MECHANISMS OF RESISTANCE TO PYRETHROIDS IN LABORATORY AND FIELD STRAINS OF *HELIOTHIS VIRESCENS*[1]

A.R. McCaffery, R.T. Gladwell, H. El-Nayir,
C.H. Walker, J.N. Perry and M.J. Miles[2]

ABSTRACT

Resistance to cis-cypermethrin was examined in a range of laboratory and field strains of *Heliothis virescens* (F.). The PEG87 laboratory strain and the DuPont field strain which was reared in the laboratory for a number of generations were both highly resistant to cypermethrin whilst the other field strains were generally less tolerant. In all but the PEG87 strain, larvae were more resistant in the first instar than in the third instar. Resistance in the field strains was especially weak in larger larvae. Use of the synergist piperonyl butoxide (PBO) indicated that the PEG87 laboratory strain and the DuPont strain possessed a resistance mechanism based on enhanced monooxygenase activity and that this was particularly strongly expressed in the PEG87 strain. The mechanism was absent from the Snook, Hearne and Itta field strains examined. A proportion of individuals from the DuPont, PEG87 and all the field strains displayed nerve insensitivity to pyrethroid action. The DuPont strain was comparatively homogeneous with respect to nerve insensitivity with a high proportion of the insects being resistant. It is concluded that nerve insensitivity is widely distributed in the field strains and confers considerable tolerance to first instar insects. The monooxygenase resistance likewise confers high tolerance to first instar insects. Larvae with a combination of both major mechanisms probably possess high tolerance to the pyrethroid throughout larval life.

INTRODUCTION

Resistance to the synthetic pyrethroids in field populations of the tobacco budworm *Heliothis virescens* has been reported from a number of locations throughout the USA (Luttrell et al. 1987, Graves et al. 1989; Plapp et al. 1989). This resistance is known to be multifactorial and involves a delayed penetration of the toxicant, increased metabolism and nerve insensitivity (Nicholson and Miller 1985, Little et al. 1989, McCaffery et al. 1989a, Gladwell et al. 1990). Regular monitoring of field populations is now carried out to give information on changes in the frequency and distribution of resistant insects (Staetz et al. 1989, Rodgers

[1]Lepidoptera: Noctuidae

[2]School of Animal and Microbial Sciences, University of Reading, Whiteknights, P.O. Box 228, Reading RG6 2AJ, UK.

et al. 1990) and is an essential feature of management of the resistance. Nevertheless, the ability to distinguish between the major mechanisms of resistance in a monitoring programme should enhance the effectiveness of any strategy to manage the insects. As part of a research programme aimed at the development of diagnostic assays for use in such resistance monitoring programmes we have examined and compared the mechanisms of resistance in a range of laboratory and field strains of the insect.

Initial detailed studies with laboratory strains emphasised the importance of the monooxygenase system (Lee et al. 1989, Little et al. 1989, Walker et al. 1990) in resistance and this has been confirmed using the inhibitor piperonyl butoxide (PBO). Here we report on the use of synergist assays with PBO to indicate the presence of this mechanism. There is some evidence for the involvement of esterases in resistance to pyrethroids in *H. virescens* (Little et al. 1989) and, in some of the strains, we have used the B-esterase inhibitor DEF as an indicator of the mechanism. The primary target of the pyrethroids in insects is the nervous system (Soderlund and Bloomquist 1989) and there is evidence that nerve insensitivity is a major mechanism of resistance in this species (Nicholson and Miller 1985, McCaffery et al. 1989a, Gladwell et al. 1990). We have used the rapid neurophysiological assay developed by Gladwell et al. (1990) to confirm its presence.

MATERIALS AND METHODS

Insects. A susceptible laboratory strain (BRC) and a resistant laboratory strain (PEG87) of *Heliothis virescens* were obtained from ICI Agrochemicals, Jealott's Hill, UK and ICI Americas Inc., Goldsboro, USA, respectively and bred in continuous culture in Reading in a similar manner to that described previously (McCaffery et al. 1989b). The PEG87 strain was selected at each generation by exposing first instar larvae to residues of cypermethrin obtained by dipping cotton leaves in aqueous solutions of 500 ppm of Cymbush (11% E.C.) as described below (see foliar residue bioassay). The selected insects were used for breeding whilst for the bioassays and tests described in this paper unselected insects were used.

A resistant field strain (DuPont) was collected from the Imperial Valley, California and reared in the laboratory for a number of generations with a selection involving topical exposure of the third instar larvae with 35 to 45 ppm cypermethrin. Pupae of this strain were then supplied by DuPont and the insects reared in Reading.

Pupae or eggs of three other strains of the insects from cotton crops were supplied to us directly from the field. The Snook strain was obtained from the Texas Brazos River Valley region and shipped to us as eggs on cotton leaves by Wellcome Environmental Health. The Hearne and Itta Bena strains were collected from cotton in Texas and Mississippi respectively and shipped to Reading as pupae by ICI Americas Inc. Except where stated the first and second laboratory strains of these insects were used for the tests described in this paper. Breeding stocks of these insects were not selected with insecticides.

Insecticides. An 11% E.C. formulation of cypermethrin ('Cymbush') was supplied by ICI Agrochemicals, Jealott's Hill, UK and technical grade cis-cypermethrin (98.4%) was supplied by Shell Research Limited Sittingbourne, UK

and ICI Agrochemicals, Jealott's Hill, UK. Piperonyl butoxide (2-(2-butoxyether)-ethyl-6-propyl piperonyl ether) was supplied both as technical (98%) material and as an 8% E.C. formulation ('Butacide') by Wellcome Environmental Health, Berkhamsted, UK). The esterase inhibitor DEF (S,S,S,-tributyl phosphorothioate) (99%) was obtained from Mobay Chemical Corporation. An emulsifier blend consisting of Fairmul no.1 and Fairmul no.3 was supplied by Wellcome Environmental Health for use as a wetter in the foliar residue assays.

Foliar Residue Insecticide Bioassay. Leaves from 4 wk old cotton plants were dipped in aqueous dilutions of the cypermethrin E.C. and allowed to dry. In all but initial assays with the BRC and PEG87 strains an emulsifier blend was added as a wetter at a final concentration of 0.02% in each dilution including controls. Newly hatched first instar larvae (< 6 h) were placed in groups of five in 30 ml plastic pots. A treated leaf was placed over the top of each pot and a snap-on lid closed over the leaf and pot. Larvae thus had access to the treated leaf disc so formed (McCaffery et al. 1989a,b). Eight replicates (each of five larvae) were prepared for each treatment and for controls. The pots were held at a temperature of 25°C and a photoperiod of 14 h. Mortality was assessed after 48 h.

Third Instar Topical Insecticide Bioassay. Serial dilutions of technical grade cis-cypermethrin in acetone were prepared such that each was one-half or one-quarter of the previous concentration and 1ul drops (of concentrations up to 500ug/ul) were applied to the mesothorax of test insects weighing between 19 and 24 mg (McCaffery et al. 1989b). In some experiments doses of 1000ug/larva were required and to achieve this 2ul of a 500ul/ul solution were applied (Solubility of cis-cypermethrin in acetone 620g/L (Worthing and Walker, 1987)). Control insects were treated with acetone alone. For each control and treatment group at least four replicates, each of ten insects were used (except where stated). The insects were fed on a bean/wheatgerm/ alfalfa diet and mortality assessed after 72 h as described previously (McCaffery et al. 1989a).

Foliar Residue Synergist Assay. The first instar foliar residue assay was performed using cotton leaves treated with combinations of 0.1% or 1.0% formulated piperonyl butoxide ('Butacide') and cypermethrin ('Cymbush') in a similar manner to that described above. Preliminary trials had shown that PBO at these concentrations was not toxic to the larvae (McCaffery et al. 1989a)

Third Instar Topical Synergist Assay. Acetone solutions of technical grade piperonyl butoxide and DEF were each prepared to give concentrations of 20ug/ul. Third instar test insects (weighing 19 to 24 mg) were treated on the mesothorax with 1ul drops (20ug) of one of the synergist solutions. Control insects were treated with acetone alone. After 30 min the insects were treated with a range of concentrations of cis-cypermethrin as described above and mortality of both synergised and unsynergised treatment groups assessed after 72 h. Pre-treatment with the synergist was used in order to be certain that the enzyme system was fully inhibited before treatment with the insecticide.

Analysis of Insecticide and Synergist Bioassays. Control mortality was rare, never exceeding 2.5%, and was corrected by using Abbott's formula (Abbott 1925). The insecticide and synergist bioassay results were examined manually using log/probit plots before being analysed by probit analysis (Finney 1971) using SAS software (SAS Institute 1985).

Neurophysiological Assay for Nerve Insensitivity. The effects of cis-

cypermethrin on the spontaneous multiunit (multiple nerve cell) activity of nerves from third instar larvae of each of the strains was measured using the technique described in detail by Gladwell et al. (1990) in which a 'cumulative dose response' assay protocol was adopted. Briefly, the assay method was as follows:

Insecticide. All the neurophysiological assays were carried out in lepidopteran saline (Weevers 1966) at 25 $\pm$ 0.5°C. A stock solution of 1mM technical cis-cypermethrin in acetone was made and diluted directly into saline to give a final range of concentrations of 10^{-9} to 10^{-7}M.

Measurement of Spontaneous Multiunit Activity. Third instar test larvae (weighing 19 to 24 mg) were decapitated, opened dorso-medially and pinned out on a layer of Sylgard (Dow Corning). The inner surface of the body wall with its associated nervous system was exposed by dissection and bathed in saline as detailed by Gladwell (1990). A peripheral nerve was picked up with a 27 gauge stainless steel, suction electrode with insulated external surfaces. This recording electrode was connected to a high gain, low noise amplifier and conditioning system (Neurolog, Digitimer Ltd.). Neural activity was monitored on an oscilloscope and recorded on magnetic tape for later analysis. Nerve action potentials were identified by amplitude discrimination (an electronic method of distinguishing true action potentials from background noise on the basis of spike height) and a microcomputer used to record their frequency as described by Gladwell et al. (1990).

Cumulative Dose Response Assay. Briefly, the number of action potentials in each successive 15 s period over a 5 min control period in cypermethrin-free saline was first recorded. The saline was then aspirated, replaced with fresh saline containing 10^{-9}M cis-cypermethrin, washed once and the recording continued. At 5 min intervals the preparation was washed and immersed in fresh saline containing increasing concentrations of the insecticide. The end point of the assay was defined as the lowest concentration at which the frequency of action potentials was over five times greater than the mean value during the pre-treatment control period (Gladwell 1990).

RESULTS AND DISCUSSION

First Instar Foliar Residue Assays. Both the PEG87 and the DuPont strains required high concentrations of residual cypermethrin to achieve equivalent levels of mortality to those seen with the susceptible BRC strain (Table 1). Resistance factors of 1000 to 1871 at LC_{50} were found with the various substrains of the PEG87 strain whilst with the DuPont strain this value was 677. The resistance factors seen at the LC_{90} level showed considerable divergence between the two resistant strains. The slope of the log dose/mortality probit line obtained with the PEG87 insect assays was considerably steeper than that found with the DuPont strain insects (Table 1), suggesting a greater degree of heterogeneity in the DuPont strain.

All three field strains showed substantial levels of resistance as indicated by the high LC_{50} and LC_{90} values and by the resistance factors (Table 2). The ranking of the strains from least to most resistant appeared to be Hearne<Itta<Snook although it was not always possible to test identical generations. A repeated assay with the third (unselected) generation of the Snook strain indicated some loss of resistance under laboratory conditions (Table 2).

TABLE 1. Mortality of First Instar Larvae of Various Laboratory Strains of *Heliothis virescens* Exposed to Dry Residues of Cymbush (11% e.c Cypermethrin) for 48 h.

Strain[a]	n	LC_{50} (ppm) (95%CI)	RF[b]at LC_{50}	LC_{90} (ppm) (95%CI)	RF[b]at LC_{90}	Slope+SE
BRCg/7	200	0.31 (0.15-0.46)	-	1.3 (0.83-4.0)	-	2.05+0.5
PEG87b/3	280	540 (350-770)	1742	5300 (3300-12000)	4077	1.29+0.18
PEG87d/1	280	310 (220-400)	1000	1800 (1200-3300)	1385	1.65+0.22
PEG87e/2	278	580 (430-760)	1871	2600 (1700-5400)	2000	1.98+0.32
PEG87g/10	290	440 (340-570)	1419	2700 (1400-3600)	2077	1.91+0.24
DuPont/1	320	210 (110-680)	677	8100 ($1800\text{-}1.4x10^5$)	6231	0.81+0.14

[a]Strain = strain name/generation number.
[b]RF = Resistance Factor = $LC_{50\ or\ 90}$ Resistant strain/$LC_{50\ or\ 90}$ Susceptible strain.

Third Instar Topical Assays. The PEG87 strain was very highly resistant in comparison to the BRC strain, especially at higher LD levels (Table 3), although there appeared to be considerable variation in the resistance between different substrains. Moderate levels of resistance were found in the DuPont strain (Table 3) which increased with selection with cypermethrin (Table 6).

The third instar topical assay with the field strains indicated only marginal levels of resistance with RF values of between 6 and 15. At the LD_{50} level the ranking of the strains from least to most resistant appeared to be Snook<Itta<Hearne (Table 4) whilst at the LD_{90} level this changed to Itta<Hearne<Snook, although these differences are not significant. Laboratory rearing of the Snook strain resulted in some loss of heterogeneity and a gradual reduction of the LD_{90} value (Table 4).

First Instar Synergist Assays. In the first trial with first instar larvae the synergistic effect obtained using 1% piperonyl butoxide was around two to three fold greater than that with 0.1% piperonyl butoxide (Table 5). The former concentration was used in the remaining tests. In each test with PEG87 larvae in which residual cypermethrin + 1% piperonyl butoxide was used the LD_{50} values were reduced by over 90% compared to those obtained with cypermethrin alone.

TABLE 2. Mortality of First Instar Larvae of a Susceptible Laboratory Strain and Various Field Strains of *Heliothis virescens* Exposed to Dry Residues of Cymbush (11% e.c Cypermethrin) for 48 h.

Strain[a]	n	LC_{50} (ppm) (95%CI)	RF[b] at LC_{50}	LC_{90} (ppm) (95%CI)	RF[b] at LC_{90}	Slope+SE
BRCg/7[c]	200	0.31 (0.15-0.46)	-	1.3 (0.83-4.0)	-	2.05+0.53
Snook/1	305	98 (76-120)	316	410 (290-680)	315	2.07+0.28
Snook/3	264	58 (46-71)	187	170 (130-250)	131	2.73+0.37
Hearne/2	320	19 (15-25)	61	110 (67-200)	85	1.72+0.19
Itta/2	240	70 (53-89)	226	310 (220-510)	238	2.01+0.27

[a]Strain = strain name/generation number.
[b]RF = Resistance Factor = $LC_{50 \text{ or } 90}$ Resistant strain/$LC_{50 \text{ or } 90}$ Susceptible strain.
[c]BRC strain against which 1989 field strains have been compared to provide RF values.

Similar synergistic effects were seen at the LD_{90} level. A rather similar degree of synergism was seen when DuPont strain first instar larvae were exposed to piperonyl butoxide/cypermethrin residues (Table 5).

Third Instar Synergist Assays. With the BRC strain, slight reductions in the LD_{50} and LD_{90} values were seen when synergists were used as compared to the values obtained with the insecticide alone (Table 6). In contrast, with the highly resistant PEG87 strain (substrain D), there was a marked synergism with PBO which resulted in synergist ratios of 228 and 590 at the LD_{50} and LD_{90} levels respectively. DEF mildly synergised the action of cis-cypermethrin in these insects.

With the DuPont strain little or no synergism of the insecticide was seen with DEF but PBO, on the other hand, gave a moderate synergism when tested with both the first and second generations of the insects.

Snook and Hearne strain third instar larvae were pre-treated with PBO or DEF (Snook only) prior to treatment with cis-cypermethrin as above. Neither material gave any synergism with this insecticide (Table 7). Similar discriminating dose tests with cypermethrin and PBO using the Itta strain (data not shown) gave similar results.

Neurophysiological Assays for Nerve Insensitivity. The neuronal response to cis-cypermethrin was examined in a range of strains and generally conformed

TABLE 3. Mortality of Third Instar Larvae of Various Laboratory Strains of *Heliothis virescens* 72 h after Topical Treatment with cis-Cypermethrin.

Strain[a]	n	LD_{50} (ug/larva) (95%CI)	RF[b] at LD_{50}	LD_{90} (ug/larva) (95%CI)	RF[b] at LD_{90}	Slope+SE
BRCb/1[c]	267	0.017 (0.013-0.022)	-	0.071 (0.046-0.14)	-	2.06+0.20
PEG87b/2	97	83 (40-1100)	4882	820 ($190-5.9x10^5$)	11549	1.29+0.43
BRCf/1[d]	174	0.013 (0.006-0.024)	-	0.16 (0.074-0.56)	-	1.16+0.18
PEG87d/3	320	910 (470-3400)	70000	[f]$6.2x10^4$ ($1.1x10^4$-$3.2x10^6$)	387500	0.70+0.14
BRCf/9[e]	234	0.009 (0.007-0.012)	-	0.040 (0.026-0.077)	-	1.95+0.25
DuPont/1	337	0.47 (0.31-0.70)	52	13 (6.0-43)	320	0.89+0.12
DuPont/2	273	0.67 (0.46-0.99)	74	10 (5.0-31)	250	1.10+0.15

[a]Strain = strain name/generation number.
[b]RF = Resistance Factor = $LD_{50\ or\ 90}$ Resistant strain/$LD_{50\ or\ 90}$ Susceptible strain.
[c]BRC strain against which 1987 tests with PEG87 have been compared to provide RF values.
[d]BRC strain against which 1988 and 1989 tests with PEG87 have been compared to provide RF values.
[e]BRC strain against which 1989 DuPont and Field strains have been compared to provide RF values.
[f]Extrapolated values.

to a consistent pattern. A variable period of latency was followed by a transient rise in the frequency of action potentials. This, in turn was followed by a decline in the frequency or by nerve block.

A total of 13 out of 14 susceptible BRC preparations became hyperactive when challenged with 10^{-9}M cis-cypermethrin (Table 8). The remaining larva responded at the next highest concentration. A considerably more heterogeneous response was obtained with the PEG87 strain preparations. One third of the larvae responded at the lowest concentration used whilst others

TABLE 4. Mortality of Third Instar Larvae of a Susceptible Laboratory Strain and Various Field Strains of *Heliothis virescens* 72 h after Topical Treatment with cis-Cypermethrin.

Strain[a]	n	LD_{50} (ug/larva) (95%CI)	RF[b] at LD_{50} (95%CI)	LD_{90} (ug/larva)	RF at LD_{90}	Slope+SE
BRCf/9[c]	234	0.009 (0.007-0.012)	-	0.040 (0.026-0.077)	-	1.95+0.25
Snook/1	321	0.064 (0.045-0.09)	7	0.59 (0.38-1.1)	15	1.33+0.14
Snook/2	243	0.050 (0.032-0.73)	6	0.6 (0.34-1.4)	15	1.19+0.16
Snook/2	281	0.081 (0.061-0.10)	9	0.42 (0.30-0.65)	11	1.80+0.19
Snook/3	246	0.070 (0.05-0.09)	8	0.34 (0.24-0.56	9	1.87+0.23
Hearne/1	114	0.10 (0.071-0.15)	11	0.43 (0.26-1.0)	11	2.01+0.34
Itta/2	320	0.098 (0.078-0.12	11	0.32 (0.25-0.45)	8	2.49+0.29

[a]Strain = strain name/generation number.
[b]RF = Resistance Factor = $LD_{50\ or\ 90}$ Resistant strain/$LD_{50\ or}$ LD_{90} Susceptible strain.
[c]BRC strain against which 1989 field strains have been compared to provide RF values.

responded at higher concentrations and 22% remained unaffected by the highest concentrations of up to 10^{-7}M. In contrast, the DuPont strain gave a much more homogeneous response profile with 10 out of 13 (77%) failing to respond at all or only at the highest concentration (Table 8).

With the Hearne strain 60% (9 out of 15) of the larval preparations responded at the two lowest concentrations whilst 20% responded only at the highest concentration (10^{-7}M)(Table 8). In contrast, the response profile for the Snook strain was flat with approximately similar numbers responding at all the concentrations across the range.

The laboratory strains examined here represent extremes of tolerance from the fully susceptible BRC strain to the very highly resistant PEG87 strain. This latter strain was created from collections from 19 sites across the United States

TABLE 5. Mortality of First Instar Larvae of Two Resistant Strains of *Heliothis virescens* Exposed to Dry Residues of Combinations of Cymbush (11% e.c Cypermethrin) and Butacide (8% e.c. Piperonyl Butoxide) for 48 h.

Materials used	n	LC_{50} (ppm) (95%CI)	SR[a] at LC_{50}	LC_{90} (ppm) (95%CI)	SR[a] at LC_{90}	Slope+SE
Trial 1 - PEG87e/2 strain[b]						
Cymbush	278	580 (430-760)	-	2600 (1700-5400)	-	1.98+0.32
Cymbush+ 0.1% PBO	280	120 (90-160)	4.8	620 (400-1300)	4.2	1.66+0.26
Cymbush+ 1.0% PBO	277	43 (25-58)	13.5	220 (150-390)	11.8	1.91+0.24
Trial 2 - PEG87f/1 strain						
Cymbush	240	690 (520-950)	-	4100 (2400-10000)	-	1.66+0.26
Cymbush+ 1.0% PBO	275	41 (33-51)	16.8	150 (110-220)	27.3	2.34+0.28
Trial 3 - DuPont/4 strain						
Cymbush	320	420 (300-620)	-	3900 (2100-10000)	-	1.33+0.17
Cymbush+ 1.0% PBO	240	17 (8.3-29)	24.7	270 (160-670)	14.4	1.07+0.17

[a]SR = Synergist Ratio = $LC_{50\ or\ 90}$ insecticide alone/$LC_{50\ or\ 90}$ insecticide + synergist.
[b]Strain = strain name/generation number.

cotton belt (from 1978 onwards) where control with synthetic pyrethroids had become increasingly difficult. In terms of tolerance, the DuPont strain, originatingfrom the Imperial Valley lies between these extremes. The other field strains examined here were generally less resistant than either the PEG87 or the Dupont strains. However, as discussed below, it is clear that these differences are not the result of simple changes in the degree of expression of a single mechanism.

With the exception of the highly resistant PEG87 laboratory strain, all of the strains examined in this study appear more resistant at the first instar than at the third instar. This is consistent with the view that the first instar foliar residue assay is an especially sensitive test for the detection of resistance in many strains as suggested previously (McCaffery et al. 1989a). The PEG87 and DuPont strains

TABLE 6 Effect of Pre-Treatment with Piperonyl Butoxide and/or DEF on Mortality of Third Instar Larvae of Various Laboratory Strains of *Heliothis virescens* 72 h after Topical Treatment with cis-Cypermethin.

Materials used	n	LD_{50} (ug/larva) (95%CI)	SR[a] at LD_{50}	LD_{90} (ug/larva (95%CI)	SR[a] at LD_{90}	Slope+SE
BRCe/3 strain[b]						
cis-cyp	331	0.003 (0.002-0.0037)	-	0.018 (0.012-0.028)	-	1.80+0.23
cis-cyp +PBO	280	0.0008 (0.0005-0.001)	3.8	0.005 (0.004-0.009)	3.6	1.60+0.21
cis-cyp +DEF	280	0.0005 (0.0003-0.0008)	6.0	0.004 (0.003-0.006)	4.5	1.65+0.23
PEG87d/3 strain						
cis-cyp	320	910 (470-3400)	-	[c]$6.2x10^4$ ($1.1x10^4$-$3.2x10^6$)	-	0.70+0.14
cis-cyp +PBO	280	4.0 (2.7-6.7)	228	110 (45-597)	590	0.93+0.16
cis-cyp +DEF	320	120 (72-240)	7.6	[c]7600 (1800-$3.1x10^5$)	8.2	0.71+0.16
DuPont/1 strain						
cis-cyp	337	0.47 (0.31-0.70)	-	13 (6.0-43)	-	0.89+0.12
cis-cyp +PBO	292	0.060 (0.040-0.090)	7.8	1.0 (0.50-3.6)	13	1.05+0.16
cis-cyp DEF	252	0.37 (0.23-0.65)	1.3	9.8 (3.6-66)	1.3	0.90+0.15
DuPont/2 strain						
cis-cyp	273	0.67 (0.46-0.99)	-	10 (5.0-31)	-	1.10+0.15
cis-cyp +PBO	246	0.063 (0.040-0.092)	11	1.0 (0.51-3.8)	10	1.11+0.18

[a]SR = Synergist Ratio = $LD_{50\ or\ 90}$ insecticide alone/$LD_{50\ or\ 90}$ insecticide + synergist.
[b]Strain=strain name/generation number.
[c]Extrapolated values.

were highly resistant at first instar and in the case of the DuPont strain this was especially so at the LD_{90} level. In contrast, third instar PEG87 strain larvae were enormously more resistant than similar DuPont larvae when compared to the susceptible BRC reference strain. Despite being very resistant at the firstinstar the tolerance of all the field strains to cis-cypermethrin in the third instar was slight with resistance factors of between 6 and 15 when compared with the BRC reference strain. The explanation for these discrepancies is likely to lie in the

TABLE 7. Effect of Pre-Treatment with Piperonyl Butoxide or DEF on the Mortality of Third Instar Larvae of Various Field Strains of *Heliothis virescens* 72 h after Topical Treatment with cis-Cypermethrin.

Materials used	n	LD_{50} (ug/larva) (95%CI)	SR[a] at LD_{50}	LD_{90} (ug/larva) (95%CI)	SR[a] at LD_{90}	Slope+SE
Snook/2 strain						
cis-cyp	321	0.064 (0.045-0.086)	-	0.59 (0.38-1.1)	-	1.33+0.14
cis-cyp +PBO	256	0.049 (0.038-0.071)	1.3	0.29 (0.17-0.79)	2.0	1.66+0.25
Snook/3 strain						
cis-cyp	246	0.070 (0.05-0.09)	-	0.34 (0.24-0.56)	-	1.87+0.23
cis-cyp +DEF	244	0.22 (0.17-0.30)	0.3	0.97 (0.61-2.2)	0.4	1.98+0.29
Hearne/1 strain						
cis-cyp	114	0.10 (0.071-0.15)	-	0.43 (0.26-1.0)	-	2.01+0.34
cis-cyp +PBO	115	0.12 (0.076-0.19)	0.8	0.77 (0.38-3.4)	0.6	1.55+0.31
Hearne/2 strain						
cis-cyp	119	0.056 (0.035-0.082)	-	0.29 (0.17-0.92)	-	1.78+0.38
cis-cyp +PBO	64	0.035 (0.015-0.057)	1.6	0.17 (0.095-0.73)	1.7	1.91+0.53

[a]SR = Synergist Ratio = $LD_{50\ or\ 90}$ insecticide alone/$LD_{50\ or\ 90}$ insecticide + synergist.
[b]Strain=Strain name/generation number.

occurrence and expression of the various resistance mechanisms and emphasises the need to monitor populations for them.

Synergist studies have been used to indicate the presence of resistances based on enhanced metabolism of the toxicant. Our use of the synergist DEF was not exhaustive but from the results here it seemed likely that metabolism ofcypermethrin by esterases was not a major resistance mechanism in the laboratory strains examined. Previous biochemical studies suggested the

TABLE 8. The Neuronal Response of Third Instar Larvae of a Susceptible (BRC), Two Laboratory Resistant (PEG87 and DuPont) and Two Field Strains (Snook and Hearne) of *Heliothis virescens* in a Cumulative Dose Response Assay with cis-Cypermethrin.

conc^{n} cis-cypermethrin (nmol/l)	Strain of larvae				
	BRC	PEG87	Hearne	Snook	DuPont
1	13	6	3	4	1
5	1	4	6	3	0
10	0	3	2	2	1
50	0	0	1	2	1
100	0	1	3	3	6
>100	0	4	0	2	4
Number of larvae treated	14	18	15	16	13

involvement of a powerful monooxygenase in resistance to cypermethrin in the PEG87 strain of *H. virescens* (Lee et al. 1989, Little et al. 1989, Walker et al. 1990). These authors showed that piperonyl butoxide effectively inhibited most of the metabolism of ^{14}C-cypermethrin in vivo. Use of PBO in bioassays with cis-cypermethrin clearly indicates that both the first instar and the third instar larvaeof the PEG87 strain have an enhanced monooxygenase. The reduction in the absolute amount of toxicant required to achieve equivalent levels of mortality of third instar PEG87 larvae when PBO was used were very large emphasising the capability of this enzyme system. Interestingly, with the DuPont strain PBO synergism at the third instar was confirmed but was comparatively weak. This is in contrast to the first instar in which PBO was almost equally effective as a synergist for cypermethrin in both the DuPont and the PEG87 strains. These results are consistent with the hypothesis that the resistance based on the PBO suppressible, enhanced monooxygenase is a major mechanism of resistance in the PEG87 strain (and at the third instar this is expressed to a high degree) whilst in the DuPont strain the mechanism may not expressed to the same degree. Nevertheless, both strains were similarly resistant and PBO similarly effective at the first instar. This may be explained by presuming that the protection afforded to first instar larvae by the presence of the monooxygenase is maximal. Further increases in the activity of the enzyme(s) merely serve to enhance the tolerance of larger larvae.

In the case of metabolic resistance such as that associated with enhanced monooxygenase activity, the rate of uptake of insecticide by different instars needs to be taken into consideration. The small first instar larvae may be expected to take up insecticide considerably more rapidly than the relatively large third instar larvae. This should hold true whether uptake is by contact (relatively large surface area/volume ratio in first instar larvae) or by ingestion (relatively rapid intake of food expressed on a body weight in first instar basis). Thus if first and third instar larvae have similar levels of monooxygenase activity, expressed in terms of their body weights, then the larger third instar larvae should appear more resistant in bioassays because the rate of uptake of the pyrethroid is slower, and less demand is made upon metabolic detoxication at any particular level of dosing. In other words, the larger third instar larvae would need to be exposed to a higher level of pyrethroid than the smaller first instar larvae to achieve a level of uptake too high for the monooxygenase to effectively detoxify. This could contribute to the apparently greater resistance of the third instar larvae compared with the first instar larvae in the present study.

The marked resistance of all the field strains at the first instar was not seen in the third instar. These larger larvae were only mildly resistant in the topical bioassay and this together with the absence of a PBO synergisable resistance component suggests the presence of another mechanism(s) which affords considerable protection to the first instar insects. We were unable to conduct synergist studies with first instar field strain larvae and so cannot be fully convinced of the absence of the mechanism at this time. However, the lack of any synergism in the third instar suggests the absence of this mechanism from these strains especially since it might be expected to be more strongly expressed in larger larvae.

Many insects are known to possess a form of nerve insensitivity which provides a high degree of resistance to pyrethroid action (Soderlund and Bloomquist. 1989). The neurophysiological assays used here indicate the presence of a range of phenotypes with respect to this nerve insensitivity in the PEG87 strain. Some individuals were indistinguishable from the susceptible BRC strain whilst others had nervous systems which tolerated very high concentrations of the toxicant. The response profile of the DuPont strain was clearly different. Most of the insects appeared to have a high degree of nerve insensitivity and only a few individuals responded at low concentrations of the insecticide. The DuPont strain would thus appear to be considerably more homogeneous with respect to nerve insensitivity than the PEG87 strain.

It is significant that at the first and the third instars with the PEG87 strain the elimination of the PBO synergisable component left up to several orders of magnitude of resistance unaccounted for. However it should be stressed that resistance mechanisms would be expected to act more than additively. Nerve insensitivity may clearly account for a large part of this resistance but possession of the mechanism in the DuPont strain did not confer high tolerance in the third instar since the resistance unaccounted for after PBO synergism was considerably less than in the PEG87 strain. The possibility that nerve insensitivity may be an important feature of tolerance in the first instar but of less importance in larger larvae is emphasised by a consideration of the incidence of nerve insensitivity in the field strains. Both the Snook and the Hearne strains were characterised by the presence of a range of phenotypes with respect to nerve tolerance. These

strains were resistant in the first instar foliar bioassay but only weakly resistant in the third instar topical bioassay. Moreover, the use of PBO suggested that enhanced monooxygenase activity could be absent from both the strains. Possession of nerve insensitivity may therefore provide less tolerance in larger larvae but may be of considerable importance to the survival of first instar insects exposed to residues of the pyrethroid.

The results suggest that all the strains examined possess nerve insensitivity and that the mechanism may be widespread within the range of *H. virescens*. Enhanced monooxygenase activity provides the basis for an extremely effective mechanism of resistance to the toxicant in both first and third instar larvae. Where both mechanisms are strongly expressed, the resultant larvae are very highly resistant. Genetic analysis will be required for a full understanding of these interactions. Payne et al. (1989) have demonstrated the genetic segregation of at least one of these resistance factors in *H. virescens*. It may be significant that the PEG87 and DuPont strains possessed both major mechanisms whilst the other field strains we examined appeared to rely on nerve insensitivity. Further, it seems possible that continued selection enhances the monooxygenase resistance and gives rise to enormously tolerant larvae. There is evidence that resistance to pyrethroids in *Heliothis armigera* in Australia is now based largely on enhanced monooxygenase activity (Daly 1988) and that the incidence of nerve insensitivity in the populations is declining (Forrester 1989). The reason for this is unknown at present but it is possibly because of the overriding efficiency of the metabolic mechanism.

Strains of insects having individuals in which all the significant mechanisms of resistance are strongly expressed are ideal for laboratory study. Moreover, where these mechanisms are based on quantitative biochemical changes, such highly resistant strains make an ideal starting point for the development of assays designed to detect resistance. Nevertheless, the continued monitoring of field strains is vital in order to gain a clear idea of the incidence of each of the major resistance mechanisms in this insect. We are of the opinion that the use of rapid assays for the detection of each mechanism will enhance the effectiveness of resistance management programmes by providing evidence for logical decisions on the use of alternative pyrethroids, other insecticides or synergists.

ACKNOWLEDGEMENT

We are grateful to the Pyrethroids Efficacy Group companies for their continued financial support of this work and for the supply of strains of the insects and chemicals. Thanks are due to the Government of Sudan for support of H.E.-N. The work was carried out under MAFF Licence No. PHF 1007/31(18) issued under the Import (Plant Health)(Great Britain) Order 1980 and the Plant Pests (Great Britain) Order 1980.

LITERATURE CITED

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Daly, J.C. 1988. Insecticide resistance in Heliothis armigera in Australia. Pestic. Sci. 23: 165-176.

Finney, D.J. 1971. Probit Analysis, 3rd edn., Cambridge University Press, UK.
Forrester, N. 1989. Countermeasures for pyrethroid resistance, part 1: synergists. Aust. Cotton Grower 10: 58-69.
Gladwell, R.T., McCaffery, A.R. and C.H. Walker 1990. Nerve insensitivity to cypermethrin in field and laboratory strains of Heliothis virescens. In: Proc. 1990 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN. (In Press).
Graves, J.B., Leonard, B.R. and A.M. Pavloff 1989. An update on pyrethroid resistance in tobacco budworm and bollworm in Louisiana. pp 343-346. In: Proc. 1989 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN.
Lee, K.-S., Walker, C.H., McCaffery, A.R., Ahmad, M. and E.J. Little 1989. Metabolism of trans-cypermethrin by Heliothis armigera and H. virescens. Pestic. Biochem. Physiol. 34: 49-57.
Little, E.J., McCaffery, A.R., Walker, C.H. and T. Parker 1989. Evidence for an enhanced metabolism of cypermethrin by a monooxygenase in a pyrethroid-resistant strain of the tobacco budworm (Heliothis virescens (F.)). Pestic. Biochem. Physiol. 34: 58-68.
Luttrell, R.G., Roush, R.T., Ali, A., Mink, J.S., Reid, M.R. and G.L. Snodgrass 1987. Pyrethroid resistance in field populations of Heliothis virescens (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985-989.
McCaffery, A.R., Little, E.J., Gladwell, R.T., Holloway, G.J. and C.H. Walker 1989a. Detection and mechanisms of resistance in Heliothis virescens. pp 207-211. In: Proc. 1989 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN.
McCaffery, A.R., King, A.B.S., Walker, A.J. and H. El-Nayir 1989b. Resistance to synthetic pyrethroids in the bollworm, Heliothis virescens from Andhra Pradesh, India. Pestic. Sci. 27: 65-76.
Nicholson, R.A. and T.A. Miller 1985. Multifactorial resistance to transpermethrin in field collected strains of the tobacco budworm Heliothis virescens F. Pestic. Sci. 16: 561-570.
Payne, G.T., Blenk, R.G. and T.M. Brown 1988. Inheritance of permethrin resistance in the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 81: 65-73.
Plapp, F.W., Frisbie, R.E. and J.A. Jackman 1989. Monitoring for pyrethroid resistance in Heliothis spp. in Texas in 1988. pp 347-352. In: Proc. 1989 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN.
Rogers, B., Feese, H.D., Riley, S.L., Staetz, C.A., Whitehead, J. and W.J. Mullins 1990. Heliothis resistance monitoring in the cotton belt: PEG-US update. Proc. 1990 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN. (In Press).
SAS Institute 1985. SAS Users guide: statistics, Version 5. SAS Institute, Cary, NC.
Soderlund, D.M. and J.R. Bloomquist 1989. Neurotoxic actions of pyrethroid insecticides. Ann. Rev. Entomol. 34: 77-96.
Staetz, C.A., Rivera, M.A., Riley, S.L., Watkinson, I.A., Whitehead, J.R., Blenk, R.J., Feese, H.D., Ross, D., Simonet, D.E. and W.J. Mullins 1989. PEG-US Heliothis virescens resistance monitoring program - 1988: Monitoring results with cypermethrin. pp 199-207. In: Proc. 1989 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN.

Walker, C.H., Lee, K.S., McCaffery, A.R. and E.J. Little 1990. Resistance to cypermethrin in the PEG87 strain of Heliothis virescens - the enzymic factor. In Proc. 1990 Beltwide Cotton Prod. Res. Conf. Nat. Cotton Council, Memphis, TN.(In Press).

Weevers, R. De G. 1966. A lepidopteran saline: Effects of inorganic cation concentrations on sensory, reflex and motor responses in a herbivorous insect. J. of Exp. Biol. 44: 163-175.

Worthing, C.R. and S.B. Walker 1987. The Pesticide Manual: A World Compendium, 8th edn., British Crop Protection Council, Thornton heath, UK 1081pp.