Pyrethroid Resistance and Mechanisms of Resistance in Field Strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT Pyrethroid resistance was found in 54 field strains of *Helicoverpa armigera* collected between 1995 and 1999 from 23 districts in seven states of India. LD_{50} values of the field strains ranged from 0.06 to 72.2 μg/larva with slopes of 0.5–3.1. Resistance was highest in regions where pyrethroid use was frequent (four to eight applications per season). Resistance to deltamethrin was exceptionally high with resistance ratios of 13,570 and 27,160 in two strains collected during February 1998 in central India. Resistance to cypermethrin, fenvalerate and cyhalothrin also was high with resistance ratios of >1,000 in four strains collected from central and southern India. Resistance ratios were below 100 in >50% of the strains tested. Pyrethroid resistance was high in strains collected from the districts in Andhra Pradesh where a majority of the cotton farmer suicide cases in India were reported. Resistance to pyrethroids appeared to have increased over 1995–1998 in most of the areas surveyed. Studies carried out through estimation of detoxification enzyme activity and synergists indicated that enhanced cytochrome p450 and esterase activities were probably important mechanisms for pyrethroid resistance in field strains. Pyrethroid nerve insensitivity also was found to be a major mechanism in some parts of the country where the use of pyrethroids was high. The information presented illustrates the importance of proper insect management programs to avoid the consequences associated with improper insecticide use.

KEY WORDS *Helicoverpa armigera*, pyrethroid resistance, India, cytochrome p450, esterases, nerve insensitivity

Helicoverpa armigera (HÜBNER) is a major pest of cotton, pigeonpea, chickpea, and several vegetable crops in India. Pyrethroid insecticides were introduced into India in 1980, primarily for emergency control of *Spodoptera litura* (F.) on cotton, which had by then become resistant to organophosphate, carbamate, and organochlorine insecticides (Ramakrishnan et al. 1984). Pyethroids became extremely popular with cotton farmers within a few years of introduction because of their rapid knockdown effect and high levels of efficacy against a wide range of cotton pests. Subsequently, pyrethroids were indiscriminately used and by 1985 had virtually replaced all other insecticides on cotton in southern India (Reddy 1987). It is not known if introduction of pyrethroids was one of the key factors, but by 1985, *H. armigera* and the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), which were only sporadic pests, emerged as the major pests of cotton. Severe outbreaks of *H. armigera* and *B. tabaci* in central and southern regions of India in 1984–1985 and in 1987 were attributed to the overuse of pyrethroids (Reddy and Rao 1989). By 1988 the situation had further deteriorated with no yield advantage being obtained with pyrethroid use in cotton (Rao et al. 1994). Poor efficacy in the field was traced to development of resistance to pyrethroids in *H. armigera* (Dhingra et al. 1988, McCaffery et al. 1989). Numerous other studies confirmed the high incidence of pyrethroid resistance in several cotton and pulse growing regions of the country (Armes et al. 1992a, 1996; Mehrotra and Phokela 1992; Sekhar et al. 1996). Armes et al. (1996) conducted an insecticide resistance monitoring survey during 1991–1995 on *H. armigera* strains, and they concluded that resistance to pyrethroids was ubiquitous across the Indian subcontinent. This article reports the results of a follow-up survey aimed at understanding the status of pyrethroid resistance and resistance mechanisms in *H. armigera* in India.

Materials and Methods

Areas Surveyed. *H. armigera* larvae were collected on cotton, pigeonpea, chickpea, and a few other crops from 23 districts of seven states (Uttar Pradesh, Punjab, Haryana, Maharashtra, Andhra Pradesh, Tamilnadu, and Karnataka) in India (Fig. 1) during the cropping seasons of 1995–1999. Together, the seven states account for ≈80% of the total cotton growing area and 70% of the total insecticides used on cotton in the country. At least 200 larvae were collected at each location. Data on insecticide use were collected

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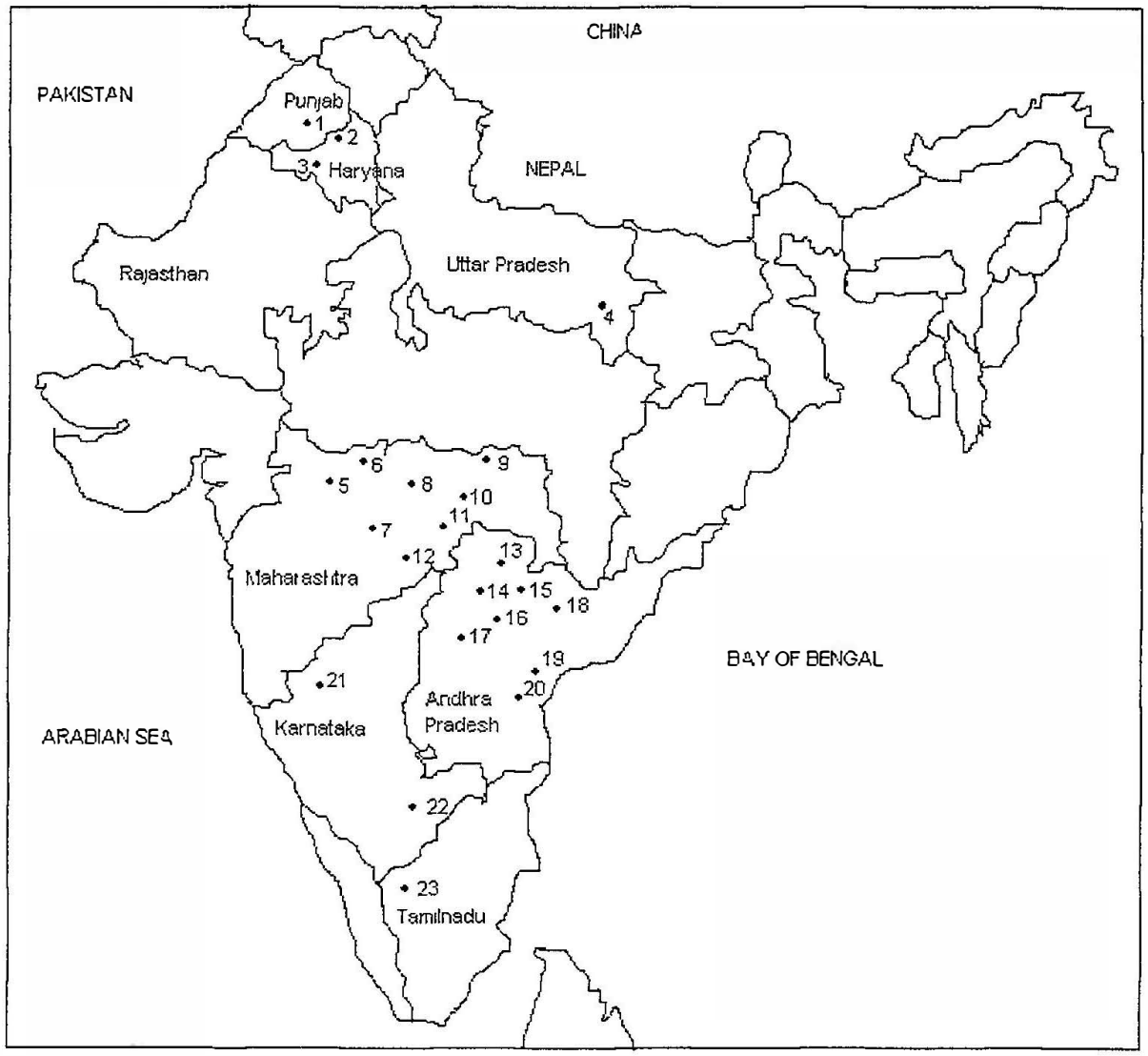

Fig. 1. Locations where *H armigera* were collected in India (1) Bhatinda (2) Dabwali, (3) Sirsa, (4) Varanasi, (5) Buldana, (6) Akola (7) Parbhani, (8) Amaravati, (9) Nagpur, (10) Wardha, (11) Yavatmal, (12) Nanded, (13) Karimnagar, (14) Rangareddy, (15) Warangal, (16) Medak, (17) Mahbubnagar, (18) Khammam, (19) Guntur, (20) Prakasam, (21) Dharwad, (22) Bangalore, (23) Coimbatore

during the survey period from at least 20 farmers in each of the districts surveyed Information collected from farmers included the total number of insecticide applications used on cotton during the season, brand names, quantity of the formulated product applied per hectare, volume application rate and date of application The data presented in this article do not include early season sprays that were not intended for bollworm control The significance of differences among mean levels of pyrethroid use in different districts was analyzed by one-way analysis of variance (ANOVA), and differences among treatment means were determined by least significant difference (LSD) test (Snedecor and Cochran 1989)

Susceptible Strain. An insecticide-susceptible strain of *H armigera* was provided by Alan McCaffery of The University of Reading, UK The Reading susceptible strain was originally collected in southern Africa and maintained at the University of Reading for at least 15 yr A colony of the susceptible strain was maintained at the International Crop Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, India, simultaneously and was found to exhibit the least interassay variability to pyrethroids (Armes et al 1996, D J, unpublished data)

Insecticides and Chemicals The following technical grade insecticides were used for bioassays *cis trans* (50 50 ratio) cypermethrin (90%, Zeneca Agrochemicals, Surrey, UK), deltamethrin (99 5%, Roussel-Uclaf, Paris, France), fenvalerate (97 6%, Sumitomo, Osaka, Japan), λ-cyhalothrin (86 4%, Zeneca Agrochemicals), profenofos (94%, Ciba-Geigy, Basel, Switzerland), and piperonyl butoxide (PBO) (90%, Gooddeed Chemical, Aylesbury, UK) All other chemicals were of high purity and obtained from either Sigma Chemicals (St Louis, MO) or Hi-media Chemicals (Bombay, India)

Bioassays. Larvae were reared individually on a chickpea based semisynthetic diet (Armes et al 1992b) in 7 5-ml cells of LINBRO 12-well tissue culture plates (ICN Pharmaceuticals, Costa Mesa, CA) Larvae were collected from cotton plants during August to March, transferred into the 12-well tissue culture plates containing semisynthetic diet, and trans-

ported to the laboratory at the Central Institute for Cotton Research, Nagpur. Larvae were reared for one generation to ensure that they were not diseased or parasitized, and F_1 progeny for testing were obtained from the laboratory cultures that were thus established for each strain from 100 to 200 of the resulting moths. Bioassays were conducted on third instars (30–40 mg) using a topical application procedure described by Armes et al. (1992a) and based on the standard *Heliothis* susceptibility test recommended by the Entomological Society of America (Anonymous 1970). Because the resistant phenotype is best expressed in the third instars of *H. armigera* (Daly et al. 1988), this stage was chosen for resistance assessment. Larvae were topically treated on the thoracic dorsum with 1-μl aliquots of acetone alone (control) or serial dilutions of the technical grade insecticide dissolved in acetone using a Hamilton repeating dispenser # PB600–1 (Hamilton, Reno, NV) and placed individually in LINBRO 12-well tissue culture plates containing semisynthetic diet. Mortality was assessed over 6 d according to Armes et al. (1996). Larvae were considered dead if they were unable to move in a coordinated manner when prodded All rearing and bioassay operations were carried out at 25 ± 2°C under a photoperiod of 12:12 (L:D) h. There were at least 12 larvae in three replicates at each of five or more doses (0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 μg/μl) plus controls (treated with acetone alone). PBO at 50.0 μg (Forrester et al. 1993) and profenofos at 0.1 μg per larva (Gunning et al. 1991, Armes et al. 1996) were used alone and as premixes with cypermethrin to determine the extent of PBO-suppressible oxidase-mediated and profenofos-suppressible esterase-mediated pyrethroid resistance, respectively. Control mortality in treatments with either acetone alone or with only synergists, was rare but, when required, corrections for control mortality were made using Abbott's formula (Abbott 1925). Dose-mortality regressions were computed by probit analysis (POLO-PC; LeOra Software 1987). Analysis of resistance ratios was done as described by Robertson and Preisler (1992).

Enzyme Preparations. Enzyme preparations were made from at least 60 fourth-instars of the susceptible and each of the field strains to understand the quantitative differences in cytochrome p450 content and esterase activity with reference to pyrethroid resistance and PBO- or profenofos-susceptible pyrethroid resistance in field strains. Midguts were dissected in ice-cold sodium phosphate buffer (100 mM, pH 7.6) containing 1.0% potassium chloride and homogenized in fresh sodium phosphate buffer containing 1 mM each of ethylene diamine tetra-aceticacid, phenyl thiourea and phenyl methyl sulfonyl flouride and 20% glycerol. The homogenate was centrifuged at 10,000 × *g* for 15 min at 0°C, and the resultant postmitochondrial supernatant was used as the enzyme source. Protein was estimated according to Lowry et al. (1951) using BSA (type V) as standard. A double beam UV spectrophotometer (U-2000, Hitachi, Tokyo, Japan) was used for protein estimation and all enzyme assays. Enzyme activity is expressed as activity per milligram of protein of the tissue supernatant. The significance of differences among mean levels of cytochrome p450 content and esterase activities were analyzed by one-way ANOVA and differences between treatment means were determined by LSD test (Snedecor and Cochran 1989). Correlation coefficients for pairwise comparison of resistance ratios with pyrethroid use, cytochrome p450, esterases, PBO synergism and profenofos synergism were calculated according to Snedecor and Cochran (1989).

Cytochrome p450 Determination. Cytochrome p450 content was determined with the dithionite reduced CO difference spectrum method described by Omura and Sato (1964) using a molar extinction coefficient of 91/mM/cm.

Esterase Determination. Esterase activity was assayed according to Kapin and Ahmad (1980) with slight modifications. Six milliliters of the reaction mixture consisting of 0.3 mM α-naphthyl acetate and 5 μg protein from tissue supernatant in 40 mM sodium phosphate buffer (pH 7.0) was incubated at 30°C for 15 min. The reaction was stopped by the addition of 1.0 ml of a freshly prepared solution containing two parts of 1% fast blue BB salt and five parts (wt:vol) of 5% sodium lauryl sulfate. Change in absorbance at 590 nm was monitored against blanks for 30 min. The enzyme activity was quantified using α-naphthol as standard and expressed as μMol/min/mg protein.

Neurophysiological Assay for Nerve Insensitivity. Nerve preparations were made from larvae of the susceptible and resistant field strains to determine the differences in neuronal sensitivity to pyrethroids. The cumulative dose–response neurophysiological assay (McCaffery et al. 1997) was used to assess the effect of *cis*-cypermethrin on the spontaneous multiunit activity of nerves from third instars (30–40 mg) to understand the extent of nerve insensitivity, now commonly referred as knockdown resistance (kdr). Larvae were dissected dorso-medially and pinned out in saline on a layer of Sylgard resin (Dow Corning, GmbH, Wiesbaden, Germany). A peripheral nerve was picked up with a 27-gauge stainless steel, suction recording electrode with an insulated outer coating. The nerve was grounded with a stainless steel entomological pin and served as a reference electrode. Extracellular neuronal activity was amplified and filtered using a high gain, low noise front-end amplifier and conditioning system (Neurolog Digitimer, UK) before being relayed for data recording and analysis (Axon Instruments 1996). Neural activity was monitored on an oscilloscope. Spontaneously occurring action potentials were discriminated from background noise above a visually adjusted threshold and recorded by computer in 15-s epochs in blocks of 5-min periods. Nerve preparations were first bathed for 5-min in saline, followed by successive 5-min perfusions of saline containing step-wise increasing concentrations of *cis*-cypermethrin. Technical cypermethrin dissolved in analytical grade acetone at one mM was diluted in saline to get final range of concentrations of 10^{-12} to 10^{-6} M. Saline containing 0.1% acetone was also tested

periodically as control. The susceptible strain was tested with a concentration range 10^{-12} to 10^{-8} M, and the field strains were tested with 10^{-9} to 10^{-6} M. The end point of the assay was defined as the lowest concentration of *cis*-cypermethrin at which the frequency of action potentials was over three times greater than the mean value during the pretreatment control period. About 25–40 individual larvae were tested for each set of assays for each strain, and EC_{50} for cypermethrin effect on nerve sensitivity was determined by probit analysis (POLO-PC, LeOra Software 1987).

Results

Bioassays. All of the four pyrethroid compounds were highly toxic to the Reading susceptible strain with high slopes of 1.9–2.0 and LD_{50} values of 0.001–0.016 μg/larva (Tables 1 and 2). LD_{50} values of the field strains ranged from 0.06 to 72.2 μg/larva with slopes of 0.5–3.1. Compared with the Reading susceptible strain, all of the 54 field strains were resistant to all four pyrethroids, indicating ubiquitous occurrence of pyrethroid resistance in the country. Resistance was low to moderate with resistance ratios below 100 in 31 of the 54 strains. These strains were from Wardha, Parbhani, Buldhana, and Nanded in central India; Bangalore, Mahboobnagar, and Dharwad in southern India; and Bhatinda, Dabwali, and Varanasi from northern India. Resistance to deltamethrin was exceptionally high in strains collected during February 1998 from Amaravati and Akola. Resistance to the other pyrethroids was also high in some strains with resistance ratios of >1,000 in strains collected from Guntur, Amaravati and Akola. High resistance ratios were recorded in strains collected from Warangal, Karimnagar, and Khammam districts of the Telangana region in Andhra Pradesh. Resistance to pyrethroids appeared to have increased over 1995–1998 in most of the areas surveyed in our study.

Insecticide Use. Almost all of the farmers interviewed had used insecticides on cotton (Table 3). Pyrethroids constituted 8–75% of the total insecticide applications for bollworm control and were used either singly or as tank mixtures with other insecticides. Cotton farmers of Bhatinda and Dabwali in northern India, and Guntur, Prakasam, Karimnagar, Khammam, and Warangal in southern India, used four to eight pyrethroid applications per season. Pyrethroid use also was high (more than five spray applications) in Akola in central India in 1997–1998; however, pyrethroid applications were about one to three per season in all other districts. The correlation between resistance ratios and the total number of applications of pyrethroid was significant ($P < 0.05$) (Table 4). Cypermethrin was the most commonly used pyrethroid, followed by fenvalerate. Both deltamethrin and cyhalothrin were used only in a few regions and at low frequencies.

PBO and Profenofos Synergists. PBO and profenophos had no significant effects on cypermethrin toxicity to the Reading susceptible strain (Table 5). Almost all field strains collected from Guntur, Bhatinda, and districts of central India had negligible PBO synergism with cypermethrin. There was significant PBO suppression of cypermethrin resistance in strains collected from other places such as Sirsa and Varanasi from northern India, the Telangana region of Andhra Pradesh, and Dharwad, Coimbatore, and Bangalore districts of southern India. There was significant profenofos suppression of cypermethrin resistance in a few strains collected from Yavatmal, Nagpur, Rangareddy, and Bhatinda.

Cytochrome p450 and Esterase Activity. Cytochrome p450 content and esterase activities were significantly higher in ≈50 and 75% of the strains, respectively. High levels of cytochrome p450 activity (>300 pMol/mg protein of the tissue supernatant) were recorded in strains from Karimnagar, Warangal, and Rangareddy districts of Andhra Pradesh, Varanasi, and Sirsa in northern India, and Coimbatore and Bangalore in southern India (Table 6). At all other sites the cytochrome p450 activity was usually <300 pMol/mg protein. Esterase activity was high (>3.0 μM/min/mg protein) in a majority of the central Indian and also Varanasi strains, but generally lower than 2.5 μM/min/mg protein in the rest of the strains. All of the Guntur strains had lower levels of detoxification enzymes associated with low PBO and profenofos synergism. Correlation between PBO synergism and cytochrome p450 content was significantly ($P < 0.05$) positive (Table 4). However, neither PBO nor profenofos synergism were significantly correlated with esterase activity. Interestingly resistance ratios were positively ($P < 0.05$) correlated with esterase activity.

Neurophysiological Assay for Nerve Insensitivity. The EC_{50}s for nerve-insensitivity of larvae to cypermethrin for six strains from central and southern India was 20.72–91.42 nM. The EC_{50} for the susceptible strain ranged from 0.028 to 0.039 nM. The highest levels of nerve insensitivity were in a strain collected from Guntur. This indicates that nerve insensitivity is a prominent resistance mechanism in the absence of synergism by either esterase or oxidase inhibitors. High levels of nerve insensitivity were also observed in strains collected from central India at Akola and Amaravati.

Discussion

We found resistance to pyrethroids in a majority of the field strains collected in India. Resistance was the highest in regions where pyrethroid use was most frequent (four to eight applications per season). This also explains the seasonal differences in pyrethroid resistance at several locations such as Nagpur, Wardha, Akola, Amaravati, Guntur, and Rangareddy, where resistance was highest by 1997–1998 when the intensity of insecticide had also increased to the highest because of the *H. armigera* outbreak in the country. Pyrethroid resistance was high in strains collected from the Andhra Pradesh districts, where a majority (174 of the 300) of the cotton farmer suicide cases were reported (Parthasarathy and Shameem 1998).

Table 1. Log-dose probit response of field strains of *H. armigera* to cypermethrin

Strain	Collection date	n^a	LD_{50}	(95% FL)[b]	LD_{90}	(95% FL)[b]	Slope ± SE	RR	(95% FL)[c]	χ^2
Reading susceptible		240	0.009	(0.007–0.011)	0.040	(0.02–0.07)	2.0 ± 0 2	—	—	—
Northern India										
Bhatinda	Nov 1998	240	0.38	(0.1–1.1)	3.62	(1.4–35.8)	1.3 ± 0.2	39	(25–59)	11.4**
Dabwali	Nov 1998	220	0.56	(0.4–0.9)	6.61	(2.9–43.5)	1.2 ± 0 2	61	(39–96)	1.1
Sirsa	Nov 1998	159	0.54	(0.2–1.0)	4.18	(1.8–27.8)	1.4 ± 0.2	56	(37–84)	10.7*
Varanasi	Oct 1993	168	0.44	(0 3–0.7)	6 58	(3.0–22.6)	1.1 ± 0.1	47	(28–80)	2.5
	Dec 1994	174	0.41	(0.3–0.7)	5.90	(2 7–19.3)	1.1 ± 0.1	44	(26–75)	2.5
Central India										
Nagpur	Sept 1995	246	0.22	(0.1–0.3)	1.53	(0.8–22.9)	1.5 ± 0.2	23	(15–37)	3.8
	Jan 1996	176	0.85	(0 4–3.9)	11.65	(2.6–1507.0)	1.1 ± 0.3	84	(46–155)	3.5
	Nov 1996	192	0.68	(0.4–0.9)	3.50	(1.6–36.6)	1.7 ± 0 3	69	(45–108)	3.3
	Jan 1997	212	0 67	(0 5–0.9)	7.99	(2.8–130.5)	1.2 ± 0.1	73	(49–108)	8.5*
	Oct 1997	176	0.88	(0.6–1.2)	2.90	(2.0–4.5)	2.5 ± 0.4	93	(66–133)	2.2
	Feb 1998	242	2.73	(1.8–4.5)	62.93	(26 7–249.8)	0.9 ± 0.1	294	(175–495)	1.3
	Nov 1998	196	1.95	(0.9–23.2)	20.77	(4.0–28,358 0)	1.2 ± 0.4	205	(94–445)	3.4
	Feb 1999	150	0.98	(0 5–3.8)	8.40	(3 9–40.4)	1.4 ± 0.2	103	(68–157)	6.4
Wardha	Feb 1995	186	0.13	(0.1–0.2)	1.05	(0 7–1.6)	1 4 ± 0.3	14	(9–22)	2.0
	Oct 1996	204	0.50	(0.2–2.3)	10.04	(4.9–41.0)	0.9 ± 0.2	51	(31–86)	1.5
	Oct 1997	396	0.85	(0.6–1.1)	9.01	(3.1–380.6)	1.2 ± 0.2	96	(65–143)	7.0
	Feb 1998	170	0.06	(0.0–0 1)	0.32	(0 2–1.4)	1.7 ± 0.5	7	(5–11)	15.9**
	Nov 1998	159	0 42	(0.2–0.8)	3.51	(1 5–13.8)	1.4 ± 0 2	45	(31–65)	17.5**
Amaravati	Oct 1997	241	6.94	(4.0–17.9)	72.41	(25.3–510 0)	1.3 ± 0.2	744	(353–1,566)	0 4
	Feb 1998	169	69.59	(23.5–926.0)	17974	(1,076 0–53,860,000.0)	0.5 ± 0.1	6978	(2,316–21,018)	10.9*
	Jan 1999	120	3.68	(1.8–7.6)	59.95	(22.5–361.0)	1.0 ± 0.3	395	(241–649)	8.4
Akola	Jan 1997	164	2.55	(1.9–3.8)	15.37	(8.9–36.0)	1 6 ± 0 2	258	(172–386)	0.9
	Feb 1998	144	72 20	(31.6–417.0)	4173 70	(918.0–55,214.0)	0.7 ± 0.2	7383	(2,870–18,990)	2.83
	Jan 1999	176	2.25	(0.9–29.2)	842.57	(147.0–1,715.0)	0.5 ± 0.1	255	(104–626)	3.8
Parbhani	Feb 1996	139	0.45	(0.3–0.9)	2.37	(1 1–9.7)	1.9 ± 0.3	49	(33–73)	3.6
	Oct 1998	166	0 80	(0.5–1.3)	9.12	(4.4–34.1)	1.2 ± 0.2	87	(57–130)	3.2
	Jan 1999	150	1 13	(0 6–4.2)	9.21	(4 3–41.4)	1.4 ± 0.2	120	(79–183)	8.2
Yavatmal	Oct 1996	129	1.16	(0.5–6.8)	25.62	(6.0–1,083.0)	0.9 ± 0.2	125	(69–226)	5.6
	Feb 1998	148	2.59	(1.7–13.2)	10.34	(6.5–22.3)	2.1 ± 0 4	256	(168–389)	0 4
	Jan 1999	132	0.94	(0.5–2.4)	13.97	(5.8–39 1)	1.1 ± 0.2	99	(60–163)	1.6
Buldana	Feb 1998	171	0.21	(0.2–0.3)	1.03	(0.6–1.9)	1.8 ± 0.2	23	(16–34)	4.4
	Jan 1999	144	0 35	(0.2–0.5)	1.84	(1.3–3.0)	1.7 ± 0.2	38	(26–56)	2.2
Nanded	Feb 1998	118	0.46	(0.3–0.8)	5.16	(2.0–39.5)	1.2 ± 0 2	50	(32–78)	6.7
	Oct 1998	165	0.35	(0.2–0.6)	5.42	(3.1–12.4)	1 1 ± 0 2	37	(23–59)	3.3
Southern India										
Warangal	Feb 1998	177	7.38	(5.1–10.5)	60.70	(35.4–130.3)	1.4 ± 0.2	789	(508–1,226)	4.8
	Nov 1998	268	6.07	(3.4–17.7)	107.10	(43 5–560.0)	1.0 ± 0.2	655	(392–1,095)	5.6
Medak	Feb 1998	210	1.08	(0.6–1.7)	9.40	(4.1–53.4)	1.4 ± 0.2	116	(76–175)	6.8
Karimnagar	Feb 1998	216	4.70	(3.1–7.1)	65.01	(18 4–1,437.0)	1.1 ± 0.2	507	(298–863)	6.9
Khammam	Feb 1998	144	18 0	(14.8–24.3)	47 07	(32.2–99.1)	3.1 ± 0 5	1934	(1,335–2,801)	1.2
Guntur	Nov 1995	176	3.41	(2.5–5.5)	11.72	(6.7–49.9)	2.4 ± 0.5	363	(255–515)	12.4*
	Dec 1995	214	3.38	(1.5–38.9)	51.50	(10.1–88,189.0)	1.1 ± 0 1	365	(224–597)	14.7**
	Oct 1997	260	1.97	(1.4–2.9)	36.20	(8.8–2135 0)	1.0 ± 0.1	228	(144–359)	7.5
	Feb 1998	192	4.70	(3.1–7.0)	48 00	(20.8–174.2)	1.3 ± 0 2	514	(339–780)	4.1
	Oct 1998	129	13.62	(6.8–59 6)	249.80	(66 7–8,929.0)	1.0 ± 0.2	1416	(628–3,193)	1 4
Prakasam	Feb 1998	144	1.18	(0.6–2.5)	10.37	(4.2–85.1)	1.4 ± 0.2	128	(84–195)	8.1
Rangareddy	Nov 1995	240	0.36	(0.3–0.4)	1.50	(0.9–4.3)	2.1 ± 0.3	39	(28–56)	3.5
	Aug 1996	216	0.33	(0.3–0.4)	1 90	(1.3–4.2)	1.7 ± 0.2	36	(25–53)	0.6
	Oct 1997	226	0.49	(0.4–0.6)	6.71	(3 2–25.7)	1.1 ± 0.1	52	(33–83)	1.6
	Feb 1998	212	0.89	(0.4–3.6)	40.60	(6.9–127,520 0)	0.8 ± 0.1	96	(56–164)	4.0
	Oct 1998	246	0 35	(0 3–0.4)	2.00	(0.9–23.1)	1.7 ± 0 3	37	(26–54)	9.0*
	Jan 1999	256	1.82	(1.2–5.1)	12.50	(4.6–153.0)	1.5 ± 0.3	196	(99–389)	1.4
Mahboobnagar	Feb 1998	220	0.82	(0 7–1.1)	2.94	(1.9–6.4)	2.3 ± 0.4	88	(61–127)	1.8
Coimbatore	Oct 1995	396	1.22	(0.6–5.4)	11.86	(3.4–968.0)	1 3 ± 0.2	133	(89–200)	24 8**
	Nov 1996	176	2.07	(1 5–2.9)	23.79	(13.2–57.0)	1.2 ± 0 1	223	(144–346)	2.2
	Mar 1998	168	0.64	(0.2–2.0)	42.76	(7.9–12,591.0)	0.7 ± 0 2	69	(39–122)	8.7
Bangalore	April 1994	212	0.66	(0 4–1.9)	7 68	(2.5–116.6)	1.2 ± 0.2	72	(41–127)	4.4
	Dec 1995	248	0.54	(0.4–0.9)	7.55	(3 4–27.6)	1.1 ± 0.1	58	(34–100)	2 0
Dharwad	Jan 1996	212	0.91	(0.5–3.7)	42.98	(9.2–3,012.0)	0.8 ± 0.2	100	(57–176)	5.4

*, Chi-square significant ($P < 0.05$), **, significant ($P < 0.01$).

[a] Number tested including controls.

[b] In micrograms cypermethrin per third instar larva.

[c] RR (resistance ratio) and 95% CL calculated by the method of Robertson and Preisler (1992) relative to the 'Susceptible Reading strain'.

Table 2. Log dose probit response of field strains of *H. armigera* to pyrethroids

Strain	Collection date	n^a	LD_{50} (95% FL)[b]	LD_{90} (95% FL)[b]	Slope ± SE	RR (95% FL)[c]	χ^2
Deltamethrin							
Reading susceptible		192	0.001 (0.001–0.002)	0.005 (0 003–0.008)	1.9 ± 0.2	—	2 2
Nagpur	Feb 1998	180	0 55 (0.2–1.6)	5.2 (2.3–25.8)	1.3 ± 0.1	574 (367–898)	8.5
Amaravati	Feb 1998	278	13.47 (3.7–1129.0)	796.0 (57.0–228,590.0)	0.7 ± 0.2	14,133 (2,071–96,443)	2.8
Akola	Feb 1998	236	27.16 (6 3–24,414.0)	841.0 (53.0–1,648,300.0)	0.8 ± 0.3	26,151 (2,455–278,514)	0.9
Warangal	Feb 1998	116	0 65 (0.3–1.2)	8 4 (3 7–36.3)	1.1 ± 0.2	648 (403–1,041)	5.7
Karimnagar	Feb 1998	189	0.52 (0.3–1.0)	4 7 (2.5–13.8)	1.3 ± 0.2	523 (334–820)	4.9
Guntur	Feb 1998	142	1.36 (0.5–10.1)	20.0 (4 3–27,363.0)	1 1 ± 0.2	1,351 (827–2,206)	21.6**
Coimbatore	Mar 1998	186	0.18 (0.0–0.5)	58 0 (7.3–117,230.0)	0 5 ± 0.1	175 (69–448)	6.5
Bhatinda	Nov 1998	154	0.09 (0.0–0.1)	0.2 (0 1–3 0)	3.1 ± 0.7	88 (60–128)	7.1
Sirsa	Nov 1998	182	0 34 (0 2–0 5)	2.8 (1 2–18.4)	1.4 ± 0.2	336 (216–521)	10.5*
Fenvalerate							
Reading susceptible		168	0.016 (0.01–0.02)	0.07 (0 05–0.13)	1.9 ± 0.3	—	2.7
Nagpur	Feb 1998	136	1.46 (0.3–11.7)	188 0 (16 7–13,155,000 0)	0.6–0 1	91 (46–182)	9.2*
Amaravati	Feb 1998	181	23.10 (14.6–55.9)	186 0 (65.3–1,708.0)	1.4 ± 0.3	1,445 (650–3,210)	0.6
Akola	Feb 1998	170	52.30 (22.2–466.0)	1,430.0 (211.0–170,450.0)	0.9 ± 0.2	3,139 (772–12,761)	1.5
Warangal	Feb 1998	178	1.91 (0.3–3.5)	17.0 (10.4–34.8)	1.4 ± 0.3	118 (77–183)	2.6
Karimnagar	Feb 1998	143	1.92 (0 9–3.6)	36 9 (17.8–110.6)	1.0 ± 0.1	121 (73–202)	2 8
Guntur	Feb 1998	140	2.59 (1.6–13.2)	10.3 (5.1–55.1)	2.1 ± 0 7	159 (109–233)	12.0**
Coimbatore	Mar 1998	120	0.35 (0.1–1.2)	2.6 (1.2–12 2)	1 4 ± 0.2	21 (14–33)	8.7*
Bhatinda	Nov 1998	164	0 16 (0.0–0.3)	0.5 (0.4–0.7)	2.7 ± 0.3	10 (7–15)	3 1
Sirsa	Nov 1998	176	1.45 (0.7–7.8)	19.6 (8.7–78.9)	1 1 ± 0 3	90 (56–144)	4.2
λ-cyhalothrin							
Reading susceptible		288	0.004 (0.002–0.005)	0 017 (0.01–0.03)	2 0 ± 0 3	—	1.3
Nagpur	Feb 1998	128	0 35 (0.1–0.7)	1.4 (1.0–2.1)	2 1 ± 0.3	85 (58–125)	3 4
Amaravati	Feb 1998	118	15.60 (4.1–2,037.0)	963.0 (62.0–1,084,800.0)	0.7 ± 0.2	3,734 (598–23,307)	2.5
Akola	Feb 1998	180	18.60 (12.5–35.6)	150.2 (54.1–1,358.0)	1.4 ± 0.3	4,477 (2,128–9,421)	0 6
Warangal	Feb 1998	136	1.39 (0.7–3 2)	8.66 (4 9–20.2)	1.6 ± 0.2	336 (214–529)	4 0
Karimnagar	Feb 1998	142	0.43 (0.3–0.7)	4.64 (1.7–55.8)	1.2 ± 0.2	106 (68–167)	12.5*
Guntur	Feb 1998	176	4.66 (2.6–14.5)	34 2 (13.8–271.0)	1.5 ± 0.3	1141 (721–1,806)	6.5
Coimbatore	Mar 1998	181	0.29 (0.2–0.5)	2.44 (1.2–10.0)	1.4 ± 0 2	72 (47–112)	7.4
Bhatinda	Nov 1998	236	0.09 (0.0–0.2)	1.51 (0 6–7.1)	1.0 ± 0.2	22 (14–36)	5.9
Sirsa	Nov 1998	140	0 25 (0.1–0.4)	2.32 (1.1–10.6)	1.3 ± 0 2	59 (38–93)	7.3

*, Chi-square significant ($P < 0.05$), **, significant ($P < 0.01$).

[a] Number tested including controls.

[b] In micrograms cypermethrin per third instar larva.

[c] RR (resistance ratio) and 95% CL calculated by the formula of Robertson and Preisler (1992) relative to the 'Susceptible Reading strain'

Previous studies in Andhra Pradesh had indicated that resistance to cypermethrin was on the rise. The resistance ratios were 40- to 750-fold during 1987 and 1988 (McCaffery et al. 1989), 7- to 2,100-fold during 1989 and 1990 (Armes et al. 1992a), and 20- to 6,500-fold between 1991 and 1994 (Armes et al. 1996). The current results showed that the pyrethroid resistance situation in Andhra Pradesh continues to be a problem with resistance ratios of 36–1,933 being recorded between 1995 and 1999. In general, insecticide use was high in almost all the regions of Andhra Pradesh, especially during the *H. armigera* outbreak year of 1997–1998. Andhra Pradesh alone accounts for >33% of the insecticides used in the country, with over 60% of this on cotton alone. Expectedly, pyrethroid resistance was also high in the state. In Coimbatore, resistance to cypermethrin was 25- to 140-fold during 1992 and 1993 (Armes et al. 1996), but despite a reduction in the use of pyrethroids in the state over the past few years resistance levels increased to 64–207 in our study. Armes et al. (1996) had reported that the most highly resistant populations were generally found in the central and southern regions of India. It was from these regions that reports of inadequate control of *H. armigera* and increased insecticide use were most frequent.

Interestingly, the highest levels of pyrethroid resistance were recorded from Akola and Amaravati districts of central India. Although pyrethroid use in these districts was high during the *H. armigera* outbreak year of 1997–1998, it was not as high as in Warangal or Guntur districts of Andhra Pradesh. Hence, higher levels of resistance in *H. armigera* to almost all the pyrethroids in central India were surprising. In sharp contrast, resistance was still at unexpectedly low levels in Bhatinda district in Punjab where pyrethroid use was reasonably high. The reasons for this are not clear. Earlier, Mehrotra and Phokela (1992) had reported low levels of cypermethrin resistance of 3- to 11-fold in strains from Ludhiana in Punjab. The insecticide use surveys (data not shown here) indicated that endosulfan was one of the most popular insecticides in Bhatinda district. As pointed out by Forrester et al. (1993) and Kern et al. (1991), the negative correlation of pyrethroid resistance with that of endosulfan may have been responsible for the low levels of pyrethroid resistance in Bhatinda as influenced by excessive use of endosulfan.

Similar to the findings of Armes et al. (1996), the current results also indicated that resistance levels varied markedly over short distances. Amaravati and Wardha, which are only 100 km apart, harbored strains

Table 3. Insecticide use reported by farmers during the survey

District	Year	No. of farmers interviewed	Mean ± SE no of spray applications by each farmer against bollworms during the season						
			Cypermethrin	Fenvalerate	Decamethrin	λ-cyhalothrin	Pyrethroids	Others	Total
Nagpur	95–96	112	0.14 ± 0.04a	0 18 ± 0.04a	0	0	0.32	3.65	3 97
	96–97	121	0 39 ± 0.07abcd	0 19 ± 0.04a	0.02 ± 0.01a	0	0 60	2.79	3.39
	97–98	39	1 36 ± 0.21ghijk	1.13 ± 0.10ghi	0.1 ± 0 05abcd	0	2.59	4.69	7 28
	98–99	26	0.65 ± 0.15bcde	0.5 ± 0.11abcde	0	0	1.15	2.69	3.84
Wardha	95–96	24	1.25 ± 0.15fghij	0.29 ± 0 09abc	0	0	1.54	3.5	5.04
	96–97	21	0.62 ± 0.17bcde	0.57 ± 0.13abcdef	0	0	1.19	5	6.19
	97–98	180	0.35 ± 0.05abcd	0.16 ± 0.03a	0	0	0.51	5.14	5 65
	98–99	54	0.17 ± 0.06ab	0.24 ± 0.06ab	0	0	0 41	2.87	3.28
Amaravati	97–98	22	1.64 ± 0.18ijkl	1 68 ± 0.23jkl	0	0	3.32	6.68	10
	98–99	28	1 03 ± 0 09efgh	0.82 ± 0.18defg	0	0	1.85	2.68	4 53
Akola	97–98	25	3.0 ± 0.30o	2.16 ± 0.23lm	0.28 ± 0.09efgh	0.04 ± 0.04a	5.48	3.44	8 89
	98–99	20	1.1 ± 0.22efghi	0.8 ± 0.22cdefg	0 05 ± 0.05ab	0	1.95	5 55	7.5
Parbhani	96–97	23	0.61 ± 0.12bcde	0.26 ± 0.09abc	0.08 ± 0.06abc	0	0.95	1.74	2.69
	97–98	19	0.58 ± 0.14bcde	0.37 ± 0 11abcd	0	0	0.98	2.21	3.19
Yavatmal	96–97	31	1 71 ± 0.24jkl	0.71 ± 0.12cdefg	0.06 ± 0.04ab	0 0	2.48	8.41	10 89
	97–98	26	0.23 ± 0 10abc	0.65 ± 0 14bcdef	0	0.04 ± 0.04a	0 92	11.94	12 84
Buldana	97–98	26	0.19 ± 0.08abc	0.11 ± 0.06a	0	0	0 3	1.77	2.07
Warangal	97–98	26	3.96 ± 0.27p	2 11 ± 0.38klm	0.92 ± 0.09j	0.04 ± 0.04a	7.03	8.34	15.37
	98–99	20	2.35 ± 0.19mn	1.65 ± 0.35jkl	0.25 ± 0.10defg	0.05 ± 0.05a	4.3	5.95	10.22
Medak	97–98	22	0.82 ± 0.19defg	0.59 ± 0.14abcdef	0.14 ± 0.07bcde	0.05 ± 0.05a	1.6	1.23	2.83
Karimnagar	97–98	26	2.07 ± 0.29lm	1.61 ± 0.25ijk	0.38 ± 0.11gh	0	4 06	4.61	8.67
Khammam	97–98	18	1 78 ± 0 29klm	1.55 ± 0 28hij	0.61 ± 0.14i	0 11 ± 0 08ab	4.05	5.0	9.03
Guntur	94–95	22	2.77 ± 0.32no	1 91 ± 0.35jklm	0 59 ± 0 14i	0	5 27	4.0	9 27
	95–96	19	4.42 ± 0.67p	3.47 ± 0.51n	0.42 ± 0 11h	0	8 31	2.84	11.15
	96–97	23	2.91 ± 0.37no	2 0 ± 0.40jklm	0 3 ± 0.10fgh	0 04 ± 0.04a	5 13	4.78	9 91
	97–98	24	1.39 ± 0.16fghijk	0.63 ± 0 13bcdef	0.12 ± 0.07abcde	0.12 ± 0 07b	2 2	3.16	5.36
Prakasam	97–98	19	2 37 ± 0.39mn	2.16 ± 0.41lm	0.21 ± 0.09cdef	0.05 ± 0.05a	4.79	6 05	10.81
Rangareddy	96–97	20	0.65 ± 0.18bcde	1.1 ± 0.22ghi	0	0	1.75	1.35	3.1
	97–98	24	1.0 ± 0.22efgh	1.5 ± 0.26hij	0	0.08 ± 0.06ab	2.58	2.46	5 04
Mahbubnagar	97–98	19	0.74 ± 0.22cdef	1.0 ± 0.22efgh	0.1 ± 0.07abcd	0	1 84	2.1	3.94
Dharwad	95–96	21	1 52 ± 0 22hijkl	0.86 ± 0.23defg	0	0	2.38	7.09	9 47
Bhatinda	97–98	22	1.91 ± 0.30lm	2.41 ± 0.34m	0	0	4 32	3.41	7 73
Dabwali	97–98	18	2.0 ± 0.25lm	2.39 ± 0.39m	0	0	4.39	3.61	8.0
Sirsa	97–98	20	0 3 ± 0.13abcd	0.4 ± 0.13abcd	0	0	0.7	1.85	2.55

Means within a column followed by different letters are significantly different ($P < 0.05$, LSD). ANOVA results Cypermethrin: $F = 36\ 2$; df = 34, 1,145; $P < 0.05$. Fenvalerate: $F = 24.9$, df = 34, 1,144; $P < 0.05$. Deltamethrin $F = 20$; df = 34, 1,146; $P < 0\ 05$ λ-Cyhalothrin· $F = 2.41$, df = 34, 1,146; $P < 0.05$.

with highly contrasting levels of 6,978 and sevenfold resistance to cypermethrin, respectively. Also, resistance ratios to cypermethrin in Buldana were only 23-fold as compared with 7,383 in Akola, which is ≈100 km away. Considering the high mobility of *H. armigera*, it is surprising that resistance was not contiguous. However, it is also possible that dispersal or migration of *H. armigera* occurs only at particular times during or after the cropping season, which eventually influences resistance patterns across the country. The high resistance ratios to deltamethrin and λ-cyhalothrin at Akola and Amaravati, despite low usage of these compounds in the two districts, indicate the likelihood of a positively correlated cross-resistance between the different pyrethroids. Similarly, resistance was reasonably high in regions, subjected to even low to moderate use of pyrethroids. The results suggest that increasing reports of poor field control of *H. armigera* with pyrethroids over large areas in India where insecticide use has been historically low could be due to gene flow through resistant immigrant moths.

Table 4. Pairwise correlation coefficient comparisons

	Pyrethroid use	Cytochrome p450	Esterases
Resistance ratios	$0\ 361^{0\ 05}$ (33)	−0.16 (14)	0.53* (14)
PBO synergism ratios	—	0.55* (14)	−0.41 (14)
Profenofos synergism ratios	—	−0 33 (14)	0.41 (14)

*, significant at $P \leq 0.05$. Degrees of freedom in parentheses.

The combined evidence of synergism bioassays and in vitro enzyme assays indicated that pyrethroid resistance in most parts of India could be due to either enhanced esterase and or monooxygenase activity. Oxidases and esterases were found to be important mechanisms mediating pyrethroid resistance in *H. armigera* in India (Kranthi et al. 1997) and Australia (Gunning 1994). The current results indicate that enhanced synergism by PBO was positively correlated with high levels of cytochrome p450. Clarke et al. (1990) showed that pyrethroid resistance in *H. virescens* was largely due to a PBO-synergizable monooxygenase and that the resistant strains possessed a sixfold greater quantity of total cytochrome p450 than the susceptible strain. However, Kennaugh et al. (1993) reported that PBO-suppressible pyrethroid resistance in *H. armigera* was due to the inhibition of a

Table 5. Influence of piperonyl butoxide (PBO) and profenophos synergists on cypermethrin resistance in field strains of *H. armigera*

District	Collection date	Cypermethrin alone					Cypermethrin + PBO					Cypermethrin + profenophos				
		n^a	LD_{50} (95% CL)[b]	Slope ± SE	RR (95% CL)[c]	χ^2	n	LD_{50} (95% CL)[b]	Slope ± SE	SR (95% CL)[c]	χ^2	n	LD_{50} (95% CL)[b]	Slope ± SE	SR (95% CL)[c]	χ^2
Reading	Dec 1995[e]	240	0 009 (0 007–0 011)	2 0 ± 0 3	—	0 6	290	0 007 (0 004–0 009)	2 4 ± 0 3	1 (0–1)	5 3	290	0 007 (0 004–0 011)	2 2 ± 0 2	1 (0–1)	6 8
	Dec 1998[e]	248	0 006 (0 005–0 008)	2 8 ± 0 5	—	2 1	253	0 006 (0 004–0 007)	3 0 ± 0 4	1 (0–1)	1 0	288	0 006 (0004–0009)	2 8 ± 0 4	1 (0–1)	6 2
	Aug 1999[e]	220	0 008 (0 004–0 014)	2 4 ± 0 3	—	10 4*	288	0 007 (0 004–0 01)	3 2 ± 0 5	1 (0–1)	7 1	286	0 008 (0 006–0 009)	2 8 ± 0 4	1 (0–1)	5 9
Nagpur	Feb 1998	242	2 73 (1 8–4 5)	0 9 ± 0 1	294 (175 495)	1 3	166	0 93 (0 5–1 7)	0 9 ± 0 1	3 (1–6)	1 2	144	0 03 (0 0–0 1)	1 7 ± 0 3	92 (47–178)	1 2
Wardha	Oct 1997	396	0 85 (0 6 1 1)	1 2 ± 0 1	96 (64–142)	7 0	178	0 05 (0 0–0 1)	1 5 ± 0 2	19 (11–34)	3 2	146	0 54 (0 3–0 8)	1 2 ± 0 1	1 (1–3)	3 8
	Feb 1998	170	0 06 (0 0–0 1)	1 9 ± 0 2	7 (5–11)	15 9**	142	0 07 (0 0 0 1)	1 2 ± 0 2	1 (0–2)	3 1	142	0 02 (0 0–0 1)	1 8 ± 0 4	3 (2–5)	0 4
Akola	Jan 1997	164	2 55 (1 9–3 8)	1 6 ± 0 2	257 (172–385)	0 9	144	1 91 (1 3–2 9)	1 5 ± 0 2	1 (1–2)	3 0	238	0 43 (0 3–0 6)	1 8 ± 0 2	6 (3–9)	3 5
	Feb 1998	144	72 2 (31 6–417 0)	0 7 ± 0 1	7,383 (2,870–18,989)	2 8	160	55 87 (18 4–3231 0)	1 1 ± 0 3	1 (0–45)	1 2	164	62 92 (18 6 6,836 0)	1 0 ± 0 3	1 (0–9)	1 8
Amaravati	Feb 1998	169	69 59 (23 5–926 0)	0 5 ± 0 1	6,977 (2,316–21,018)	10 9*	154	12 48 (6 2–40 9)	0 9 ± 0 2	5 (1–20)	2 6	166	16 29 (8 5–58 4)	1 1 ± 0 2	4 (1–16)	1 9
Yavatmal	Feb 1998	148	2 59 (1 6–13 2)	2 0 ± 0 3	255 (168–389)	0 4	212	2 52 (1 5–4 8)	1 1 ± 0 2	1 (0–2)	2 1	174	0 10 (0 1–0 2)	1 6 ± 0 2	23 (13–39)	2 2
Dharwad	Jan 1996	212	0 91 (0 5–3 7)	0 8 ± 0 1	100 (57–175)	5 4	132	0 08 (0 0–0 1)	1 3 ± 0 2	12 (6–25)	3 9	148	0 25 (0 1–0 4)	1 3 ± 0 2	4 (2–7)	6 7
Bangalore	April 1994	212	0 66 (0 4–1 9)	1 2 ± 0 2	72 (41–127)	4 4	124	0 04 (0 0–0 1)	1 9 ± 0 3	16 (8–31)	2 3	144	0 17 (0 1–0 3)	1 7 ± 0 2	4 (2–7)	4 4
	Dec 1995	248	0 54 (0 4–0 9)	1 1 ± 0 1	58 (34–100)	2 0	182	0 05 (0 0–0 1)	2 8 ± 0 6	10 (6–18)	2 6	168	0 15 (0 1–0 2)	1 5 ± 0 2	3 (2–7)	3 5
Coimbatore	Nov 1996	176	2 07 (1 5–2 9)	1 2 ± 0 1	223 (144–346)	2 2	196	0 04 (0 0–0 1)	2 5 ± 0 5	52 (32–83)	0 2	180	1 23 (0 8 1 9)	1 3 ± 0 2	2 (1–3)	3 5
	Mar 1998	168	0 64 (0 2–2 0)	0 7 ± 0 1	69 (39–122)	8 7	160	0 02 (0 0–0 1)	1 8 ± 0 4	38 (18–79)	0 1	172	0 24 (0 1–0 4)	1 4 ± 0 2	3 (1 5)	5 5
Guntur	Nov 1995	176	3 41 (1 7–5 6)	2 4 ± 0 3	362 (255–515)	12 4**	168	1 73 (1 1–2 8)	1 2 ± 0 2	2 (1–3)	1 9	166	3 40 (2 1–6 8)	1 1 ± 0 2	1 (0 2)	1 9
	Dec 1995	214	3 38 (1 5 38 9)	1 1 ± 0 1	365 (224–597)	14 7**	176	2 13 (1 3–3 8)	1 2 ± 0 2	2 (1–3)	3 2	218	2 27 (1 5–3 6)	1 5 ± 0 2	1 (1–3)	0 9
	Oct 1997	260	1 97 (0 9–8 5)	1 0 ± 0 1	227 (144–780)	7 5	160	0 85 (0 5–1 4)	1 4 ± 0 2	2 (1–4)	6 3	196	1 32 (0 8–2 2)	1 2 ± 0 2	2 (1–3)	2 7
	Feb 1998	192	4 70 (3 1–7 0)	1 3 ± 0 2	514 (338–779)	4 0	164	3 52 (2 4–5 9)	1 5 ± 0 2	1 (1–2)	2 3	126	3 54 (2 4 5 8)	1 4 ± 0 0	1 (1–3)	5 4
Kammagar	Feb 1998	216	4 70 (3 1–7 1)	1 1 ± 0 1	507 (297–863)	6 9	148	0 09 (0 0–0 1)	1 7 ± 0 2	51 (27–95)	1 2	196	2 18 (1 4–3 5)	1 4 ± 0 2	2 (1–4)	1 8
Khammam	Feb 1998	144	18 0 (14 8 24 3)	3 1 ± 0 5	1,934 (1,335–2,801)	1 2	144	0 26 (0 1–0 4)	0 9 ± 0 1	67 (38–116)	4 3	224	9 53 (5 3–32 2)	1 3 ± 0 3	2 (1–4)	0 5
Warangal	Feb 1998	177	7 38 (5 0–10 5)	1 4 ± 0 2	789 (508–1,225)	4 8	178	0 29 (0 2–0 5)	1 1 ± 0 1	25 (14–44)	4 6	196	4 95 (3 5–8 1)	1 7 ± 0 3	1 (1–2)	2 9
Prakasam	Feb 1998	144	1 20 (0 6–2 5)	1 4 ± 0 1	128 (84–194)	8 1	182	0 04 (0 0 0 1)	1 7 ± 0 3	27 (15–47)	1 3	180	0 18 (0 1–0 3)	1 5 ± 0 2	7 (4–11)	1 7
Medak	Feb 1998	210	1 08 (0 6–1 7)	1 4 ± 0 1	115 (76–175)	6 8	148	0 05 (0 0–0 1)	1 8 ± 0 3	19 (11–32)	4 7	242	0 21 (0 1–0 3)	1 3 ± 0 2	5 (3 9)	0 8
Rangareddy	Oct 1998	246	0 35 (0 3–0 4)	1 7 ± 0 3	37 (25–54)	9 0*	188	0 03 (0 0 0 1)	1 3 ± 0 2	10 (5 18)	1 6	160	0 32 (0 2–0 5)	1 7 ± 0 2	1 (1–2)	3 5
	Jan 1999	256	1 82 (1 2–5 1)	1 5 ± 0 3	196 (99 389)	1 4	174	0 88 (0 6–1 3)	1 4 ± 0 2	2 (1–4)	4 9	184	0 10 (0 1 0 2)	1 8 ± 0 3	18 (9–38)	2 5
Sirsa	Nov 1998	159	0 54 (0 2 1 0)	1 4 ± 0 1	56 (37–84)	10 7*	160	0 06 (0 0–0 1)	1 2 ± 0 2	9 (5–17)	2 7	168	0 11 (0 1–0 2)	1 2 ± 0 1	5 (3 8)	2 1
Varanasi	Oct 1993	168	0 44 (0 3–0 7)	1 1 ± 0 1	47 (27–80)	2 5	154	0 05 (0 0–0 1)	1 3 ± 0 2	9 (0–181)	3 6	140	0 12 (0 1–0 2)	1 3 ± 0 2	4 (2–7)	2 7
	Dec 1994	174	0 41 (0 3 0 7)	1 1 ± 0 1	44 (26–74)	2 5	160	0 04 (0 0–0 1)	1 5 ± 0 2	11 (6–22)	2 5	140	0 10 (0 0–0 2)	1 3 ± 0 2	4 (2–8)	5 4
Bhatinda	Nov 1998	240	0 38 (0 1–1 1)	1 3 ± 0 1	39 (25 59)	11 4**	148	0 28 (0 1–0 5)	1 1 ± 0 2	1 (1–2)	5 1	224	0 03 (0 0 0 1)	2 5 ± 0 5	11 (7–18)	0 8

*, Chi square significant ($P < 0.05$), **, significant ($P < 0.01$)

[a] Numbers tested

[b] In micrograms cypermethrin per third instar larva

[c] RR (resistance ratio) and 95% CL calculated by the method of Robertson and Preisler (1992) relative to the 'Susceptible Reading strain-Dec '95'

[d] SR (synergism ratio, LD of insecticide alone divided by LD of insecticide plus synergist) and 95% CL calculated by the method of Robertson and Preisler (1992)

[e] Date tested

Table 6. In vitro enzyme titres and nerve insensitivity of *H. armigera* field strains

District	Collection date	Mean ± SD In vitro enzyme titres		Cypermethrin nerve insensitivity				
		Cytochrome p450 (p Mol/mg protein)	Esterases (μMol/min/mg protein)	n^a	EC_{50} (nM, 95% FL)[b]	Slope ± SE	χ^2	RR (95% CL)[c]
Reading	Dec 1998[d]	183 ± 24d	1 24 ± 0.1d	25	0 039 (0 02–0 06)	2.1 ± 0.4	0 07	—
	Aug 1999[d]	187 ± 22d	1 33 ± 0 0d	40	0 028 (0 02–0.04)	2 3 ± 0.3	0.02	—
Nagpur	Feb 1998	198 ± 7cd	5 30 ± 1 1a	40	2325 (7 08–126 00)	0 7 ± 0.1	5 98	622 (271–1,429)
Wardha	Sept 1993	362 ± 14ab	2.76 ± 0 6bc					
	Nov 1993	231 ± 15cd	3 73 ± 0.5b					
	Oct 1994	182 ± 11d	1 93 ± 0.5cd					
	Nov 1995	237 ± 23cd	2.95 ± 0.5bc					
	Feb 1998	237 ± 19cd	2 71 ± 0.6c					
Akola	Sept 1997	294 ± 18bc	4.77 ± 0 8a					
	Feb 1998	214 ± 15cd	5 62 ± 0.7a	40	68 06 (19.24–13,68 0)	0.6 ± 0 1	5.94	1,771 (637–4,923)
Amaravati	Feb 1998	269 ± 36bc	5.12 ± 0 8a	40	38.08 (11 90–272 36)	0.6 ± 0.1	5 39	994 (396–2,495)
Yavatmal	Feb 1998	193 ± 26cd	3 88 ± 0.4ab					
Bangalore	April 1994	352 ± 34ab	2.22 ± 0 5cd					
	Dec 1995	353 ± 38ab	4 60 ± 0.7ab					
Coimbatore	April 1994	354 ± 24ab	2.80 ± 0.1bc					
	Nov 1994	360 ± 36ab	1.95 ± 0 3cd					
	Sept 1995	382 ± 14ab	1 76 ± 0 3cd					
	Nov 1996	394 ± 22ab	2 99 ± 0 3bc					
Guntur	Dec 1993	212 ± 21cd	1 67 ± 0.4cd					
	Nov 1994	193 ± 20cd	1 39 ± 0 2d					
	Dec 1995	172 ± 19d	1 39 ± 0.3d					
	Feb 1998	206 ± 23cd	1 45 ± 0.2d	40	91 42 (26 78–1,421.80)	0 7 ± 0 1	8 20	2,414 (840–6,940)
Karimnagar	Feb 1998	339 ± 20ab	1.78 ± 0 1cd	40	27 74 (6 13–351 47)	0 6 ± 0 1	7 16	724 (271–1,933)
Khammam	Feb 1998	259 ± 31c	2.33 ± 0 6cd					
Warangal	Feb 1998	328 ± 24b	1 98 ± 0 8cd	40	20 72 (6 62–67.98)	0.9 ± 0 1	6 33	503 (241–1,051)
Prakasam	Feb 1998	278 ± 19bc	2 44 ± 0 3c					
Medak	Feb 1998	249 ± 26cd	2 76 ± 0.2bc					
Rangareddy	Oct 1993	397 ± 18a	2.50 ± 0 3c					
	Dec 1993	233 ± 20cd	3.34 ± 1.4bc					
	Mar 1994	285 ± 19cd	4.70 ± 0.7ab					
	Oct 1994	359 ± 22ab	1 94 ± 0 5cd					
	Nov 1995	218 ± 19cd	2 32 ± 0 5cd					
	Aug 1996	381 ± 31ab	2 63 ± 0 3c					
	Feb 1998	252 ± 13c	3 77 ± 1.0b					
Sirsa	Nov 1993	378 ± 46ab	1 87 ± 0.5cd					
Varanasi	Oct 1993	313 ± 25bc	3 08 ± 0 4bc					
	Dec 1994	204 ± 18cd	4.38 ± 1.1ab					

ANOVA results Cytochrome p450. $F = 9\ 22$; df = 34, 70, $P < 0.05$ Esterase $F = 120$, df = 34, 70, $P < 0\ 05$. No χ^2 values significant at $P = 0\ 05$ level

Means within a column followed by different letters are significantly different ($P < 0\ 05$, LSD)

[a] Numbers tested

[b] EC = effective concentration expressed as nM/larva.

[c] RR (resistance ratio) and 95% CL calculated by the method of Robertson and Preisler (1992) relative to the 'Reading strain–Dec '98'

[d] Testing date

cytochrome p450-dependent penetration resistance and was not associated with enhanced cytochrome p450 content. Hence, it was argued that PBO-suppressible pyrethroid resistance was not necessarily an indication of cytochrome p450-mediated resistance. This view was further strengthened by Gunning et al. (1998) who demonstrated that PBO could also suppress esterase-mediated pyrethroid metabolism in *H. armigera*. We could not find a positive association between PBO-suppressible pyrethroid resistance and esterase activity in the resistant field strains. Hence we are inclined to infer that PBO-suppressible resistance indicates the importance of at least cytochrome p450 mediated metabolism in pyrethroid resistant *H armigera* strains. Profenofos-suppressible pyrethroid resistance was correlated with esterase activity. The nonsignificance of the correlation was because of some central Indian strains, which possessed nonsynergizable pyrethroid resistance but had the highest esterase activity. Esterase activity was significantly correlated with resistance ratios, and may be used as an indicator of pyrethroid resistance in field populations. Gunning et al. (1996) reported that resistant in *H. armigera* was positively correlated with esterase titers and that increasing resistance was accompanied by increasing esterase activity. They also showed that pyrethroid-resistant *H. armigera* had approximately up to 50-fold higher esterase activity compared with susceptible populations.

Interestingly, a few strains from the same location but collected at different times in the year, exhibited different mechanisms. PBO synergism was inconsistent over a period of time in some regions. It was reported earlier that PBO synergism decreased toward the end of cropping season in the Hyderabad region (Armes et al. 1996) and central India (Kranthi et al. 1997). Though synergism bioassays and in vitro enzyme assays indicated that metabolic detoxification

was an important pyrethroid resistance mechanism, the fact that full suppression of resistance was never achieved in any of the strains suggests that metabolic detoxification was probably only one of two or more mechanisms conferring pyrethroid resistance. A non-PBO-synergisable component in pyrethroid-resistant *H. armigera* was attributed to the presence of nerve-insensitivity or penetration mechanisms or a combination of both (Gunning et al. 1995). As penetration resistance usually only confers a low order resistance (Gunning et al. 1995), it is likely that nerve-insensitivity is the major component of the nonsynergisable resistance. High levels of nerve insensitivity in the Guntur, Amaravati, and Akola strains were associated with nonsynergisable resistance. Nerve insensitivity in *H. armigera* was also demonstrated to occur at varying degrees in *H. armigera* strains collected in 1992 from Maharashtra and Andhra Pradesh (West and McCaffery 1992), China (McCaffery et al. 1997), and Australia (Gunning et al. 1995).

The frequency of the nerve-insensitivity gene is expected to increase in field populations with continuous pyrethroid selection pressure. Because this mechanism is the most difficult to eradicate, unless appropriate management strategies are devised to further reduce selection pressure, pyrethroid resistance may become more unmanageable in the foreseeable future. Reports from Australia (Forrester et al. 1993) point out that a significant reduction in pyrethroid selection pressure resulted in a shift in pyrethroid resistance mechanisms from nerve insensitivity to oxidative metabolism. Thus, reduction in pyrethroid selection pressure on *H. armigera* could play an important role in diluting the contribution of nerve-insensitivity to pyrethroid resistance in India as well.

The development of resistance calls for a management strategy to restrict pyrethroid use and to promote greater emphasis on the use of alternatives to insecticides. Much of the pest management problem in India is due to the ever-increasing number of insecticide brands, spurious insecticide use, and lack of proper recommendations (Armes et al. 1994) that put farmers in a quandary. In addition, resistance to insecticides compounds the problem by increasing the need for repeated spray applications, which destabilizes the cotton ecosystem. Farmers attribute poor pest control to sub-standard or spurious insecticide formulations. Providing timely information on resistance certainly can help curtail the development of resistance to insecticides in regions where the problem is more acute. Because LD_{50} slopes of probit regression lines of the field strains indicate a high level of heterogeneity in population response to pyrethroids, it is anticipated that the frequency of resistant individuals would increase rapidly in field populations after only a few pyrethroid applications. Thus, avoidance of pyrethroids on the first few generations of *H. armigera* in cotton and restricting use to later generations of bollworms may help in preventing the resistance problem in India.

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