# Diamondback moth resistance to Bt: relevance of genetics and molecular biology to detection and management

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## Abstract

Diamondback moth is the only species that has evolved resistance to *Bacillus thuringiensis* in open field populations. Resistance has appeared several times and involves more than one mechanism, yet one type of resistance designated "Mode 1" occurs often in diamondback moth and other lepidopteran species. This type of resistance is very potent, shows recessive inheritance, displays a characteristic cross-resistance spectrum and is associated with reduced toxin binding to the midgut; thereby exhibiting many properties expected of a mutation in the toxin binding target. Recently, the molecular mechanism causing "Mode 1" resistance to Cry1A toxins in another lepidopteran was shown to be due to a knockout of a cadherin-superfamily gene, implying that the cadherin is an important Bt toxin binding target. If it eventually becomes possible to diagnose this type of resistance using DNA-based tests for the cadherin gene, it is important to develop methods that can provide growers with timely information so that Bt deployment strategies can change as needed. Actual implementation of a resistance management plan still remains the major challenge in ensuring continued efficacy of Bt-based control strategies, whether in spray formulations or transgenic plants.

## Introduction

Insecticidal toxins from the bacterium *Bacillus thuringiensis* Berliner (Bt) have played an important role in controlling crop damage by the diamondback moth (DBM, *Plutella xylostella* (L.), Lepidoptera: Plutellidae) and other pests. Unlike most chemical insecticides, which target the nervous system, Bt toxins have a unique mode of action. Upon sporulation, certain strains of Bt produce crystalline (Cry) protein inclusions. More than 100 Cry proteins have been identified. When a susceptible insect larva ingests a Bt crystal, the crystal dissolves and the Cry protoxins go into solution in the midgut lumen. Partial digestion by the insect's own proteases is required to convert the protoxins to activated toxins. These bind to certain sites on the brush border membrane of the midgut, causing formation of pores in the membrane, lysing the epithelial cells and eventually killing the insect.

The earliest Bt formulations for pest control were spore/crystal mixtures isolated from large-scale fermentation cultures and are still in use. More recently, genes encoding the Cry proteins have been inserted into crop plants, which produce the toxin in their tissue and are thereby protected from insect feeding damage. Most Cry proteins have a limited host range which is believed to be due to the specificity of the binding step. Because Cry proteins are not toxic to predatory or parasitic insects or to mammals, they have many environmental advantages over chemical insecticides. The single most important threat to their continued efficacy is the evolution of resistance in insect pests and this consideration has dictated deployment strategies for Bt-transgenic plants.

As DBM is still the only species with Bt resistance in open field populations, it has a very rich literature on the topic. Tabashnik (1994) provides an overview of the earliest Bt resistance studies in insects and Ferré *et al.* (1995) and Ferré and Van Rie (2002) focus on physiological and genetic mechanisms of resistance. Talekar and Shelton (1993) cover general pest control strategies for DBM, Tabashnik *et al.* (1996) deal specifically with Bt resistance in DBM and Tabashnik *et al.* (1998) introduce the concept of "Mode 1" resistance. Here we provide an overview of some of the key studies on Bt resistance in DBM from 1990 to 2001. We also evaluate the prospects for application of DNA-based markers for field detection of resistance and its application for Bt resistance management in DBM.

## Resistance in field populations

Most Bt spray formulations used on DBM are spore-crystal mixtures derived from *B. thuringiensis* subsp. *kurstaki* (or Btk, with Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, and Cry2B toxins) and *B.thuringiensis* subsp. *aizawai* (or Bta, with Cry1Aa, Cry1Ab, Cry1C, and Cry1D and Cry2B). A few are based on a single toxin such as Cry1Ac. Bt spray formulations were widely adopted for DBM control in the late 1980s and were initially quite successful. Loss of effectiveness was noted in many locations where field applications were most intensive and this was eventually shown to be due to resistance.

Resistance is defined as a genetically-based decrease in susceptibility of a population over time, in response to long-term exposure to an insecticide. Establishing that resistance has occurred (and not faulty insecticide application, for example) requires determination of insecticide susceptibility under laboratory conditions. DBM is the first (and still the only) species to evolve Bt resistance in open field populations. Early reports include Hawaii (Tabashnik *et al.* 1990), the Philippines (Ferré *et al.* 1991), Florida (Shelton *et al.* 1993) and Japan (Morishita *et al.* 1992). More recently Malaysia (Verkerk & Wright 1997), Central America (Pérez & Shelton 1997), and Mexico (Díaz-Gomez *et al.* 1994, Díaz-Gomez *et al.* 2000) have been added to the list. Resistance to Btk products dates from the earliest reports; loss of Bta efficacy is more recent but increasing.

The evolution of Bt resistance in DBM followed the classical pattern seen with chemical insecticides in this species. Over-reliance on a single type of control, combined with year-round growing conditions and a rapid generation time have provided the optimal conditions for resistance evolution time and time again. In retrospect this should not have been surprising, but it must be remembered in the 1980s Bt was still regarded in some quarters as “naturally resistance-proof” because it was derived from a micro-organism that had been in the environment for a very long time as opposed to recently-developed chemical insecticides. If not for the “reality check” of Bt resistance popping up all over the globe in DBM, it is unlikely that the development of pro-active resistance management plans for Bt-transgenic crops would have been pursued with as much enthusiasm. For this reason, DBM has been called “the moth heard 'round the world” (Tabashnik *et al.* 1996).

## Laboratory studies of resistant strains

The ready availability of so many resistant DBM field populations has provided an unparalleled opportunity for laboratory studies. Initially it was not clear whether resistance was due to a physiologically-based decrease in susceptibility or a newly-acquired ability to avoid Bt in the environment. A number of studies tested female oviposition and larval feeding preferences. Although there is a tendency for larvae (resistant or susceptible) to avoid food containing Bt if given a choice, to date there is no evidence that behavioural avoidance is a mechanism of Bt resistance in DBM.

Early studies showed how DBM responded to continuation and relaxation of selection with Bt in the laboratory. Of primary interest was the speed of further resistance evolution and the maximum level attainable under continued, controlled selection in the laboratory, which could establish a “worst-case” scenario for future behaviour of field populations. Once a certain resistance level was attained by selection, its stability was also assessed, to determine whether that level could be maintained without further selection, or would spontaneously decline. Representative studies include populations from Hawaii (Tabashnik *et al.* 1990, Tabashnik *et al.* 1994a), the Philippines (González-Cabrera *et al.* 2001), Malaysia (Iqbal *et al.* 1996, Sayyed *et al.* 2000a), Japan (Maruyama *et al.* 1999) and the continental USA (Zhao *et al.* 2000). In all cases studied, laboratory selection of field-collected resistant strains indicated that much higher resistance levels were attainable, but that susceptibility usually increased when selection was halted. We now turn to possible reasons for this decline.

## Fitness costs in resistant strains

If resistance involves a physiological change that decreases susceptibility, this may entail a fitness cost. Susceptibility decreases slowly at first under selection because resistance genes are initially rare; presumably because they are selectively disadvantageous in the absence of the insecticide and only reappear in the population by mutation. Once field-collected resistant strains are established in the laboratory, they usually increase in susceptibility if Bt selection is not applied, showing that the initial decrease is reversible. This “instability” suggested a fitness cost, so it was of great interest to find which components of the life cycle were most affected. The practical implications would be that resistance might be delayed or reversed

in the field as well by withholding Bt applications for long enough for the population to return to a susceptible level.

Several fitness components appear to be reduced in some, but not all, resistant strains. Egg production, hatchability and adult viability were lower in resistant Hawaiian strains (Groeters *et al.* 1994). A strain from Japan showed lower egg hatchability, longer larval and pupal stage duration, lower larval, pupal, and adult survivorship, and lower fecundity (Shirai *et al.* 1998). However, selected and unselected sub-lines of a Malaysian strain were found to vary in fecundity, egg viability, growth rate and development time, but the variation was compensatory such that the intrinsic rate of increase of all the strains was the same (Sayyed & Wright 2001).

Mechanisms of resistance

Much effort has been expended on determining the biochemical or physiological changes underlying Bt resistance. Two early papers, one on Indianmeal moth (Van Rie *et al.* 1990) and the other on DBM (Ferré *et al.* 1991), established reduced toxin binding to the midgut epithelium as an important resistance mechanism. Although most attention since those studies has been paid to reduced binding, a few other studies, e.g. (Liu *et al.* 2000, Sayyed *et al.* 2001b) provide evidence of reduced proteolytic activation as well; based on the observation that susceptibility of the activated toxin is much more than to the protoxin.

Studies on Philippine strains were the first to establish the connection between high levels of resistance and reduced toxin binding in DBM (Ferré *et al.* 1991). The BL strain, derived from a field population exposed to Btk, was 200-fold resistant to Cry1Ab, but not Cry1B or Cry1C. Binding of midgut BBMVs (brush border membrane vesicles) to Cry1Ab was greatly reduced in BL relative to a susceptible strain, while Cry1B and Cry1C binding were the same in the two strains. Strains from Hawaii confirmed and extended this correlation. Originally resistant strains that had reverted to susceptibility while being maintained in the laboratory without selection, showed levels of BBMV binding to Cry1Ac equivalent to susceptible strains with no prior exposure to Bt. BBMVs prepared several generations later, after renewed laboratory selection with Bt had restored high levels of resistance, no longer bound to Cry1Ac (Tabashnik *et al.* 1994b). Strains from Florida (Tang *et al.* 1996) and Malaysia (Wright *et al.* 1997, Sayyed *et al.* 2000b) also showed reduced binding, and this phenomenon (with some variations) is often found with resistance to the Cry1A-type of toxins. In contrast, Cry1C-resistant strains have not generally displayed marked reduction of BBMV binding (Wright *et al.* 1997, Zhao *et al.* 2000).

A series of competitive binding experiments and previous results have been integrated into a model of four Cry1 toxin binding sites and their properties in DBM (Ballester *et al.* 1999, Ferré & Van Rie 2002). Site 3 (Cry1B-binding) and Site 4 (Cry1C-binding) are distinct and do not interact with other toxins. Site 1 binds Cry1Aa only; and Site 2 binds Cry1Ab, Cry1Ac, Cry1F and, to a much lesser extent, Cry1Aa (Granero *et al.* 1996). Two distinct types of Site 2 modification have occurred in resistance: "Type 1" reduces only Cry1Ab binding, while "Type 2" abolishes binding to Cry1Aa, Cry1Ab, Cry1Ac and probably Cry1F. Both types may correspond to the "Mode 1" syndrome defined by Tabashnik *et al.* (1998).

Most studies measure equilibrium or endpoint toxin binding to BBMV in suspension. Other methods (surface plasmon resonance, Masson *et al.* 1995; or binding to tissue sections, Escriche *et al.* 1995), while sometimes corroborating the BBMV results, often fail to show binding and the significance of these discrepancies is not clear.

Studies in other Lepidoptera have identified one class of Cry1A-binding proteins from larval midguts to be aminopeptidase N (APN), digestive enzymes that cleave amino acids from the N-terminus of proteins. The first APN to be cloned on the basis of its Bt toxin-binding ability was from *Manduca sexta* (L.) (Lepidoptera: Sphingidae) (Ms-APN1, (Knight *et al.* 1995)). Subsequently, a 120 kDa Cry1Ac-binding APN was identified in DBM (Luo *et al.* 1997). However, the protein isolated from resistant (NO-QA) and susceptible strains bound Cry1Ac equally well, making it unlikely to be involved in resistance. Denolf *et al.* (1997) cloned the first APN from DBM (Px-APN1) and the second from *Manduca* (Ms-APN2). A second APN (Px-APNA) was cloned from DBM (Chang *et al.* 1999); its discovery provided evidence of family of aminopeptidases in Lepidoptera and several other genes have now been cloned. A third APN from DBM (Px-APN2) has been cloned (GenBank AJ222699). It is not known whether any of the three cloned APNs correspond to the 120 kDa protein studied by Luo *et al.* (1997).

## Cross-resistance

Cross-resistance is a decreased susceptibility of a population to an insecticide, caused by selection with a different insecticide. Significant progress in Bt cross-resistance studies was made only after individual toxins were purified, often at great labour and expense, and tested separately. The practical application of measuring cross-resistance patterns is to predict whether selection with one particular toxin or formulation will endanger the future efficacy of other toxins or formulations.

The early use of formulations based on the two subspecies, Btk and Bta, gave rise to the first observations of cross-resistance. In Hawaii, strains selected with Btk to >1,000-fold resistance levels were resistant to its constituent toxins Cry1Aa, Cry1Ab, Cry1Ac, and Cry2A, but not to Cry1C which is absent from Btk (Tabashnik *et al.* 1993a). Three-fold cross-resistance to Bta was observed, and suggested to be due to the presence of Cry1Aa and Cry1Ab in both formulations. Some strains from Malaysia selected with Bta exhibited slight cross-resistance to Btk, but not *vice-versa* (Iqbal *et al.* 1996); however other strains selected with Btk or Bta had no apparent cross-resistance to the other formulation (Wright *et al.* 1997). In strains selected to a high level of Cry1C resistance; if Cry1A resistance is also present, it appears to be under different genetic control (Liu *et al.* 1996, Zhao *et al.* 2001).

Generalising the results from these and other experiments; resistance to one of the Cry1A toxins is often accompanied by resistance to the others and to Cry1F and Cry1J, but not Cry1C; and resistance to Cry1C does not confer high cross-resistance to the others. An interesting Philippine strain provided a variant on the first statement, in which resistance to Cry1Ab but not Cry1Aa or Cry1Ac was (at least initially) the case. Cry1C resistance may confer a slight amount of cross-resistance to Cry1A, enough to respond to selection, but has a different genetic mechanism.

## Synergism

Another practical matter is whether a given Bt resistance mechanism can be overcome by other pest control methods or compounds such as synergists. Synergism occurs when the combined potency of two compounds is greater than would be predicted by their separate potencies when administered singly. There are two issues here: whether a given compound synergise Bt against susceptible DBM and whether this synergistic action is enhanced or reduced in resistant strains. Enhancement should occur if the synergist specifically interferes with the resistance mechanism.

Since proteolytic activation of the protoxin is required for toxicity, one might expect that protease inhibitors would synergise Bt toxins. But in an early study, two serine-protease inhibitors failed to synergise Bt in either susceptible or resistant Hawaiian strains (Tabashnik *et al.* 1992a). Transgenic *Arabidopsis* expressing potato protease inhibitor PI2 suffered more feeding damage by Bt-susceptible DBM because they increased their consumption rate to compensate for the lower protein intake. This actually reduced the efficacy of Bt in *Arabidopsis* expressing both proteins (Winterer & Bergelson 2001).

In addition to the Cry proteins, other compounds produced by Bt have toxic effects. The Cyt1A protein of *B. thuringiensis* subsp. *israelensis* has no structural similarity to the Cry proteins and appears to have a different mode of action. In resistant strains of a mosquito and a beetle, Cyt1A synergised certain Bt toxins (Wirth *et al.* 1997, Federici & Bauer 1998). Similar synergism of Cyt1A against resistant DBM occurred in one study (Sayyed *et al.* 2001a), but not in another (Meyer *et al.* 2001).

Ungerminated Bt spores, when added to purified Cry proteins, increase their toxicity. The response depends on many factors; including the specific Bt toxin, spore type, and DBM strain. In one study, HD-1 spore/crystal preparations synergised Cry1A toxins in the resistant strain and Cry1C in resistant and susceptible strains, but not Cry2A in either strain (Tang *et al.* 1996). In another study, Btk spores synergised Btk crystals against both susceptible and resistant larvae; and the latter effect could be blocked by addition of streptomycin to inhibit spore germination. However, Bta spores did not synergise Bta crystals; and Btk spores synergised Cry1C against both resistant and susceptible strains, but synergised Cry2A against susceptible larvae only (Liu *et al.* 1998).

## Inheritance of resistance

After the response to laboratory selection with Bt has stabilised, a strain is likely to be fairly homogeneous genetically and the inheritance of resistance can then be characterised. The first step requires crossing

resistant (R) and susceptible (S) strains to produce an $F_1$ generation, and measuring its concentration-mortality curve. If reciprocal $F_1$ curves coincide with each other and with the S curve, resistance is autosomal and recessive. If the $F_1$ and R curves are coincident, resistance is dominant. Usually the situation is more complex and terms like "incompletely recessive" are used when the $F_1$ curve is substantially closer to the S curve; however these concepts can become quite complex when the concentration administered and other environmental factors influence the response (Bourguet *et al.* 2000). To estimate the number of different genes contributing to resistance, the concentration-mortality response of backcross or $F_2$ generation is compared with predicted mortality predicted under various hypotheses. If there is a good chi-squared fit to the simplest model, most investigators conclude that resistance is monogenic. A more rigorous approach is to compare the fit among models with 1, 2, or more genes, but it can be difficult to discriminate among such models with bioassay data alone (Tabashnik 1991).

Generally, DBM resistance to the Cry1A toxins has been found to be completely or partially recessive (Hama *et al.* 1992, Tabashnik *et al.* 1992b, Martínez-Ramírez *et al.* 1995, Tabashnik *et al.* 1997a, Tang *et al.* 1997, Sayyed *et al.* 2000a). It has never been found to be dominant; although a Thai strain appeared to have polyfactorial control of resistance (Imai & Mori 1999). Crosses between three strains with recessive resistance to Cry1Ab (from Pennsylvania, the Philippines and Hawaii) enabled a three-way test for allelism revealing a common Cry1Ab resistance gene. This was the first evidence that Bt resistance arising in different geographical areas could have the same genetic basis. The Philippine strain also had a second gene controlling Cry1Ab resistance (Tabashnik *et al.* 1997b).

Recessivity of Cry1C resistance appears to show more dependence on the concentrations tested. A Hawaiian strain displayed achieved 60-fold resistance under autosomal control, with more pronounced dominance at lower concentrations. A strain from South Carolina selected to >60,000-fold resistance to Cry1C displayed autosomal, incompletely recessive inheritance when evaluated by a leaf-dip bioassay and completely recessive behaviour on Cry1C-expressing transgenic broccoli (Zhao *et al.* 2000).

### Experimental tests of IRM strategies with resistant DBM strains

Insecticide resistance management (IRM) aims to use a combination of control strategies to retard or contain the spread of resistance in the field. The large number of potential strategies includes mixtures of different toxins, incorporation of synergists, application in spatial mosaics, temporal rotations of toxins, ultrahigh concentrations to ensure all heterozygotes are killed and refuges that permit a certain fraction of the population to escape selection by the toxin (Tabashnik 1994). There is a large modelling literature addressed to the advantages and disadvantages of these, but very few experimental tests. The availability of Bt resistant strains in the field is a unique advantage of DBM in providing data directly relevant to these issues.

Resistant strains have been used for two main purposes. The first is as a standard—a realistic upper bound to accurately compare and calibrate the results of laboratory and field studies (Tabashnik *et al.* 1993b, Pérez *et al.* 1997a). This includes the first laboratory experimental test of the $F_2$ screen for rare resistance alleles (Zhao *et al.* 2002). The second main use of Bt-resistant strains is in experiments that compare different IRM plans. In a laboratory selection experiment designed to simulate the rate of resistance evolution under various conditions, the presence of refuges was shown to delay the rate of increase of resistance to Bta (Liu & Tabashnik 1997). In field cage tests in Honduras (Pérez *et al.* 1997b), the effects of different rates of Btk application and the presence or absence of a 25% refuge (unsprayed row) was examined on the rate of resistance evolution, mortality and fraction of marketable cabbage produced. Open-field tests with different deployment strategies of transgenic Cry1Ac-expressing broccoli and field-collected resistant insects explored the effects of different physical placement of refuge (nontransgenic) plants (Shelton *et al.* 2000). Greenhouse cage experiments employing different refuge sizes with transgenic Cry1Ac-expressing broccoli and different DBM resistance allele frequencies showed that larger refuges delayed resistance more, compared separate refuges with mixed ones and highlighted the importance of larval movement (Tang *et al.* 2001). Recent greenhouse cage tests indicated that, compared with sequential or mosaic deployment of Cry1Ac and Cry1C toxins in transgenic broccoli, pyramided two-gene plants could significantly delay resistance development to each or both toxins while providing good control of the DBM population (JZ Zhao & AM Shelton, unpublished data).

### A genetic mapping approach to Bt resistance in diamondback moth

It would be very useful to identify the genes responsible for the resistance phenomena discussed here, for basic and applied reasons. It would resolve some of the conflicting data from different approaches and

enhance our basic understanding of the fundamental mode of action of Bt toxins. It would also enable the development of DNA-based diagnostic tests for resistance in the field. Resistance genes found in one species of Lepidoptera could well be relevant to other species. Recently the gene conferring "Mode 1" resistance (Tabashnik *et al.* 1998) in tobacco budworm *Heliothis virescens* F. (Lepidoptera: Noctuidae) was identified using a genetic approach. An 11-domain cadherin protein, homologous to proteins from other Lepidoptera that bind Cry1A toxins, mapped to exactly the same chromosomal location as the resistance gene. Cloning of the cadherin gene from a resistant strain showed that it was interrupted by a retrotransposon insertion, preventing synthesis of a full-length protein capable of binding Bt toxins. Thus a knockout of the cadherin conferred resistance, strongly implicating it as the major binding protein of Cry1A toxins in *H. virescens* (Gahan *et al.* 2001).

We are now applying the same methods to identify resistance genes by linkage mapping in DBM. Using about 200 AFLP markers, sufficient to mark all of the 31 chromosomes, we showed that a single gene accounted for most of the Cry1Ac resistance in the NO-QA strain from Hawaii (Heckel *et al.* 1999). By cloning and sequencing one of the AFLPs near that gene we developed a polymorphic marker that can be used for linkage tests in other strains. The aminopeptidase Px-APNA was mapped to a different linkage group, excluding it as a candidate for the resistance gene. We are now testing additional APNs from DBM, as well as the homologue of the *H. virescens* cadherin, to see if they map to the same linkage group as resistance.

We are also analysing Cry1Ac and Cry1C resistance mechanisms in a South Carolina strain (Zhao *et al.* 2001). The same linkage group associated with Cry1Ac resistance in NO-QA is the only one associated with Cry1Ac resistance in this strain. It is intriguing to speculate that this linkage group might be homologous to the one in *H. virescens,* as Herrero *et al.* (2001) have shown an association between the marker MPI (linked to resistance in *H. virescens*) and Cry1Ab resistance in a Philippine strain of DBM. In the South Carolina strain, two different linkage groups are associated with Cry1C resistance, confirming earlier indications of a different genetic basis to resistance against the two toxins. So far, no APNs have been found to map to these linkage groups (S. Baxter, unpublished data).

Based on this progress, it is likely that molecular methods of diagnosing Bt resistance in field populations of DBM will be available soon. DNA-based methods will not replace conventional bioassay methods, but instead offer a valuable complement to them. Only larvae can be bioassayed, but any life stage can provide DNA for analysis. Living, healthy individuals are required for bioassay, but frozen or ethanol-preserved specimens can provide DNA. Bioassay can only detect resistance in a population if the resistance allele frequency is high enough to shift the concentration-mortality curve of the entire population; but the DNA detection basis works on an individual basis and could detect resistant alleles at a frequency of one in ten thousand; if ten thousand individuals were screened and one positive were found. When resistance is recessive, bioassays can only detect resistant homozygotes, but DNA-based methods can detect heterozygotes as well. The detection of resistance at low frequencies will probably be the most useful aspect of DNA-based methods. An independent approach at detecting rare recessive alleles, the $F_2$ screen, indicates that their frequency is likely to be less than 0.001 in an Australian population of DBM (Ahmad & Roush 1999).

## Conclusion

### Applications to Bt resistance management in the field

The potential benefits of DNA diagnostic methods for detection of resistance to Bt-transgenic crops are constrained by several factors in some systems. Bt-expressing maize and cotton in large monocultures present a strong, uniform selection pressure which the target pests have not yet overcome. The goal is to prevent resistance occurring in the field. The "high-dose/refuge strategy" attempts to do this by ensuring that a certain fraction of the crop does not express Bt. This "refuge" from Bt selection allows the production of susceptible individuals which mate with the few Bt resistant survivors of the transgenic crop, producing heterozygous offspring that are killed by the high dose in the next generation. DNA-based detection would be most useful for validating that strategy or giving an early warning if it starts to fail. Predicting which of many possible resistance mutations will occur in the field first, based on laboratory studies, can be difficult (but not impossible, as shown by studies in *H. virescens* (Gould *et al.* 1997, Gahan *et al.* 2001). If resistance is detected early by the DNA methods, "soft" options such as increasing the refuge size or refraining from planting transgenic crops must be delayed to the next planting season; although the "harder" responses of crop destruction or massive chemical control could be implemented immediately.

In contrast to pests of transgenic cotton and maize, application of DNA diagnostic methods in DBM may be less constrained and more flexible for two reasons. The first relates to the existing distribution of resistance genes. The worldwide selection pressure on DBM has provided a variety of different outcomes and the goal is to manage resistance mechanisms that already occur in the field rather than to anticipate the unknown. Genetic characterisation of these mechanisms is therefore possible because they already exist and are thus guaranteed to be relevant. Some, but not all, of these will be the same in different populations, facilitating screening efforts. Migration will have already distributed resistance genes over a wide area. Overall, historical effects will be less important, although not negligible. The prudent approach will be to supplement DNA methods with bioassays at first, with each new geographical area to be screened.

The second factor in favour of the DNA approach for DBM is the manner in which Bt is used still exclusively in spray formulations. This enables a much more flexible response to any findings of DNA monitoring than possible with transgenic plants. Changing the frequency or timing of sprays, or switching to a different Bt formulation with other toxins, or resorting to chemicals with an entirely different mode of action, are all possible. If contingency plans were put into place in advance, switching strategies might be accomplished very rapidly. If Bt were one component in a pre-determined rotating series of different compounds, DNA monitoring could verify that susceptibility had returned to a level sufficient to obtain effective control when Bt was ready to be used again.

On the other hand, some factors weigh against the utility of DNA monitoring in DBM. The technology is too complicated for most growers and consultants, and diagnostics would have to be centrally done. Resistance management is less effective when resistance is already common; but the advantage of DNA approaches is greater when resistance is rare. Economically, the stakes are lower with sprays than with transgenic plants. Moreover, in many cases Bt sprays have been a relatively minor component in crop protection overall because of their low field persistence and difficulty of getting the insecticide to the insect, compared with other options.

Although Bt-transgenic plants are not currently in commercial use for DBM control, future adoption may enhance some opportunities for resistance management and the concomitant benefits of DNA diagnostics. The high-dose/refuge strategy, or “pyramided” plants expressing two different toxins, could be very effective in both pest control and resistance management. Alternating between different Bt genes in plants within a region could also be done on a yearly basis, similar to the window strategy with foliar sprays. Industry control of availability of seeds expressing different Bt toxins may be a more effective way of promoting grower compliance with such a rotational strategy than control of availability of different sprays, which can be stored and used whenever desired.

The biggest challenge of DNA diagnostics remains to provide the information to growers, consultants and the pest control industry in a timely and useful manner so that it can be used in decision-making in the context of a previously devised and accepted integrated pest management plan with a variety of options. Ideally, an area-wide Bt resistance monitoring program (e.g. Carrière *et al.* 2001) would be coordinated with a long-term assessment of DBM movement, population structure and gene flow. The use of DNA-based markers for both purposes would be an efficient use of sampled individuals and could be extended to screening for chemical insecticide resistance by examining genes for targets of the pyrethroids, cyclodienes and organophosphates.

In summary, just as diamondback moth has furnished the greatest variety of resistance phenomena to *Bacillus thuringiensis,* so may it provide the greatest number of opportunities to successfully manage the resistance problem. Keeping Bt as an active ingredient in the arsenal against this cosmopolitan pest is achievable; only time will tell whether it is achieved.

## Acknowledgements

Our research described herein was supported by USDA NRI Competitive Grant 96–35302–3470, USDA Western Regional Pesticide Impact Assessment Program Grant 97RA1810/WR337, CSRS-TSTAR Program Grant 95–34135–1771 and ARC SPIRT Grant C00106849. Manuscript preparation was supported by CESAR—the Centre for Environmental Stress and Adaptation Research, a Special Research Centre funded by the Australian Research Council.

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