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Resistance to insecticides in heliothine Lepidoptera: a global view

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The status of resistance to organophosphate, carbamate, cyclodiene and pyrethroid insecticides in the heliothine Lepidoptera is reviewed. In particular, resistance in the tobacco budworm, *Heliothis virescens*, and the corn earworm, *Helicoverpa zea*, from the New World, and the cotton bollworm, *Helicoverpa armigera*, from the Old World, are considered in detail. Particular emphasis has been placed on resistance to the most widely used of these insecticide groups, the pyrethroids. In each case, the incidence and current status of resistance are considered before a detailed view of the mechanisms of resistance is given. Controversial issues regarding the nature of mechanisms of resistance to pyrethroid insecticides are discussed. The implications for resistance management are considered.

Keywords: insecticide resistance; Heliothinae; *Heliothis virescens*; *Helicoverpa armigera*; mechanisms of resistance

1. INTRODUCTION

Lepidopteran species in the genera *Heliothis* and *Helicoverpa* are grouped together in the Trifine subfamily Heliothinae of the family Noctuidae (Hardwick 1965; Mitter *et al.* 1993). The biology and ecology of the species within this complex have recently been reviewed by Fitt (1989), Zalucki (1991) and King (1994). It is significant that within the group there exists a large number of highly destructive crop pests against which an unparalleled variety and quantity of insecticides have been used. The polyphagous nature of a number of these species, their wide geographic range and their ability to adapt to diverse cropping systems have contributed to this pest status. Moreover, the ability of certain species within the complex to develop resistance to insecticides has placed the heliothine Lepidoptera among a handful of the world's most significant crop pests.

The genus *Helicoverpa* (designated *Heliothis* for a period) includes the Old World species *Helicoverpa armigera*, generally considered to be the most important species within this group. Commonly known as the cotton bollworm, gram podborer or American bollworm, *H. armigera* occurs in Africa, Asia, southern Europe and Australia and is a major pest of cotton, maize, sorghum, pigeonpea, chickpea, soyabean, groundnut, sunflower and a range of vegetables. It, above all others in this genus, has developed resistance to virtually all of the insecticides that have been deployed against it in any quantity. *Helicoverpa punctigera* is a pest of cotton, sunflower, lucerne, soyabean, chickpea and safflower in Australia and is commonly found alongside *H. armigera*. Interestingly, until recently *H. punctigera* had not developed resistance to insecticides (Gunning & Easton 1994; Gunning *et al.* 1994), and this may have been because the pool of unsprayed insects is so vast that the treated proportion of the total population is only trivial (Forrester *et al.* 1993). Any resistance genes would be swamped by susceptible genes in the unsprayed refugia. Nevertheless, a field population of *H. punctigera* from New South Wales was recently shown to have developed resistance to a pyrethroid (Gunning *et al.* 1997). The oligophagous species *Helicoverpa assulta* feeds on tobacco and other solanaceous plants and is found in Africa, Asia, parts of Australasia and the South Pacific. It is normally considered to be a minor pest and there is no evidence of it having developed resistance anywhere within its range since it is not subject to any significant insecticide treatment (Armes *et al.* 1996). In the New World this genus is represented by the corn earworm, *Helicoverpa zea*, a key pest of maize, sorghum, cotton, tomato, sunflower and soyabean. It too has developed resistance to a number of the insecticide groups used against it (Sparks 1981; Wolfenbarger *et al.* 1981; Stadelbacher *et al.* 1990), although not all the crops it attacks are sprayed and the species remains reasonably amenable to control with insecticides.

Within the genus *Heliothis* there are two species of note. The New World representative, *Heliothis virescens*, is distributed throughout the Americas, is commonly known as the tobacco budworm and is a major pest of cotton, tobacco, tomato, sunflower and soyabean. Like *H. armigera* above, it has developed resistance to all the insecticides that have been used against it in significant quantities (Sparks 1981; Wolfenbarger *et al.* 1981; Sparks *et al.* 1993). The polyphagous species, *Heliothis peltigera*, has a broad distribution across central and southern Europe, the Canary Islands, Asia Minor and India and it is a pest of safflower, tobacco, cotton, chickpea, fodder crops, grapevines and various fruit trees. Resistance to insecticides has not been reported in this species and this is presumed to be due to lack of intense selection.

It is clear that within the Heliothinae there are two very significant pest species that have been subjected to intense selection with a range of insecticides and which have developed significant levels of resistance to insecticides: *H. armigera* and *H. virescens*. This review will therefore concentrate on resistance in these species and will attempt to compare and contrast the phenomenon, particularly with respect to the mechanisms of resistance. There are a large number of reviews that document the historical development of resistance in these species and it is not the intention of the present author to rehearse all of this literature here. The reader is directed to earlier works by Sparks (1981), Wolfenbarger *et al*. (1981) and Sparks *et al*. (1993). Most studies on resistance in heliothine insects in the past 15 years have concerned the pyrethroid insecticides and it is for this reason that this review will place special emphasis on this group, although other insecticide groups will also be considered.

An understanding of the mechanisms underlying resistance is central to an ability to continue to effectively use existing insecticide chemistry to which resistance has already developed. A knowledge of the mechanisms of resistance enables one to understand not only the cross-resistance patterns within insecticide groups but also those between them. Thus, the mechanisms of resistance determine the use of 'resistance-breaking' compounds and areas of new insecticide chemistry. Such considerations are crucial in resistance management. A detailed knowledge of resistance mechanisms could also be considered as essential in the formulation of diagnostics for use in resistance management although, as will be emphasized later, the very diversity of response to selection in these insects could make the practical use of such diagnosis especially difficult. The design of expression systems for use in insecticide discovery might also be usefully influenced by such information. This review therefore places considerable emphasis on the mechanisms of resistance and compares them in heliothine populations around the world.

2. RESISTANCE TO ORGANOPHOSPHATES

(a) Heliothis virescens

After the development of resistance to DDT and toxaphene, organophosphates (OPs), particularly methyl parathion, were introduced into the USA to control *H. virescens* and *H. zea*. Resistance developed in *H. virescens* within a few years of OP introduction (Wolfenbarger & McGarr 1970; Harris 1972), and had become widespread throughout the southern states of the USA by 1980 (Sparks *et al*. 1993). Numerous reports have detailed the progress of resistance to OPs including methyl parathion, sulprofos and profenofos both within and between seasons (e.g. Wolfenbarger 1981; Elzen *et al*. 1992; Kanga & Plapp 1992, 1995; Sparks *et al*. 1993; Graves *et al*. 1994; Kanga *et al*. 1995; Martin *et al*. 1995, 1997). Outside the USA, a low level of resistance to monocrotophos was noted in Colombia (Ernst & Dittrich 1992).

(b) Helicoverpa zea

Resistance to methyl parathion was reported in some states of the USA and Central America (Wolfenbarger *et al*. 1981), although there is little supporting information in the literature that resistance to OPs is a significant problem in the control of this species (Sparks *et al*. 1993).

(c) Heliothis armigera

H. armigera in Australia have generally been considered to be relatively susceptible to OP insecticides. Gunning & Easton (1993) found no evidence of resistance to methyl parathion and today only low levels of resistance are found to profenofos, chlorpyrifos and methyl parathion (N. W. Forrester, personal communication). In contrast, high levels of resistance to monocrotophos and low levels of resistance to chlorpyrifos and profenofos have been recorded in populations of *H. armigera* in Pakistan (Ahmad *et al*. 1995), although resistance to profenofos is continuing to rise as growers opt to use OPs rather than the pyrethroids, to which there is resistance. Low-to-moderate resistance was found to quinalphos in Indian and Pakistani populations of *H. armigera* (Armes *et al*. 1996), but there was no evidence of significant resistance to monocrotophos. Since 1980, phoxim has been the most widely used OP for the control of *H. armigera* in China. It was highly effective until 1990, when it failed to control populations in North China. Bioassays with insects collected from different geographical areas of China during 1994 and 1995 showed resistance to phoxim to be widespread (Wu *et al*. 1997). No resistance to monocrotophos was observed in 1992 and 1993 (Wu *et al*. 1995), but higher levels were recorded in 1995 (Wu *et al*. 1996). No resistance to OPs was detected in *H. armigera* in Thailand (Ahmad & McCaffery 1988).

3. MECHANISMS OF RESISTANCE TO ORGANOPHOSPHATES

(a) *Insensitive acetylcholinesterase: target-site resistance to organophosphates*

The enzyme acetylcholinesterase resides on the postsynaptic membrane of cholinergic synapses and is responsible for the breakdown of acetylcholine after stimulation of nicotinic acetylcholine receptors on the postsynaptic neuron. Both organophosphate and carbamate insecticides prevent the breakdown of acetylcholine by inhibiting the activity of this enzyme. The increased residence time of acetylcholine in the synapse causes repeated stimulation of the postsynaptic neuron and hence neuronal hyperactivity. Commonly, resistance to OPs involves the selection of mutants that possess a form of the enzyme insensitive to inhibition.

A large number of reports have shown that resistance to OPs in *H. virescens* may be due, at least in part, to a target-site resistance involving decreased sensitivity of acetylcholinesterase to inhibition (Brown & Bryson 1992; Kanga & Plapp 1995; Brown *et al*. 1996*a*; Harold & Ottea 1997). In resistant strains of *H. virescens*, Brown & Bryson (1992) and Gilbert *et al*. (1996) demonstrated the presence of acetylcholinesterase insensitive to inhibition by methyl paraoxon and G. Zhao *et al*. (1996) demonstrated acetylcholinesterase insensitive to paraoxon. Although this mechanism may be common in OP-resistant insects it may not be universal within field populations of *H. virescens*, as shown by Harold & Ottea (1997).

(b) *Metabolic mechanisms of resistance to organophosphates*

Metabolic resistance to organophosphate insecticides in heliothine insects has been thought to be due to elevation in the activity of number of detoxification systems. Most frequently, resistance to these insecticides has been correlated with elevated esterase activity, especially when the model substrate 1-naphthyl acetate (1-NA) is used; this result suggests a strong association between these enzymes and OP resistance. Esterase synergists such as TBPT and EPN were shown to be effective against methyl parathion resistance in *H. virescens* (Payne & Brown 1984). Importantly, in this New World species, elevated esterase activities were shown to be responsible for resistance to OPs such as methyl parathion, profenofos and azinphosmethyl and for cross-resistance between carbamate, OP and pyrethroid insecticides (Goh *et al.* 1995; G. Zhao *et al.* 1996). Higher phosphotriester hydrolase activity was reported to be involved in resistance to methyl parathion in *H. virescens* from North Carolina (Konno *et al.* 1989). In a recent study, high frequencies of profenofos resistance were recorded in larvae of all of a number of field strains of *H. virescens* collected from Louisiana in 1995 and were strongly correlated with esterase activity (Harold & Ottea 1997).

Glutathione S-transferases have also been frequently associated with resistance to OPs and thought to be responsible for metabolism of these compounds (Whitten & Bull 1978). Resistance to profenofos was shown to be only moderately correlated with glutathione S-transferase activity towards 1-chloro-2,4-dinitrobenzene (CDNB) and had no correlation with glutathione S-transferase activity to 1,2-dichloro-4-nitrobenzene (DCNB) (Ibrahim & Ottea 1995; Harold & Ottea 1997). This correlation of profenofos resistance with activity of GST towards CDNB but not DCNB suggests that these GST enzymes have different identities and therefore likely contributions to profenofos resistance. No differences in GST activity were observed by Konno *et al.* (1989).

Metabolism of OP insecticides by P450 monooxygenases was reported in a number of early studies (Whitten & Bull 1974; Reed 1974; Brown 1981; Bull 1981). Martin *et al.* (1995) identified low to moderate levels of resistance to profenofos and sulprofos in Louisiana, Mississippi and Texas and later showed that profenofos was synergized by PBO in populations of *H. virescens* from Texas, Mississippi and Oklahoma (Martin *et al.* 1997). Other research suggested that monooxygenases might not be involved in the elimination of OPs (Gould & Hodgson 1980; Payne & Brown 1984; Konno *et al.* 1989). Most recently, Harold & Ottea (1997) found no correlation between profenofos resistance and P450 monooxygenase activity towards the model substrate *p*-nitroanisole.

4. RESISTANCE TO CARBAMATES

(a) Heliothis virescens

Resistance to the oxime carbamates thiodicarb and methomyl has been recorded a number of times in populations of *H. virescens* from Louisiana, Mississippi, Texas and Arkansas (Sparks 1981; Elzen *et al.* 1992; Martin *et al.* 1992, 1995; Sparks *et al.* 1993; Kanga & Plapp 1995), and also in populations from Mexico (Roush & Wolfenbarger 1985).

(b) Helicoverpa zea

There are no reports of significant resistance to carbamates in *H. zea*.

(c) Helicoverpa armigera

Thiodicarb and methomyl are the carbamates most widely used against *H. armigera* in Australia. Methomyl resistance was noted in 1986 but the insect remained susceptible to thiodicarb for a number of years more (Gunning *et al.* 1992). Resistance to thiodicarb was detected in New South Wales in 1993 and this gave cross-resistance to methomyl (Gunning *et al.* 1996*b*). Since then resistance to thiodicarb has increased and moderate resistance to carbamates is now common (N. W. Forrester, personal communication). In China, significant resistance to methomyl was recorded in strains of *H. armigera* from Shandong province (Wu *et al.* 1995, 1996). Low-level resistance to thiodicarb was seen in *H. armigera* from Pakistan (Ahmad *et al.* 1995). Substantial resistance to methomyl was recorded in populations from cotton-growing areas of Andhra Pradesh, India (Armes *et al.* 1992, 1996), with lower levels being more typical of other locations including Nepal, Gujarat and Maharashtra.

5. MECHANISMS OF RESISTANCE TO CARBAMATES

(a) *Insensitive acetylcholinesterase: target-site resistance to carbamates*

Target-site resistance to carbamates is similar to that found with organophosphates (see above). Acetylcholinesterase insensitive to inhibition by propoxur and methomyl was observed in a selected strain of *H. virescens* (Brown & Bryson 1992). More recently, insensitive acetylcholinesterase was shown to be a major mechanism of resistance to methomyl and carbaryl in strains of *H. virescens* and to thiodicarb in a thiodicarb- and pyrethroid-resistant strain (G. Zhao *et al.* 1996). In Australia the recently developed resistance to thiodicarb in *H. armigera* has been shown to be due to a form of acetylcholinesterase that is insensitive to both thiodicarb and methomyl.

(b) *Metabolic resistance to carbamates*

Both enhanced esterase and enhanced monooxygenase activity have been found to be significant mechanisms of resistance to carbamates. In one recent study, substantially increased esterase activity was observed and thought to be responsible for resistance in a thiodicarb-resistant (and pyrethroid-resistant) strain of *H. virescens* (Goh *et al.* 1995). Rose *et al.* (1995), using a similar strain, found high levels of P450 monooxygenase activity as well as increased esterase activity. The involvement of P450 monooxygenases was considered likely by G. Zhao *et al.* (1996), who showed significant synergism of thiodicarb with PBO. They also inferred the involvement of enhanced esterase activity in this resistance. Very recently, PBO was shown to synergize the action of methomyl and thiodicarb in a number of field strains although it antagonized the action of thiodicarb in some strains (Martin *et al.* 1997).

6. RESISTANCE TO CYCLODIENES

(a) Heliothis virescens

Resistance to endosulfan has been demonstrated in strains of *H. virescens* from Louisiana, Mississippi, Texas

and Arkansas (Elzen *et al*. 1992; Kanga *et al*. 1995; Martin *et al*. 1995).

(b) Helicoverpa zea

Increased tolerance to endosulfan was found in field populations of *H. zea* from Texas in 1994 (Kanga *et al*. 1996). There appear to be no other records of significant resistance to endosulfan in this species.

(c) Helicoverpa armigera

Resistance to endosulfan in *H. armigera* has recorded in Australia since the early 1970s and a number of reports have demonstrated the substantial and continuing nature of this problem (Kay 1977; Forrester *et al*. 1993; Gunning & Easton 1994). Current levels of resistance to endosulfan in Australia are moderate. Relatively low levels of resistance were characteristic of *H. armigera* in various regions of India from 1988 to 1992 (McCaffery *et al*. 1989; Armes *et al*. 1992). Rather higher levels of resistance to this compound were found in later years by Armes *et al*. (1996), who suggested that incipient resistance to endosulfan was present in this species in India, Nepal and Pakistan. Low resistance to endosulfan characterized populations of *H. armigera* from Pakistan between 1991 and 1993, but thereafter resistance rose to peak frequencies in 1995, falling back somewhat in later years (Ahmad *et al*. 1995, 1998). Populations collected from Indonesia in 1987 and 1988 were reported to be resistant to pyrethoids (McCaffery *et al*. 1991*a*).

7. MECHANISMS OF RESISTANCE TO CYCLODIENES

(a) *Altered GABA receptor: target-site resistance to cyclodienes*

The GABA-gated chloride-ion channel receptor complex is generally considered to be the target for cyclodiene insecticides such as endosulfan. These compounds act as GABA antagonists and hence, because they suppress the inhibitory transmitter action of GABA, their action results in increased postsynaptic neuronal activity. Although no direct evidence has been obtained with heliothines, target-site insensitivity to cyclodiene action has been inferred in adult *H. virescens* on the basis of highly correlated toxicities of dieldrin and endosulfan (Kanga & Plapp 1995).

8. RESISTANCE TO PYRETHROID INSECTICIDES

(a) *Introduction*

The pyrethroid insecticides were introduced to replace the resistance-prone and environmentally unsuitable organochlorines (OCs), cyclodienes and organophosphates (OPs) (Morton & Collins 1989). They clearly had a number of distinct advantages over insecticides used previously. They possessed an inherently high activity and could be applied at extremely low doses for the control of a huge range of public health and agricultural pests. Their high activity meant that effective foliar profiles were maintained for considerable periods. They were safe to mammals, had low environmental impact and were immobile in the soil (Elliott 1989). The pyrethroids were especially useful in cotton, where their contact activity and good efficacy enabled the grower to regain control of pest species that had become resistant to previously used insecticides. The global demise of the effectiveness of pyrethroids has provoked a huge research effort directed at understanding the nature of this resistance and hence alternative control strategies.

(b) *Resistance to pyrethroid insecticides around the world*

(i) Heliothis virescens *in the USA*

Following the development of resistance to DDT, methyl parathion and a growing number of other OPs (Sparks *et al*. 1993), the pyrethroid insecticides were introduced into the USA and became available for use on cotton in 1978, quickly becoming the insecticides of choice. A small number of studies had inferred a degree of cross-resistance to pyrethroids in methyl parathion-resistant strains of the tobacco budworm, although analysis of these data revealed no significant trends. Nevertheless, susceptibility to pyrethroids was correlated with that to methyl parathion (Sparks *et al*. 1993), and suggested that differences in susceptibility were already present in populations of the tobacco budworm in cotton.

Numerous studies have documented resistance to pyrethroids in *H. virescens* in the USA and the reader is directed to the comprehensive review by Sparks *et al*. (1993) for more details. Although significant changes in susceptibility had been noted in the Imperial Valley of California in the early 1980s (Twine & Reynolds 1980; Martinez-Carrillo & Reynolds 1983), these were not considered to have led to any field failure. The first reports of significant resistance appeared in 1985 in west Texas (Plapp & Campanhola 1986) and these were quickly followed by a range of similar findings throughout the cotton-belt states of the southern USA, including Alabama (Mullins *et al*. 1991), Arkansas (Plapp *et al*. 1987, 1990), Louisiana (Leonard *et al*. 1988; Plapp *et al*. 1990; Elzen *et al*. 1992), Mississippi (Luttrell *et al*. 1987; Plapp *et al*. 1990; Elzen *et al*. 1992; Ernst & Dittrich 1992), Oklahoma (Plapp *et al*. 1990) and Texas (Plapp *et al*. 1987, 1990). In many cases this resulted in considerable cross-resistance between pyrethroids and this was thought to imply the presence of a target-site mechanism of resistance (Martin *et al*. 1992; Graves *et al*. 1993; Sparks *et al*. 1993). Because a complete loss of pyrethroids was feared, resistance monitoring programmes were instituted (Plapp *et al*. 1987), and management plans organized in Texas and the mid-south in an effort to provide pyrethroid-free windows during the cotton-growing season (Sparks *et al*. 1993). Interestingly, the continued used of pyrethroids in the USA has led to what appears to be a shift in the mechanisms of resistance to pyrethroids, as detailed below.

(ii) Heliothis virescens *in Mexico*

H. virescens is a common pest of cotton in Mexico and pyrethroids have been extensively used for its control since the early 1980s. Monitoring for resistance to pyrethroids has been conducted in agricultural regions of northwestern Mexico since 1984, when resistance was first noted. High levels of resistance were recorded in 1987 from populations from the Yaqui and Mexicali valleys and in the 1988 season from the Costa de Hermosillo and Region de Caborca (Martinez-Carrillo 1991, 1995). These high levels of resistance prompted the introduction, in 1989, of a strategy to

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reduce pyrethroid selection pressure in the Yaqui Valley. As a result, pyrethroid resistance decreased in this area in 1988 and 1989 and has remained stable since 1990 (Martinez-Carrillo 1995). In contrast, levels of resistance in the northeast of the country are high and would be expected to cause control problems.

(iii) Heliothis virescens *in Colombia*

Pyrethroids became available for use in cotton in the late 1970s and early 1980s and were very extensively used, to the exclusion of other products. Very substantial resistance to cypermethrin in the tobacco budworm was noted from 1985 and has been documented by Ernst & Dittrich (1992) and confirmed by McCaffery (1994).

(iv) Helicoverpa zea *in the Americas*

Very extensive resistance to DDT was a feature of early control of *H. zea* in the USA (see, for example, Graves *et al.* 1963; Wolfenbarger *et al.* 1981; Sparks *et al.* 1993). The first substantial report of resistance to pyrethroids in *H. zea* was that of Stadelbacher *et al.* (1990). Following this, a number of other authors noted a loss of susceptibility to pyrethroids in this species (Graves *et al.* 1993; Abd-Elghafar *et al.* 1993; Kanga *et al.* 1996; Bagwell *et al.* 1997). Despite this loss of susceptibility, pyrethroid insecticides presently remain effective for the control of *H. zea* in US cotton, even at low field application rates. In one of the few studies on this species conducted outside the USA, strains of *H. zea* from the Tiquisate area of Guatemala and the Leon area of Nicaragua were found to be very substantially resistant to cypermethrin (Ernst & Dittrich 1992).

(v) Helicoverpa armigera *in Australia*

Before the introduction of pyrethroids in 1977 in Australia, *H. armigera* had developed severe resistance to DDT in the Ord River Valley (Wilson 1974), New South Wales (Goodyer *et al.* 1975; Goodyer & Greenup 1980) and Queensland (Kay 1977). Resistance to endosulfan (Kay 1977; Kay *et al.* 1983; Gunning & Easton 1994), OPs (Goodyer & Greenup 1980; Kay *et al.* 1983) and carbamates (Gunning *et al.* 1992) was also known to be present. Resistance to pyrethroids first appeared in 1983 (Gunning *et al.* 1984), and immediately a resistance management strategy was implemented, which restricted the use of pyrethroids to a 42-day window during January–February (from 1990 they were restricted to a 35-day window) (Forrester 1990; Forrester *et al.* 1993). Endosulfan use was also limited. An effective weekly monitoring scheme based on survival of fourth-instar larvae of *H. armigera* after treatment with a diagnostic dose of fenvalerate was initiated and much data accumulated on the effects of selection and survival of resistant individuals. Later monitoring also determined the likely presence of a metabolic resistance based on enhanced monooxygenase activity by treating larvae with both fenvalerate and the metabolic inhibitor piperonyl butoxide (PBO) (see below). Based on these results, PBO could be added to the last of the three (maximum) sprays in the pyrethroid window. This strategy undoubtedly held pyrethroid resistance in check for a number of years although there appeared to be a steady rise in the proportion of the population that was resistant to pyrethroids (Forrester *et al.* 1993). *H. armigera* in unsprayed refugia readily became contaminated with resistant individuals (Gunning & Easton 1989; Forrester *et al.* 1993), and similar levels of resistance were found in other crops, such as maize (Glenn *et al.* 1994). This gradual loss of pyrethroid efficacy together with the development of an immunodiagnostic to distinguish the eggs of *H. armigera* from those of *H. punctigera*, the use of *Bacillus thuringiensis* (*Bt*) and other insecticides and the advent of *Bt*-transgenic cotton led to a complete reorganization of the strategy and a relaxation on the use of the now less useful pyrethroids. The situation is continuing to deteriorate, with resistance to pyrethroids increasing steadily (N. W. Forrester, personal communication).

(vi) Helicoverpa armigera *in New Zealand*

A programme to monitor resistance to fenvalerate in *H. armigera* was initiated in 1991 in tomato, maize and lucerne crops in New Zealand. A significant trend of declining mortality from 1992 to 1994 was seen and this suggests an increase in the frequency of resistance to the pyrethroids (Cameron *et al.* 1995; Suckling 1996). Management strategies have been devised to counter this problem (Suckling 1996).

(vii) Helicoverpa armigera *in Thailand*

Wangboonkong (1981) first reported inadequate control of *H. armigera* in Thailand soon after the introduction of pyrethroids, but it was not known whether resistance was the cause. Significant resistance to pyrethroids was found in populations of *H. armigera* from the Tak Fa area of Nakonsawan in Thailand in 1985 (Ahmad & McCaffery 1988). These insects were also resistant to DDT and carbaryl. Pyrethroid resistance was again noted in Thai populations of the insect by Ernst & Dittrich (1992).

(viii) Helicoverpa armigera *in Indonesia*

After the introduction of pyrethroids in the 1980s, resistance to was found in populations of *H. armigera* collected from the cotton-growing areas of South Sulawesi, Indonesia, in 1987 and early 1988 (McCaffery *et al.* 1991*a*). These populations were also resistant to endosulfan and DDT.

(ix) Helicoverpa armigera *in China*

Almost all groups of conventional insecticides have been used to control *H. armigera* in China. DDT resistance was first detected in *H. armigera* in Henan province (Anon 1974), and subsequently in Jiangsu and Hebie provinces (Zhu *et al.* 1982), together with resistance to carbaryl. Pyrethroids such as fenvalerate and deltamethrin have been widely used since 1983 with others such as cyhalothrin, cypermethrin, esfenvalerate, fenpropathrin and cyfluthrin being used from the mid- to late-1980s. There were no substantial changes in susceptibility until around 1989, but in the following years resistance to pyrethroids was widely detected in a number of areas including Jiangsu, Henan and Shandong provinces (Tan *et al.* 1987; Shen *et al.* 1991, 1992, 1993; Wu *et al.* 1996, 1997*b*). The development of this resistance led to calls for a resistance management strategy to restrict pyrethroid use, to promote greater emphasis on the use of alternations with other insecticides and to promote the use of biological control (Shen *et al.* 1992). Although levels of resistance to pyrethroids are still high,

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recent lower populations have alleviated the problem to some degree (Y. Wu, personal communication).

(x) Helicoverpa armigera *in Central Asia*

High levels of resistance to pyrethroids (as well as to OCs and OPs) have been found in *H. armigera* from Tajikstan and Azerbaijan (Sukhoruchenko 1996). In a similar study, resistance to pyrethroids was found to be present in populations of *H. armigera* from Russia (Leonova & Slynko 1996).

(xi) Helicoverpa armigera *in India*

Pyrethroid insecticides were first used in India in 1980 for the control of a number of pests, including *H. armigera*. In 1987 resistance to pyrethroids was first noted in India in Andhra Pradesh (Dhingra *et al.* 1988; McCaffery *et al.* 1988, 1989; Phokela *et al.* 1989) in populations that were also resistant to DDT and slightly resistant to endosulfan (McCaffery *et al.* 1989). Numerous other studies confirmed the high incidence of pyrethroid resistance, especially in the cotton- and pulse-growing regions of central and southern India, and also confirmed its gradual spread to other regions of the country (see, for example, Phokela *et al.* 1990; Mehrohtra & Phokela 1992; Armes *et al.* 1992, 1996; Sekhar *et al.* 1996; Jadhav & Armes 1996). Pyrethroid resistance has recently been found in the Punjab close to populations over the border in Pakistan, leading Armes *et al.* (1996) to the conclusion that pyrethroid resistance is ubiquitous in *H. armigera* in the Indian subcontinent. Resistance to pyrethroids is frequently accompanied by resistance to endosulfan, to OPs such as quinalphos and monocrotophos, and to the oxime carbamate methomyl (Armes *et al.* 1992, 1996).

(xii) Helicoverpa armigera *in Pakistan*

As a result of pyrethroid use since the early 1980s, moderate to high levels of resistance to pyrethroids were found in populations of *H. armigera* collected from various regions of Pakistan from 1991 onwards (Ahmad *et al.* 1995). These insects were also resistant to the OP monocrotophos, showed moderate resistance to endosulfan and had low-level resistance to the OPs chlorpyrifos and profenofos and the carbamate thiodicarb. Interestingly, in a subsequent study these authors showed variations in resistance to pyrethroids depending on their structure. Although resistance varied from location to location, the general trend was for moderate to high resistance to chemicals like cypermethrin, a low-to-moderate resistance to compounds like deltamethrin and comparatively low resistance to others like lambda-cyhalothrin (Ahmad *et al.* 1997). With the loss of efficacy of the pyrethroids farmers have begun to use other non-pyrethroid compounds, with the result that levels of pyrethroid resistance were lower in 1997 than in previous years (Ahmad 1998).

(xiii) Helicoverpa armigera *in Africa*

In the Ivory Coast pyrethroids have been applied for 15 years to control *H. armigera* and other bollworms. These pyrethroids were always mixed or rotated with organophosphate insecticides in an effort to prevent or delay resistance in bollworms (Alaux *et al.* 1997). Ernst & Dittrich (1992), in a comparative survey of resistance in heliothines around the world, could find no evidence for resistance to pyrethroids in the Ivory Coast. Vassal *et al.* (1997) confirmed that before 1992 there was no change in resistance to pyrethroids but in subsequent years susceptibility decreased and by 1995 and 1996 significant resistance was recorded. This is the first documented evidence for resistance to pyrethroids in bollworms in West Africa. No resistance to pyrethroids was found in populations of *H. armigera* from Chad, although some changes in tolerance were believed to be occurring (Martin & Renou 1995).

(xiv) Helicoverpa armigera *in Turkey*

Resistance to synthetic pyrethroids was found in populations of *H. armigera* in 1984, after their initial use around 1980 (Anon 1986). Similar findings were reported by Ernst & Dittrich (1992).

(xv) Helicoverpa armigera *in Israel*

Since 1987 a strictly observed insecticide resistance management strategy has been in place in cotton fields in Israel. This is designed to maintain susceptibility to a range of insecticides, including pyrethroids, in *H. armigera* and other cotton pests. Monitoring studies show that, despite slight fluctuations during the season, susceptibility to cypermethrin did not alter during the period 1987–1991 (Horowitz *et al.* 1993); control continued to be achieved despite a very marked decline in the number of sprays applied (Horowitz *et al.* 1995).

(xvi) Helicoverpa punctigera *in Australia*

A population of *H. punctigera* collected from New South Wales in 1994 was shown to be resistant to fenvalerate (Gunning *et al.* 1997). This is the first report of significant resistance in this species.

9. MECHANISMS OF RESISTANCE TO PYRETHROIDS

(a) *Nerve insensitivity: target-site resistance to pyrethroids*

The principal site of action of DDT and pyrethroids is the voltage-gated sodium channel of nerve cells (Soderlund & Bloomquist 1989; Narahashi 1992; Bloomquist 1996). These insecticides alter the gating kinetics of the sodium channel so that the open time of the channels is increased after the passage of the depolarizing pulse of an action potential. This inhibition of sodium-channel inactivation leads to the development of prolonged sodium currents and accounts for the prolonged depolarizing after-potential. This action causes the repetitive firing of neurons that is typically found in pyrethroid-poisoned insects. Pyrethroids also cause membrane depolarization due to the prolonged opening of sodium channels. Type II pyrethroids, which contain a cyano group at the α position, are generally more potent in this respect than type I pyrethroids, which lack this α-cyano group. Thus sensory neurons are stimulated as a result of membrane depolarization. Membrane depolarization at nerve terminals causes massive release of neurotransmitter, resulting in severe disruption of synaptic transmission.

(i) *Indirect evidence for nerve insensitivity*

Evidence for the involvement of a *kdr*-like mechanism of resistance in heliothine insects has not been easy to obtain. There is much indirect evidence that implies the

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involvement of a target-site mechanism of resistance to DDT and pyrethroids, although such evidence is never wholly reliable. The most frequently used criterion has been the lack of synergizable resistance. Weekly estimates of the survival of third-instar *H. armigera* treated with a discriminating dose of fenvalerate both with and without PBO have been used in Australia to provide an estimate of the percentage of non-synergizable resistance (Forrester *et al.* 1993). It is inferred that this residual resistance is due to other mechanisms, including a target-site resistance. Similar findings were presented by Armes *et al.* (1996) working with Indian populations of *H. armigera*. In a study in Maharashtra State in India, it was suggested that the residual non-metabolic resistance remaining after synergism with both the monooxygenase synergist piperonyl butoxide (PBO) and the esterase synergist *S,S,S,*-tributyl phosphorotrithioate (DEF) was likely to be due to target-site insensitivity (Kranthi *et al.* 1997), and this is being verified now. Given that the principal mechanisms of resistance in these insects are considered to be enhanced monooxygenase activity and/or esterase activity and a target-site resistance of the *kdr* type, this approach might appear reasonable. Nevertheless, as indicated below, PBO may not be a reliable synergist for monooxygenases and it may indeed synergize other forms of metabolic resistance as suggested by Gunning *et al.* (1996*a*). Moreover, non-synergizable penetration resistance also contributes to this residual resistance. Given such considerations the premise that non-synergizable resistance represents target-site resistance is at best equivocal.

The presence of cross-resistance between DDT and pyrethroids is frequently used as evidence for the involvement of resistance at the target site. *H. armigera* from Thailand were shown to possess high levels of resistance to DDT and cypermethrin; this observation implies a common mechanism. Lack of synergism by the DDT-dehydrochlorinase synergist FDMC reinforced this view (Ahmad & McCaffery 1991); as discussed below, the insects were indeed shown to possess a nerve insensitivity (Ahmad *et al.* 1989). Recent studies with strains of *H. armigera* selected from field collections from Jiangsu province in China have shown highly significant, non-synergizable cross-resistance between DDT and fenvalerate (J. Tan & A. R. McCaffery, unpublished results). This resistance has subsequently been shown to be due to nerve insensitivity and its molecular basis is being studied presently (see below). The inability to identify metabolites of pyrethroids in biochemical studies of pyrethroid metabolism has also been used to imply that resistance may be due to target-site resistance, although it is clear that the common involvement of both metabolic and non-metabolic mechanisms in the same individuals in resistant strains makes such an approach difficult.

Finally, because individuals with target-site resistance might theoretically be able to withstand higher internal concentrations of insecticide than their susceptible counterparts, it has been thought that the presence of high titres in insects that survive such treatments indicates the presence of this mechanism. The nervous system of resistant third-instar larvae of the PEG87 strain of *H. virescens* was shown to contain up to tenfold greater concentrations of *cis*-cypermethrin than those of susceptible larvae of the BRC strain (Wilkinson & McCaffery 1991). In addition, the behavioural responses of these intoxicated insects suggested that comparable symptoms of intoxication occurred at higher concentrations in larvae of the resistant strain than in larvae of the susceptible strain. This was again taken to imply a decreased interaction of the pyrethroid with its target site. At best, such evidence is tenuous. Such considerations form the basis of behavioural assays for nerve insensitivity, typified by the hot-needle assay developed by Bloomquist & Miller (1985) and a locomotory assay developed by Gunning (1996).

(ii) *Direct evidence for nerve insensitivity*

There now exists a large body of direct evidence that a form of nerve insensitivity contributes substantially to many cases of resistance to DDT and pyrethroids in heliothine insects. Nicholson & Miller (1985) first demonstrated this neurophysiologically in a resistant strain of *H. virescens* collected from cotton-growing areas of southern California. A similar technique was used to demonstrate nerve insensitivity in a pyrethroid- and DDT-resistant Thai strain of *H. armigera* (Ahmad *et al.* 1989). At the onset of pyrethroid resistance in Australia in 1983 a strong *super-kdr*-like mechanism was demonstrated by using a simple single-dose neurophysiological technique (Gunning *et al.* 1991), but in a survey during the period from 1997 to 1990 no evidence was found for the presence of this *super-kdr*-like mechanism. Instead, another distinct *kdr*-type mechanism with little or no toxicological significance was found. By means of a cumulative dose–response neurophysiological assay for spontaneous neuronal activity, nerve insensitivity to cypermethin was demonstrated in resistant laboratory strains of *H. virescens* (Gladwell *et al.* 1990) and in field strains collected from various parts of the US cotton belt (McCaffery *et al.* 1995; Ottea *et al.* 1995). Since monitoring of pyrethroid resistance in the USA has been based upon the adult vial test (Plapp *et al.* 1987), it is significant that nerve insensitivity in adult stages of resistant strains of *H. virescens* was correlated with that in larval stages (Holloway & McCaffery 1996). Modifications of this technique have also been used to demonstrate high levels of nerve insensitivity to pyrethroids and DDT in *H. armigera* from Andhra Pradesh state in India (West & McCaffery 1992) and from various parts of China (Y. Zhao *et al.* 1996; McCaffery *et al.* 1997; Ru *et al.* 1997; Zhang *et al.* 1997), and *H. zea* from the USA (Holloway *et al.* 1997).

(iii) *Molecular basis of nerve insensitivity resistance to pyrethroids*

Although pyrethroids may interact with a number of sites within the nervous system and although a range of effects may be produced by these interactions, the principal site of action is considered to be the voltage-gated sodium channel. For this reason efforts to determine the molecular basis of resistance to pyrethroids in heliothine insects have centred on changes in sodium channels and have followed similar pioneering studies on house flies and cockroaches. Experiments conducted by Church & Knowles (1992) on binding to neural membranes of saxitoxin, a high-affinity neurotoxin binding to site 1 on the sodium channel, suggest that there is no difference in the number of sodium channels between pyrethroid-resistant and -susceptible strains of *H. virescens*. Further work

has shown that binding of batrachotoxin, a sodium-channel neurotoxin, is enhanced by pyrethroid binding; by means of this assay these authors have provided evidence that the affinity for pyrethroids on the sodium channels is considerably reduced in resistant *H. virescens* compared with susceptible counterparts (Church & Knowles 1993). Taken together these studies imply that reduced affinity of binding is responsible for resistance to pyrethroids at the sodium channel in this species.

The evidence reviewed above suggested that resistance to pyrethroids and DDT might be expected to result from the selection of genetic mutants with altered sodium channels. Molecular genetic studies on sodium channels would clearly be essential to understand the basis of this resistance. By using degenerate oligonucleotide primers based on conserved amino-acid sequences in sodium channels of *Drosophila melanogaster*, *para*-homologous sodium-channel genes were isolated from a range of insects including *H. virescens* (Doyle & Knipple 1991). The polymerase chain reaction was used to amplify sequences from genomic DNA from the PEG87 strain of *H. virescens* by using degenerate primers homologous to the fourth transmembrane domain of the α-subunit locus *para* of *D. melanogaster* (Taylor *et al.* 1993). One genomic clone encoding a putative sodium channel in *H. virescens* was obtained and designated *hscp* (*Heliothis sodium channel para* homologue). In a subsequent experimental analysis, markers for *hscp* were found to be linked to resistance phenotypes and this provided the first molecular genetic evidence for such a link in any heliothine.

Sequence comparisons between resistant and susceptible genotypes of house fly have revealed the presence of a single leucine-to-phenylalanine mutation (L1014F) in transmembrane segment 6 of domain II associated with *kdr* resistance, and an additional methionine-to-threonine mutation (M918T) associated with *super-kdr* resistance (Williamson *et al.* 1996). Park & Taylor (1997) examined *H. virescens* in a similar manner and revealed the existence of a leucine-to-histidine change (L1029H) associated with resistance to pyrethroids and located at a position homologous to that in *kdr* strains of the house fly. No mutation homologous to that found in *super-kdr* flies was found in *H. virescens* (Park & Taylor 1997). Interestingly, the resistant PEG87 strain of this insect was not found to carry this mutation; this observation leads to the suggestion that more than one sodium-channel mutation may be contributing to pyrethroid resistance in field populations of *H. virescens*. This contrasts with the situation in *Musca domestica*, *Blattella germanica*, the diamondback moth, *Plutella xylostella*, and the peach–potato aphid, *Myzus persicae*, in which the leucine-to-phenylalanine substitution is always consistently present in resistant genotypes (Martinez-Torres *et al.* 1997). More recently, Park *et al.* (1997) reported a valine-to-methionine (V421M) substitution in transmembrane segment 6 of domain I (IS6) of the *hscp* locus of individuals of the homozygous resistant strain used by Taylor *et al.* (1993) for linkage analysis. More recently still, Head *et al.* (1998) made sequence comparisons between resistant and susceptible strains of both *H. virescens* and *H. armigera* and showed consistent aspartic acid-to-valine (D1561V) and glutamic acid-to-glycine (E1565G) substitutions in the cytoplasmic linker region between domains III and IV (III–IV) of the *para*-homologous sodium-channel sequence of neurophysiologically resistant insects of both species; this region is involved in channel inactivation. A further mutation in the IIS5–IIS6 linker region was again consistently found in both resistant *H. armigera* and resistant *H. virescens* (Head 1998). All these findings emphasize the likelihood that a number of possible mutations can confer resistance at the sodium channel, although the function of these mutations clearly remains to be ascertained. The development of diagnostic technologies based on mutations that unequivocally indicate the *kdr*-like nerve insensitivity resistance to pyrethroids and DDT is a clear aim of such studies. The successful deployment of technology of this type would provide a degree of precision and refinement that has so far been lacking in the monitoring of resistance-gene frequency in heliothine pests.

(b) *Metabolic mechanisms of resistance to pyrethroids*

Studies on the metabolism of pyrethroids in heliothine insects have been characterized by a degree of contradiction, which has centred on the relative roles of the principal systems of enzymic detoxication: oxidation by the microsomal P450-dependent monooxygenases (or mixed-function oxidases) and hydrolysis by esterases. Glutathione S-transferases do not appear to be involved in resistance to pyrethroids. The traditional use of synergists to give preliminary indications of the type of metabolism involved in resistance has been critically questioned in relation to resistance to pyrethroids in view of findings, discussed below, which suggest that specific synergists are no longer (or possibly never were) effective at suppressing the enzyme systems with which they have traditionally been associated. Other studies suggest that some synergists inhibit enzyme systems alternative to those with which they are normally associated. The use of model substrates is also an area of some uncertainty: the isozymes responsible for detoxication of specific insecticides may not necessarily be those involved in model substrate metabolism. The latest and most comprehensive view of this field would suggest that both oxidative and hydrolytic activity is involved in resistance to pyrethroids in heliothines and that, indeed, these species seem likely to be able to use both types of metabolism in response to appropriate selection. Such a view has considerable implications for resistance management.

(i) *Metabolic resistance in* Heliothis virescens

Initial studies on metabolism of pyrethroids in *H. virescens* suggested that monooxygenases were involved in tolerance to *trans*-permethrin (Bigley & Plapp 1978). In field strains of this insect collected from the Imperial Valley in California, enhanced metabolism of *trans*-permethrin was a shown to be a mechanism of resistance (Nicholson & Miller 1985) and it was thought that this was likely to be due to oxidative hydroxylation. Dowd *et al.* (1987) brought insects from this location into the laboratory and selected them with flucythrinate. In contrast to the findings above, they demonstrated both a qualitative and a quantitative enhancement in the ability of larvae to hydrolyse pyrethroids compared with a susceptible strain.

The PEG87 strain of *H. virescens* has been used extensively in research on resistance to pyrethroids in this species and was derived from the US83 strain, itself

assembled from a series of 19 collections across the US cotton belt where control with pyrethroids had become increasingly difficult. Despite effectively being a laboratory strain, it was considered to possess the mechanisms of resistance most likely to be representative of those in the field. By using this strain it was shown that resistance to *trans*-cypermethrin and *cis*-cypermethrin was largely due to a PBO-synergizable monooxygenase, which resulted in hydroxylation of the pyrethroid in the 4′; and 2′; positions on the phenoxybenzyl moiety (Lee *et al.* 1989: Little *et al.* 1989; Clarke *et al.* 1990; McCaffery *et al.* 1991*c*) and later elimination of conjugated metabolites. Further studies on this mechanism showed that the resistant strain possessed a sixfold greater quantity of total cytochrome P450 and a fourfold greater quantity of cytochrome P450 reductase than did the comparable susceptible strain (Clarke *et al.* 1990). Activity was shown to be NADPH-dependent and PBO-suppressible. Significantly, it was shown in these studies that the major hydroxy-metabolites were likely to be better substrates for hydrolysis than the parent compound. This finding was considered to explain the PBO-suppressible, NADPH-dependent appearance of acid metabolites, although carboxylesterase action was considered to play a minor role in the direct hydroxylation of the pyrethroid (Clarke *et al.* 1990). Using the PEG87 strain of *H. virescens* Abd-Elghafar *et al.* (1994) presented similar evidence for oxidative metabolism of fenvalerate.

Despite the early demonstration of enhanced metabolism in the field strain from California noted above (Nicholson & Miller 1985; Dowd *et al.* 1987), metabolic resistance was considered to be rare or absent in field populations of *H. virescens* for many years. Accordingly, a number of studies showed that pyrethroid resistance was not synergized by PBO or DEF (McCaffery *et al.* 1991*b*; Clower *et al.* 1992), and it was considered that the majority of the resistance was likely to be due to target-site resistance of the *kdr* type. With continued use of pyrethroids, evidence for PBO-synergizable resistance began to appear in the early 1990s in various US cotton-belt states including Louisiana, Mississippi, Texas and Oklahoma (Graves *et al.* 1991; McCaffery & Holloway 1992; Elzen *et al.* 1993; Kirby *et al.* 1994; Martin *et al.* 1994, 1997; G. Zhao *et al.* 1996), suggesting a widespread and growing resistance problem based on enhanced monooxygenase activity as had been found with *H. armigera*. The existence of enhanced resistance to cypermethrin through selection with the oxime carbamate thiodicarb, and the existence of PBO synergism of these insecticides, was strongly indicative of the involvement of oxidative metabolism (G. Zhao *et al.* 1996).

A number of other findings suggest that this might not be wholly representative of the status of this mechanism. Martin *et al.* (1997) showed that application of PBO delayed penetration of pyrethroids and suggested that PBO could influence toxicity in other ways. Some strains of *H. virescens* believed to possess enhanced monooxygenase activity were shown to be entirely unresponsive to the action of PBO and instead were synergized by propynyl ethers such as TCPB (Brown *et al.* 1996*b*) (see below). Nevertheless, many later biochemical studies demonstrated the importance of oxidative attack in resistance to pyrethroids in *H. virescens* (Ottea *et al.* 1995; Ibrahim & Ottea 1995; G. Zhao *et al.* 1996). In a recent study with the metabolically blocked pyrethroid fenfluthrin, a number of other structurally modified pyrethroids and several synergists Shan *et al.* (1997) confirmed that P450 monooxygenases were associated with pyrethroid resistance in this species. Using a pyrethroid- (and thiodicarb-) resistant strain of *H. virescens* strain originally collected from fields in Louisiana where field failures with cypermethrin and thiodicarb had been recorded, Rose *et al.* (1995) examined monooxygenase, esterase and glutathione S-transferase activity. Up to 4.4-fold higher quantities of cytochrome P450 were found in the gut, fat body and carcass of the resistant strain than in those of the susceptible strain and it was thought likely that these increased P450 levels represented the sum of several P450 isozymes, each of which may possess specific yet overlapping substrate specificities. Esterases and transferases were thought to be less important in conferring resistance in this strain, although transferases may be important in the production of conjugates, which form the bulk of excreted metabolites in monooxygenase-resistant *H. virescens* (Little *et al.* 1989). Interestingly, Rose *et al.* (1995) obtained incomplete synergism with PBO in this strain and offered the suggestion that isozymes involved in pyrethroid resistance might be made unresponsive to synergists by selection pressure with PBO or other insecticides, as appears to be the case in other insects. Incomplete synergism with PBO was also obtained in another *H. virescens* strain derived from field collections in Louisiana (Shan *et al.* 1997) although it could be completely synergized by the propynyl ether TCPB. The effectiveness of TCPB as a monooxygenase synergist for pyrethroid resistance in *H. virescens* was first shown by Brown *et al.* (1996*b*), who concluded that different classes of P450 monooxygenases were involved in resistance-associated metabolism of pyrethroids. Such a finding casts considerable doubt on the validity of previous synergism studies using PBO; the absence of PBO synergism should perhaps not be taken as an indication of the absence of enhanced oxidative metabolism.

The doubts about the efficacy of PBO and the involvement of monooxygenases are compounded by renewed interest in the role of esterases in pyrethroid resistance in field strains of *H. virescens*. Graves *et al.* (1991) had initially found evidence for synergism of pyrethroids with DEF, inferring the involvement of esterases and confirming earlier observations (Dowd *et al.* 1987). Martin *et al.* (1997), however, showed antagonism of the esterase synergist TPP to cypermethrin action. The larval stages of a strain of *H. virescens* originally obtained from the field in Louisiana, where control with cypermethrin and thiodicarb had failed, were examined for esterases associated with resistance to these two compounds (Goh *et al.* 1995). Esterase activity against the model substrate 1-naphthyl acetate (1-NA) was elevated in whole-body homogenates of resistant insects compared with those of susceptible insects. Increased esterase activity was attributed to three esterases, A1, B1 and C1, which were purified and compared by means of immunoblotting techniques. The most significant of these, esterase A1, was considered to share common epitopes with the resistance-associated esterase of other insects, although its role in insecticide resistance in the tobacco budworm was not

entirely clear. In a very recent study, G. Shan and J. A. Ottea (personal communication) have shown that metabolism of cypermethrin in *H. virescens* larvae occurs by both oxidative and hydrolytic pathways but that the hydrolytic route appears to be the major resistance mechanism. The production of metabolites of hydrolysis in laboratory and field strains, as well as observations that suggest that both cypermethrin and 1-NA inhibit esterases in a concentration-dependent manner, provide further evidence that esterases are the major metabolic mechanisms of resistance to pyrethroids. Moreover, inhibition experiments with PBO and paraoxon and studies with 1-NA all suggest that the monooxygenase inhibitor also inhibits esterases. Such studies clearly concur with the findings of Gunning *et al.* (1996*a*) working with *H. armigera* (see below) and cast yet further doubt on the validity of using PBO as a synergist. Urgent re-evaluation of the action and usefulness of these synergists is required.

(ii) *Metabolic resistance in* H. armigera

Australia too has been a focus of considerable debate regarding the relative roles played by esterase- and cytochrome P450-mediated pyrethroid metabolism. In 1983, with the onset of resistance to synthetic pyrethroids in *H. armigera* in Australia (Gunning *et al.* 1984), three mechanisms of resistance were thought to be involved. Both a strong nerve insensitivity (*super-kdr*) and a penetration resistance (*Pen*) were believed to be present, together with a third factor overcome by PBO (*Pbo*) (Gunning *et al.* 1991). Between 1987 and 1990 these insects were again examined to determine which mechanisms were present. Both the *Pen* and *Pbo* mechanisms had increased in importance, although they conferred only a low-order resistance of around 20-fold (Gunning *et al.* 1991). The ability of PBO to completely suppress resistance to pyrethroids in strains of the insects homozygous for a metabolic detoxication mechanism was presumed to be evidence of the involvement of P450-mediated metabolic resistance (Forrester *et al.* 1993). Moreover, the relative metabolic resistance-suppressing activity of a range of 65 synergists including TCPB provided further strong evidence of the involvement of P450-mediated metabolism. Field populations of the insects were regularly tested with a discriminating dose of fenvalerate both with and without PBO. The evidence from this monitoring suggested that the PBO-suppressible resistance component was always predominant. As discussed earlier, the non-synergizable component was assumed to represent other mechanisms, in particular nerve insensitivity. Further convincing evidence that the great majority of the resistance to pyrethroids seen Australian *H. armigera* was due to enhanced oxidation came from an important examination of the structure–activity relations of a large range of pyrethroid analogues with varying acid and alcohol structures and a range of substitutions. Alterations in the alcohol moiety of the pyrethroid structure could overcome most, if not all, resistance. The nature of these changes in countering resistance provided strong evidence that the resistance was due to oxidative metabolism. All of these findings would seem to provide overwhelming, if somewhat indirect, evidence that resistance to pyrethroids in *H. armigera* in Australia was based on enhanced P450-mediated metabolism. Similar evidence has been put forward for enhanced monooxygenase activity being a mechanism of resistance to pyrethroids in *H. armigera* from India (Phokela & Mehrohtra 1989; Kranthi *et al.* 1997) and China (Wu *et al.* 1997*a*).

This conventional view was challenged by Kennaugh *et al.* (1993) using a strain of *H. armigera* derived from field collections and subsequently backcrossed and selected. Although the resistant strain was 19- to 33-fold resistant to fenvalerate and this resistance could be eliminated with PBO, these authors could find no increased levels of P450 in the midguts of the resistant strain compared with those of the susceptible strain. Further, there was no evidence for increased permethrin detoxication in the resistant strain. Significantly, PBO increased the rates of metabolism in both susceptible and resistant strains. Evidence was obtained which suggested the involvement of a cytochrome P450 in the process of penetration of the insecticide through the insect cuticle. The action of PBO would thus be to inhibit a P450-dependent penetration resistance (Kennaugh *et al.* 1993). These findings were corroborated by Gunning *et al.* (1995), who examined esfenvalerate metabolism in a resistant strain of *H. armigera*, in which the resistance was suppressed by PBO and which lacked any nerve insensitivity. It was shown that esfenvalerate metabolism was only slightly enhanced in this resistant strain and that PBO did not inhibit this metabolism. The authors concluded that reduced penetration appeared to be an important mechanism of esfenvalerate resistance in this strain.

An important study was then published, which suggested that pyrethroid-resistant *H. armigera* in Australia have enhanced esterase activity that is due to increased production of enzymes (Gunning *et al.* 1996*a*). The most resistant individuals were shown to have an approximately 50-fold increase in esterase activity compared with susceptible populations. Moreover, resistant strains were shown to have additional esterases not detectable in susceptible populations and increased esterase hydrolysis of 1-NA was correlated with the esfenvalerate resistance factor. Furthermore, evidence was obtained which suggested that the esterase had a poor catalytic activity towards the pyrethroids and that esterases were also acting as insecticide-sequestering agents. It was concluded that detoxification by hydrolysis together with sequestration would give *H. armigera* the ability to detoxify significant quantities of fenvalerate, consistent with the large resistance factors involved. Together these findings imply that detoxication via monooxygenases is no longer, or was never, a significant mechanism of resistance to pyrethroids in *H. armigera*, a situation that is paralleled in *H. virescens* in the USA. To further emphasize this revised view of metabolic resistance to pyrethroids, Gunning *et al.* (1996*b*) have shown that PBO can suppress esterase-mediated metabolism. This crucial observation defies the conventional assumption that PBO uniquely suppresses metabolic resistance mediated by cytochrome P450 and is again mirrored in recent studies in the USA on *H. virescens* (J. A. Ottea, personal communication). More recently Gunning *et al.* (1997) showed that fenvalerate toxicity in *H. punctigera* was synergized by the esterase inhibitors DEF and profenofos and that the resistant insects had increased esterase activity to 1-NA.

(iii) *Molecular studies on metabolic resistance in heliothines*

The conflicting nature of these findings both in Australia and in the USA emphasizes that a definitive role in metabolic resistance for specific P450s or esterases is likely to come only from expression studies using genes cloned from pyrethroid-resistant strains.

The complete coding sequence and parts of the 3′ and 5′ non-coding regions of a mRNA coding for a cytochrome P450 from *H. armigera* was obtained (Wang & Hobbs 1995). The sequence is most similar to member of the CYP6 family and has been designated CYP6B2. The cDNA hybridizes to two major mRNAs, the larger of which is inducible by permethrin, although the levels of induction are generally low. These same authors have demonstrated much higher quantities of the larger mRNA in individual, pyrethroid-resistant larvae collected directly from the field; this result implies the involvement of this P450 in resistance. In a separate study, RT–PCR was used to clone P450 gene fragments from the RNA of a pyrethroid-resistant strain of *H. armigera* (Pittendrigh *et al.* 1997). By this method eight new P450 genes were isolated, seven from the *CYP4* family and one *CYP9*. One of these genes, *CYP4G8*, was twofold overexpressed in the resistant strain. Although no difference in expression was noted in resistant strains, *CYP9A3* appeared to be a homologue of the putatively resistance-associated *CYP9A1* of *H. virescens* (Rose *et al.* 1997) (see below). Further, the authors found non-detectable levels of expression of the *CYP6B2* isolated by Wang & Hobbs (1995) and reportedly overexpressed in resistant strains. In *H. virescens* Rose *et al.* (1997) isolated a P450 gene designated *CYP9A1*, the first member of family 9, from a pyrethroid-resistant strain. These studies indicate that both qualitative and quantitative strain-to-strain variations in P450 expression levels are important and that recombinant expression will be necessary in order to precisely define the substrate specificities and pyrethroid-metabolizing abilities of individual P450s. The ability to define the characteristics of the detoxification systems of resistant strains of insects would lead to a significant refinement in cross-resistance studies. On the basis of substrate specificity it should prove possible not only to objectively select insecticides between chemical groups but also to select more efficacious analogues from within groups.

10. DISCUSSION

As summarized in this review, *H. armigera*, *H. virescens*, and to some extent *H. zea*, have developed substantial and often uncontrollable levels of resistance to virtually all the neurotoxic insecticides that have been directed against them. Ecological and physiological aspects of the biology of these insects have made possible the emergence of pest species, which have often proved difficult to control. Continued selection with insecticides has allowed the survival of resistant populations, which have generally proved exceedingly difficult or impossible to control.

Despite this there are examples of cropping systems in which resistance is absent or in which resistance is a minor problem. The resistance-management strategy initiated in cotton in Israel to control a range of pests has left *H. armigera* there very largely susceptible to all insecticides, despite, or more probably because of, the use of a very small number of applications (Horowitz *et al.* 1995). In other areas such as the Ivory Coast, low or restricted use of insecticides has allowed a great many years of resistance-free pest control and only now are levels of resistance beginning to rise.

Species such as *H. punctigera*, which have, by virtue of their biology and ecology, been considered capable of escaping the development of resistance, have now been shown to do so (see, for example, Gunning *et al.* 1994, 1997). It is essential that resistance-management strategies are formulated in ways that do not enhance the resistance status of such species. In the USA, where pyrethroids have been very widely used, it is generally accepted that the key feature that has prevented development of resistance in the maize earworm, *H. zea*, is its wider range of unsprayed hosts: *H. zea* attacks maize and soyabeans whereas *H. virescens* does not attack maize and prefers cotton to soyabeans. Nevertheless, *H. zea* has recently developed significant resistance to these compounds and consideration must be given to the implications of this.

The most highly imitated resistance management strategy for heliothines is that which was set up for the control of pyrethroid- and endosulfan-resistant *H. armigera* in cotton (and other crops) in Australia (Forrester *et al.* 1993). The obvious success of this highly acclaimed scheme was that control was maintained for well over ten years with insecticides to which resistance had already developed. This was achieved largely through strict observance of restrictions in use. The gradual loss of the pyrethroids to resistance and the advent of new insecticides, *Bt* and *Bt*-transgenic cotton has allowed a relaxation of the pyrethroid use strategy and control is now based on a broad range of chemical, biological and cultural methodologies. Similar types of strategy have been initiated elsewhere with varying degrees of success, as noted above.

The development of pyrethroid resistance in heliothine species in various countries around the world continues unceasingly, even in countries with management strategies, although the rate of loss of efficacy is generally slower in controlled situations. That pyrethroid-resistant insects can be found in unsprayed refugia in Australia (Forrester *et al.* 1993), and pyrethroid-susceptible insects are absent or exceedingly hard to find anywhere in countries such as Pakistan and India, does not bode well for much further use of these compounds unless new chemistry is deployed or severe restrictions on use are instigated. Such actions have complex economic and political implications. Moreover, questions regarding the fitness costs of these resistances need to be addressed urgently since there are important considerations for insecticide-resistance management. Although new technology and new chemistry will enable the selection pressure from older insecticides to be relaxed, it is likely that the use of conventional insecticide chemistry will continue for many years.

This review has placed considerable emphasis on the mechanisms of resistance to the insecticide groups considered. This is because patterns of cross-resistance within and between insecticide groups are entirely dependent on the biochemical and molecular nature of the resistance mechanism. Even within the conventional insecticide groups there are many areas of susceptibility to existing chemistry, which can be exploited for control. In a previous brief review this author considered that

target-site resistance to pyrethroids had developed in many heliothines before the later emergence of metabolic resistance (McCaffery 1994). Although this may still hold true to some degree, the ability of these species to diversify their mechanisms of resistance under selection pressure is noteworthy. The current debate over the nature of metabolic resistance in heliothines is confused by technical controversy over the use of some of the basic tools, such as synergists and model substrates, and these arguments have been considered in detail in this review. Likewise, the inconsistencies found in pyrethroid target-site mutations are at variance with those found in other insects. It is the view of this author that the heliothines are especially flexible in the use of a variety of modifications in all of their resistance mechanisms. Thus, even when a similar system of enhanced enzyme activity is involved in resistance to the same group of compounds, such as monooxygenases, different P450 forms seem likely to be found in individual populations of the same species.

It could be argued that an ability to diagnose the precise nature of the mechanisms of resistance would be a key component of the management of resistance in the heliothines. However, as emphasized in this review, the very diverse nature of the modifications found so far makes this enormously less easy than might otherwise be so and possibly renders such an approach not practicable. It might be considered that this diversity would allow the use of resistance-breaking molecules within existing conventional insecticide groups. The existence of such compounds has been illustrated for both *H. armigera* (Forrester *et al.* 1993; J. Tan and A. R. McCaffery, unpublished observations) and *H. virescens* (Shan *et al.* 1997), but the usefulness of such an approach again depends entirely on an ability to correctly diagnose subtle changes in resistance mechanisms in field populations. As highlighted above, this might prove difficult in reality and it is disappointing that such approaches have not yet resulted in commercially viable products. A knowledge of the mechanisms of resistance existing in these insects is clearly of value in devising new insecticides to control them. The advent of new areas of insecticide chemistry such as *Bacillus thuringiensis*, pyrroles, phenyl pyrazoles, spinosad, nicotinyls and insect growth regulators (IGRs) should make control of heliothines (Tabashnik *et al.*, this issue) considerably more effective and release selection on existing resistance mechanisms. Nevertheless, incipient resistance to some of these materials in a number of artificial laboratory strains of Heliothinae is surely a stimulus to effective use of new materials and an ever-watchful study of the development and nature of possible resistances to them.

In conclusion, it is perhaps significant that, to date, the most successful resistance-management programme that has been developed for these insects has been instituted in cotton in Israel. Key features of this programme have been a dramatic reduction in the number of sprays directed at a number of pests, including *H. armigera*, together with the considered use of a range of integrated pest management (IPM) techniques, It would appear that, despite our rapidly increasing knowledge of the biochemical and molecular nature of this problem, the most effective means of managing resistance to insecticides in the Heliothinae remains a strict control of insecticide use.

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