels rose rapidly from September onwards, probably as a result of increased selection for resistance through intensive spraying of insecticides on cotton and pulse crops in the region, and resistance remained high throughout the remainder of the cropping season. Prospects for insecticide resistance management of *H. armigera* in Andhra Pradesh are briefly discussed.**

## INTRODUCTION

Historically, the spotted bollworm, *Earias vittella* (F.) and spiny bollworm, *Earias insulana* (Boisd.) are the most important pests on cotton in southern India (Agarwal and Gupta, 1983). However, in the late 1970s they became secondary to the tobacco caterpillar, *Spodoptera litura* (F.) which by the early 1980s had become resistant to carbamate, organochlorine and organophosphate insecticides in Andhra Pradesh (Ramakrishnan *et al.*, 1984). Partly as a result of poor control of *S. litura* with conventional insecticides, synthetic pyrethroids were introduced for use on cotton during the 1982-83 cropping season. It is not known whether or not the introduction of pyrethroids was the cause but, *S. litura* and *Earias* spp., virtually disappeared from cotton and were quickly replaced by the whitefly, *Bemisia tabaci* (Genn.) and gram podborer, *Helicoverpa armigera* (Hübner) as the dominant pests (Anonymous, 1989a; Reed and Pawar, 1982). Severe outbreaks of *B. tabaci*, attributed to the excessive use of pyrethroids, were recorded during the 1984-85 and 1985-86 cotton seasons (Gour 1986), but its importance has declined in recent years. *H. armigera* is now the dominant pest on cotton and pulses in most regions of India. The development of pyrethroid resistance in *H. armigera* was probably inevitable in view of the heavy dependence on insecticides for pest control on cotton in Andhra Pradesh, which accounts for nearly 40% of pesticide sales in the country (Anonymous 1990), with synthetic pyrethroids comprising 50-70% of insecticide applications on cotton (Jayaswal 1989). Resistance to synthetic pyrethroids was first detected in *H. armigera* in the postrainy season of 1987 (Dhingra *et al.*, 1988; McCaffery *et al.*, 1988, 1989). Widespread field control failures were reported from September to December over large areas of the coastal Andhra Pradesh cotton growing belt, comprising Krishna, Guntur and Prakasam Districts. As a result, average yields of cotton lint for the region declined from 436 kg/ha in 1986-87 (Anonymous, 1989b) to 168 kg ha in 1987-88 (Anonymous, 1989c).

The development and implementation of Integrated Pest Management (IPM) and Insecticide Resistance Management (IRM) strategies to reduce the selection pressure for insecticide resistance in *H. armigera*, and to effectively control this pest, is probably the greatest challenge for entomologists, plant protection specialists, agronomists and extension workers in India in the 1990s.

The Natural Resources Institute (NRI), UK, in collaboration with ICRISAT Center, has been monitoring resistance in field populations of *H. armigera* since 1986. This paper describes the techniques used, summarizes and compares the data obtained from the coastal cotton area (Guntur and Krishna Districts) and ICRISAT (Medak District), and discusses the future prospects for resistance management of *H. armigera* in India.

## MATERIALS AND METHODS

### Dose/Response Monitoring

Second to sixth instar *H. armigera* larvae were collected from fields of sorghum, pigeonpea and chickpea at ICRISAT, and from cotton, pigeonpea and chickpea in Guntur and Krishna districts. Samples were collected by sampling the whole field in order to ensure that the progeny from as large a genetic mix of moths as possible were collected. Samples from different fields were kept separate in order to correlate insecticide use with larval susceptibility to pyrethroids, as insecticide spray regimes varied markedly from field to field and crop to crop. Larvae were reared on a chickpea ('*kabuli*' type) flour based artificial diet (N.J. Armes, unpublished) at 26 ± 1°C, under natural photoperiod (approx., 13L:11D) at ICRISAT and constant photoperiod (14L:10D) in the UK. Insecticide bioassays were conducted on the resulting first generation of larvae.

Fifty percent serial dilutions of technical grade *cis / trans* (approx., 1:1 ratio) cypermethrin (ICI Agrochemicals, U.K.), in analytical grade acetone were prepared and a 1-l drop applied to the thoracic dorsum of each 3rd - 4th instar larva in the weight range 30-40 mg during 1986-88 and 30-50 mg during 1989-92. The 30-40 mg range recommended by Anonymous (1970) was followed by McCaffery *et al.*, (1988, 1989), however subsequent bioassays at NRI on a pyrethroid susceptible strain using the 30-50 mg range produced similar bioassay statistics to the Reading University susceptible strain using the 30 - 40 mg range (Table 1). We felt therefore that any slight loss in precision was more than compensated for by the larger number of larvae that could be obtained within a 20 mg range, particularly where staff resources and facilities were constrained. With few exceptions, at least 40 larvae were treated at each of five or more concentrations plus control (acetone alone). Larvae were held individually in 30 ml plastic cups with fresh artificial diet at 26 ± 1°C. Mortality was assessed 72 h after treatment. A larva was considered dead if it was unable to move in a co-ordinated manner when prodded and no significant growth had occurred since the time of treatment. Log dose probit (ldp) statistics were computed by probit analysis (Finney, 1971), using Maximum Likelihood Program (version 3.08) software (Ross, 1987).

### Discriminating Dose Monitoring

Two laboratory strains were used to calibrate a cypermethrin discriminating dose for 30-50 mg *H. armigera* larvae. One, the NRI strain was considered pyrethroid susceptible and was originally collected from Kasala Province in the Sudan where few insecticides are used. The second, Delhi strain was obtained from the Indian Agricultural Research Institute (IARI), New Delhi. This strain was re-established annually from larvae collected from unsprayed pigeonpea at the IARI farm, in an area



where pyrethroid resistance had not been reported at the time of testing (K.N. Mehrotra, *perso communication*).

Both strains were bioassayed in 1989 and 1990 and ldp statistics determined following methodology for dose/response monitoring.

Discriminating dose test methodology was evaluated at ICRISAT Center. Samples eggs and first - second instar larvae (samples generally comprised 150-400 eggs and/or larvae) w collected from infested host plants every 1 - 2 weeks during the 1991-92 cropping season. Monitor commenced at the start of the rainy season in June with the appearance of the first *H. armig* generation on weeds, up-to mid April when a closed season is implemented on the farm and further crops are grown during the summer period (March - June). The plants sampled over season were *Lagascea mollis* Cav. (wild host plant), mung bean (*Vigna radiata* L.), sorgh pigeonpea and chickpea. Field collected eggs/larvae were reared to the 30-50 mg stage on chick flour based artificial diet. When larvae reached the 30-50 mg weight range, they were treated topical application with the cypermethrin discriminating dose (calibrated at 1-g/larva). Cont larvae were treated with acetone alone. Larvae were held at 26 ± 1°C and mortality assessed 7 after treatment.

## RESULTS AND DISCUSSION

### Dose/Response Monitoring

Average monthly $LD_{50}$ values determined for strains collected from ICRISAT Center and the And Pradesh coastal cotton growing belt from July 1986 to March 1992 are given in Figure 1. Pyreth resistance varied substantially between and within years at both locations. In July 1986 there was evidence for tolerance to cypermethrin at ICRISAT Center. By October 1987, failures to control *armigera* were reported from the cotton belt, and control problems were experienced at ICRIS Center. These are reflected in the high $LD_{50}$ values recorded for strains collected during Octob November from cotton in Krishna district and pigeonpea at ICRISAT Center (McCaffery *et* 1989) (Fig. 1). In general, resistance levels over the five cropping seasons from 1987-92 incre as each season progressed. This is particularly evident for the 1991-92 season for which most are available. In the cotton belt in particular, these increases can most likely be attributed to l selection for resistant genotypes resulting from application of pyrethroids to cotton and pigeon crops. At ICRISAT Center however the situation is not so clear as pyrethroids were not extensively on the farm (pyrethroids constituted < 2% of insecticide applications on field c during the growth stages susceptible to *H. armigera* attack during the 1991-92 season). It is likely therefore that seasonal increases in pyrethroid resistance at ICRISAT Center largely r from immigration of resistant moths into the locality from sprayed areas of cotton and pigeonpe this respect, it is interesting to note that pyrethroid resistance levels recorded at ICRISAT Ce generally mirrored those recorded in the cotton belt in each season. This provides further suppo the theory that long-distance dispersal of resistant moths from the cotton belt to the Hyderabad may take place on the prevailing NE to E winds between October and December (Pedgley *et* 1987; McCaffery *et al.*, 1989). Migration may explain the sudden appearance of pyrethroid resis populations at ICRISAT Center in 1987, as synthetic pyrethroids were used infrequently on ICRISAT farm at that time. From the information we have to date, it appears that geographic temporal changes in pyrethroid resistance in *H. armigera* in Andhra Pradesh arise from dyn interactions between local selection pressure and immigration of resistant and susceptible *H. armi* populations at certain times of the year.

It should be noted that high pyrethroid resistance levels recorded in laboratory bioassays are not necessarily indicative of field control failures. During the 1989-90 season for example, 2100-fold and 830-fold resistance to cypermethrin was recorded in the cotton belt and at ICRISAT Center, respectively (Armes *et al.*, 1992), but in the field, these populations went largely unnoticed as the pest pressure was low, and despite poor control damage was tolerable.

**Discriminating Dose Monitoring**

The use of ldp statistics for resistance monitoring is considered inefficient compared to discriminating dose tests, particularly for heterogeneous insect populations. Ideally, monitoring should be able to detect resistant individuals at a phenotypic frequency of close to 1%, and in this respect, dose/response bioassays are not capable of detecting such low frequencies in a population (Roush and Miller, 1986). In addition, dose/response bioassays require large numbers of insects and to achieve this, it is generally necessary to rear cultures in the laboratory and test the $F_1$ progeny for insecticide resistance. This results in a substantial time-lag between field sampling and determining resistance levels, which is not acceptable in a reactive IRM strategy.

We are working towards calibrating discriminating doses for the synthetic pyrethroids commonly used for the control of *H. armigera* in India. We have not however been able to isolate a homogeneous pyrethroid susceptible field strain in India. To date, the Delhi strain is the most susceptible *H. armigera* field strain recorded, but in both years of testing, the Delhi strain exhibited significantly greater tolerance to cypermethrin than the NRI strain (Resistance Factor at $LD_{50}$ was 21-fold in 1989 and 13-fold in 1990). In both years, the ldp line slopes recorded for the NRI strain (2.0 and 2.5) were higher than the Delhi strain (1.7 and 1.9) (Table 1 and Fig. 2). The Delhi strain was therefore not fully susceptible, nor homogeneous with respect to pyrethroid resistance and probably included a significant proportion of resistant genotypes at the time of testing. The presence of pyrethroid tolerance in the Delhi area where pyrethroids were not used to any great extent (K.N. Mehrotra *personal communication*) suggests that either gene flow throughout the Indian subcontinent had been sufficiently widespread to have contaminated north Indian populations with resistant genotypes from the south, or pyrethroid resistance had developed independently over large areas, particularly in the cotton regions of the Punjab, Haryana and Rajasthan.

Low ldp line slopes for all Indian field strains of *H. armigera* tested has made the determination of a discriminating dose for cypermethrin, with narrow error estimates, problematic. Gunning *et al.* (1984) and N.W. Forrester (*personal communication*) obtained high slopes (average 3.0) for Australian susceptible strains bioassayed with fenvalerate, and were able to confidently determine a discriminating dose for *H. armigera* (99.1 ± 0.3% kill for 30-40 mg larvae with 0.2-g fenvalerate). However, they found that susceptible strains bioassayed with deltamethrin gave a more variable response (not dissimilar to our results for cypermethrin) and much lower slopes (average 1.7). Because of this, they did not attempt to calibrate a discriminating dose for deltamethrin despite it being the most popular pyrethroid in commercial use at the time (Forrester, *personal communication*). McCaffery *et al.* (1989) obtained low slopes of 1.3 for cypermethrin and 1.8 for fenvalerate with the Reading susceptible strain which had been maintained in their laboratory for several years. However, Gunning *et al.* (1984) working with *H. armigera* and Leonard *et al.* (1988) with *Heliothis virescens* (Fab.) and *Helicoverpa zea* (Boddie), obtained higher slopes (2.3-4.4) with cypermethrin topically applied to third-instar larvae.



Under our test conditions, the NRI strain consistently recorded higher slopes (2.0-2.5) when bioassayed with cypermethrin. However, calibrating a discriminating dose on the basis of this strain derived from Africa could overestimate pyrethroid resistance in Indian populations of *H. armigera*, as there can sometimes be substantial differences in tolerance between insect strains unexposed to insecticides (Sawicki, 1987). This may be particularly true for strains from different continents.

In the absence of a more susceptible Indian strain we opted to use the Delhi strain as the baseline in view of the fact that pyrethroids were not used locally to any great extent, and where used were considered to give effective field control of *H. armigera* larvae (K.N. Mehrotra, *personal communication*). We therefore set a provisional discriminating dose for *cis / trans* cypermethrin topically applied to 30-50 mg larvae at 1.0 g/larva (Fig. 2). On the basis of the 1990 results, this would kill 95% of the Delhi strain. In view of the low slope for the Delhi strain, there is significant overlap in the ldp lines with resistant field populations collected in southern India (Armes *et al.*, 1992). It is not therefore possible to accurately determine the frequency of resistance (resistant vs. susceptible) on the basis of a discriminating dose calibrated for the Delhi strain, but it does however provide a relative measure of changes in resistance.

Results obtained so far would appear to vindicate 1 g cypermethrin as a suitable monitoring dose, because on the basis of present resistance levels recorded at ICRISAT Center, this gives ample scope for future changes in percentage of larvae surviving the discriminating dose (Fig. 3). If in the future, pyrethroid resistance in *H. armigera* is brought under control through the adoption of IRM in cotton and pulse crops, then it may at that point be appropriate to use a lower monitoring dose based on the $LD_{99}$ of true pyrethroid susceptible *H. armigera* (Fig. 2). However, while resistance is 'free-running' and no controls are being exerted on pesticide use on field crops then the 1 g cypermethrin dose appears to be appropriate.

At ICRISAT Center, resistance in the first *H. armigera* generation during rainy season in late June - late July was high, probably as a result of intensive use of insecticides during the March - May summer period on irrigated vegetables and cash crops (e.g., tomatoes, sunflower, cotton and chillies) in the region (Fig. 3). Resistance declined significantly in the first week of August and this could have resulted from early rainy-season *H. armigera* build-up on weed hosts and mung bean (*Lagascea mollis* is a common wild host in the region at this time which is never sprayed, and mung bean which is rarely treated with insecticides). With no selection pressure, resistance levels would probably decline (May and Dobson, 1986) and dilute the residual resistant summer carry-over populations. However, by the first week of September, resistance started to increase and by late October had attained the early rainy-season level (average 45% resistance). The rise in resistance at this time synchronized closely with the appearance of the first generation of moths from early rainy season cotton in the region; as larvae they could have received 2-3 applications of insecticide and therefore subject to intense selection pressure. Resistance continued to rise steadily over the postrainy season (October - February), when an overlapping succession of host crops were available (*viz.* sorghum, cotton, pigeonpea, chickpea, sunflower, tomatoes), with legumes and tomatoes in particular receiving 1-6 sprays of insecticides targeted against *H. armigera*. Resistance remained high up to the end of the cropping season in early April.

## PROSPECTS FOR INSECTICIDE RESISTANCE MANAGEMENT IN ANDHRA PRADESH

For IRM to be successful it must be conducted on an area-wide basis. This is particularly true for highly mobile pests such as *H. armigera*, which as adults are capable of dispersing over large

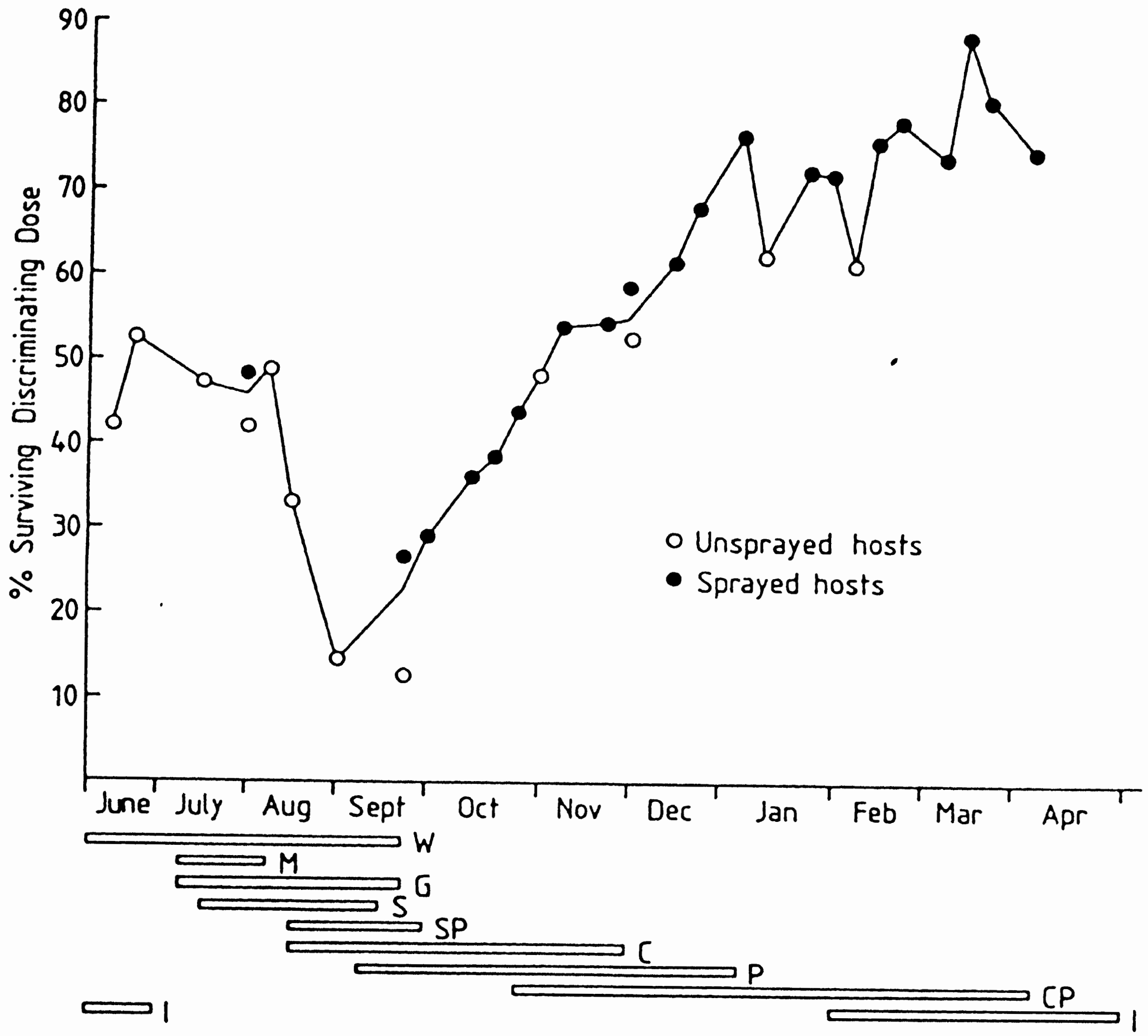


FIGURE 1. Seasonal changes in pyrethroid resistance at ICRISAT Center (open columns) and the Andhra Pradesh coastal cotton belt (shaded columns) based on average monthly $LD_{50}$ values recorded for topically applied cypermethrin to 30-50 mg larvae of *H. armigera* between 1986-92. (Data for 1986-88, 1988-89, 1989-91 and 1991-92 from McCaffery *et al.* (1989), King and Sawicki (1990), Armes *et al.* (1992) and Armes (unpublished), respectively).

***Errata:*** The legend of Fig.1 may be read for Fig.2; of Fig.2 may be read for Fig.3 and of Fig.3 may be read for Fig.1.

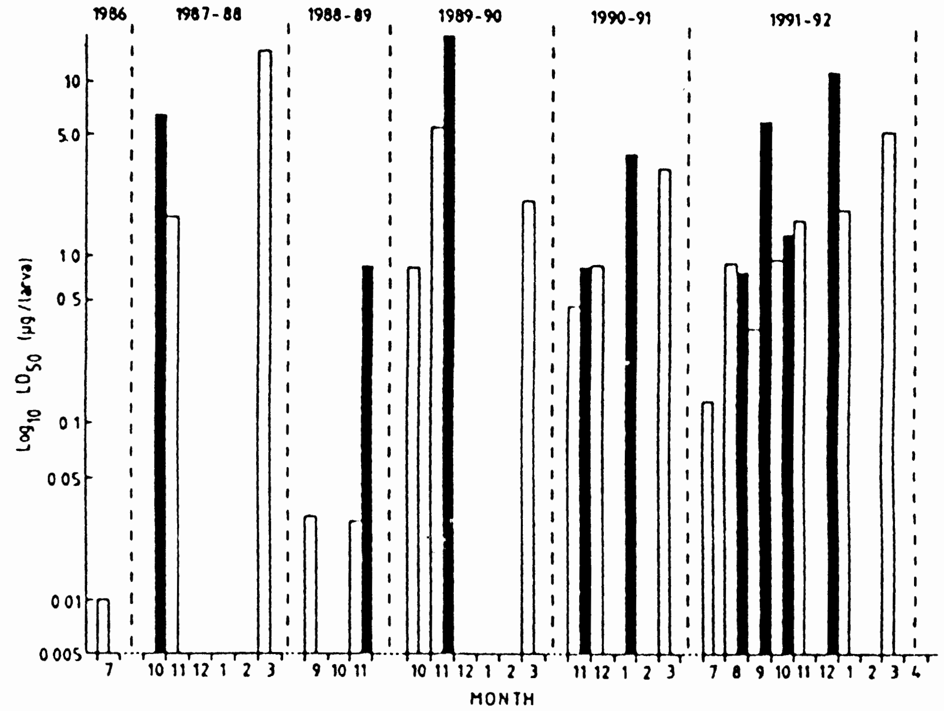


FIGURE 2. Response to cypermethrin of the NRI laboratory strain and Delhi strain of *H. armigera*.

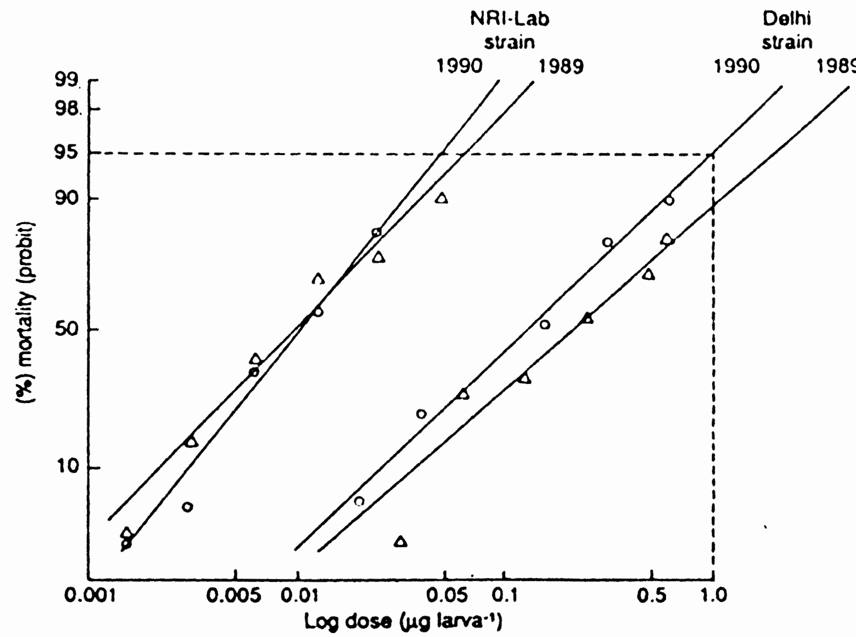


FIGURE 3. Changes in pyrethroid resistance in *H. armigera* at ICRISAT Center during the 1991-92 cropping season based on percentage of 30-50 mg larvae surviving the 1 g cypermethrin discriminating dose. Bars indicate approximate timings of plant susceptibility to *H. armigera* infestation for the major host crops in the ICRISAT locality, where: W = weeds, M = mung bean, G = groundnut, S = sorghum, SP = short duration pigeonpea, C = cotton, P = medium and long duration pigeonpea, CP = chickpea and I = irrigated [illegible] (mainly tomato).

distances (Armes and Cooter, 1991; Riley *et al.*, 1992). In Australia, a "window" strategy has been in operation since 1983, where the use of pyrethroids and endosulfan are restricted to defined periods during the growing season (Forrester and Cahill, 1987). The strategy has been successful in preventing field control failures despite steadily increasing levels of resistance (Daly, 1988). Major factors contributing to the success of the Australian strategy are that only a relatively small number of large farms are involved, farmers comply voluntarily through common interest to contain resistance buildup, infrastructure for research and extension is well established and pesticide producers have given full support from the outset. However, despite adherence to the strategy, pyrethroid resistance continues to rise annually; field control is maintained with pyrethroid products because of careful targeting of sprays against eggs and first instar larvae through regular field scouting and rapid mobilization of spray teams.

In Andhra Pradesh, farming systems are diverse and area-wide management is likely to be extremely difficult in view of the large number of farmers involved and the wide range of *H. armigera* susceptible crops grown. Planting dates for all crops are highly variable, so a window strategy on the lines of that in operation in Australia would seem to be inappropriate.

Farmers are generally ill-informed as to the most appropriate pest control practices for their crops, and most rely on pesticide retailers for advice on control of insect pests and choice of insecticides. Application of insecticides is frequently poor. In the cotton belt for example, locally manufactured hand-operated knapsack sprayers and slide-sprayers, out-dated and of poor quality, are used during the early part of the season and motorized mist blowers once the canopy has closed over. Farmers frequently tank mix different chemicals at higher than recommended concentrations, thereby increasing risks of toxicity to operators and causing environmental pollution. Spray application is often uneven and the purity of some agrochemicals marketed, particularly by small formulators, has been questioned (Anonymous, 1990). These factors increase the likelihood of a significant proportion of larvae on treated crops receiving sub-lethal doses of insecticide, thereby augmenting the rapid buildup of resistance.

**Table 1. Toxicity of topically applied cypermethrin to 30-50 mg larvae of laboratory strains of *H. armigera* (Reading strain data from McCaffery *et al.*, 1989)**

| Strain/ year tested | $LD_{50}$ (95% F.L.) (g / larva) | $LD_{90}$ (g / larva) | Slope (±S.E.) |
|---|---|---|---|
| Reading/1987 | 0.020 (0.017-0.024) | 0.050 | 1.31 (0.14) |
| NRI/1989 | 0.010 (0.008-0.012) | 0.042 | 2.00 (0.22) |
| NRI/1990 | 0.010 (0.009-0.013) | 0.033 | 2.53 (0.32) |
| Delhi/1989 | 0.21 (0.17-0.27) | 1.2 | 1.70 (0.21) |
| Delhi/1990 | 0.13 (0.09-0.18) | 0.59 | 1.91 (0.29) |

Commonly, farmers do not scout their fields for insect eggs and only perceive *H. armigera* as a pest problem when the larvae have reached the conspicuous 3rd-6th instar stages. Much higher doses of insecticide are required to kill large larvae even if they are susceptible to insecticides. As both resistant and susceptible larvae are equally and most easily killed within the first four days after



hatching (Daly *et al.*, 1988), spray decisions should be based on the presence of eggs in the crop. Clearly, there is an urgent need to educate farmers to target insecticides against eggs and neonate larvae based on scouting and economic thresholds on the lines of the simple scouting systems developed for cotton farmers in Africa in the 1960s (Matthews and Tunstall, 1968; Beeden, 1972).

In southern India, summer season (March - June) survival presents a potential weak-link in the *H. armigera* life cycle as less than 2% of pupae diapause at this time (D. R. Jadhav, unpublished). The present trend of increasing irrigation facilities for growing summer crops is undoubtably augmenting population carry-over between the end of the postrainy season and start of the next rains, and could be a contributory factor in the emergence of *H. armigera* as a major pest over the past ten years. Because of high temperatures, larval and pupal development is rapid and 2-4 overlapping generations can be completed during the summer. Insecticides are used liberally to control these populations and hence intense selection pressure for insecticide resistance occurs. If a break period for cropping were to be implemented during April - May then only low density populations on wild plants in wet areas and the small proportion of diapause pupae would survive and population increase in the following rainy season crops would be low. However, in view of the importance of summer vegetables such as okra, eggplant and tomato to the economics of Andhra Pradesh farmers, it is unlikely that summer cropping could be discouraged and therefore the next best course of action would be to develop IPM procedures suitable for summer crops in order to reduce *H. armigera* survival at this time.

Implementing curative IRM for *H. armigera* in Andhra Pradesh represents a major challenge for the Indian government, scientists and the agrochemical industry alike. Clearly there is a need to reduce pesticide use immediately and this can only be achieved by educating farmers in the appropriate selection and application of insecticides, developing farmers confidence in sound IPM practices and enforcing tighter regulations on the manufacture, quality control and sale of agrochemicals.

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