

Insecticide Resistance in Diamondback Moth

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Abstract

More cases of insecticide resistance have been reported in many parts of the world and more information regarding the resistance mechanisms has been obtained through research since 1985. This paper, in addition to giving a brief account of the overall situation, places more emphasis on recent findings of diamondback moth, *Plutella xylostella* (L.), resistance to various categories of insecticides, especially the chitin synthesis inhibitors, with an attempt to integrate these results into recommendations for the resistance management of this insect pest of crucifers. Glutathione S-transferase degradation has been found responsible for diamondback moth resistance to parathion and methyl parathion. Very limited cross resistance from these two organophosphorus (OP) compounds to some other OPs is observed. Diamondback moth with high microsomal monooxygenase activity converts more parathion to paraoxon, the potent anticholinesterase metabolite; and paraoxon and methyl paraoxon are conjugated at much reduced rates by glutathione S-transferase. Components of microsomal monooxygenases have been observed and studied for the first time in diamondback moth larvae. Both qualitative and quantitative differences have been observed among the susceptible and resistant strains. Implications of these observations in resistance management are elaborated.

Introduction

Significantly more research efforts on diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), resistance to insecticides have been made since the First International Workshop on DBM Management was held in 1985. Occurrence of insecticide resistance in this insect pest has been reported, since then, in several countries outside Southeast Asia, e.g., Japan (Hama 1986), U. S. (Tabashnik et al. 1987), Honduras (Secaira 1988), Australia (Altman 1988); resistance to benzoylphenyl ureas (BPUs) or so-called insect growth regulators (IGRs), which did not exist then, has been detected in some regions (Lin et al. 1989; Vattanatangum 1988); and for the first time, DBM resistance to a microbial insecticide, *Bacillus thuringiensis* Berliner, has been observed in the field in Hawaii (Tabashnik et al. 1990).

In the meantime, rapid assays to detect resistance in some insects, such as *Myzus persicae* (Sulzer), have been developed (Field et al. 1989); insecticide resistance management strategy has been adopted and assessed in the control programs of insect pests on some crops, such as *Heliothis* spp. on cotton (Sawicki 1989; Plapp et al. 1990). Thus it appears appropriate and timely for us to reflect upon the resistance management of DBM, despite the paucity of technical data and the pessimistic view expressed by some that this phenomenon might not be manageable.

This paper will first review our work on DBM resistance to major categories of insecticides and some synergists. Then current insecticide resistance management programs on cotton will be examined briefly to illustrate the influence of economic importance and farming scale on their implementation. Finally, guidelines based on existing information for DBM resistance management will be formulated.

What We Know

Our current understanding of DBM resistance to carbamates, organophosphorus insecticides (OP), pyrethroids, synergists, BPU, and abamectin is summarized in Table 1.

Among these major groups of insecticides, carbamates are the least effective against even the susceptible DBM larvae (Liu et al. 1982). Common mechanisms of resistance with OPs (acetylcholinesterase insensitivity) (Sun et al. 1986) and pyrethroids (oxidative detoxication) (Liu et al. 1984; Chen and Sun 1986) make them less useful while designing management strategies of insecticide mixing or alternations.

On the other hand, OPs have been and will continue to be an important group of compounds for the control of DBM. Enough variations in chemical structures within OPs have contributed to the wide spectrum of efficacy and varied levels of resistance observed in DBM (Liu et al. 1982). While target insensitivity is found to be one resistance mechanism (Sun et al. 1986), its contribution to overall OP resistance is not fully investigated and appears probably additional to that of the metabolic resistance mechanisms (Kao and Sun 1991).

Glutathione S-transferase conjugation appears to be a major detoxication mechanism for parathion and methyl parathion (Kao and Sun 1991). Considerably higher degradation mediated by this detoxifying enzyme is associated with resistance to these two OPs. Existence of isozymes has been proposed. Involvement of this enzyme in DBM resistance to other OPs or other kinds of insecticides is not clear and remains to be assessed. Carboxylesterase hydrolyzes only malathion to a significant extent. A recent *in vitro* experiment reveals that microsomal P₄₅₀ monooxygenation is probably not related to DBM resistance to some OPs, e.g., diazinon, azinphosmethyl, prothiophos, and tetrachlorvinphos (Kao and Sun 1991). This is in accordance with previous observations that no significant cross resistance existed between OPs and pyrethroids (Chen and Sun 1986).

Three mechanisms have been proposed for DBM resistance to pyrethroids. Reduced penetration is suggested by Noppun et al. (1989) as a major mechanism for fenvalerate resistance. It is nevertheless difficult to assess the importance of this mechanism as well as that of reduced nerve sensitivity (Liu et al. 1982; Hama et al. 1987) in overall resistance phenomenon. We believe that microsomal P₄₅₀ monooxygenase detoxication constitutes the major factor in pyrethroid resistance of this insect pest (Yao et al. 1988; Hung and Sun 1989). All pyrethroids are significantly synergized by monooxygenase inhibitors, e.g., piperonyl butoxide (PB), MGK 264 and sulfoxide, in terms of killing (Liu et al. 1984; Chen and Sun 1986) as well as knockdown (Chen et al. 1985) effects against DBM larvae and adults.

However, DBM may become resistant rapidly to a combination of pyrethroid and synergist, rendering the latter totally ineffective to enhance the toxicity of the former (Chen and Sun 1986). This resistance to synergist appears unstable and upon the termination of synergist usage, susceptibility may recover to a considerable extent within a short period of time. Limited data indicate that resistance to one synergist will not affect the synergistic effect of the others mentioned above.

Before BPU were introduced to Taiwan, a survey was made to determine the susceptibility baseline of DBM. One important observation is that there is no cross resistance to this new group of compounds from high levels of existing OP and pyrethroid resistance (Perng and Sun 1987; Perng et al. 1988). This phenomenon, of course, was the basis for the remarkable performance of teflubenzuron and chlorfluazuron when they were first put on the markets. To no one's surprise, DBM mustered up its defense weapons within 1 year and control failures started to appear (Lin et al. 1989). Microsomal P₄₅₀ detoxication again has been shown responsible for the resistance in both laboratory-selected and field-collected strains. Further studies show that different forms of cytochrome P₄₅₀s are probably involved in the oxidative degradation of BPU and pyrethroids (Lin et al. 1989; Perng and Sun 1987). This also suggests that DBM possesses a tremendously active and versatile microsomal monooxygenase system, which is ready to cope with whatever compounds having chemical structures vulnerable to oxidation (Chen and Sun 1986; Hung et al. 1990). In this regard, we have observed a definite synergism

Table 1. A summary of insecticide and synergist resistance in DBM.

Insecticide/range	Mechanism	Comment
Carbamates 150-2000xstable	Acetylcholinesterase (AChE) insensitivity Oxidative detoxication	Cross resistance with some OPs Cross resistance to/from PYs
Organophosphorus (OP) 100-50000xunstable in some cases	AChE insensitivity (K _i 20-50x difference) Microsomal oxidation probably not involved Glutathione S-transferase degradation for some OPs Carboxylesterase hydrolysis for malathion	Insufficient to account for the high resistance observed No cross resistance from high levels of PY resistance to some OPs
Pyrethroids (PY) 150-2000xstable	Reduced penetration Nerve insensitivity Microsomal oxidation: major mechanism	Difficult to assess the importance Difficult to assess the importance Strong synergism by piperonyl butoxide (PB), MGK 264, sulfoxide Insignificant cross-resistance to some BPUs Knockdown of both larvae and adults synergized by PB
Piperonyl butoxide (PB) > 12xunstable MGK264 > 6x unstable	Microsomal oxidation Probably microsomal oxidation	Insignificant cross-resistance to MGK 264 and sulfoxide Insignificant cross-resistance to PB and sulfoxide
Benzoylphenyl ureas (BPU) 40-700x unstable (?)	Microsomal oxidation: major mechanism	Unusually high activities of some microsomal oxidations Forms of cytochrome P ₄₅₀ s probably different from those involved in PY oxidation
Abamectin 125x (lab.) stability unknown	Microsomal oxidation	

of abamectin by PB in a laboratory-selected resistant strain (resistance ratio of 125) of DBM.

Implications of Studies on Insecticide Resistance and its Management of Other Insect Pests

In a recent paper Devonshire (1989) reviewed the work on insecticide resistance in *M. persicae* for the past 20 years in Rothamsted Experimental Station. Through a detailed understanding of the biochemistry and molecular genetic bases of the resistance mechanism, sensitive and reliable methods for monitoring resistance in field populations have been developed. In terms of overall advancement, this has been the most sophisticated work on insect resistance to insecticides up to now. One significant aspect underlying all these accomplishments is that increased production of esterase E4 through gene amplification is the sole mechanism responsible for *M. persicae* resistance to carbamates, OPs as well as pyrethroids (Devonshire and Moores 1982). This is in sharp contrast with what we know about DBM. Resistance of DBM to various groups of insecticides is attributable to almost all known mechanisms, metabolic and non-metabolic. This phenomenon undoubtedly will render the development of rapid biochemical assays for resistance monitoring in the field and resistance management program for DBM a much more complicated task.

Resistance research over the past four decades has identified more than a dozen factors that can influence the development of resistance (Georghiou 1983). Therefore, designing a scientifically valid and economically acceptable resistance management program for a particular pest, such as DBM, is a very complex job. Table 2 lists several programs of pyrethroid resistance management of cotton pests that have been implemented (Sawicki 1989; Plapp et al. 1990). They all have been established without detailed and quantitative knowledge of resistance mechanisms, genetics or ecology of the pests. Whether mandatory or voluntary, curative or preventive, these programs consist of simple guidelines, such as pyrethroids are allowed only in certain periods of time, or certain types of insecticides should precede or follow pyrethroids. Nevertheless, they have been considered in general to be quite effective.

However, I would like to point out some special features of these programs and try to put DBM resistance management in perspective. The implementation of these programs obviously depends on the fact that cotton farming is of such a large scale and commercial value that pest control measures for this crop can dictate spray schemes on all other crops. In addition, simple culture system (one crop per year and somewhat uniform farming calendar) and relatively longer growing season make possible synchronization of spray schedules and full manifestation of management results. In contrast, cruciferous vegetables are often grown by small landholders around urban centers and in highlands in Asia and many other regions. These vegetables are usually grown year-round as their growing seasons vary and are relatively short. Many generations of DBM (>20 in Taiwan) occur each year and generation overlapping is common. In this connection, compliance with any management program appears to be a challenge very difficult for vegetable growers to meet.

In view of the current programs on cotton, which have been based on very limited knowledge, consisting of simple guidelines and generally considered effective, there is no reason why we cannot put resistance management of DBM to work. With existing knowledge, guidelines can be proposed without making serious errors in recommendation. As new data become available, it can be further fine-tuned.

What We Can Do

Five years ago in the First Workshop we proposed our first set of recommendations with regard to the management of insecticide resistance (Sun et al. 1986). The following represents an updated version.

Table 2. Current insecticide management programs in cotton.

Acaricide resistance Zimbabwe 1975	Curative measure	<p>Voluntary program</p> <p>Country divided into three regions; same two acaricides (from different chemical groups) used in each region for 2 years; change to next pair of compounds for 2 years; and so on Successful for 14 years</p>
Pyrethroid resistance Zimbabwe 1978	Preventive measure <i>Heliothis armigera</i>	<p>Voluntary program</p> <p>Pyrethroid spray scheme on all crops dictated by pest control measures for cotton</p> <p>Pyrethroids allowed only in mid summer, 9-week period (peak flowering and fruiting in cotton season)</p> <p>Only those pyrethroids with effective acaricide activity are recommended</p> <p>Has worked well, but not sure if due to the beneficial effects of the program or to a historical lack of insecticide resistance in <i>H. armigera</i></p>
Egypt 1978	Preventive measure <i>Spodoptera littoralis</i>	<p>Mandatory program</p> <p>Pyrethroids used solely on cotton and only against <i>S. littoralis</i></p> <p>First spray of OP/IGR mixture, followed by a single spray of OPs or carbamates alone or with IGRs</p> <p>Control failure rising in 1986-87; increasing resistance levels detected</p> <p>Spraying becoming unsynchronized; pyrethroids used on other crops</p>
Australia 1983	Curative measure <i>H. armigera</i>	<p>Voluntary program applied to all host plants</p> <p>Cotton season divided into three stages, maximum three applications of pyrethroids only in stage 2, a 6-week period, one application with synergist PB</p> <p>Selection of only one out of four or five generations</p> <p>Proper timing of spray, pyrethroid being selective only on older (> 4 day) larvae</p> <p>First 3 years working well, pyrethroid resistance frequency returning to average in stage 1</p> <p>Resistance frequency high at beginning of season of fourth year, many localized pyrethroid failures at stage 2</p> <p>Fifth year lower resistance frequency at beginning; <i>H. armigera</i> occurrence low; fewer pyrethroid applications; control failures rare</p>

1. Start management before resistance is detected.
2. Avoid continuous cultivation of crucifers.
3. Observe the economic threshold while spraying insecticides.
4. Do not mix insecticides. It usually cannot delay the onset of resistance and often accelerates and complicates it.
5. Always rotate different groups of compounds, i.e., OPs, pyrethroids, BPUs and *B. thuringiensis*.
6. Limit the use of pyrethroids and BPU. If OPs are still useful, do not use pyrethroids. Do not introduce BPU if OPs and pyrethroids, alternatively, can still control DBM.
7. Use BPU on crucifers of longer growing period in order to manifest their toxicity fully. Use BPU early in the season, just once if possible.
8. Synergists, such as PB, may be used with pyrethroids and BPU. But do not use PB continuously.

Publications and information available after this Workshop have shown that DBM has developed in the field several hundred fold resistance to *B. thuringiensis*, and resistance mechanism identified in DBM is similar to that found in *Plodia interpunctella*, i.e., reduced affinity of midgut brush border membrane to *B. thuringiensis* toxin (Gould 1991; Van Rie et al. 1990). Cross resistance within *B. thuringiensis* preparations from the same serotype occurs in DBM and this insect could develop resistance to mixtures of *B. thuringiensis* toxins (Tabashnik et al. 1991). Therefore, it appears that resistance to *B. thuringiensis* will occur despite all our wishful thinking, and immediate measures should be taken to manage DBM resistance to this microbial insecticide.

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