PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The influence of the molecular basis of resistance on insecticide discovery

M. D. Broadjurst

Phil. Trans. R. Soc. Lond. B 1998 **353**, 1723-1728
doi: 10.1098/rstb.1998.0324

THE ROYAL SOCIETY

The influence of the molecular basis of resistance on insecticide discovery

Michael D. Broadhurst

Zeneca Ag Products, Western Research Center, 1200 South 47th Street, Richmond, CA 94804, USA

This paper focuses on the process of invention and development of new insecticides and the impact of current research in resistance mechanisms on that process. The topic is introduced in the context of (i) the critical need to develop new insect-control agents to ensure a continued supply of high-quality food and fibre; (ii) how resistance development will continue to influence the potential to ensure the supply of these essentials; and (iii) why new insect-control technology is welcomed by growers.

The main section of the paper describes a generic agrochemical invention process and discusses the impact that an understanding of the molecular basis of resistance will have on the various stages of this process, using specific examples to illustrate these points. By focusing on insecticide invention, this paper provides a context in which other information more specific to insecticide resistance from this issue can be understood.

Keywords: resistance; insecticide; invention; discovery; agriculture

1. INTRODUCTION

Crop protection is a research-based business where a number of drivers demand a continual flow of new products. Although reasonably effective products exist in most market segments, new products that allow growers to produce their crop in a safe and economical manner are always welcomed. These new products must conform to high standards of safety with respect to their potential to affect the health of those who use them, the quality of the food we eat and the environment. These factors, together with the need to provide an adequate return to those who invest in the companies that conduct the research and development required to discover new crop-protective agents, impose many diverse scientific and commercial challenges on the invention process.

Development of resistance to existing products is an important driver for new methods to control insects, plant pathogens and weeds in many market segments. This paper focuses on the impact of our increasing understanding of the detailed mechanisms of resistance to insect-control agents at the molecular level on the process of invention of suitable new chemistry.

2. BACKGROUND

One study predicts that the world population will continue its present growth to approximately 11 billion during the period between 2025 and 2050 and then level off (UNFPA, unpublished). Associated with this population growth, the amount of land available to grow food will continue to diminish (UNFPA, unpublished). In addition, individuals are becoming more sophisticated in their tastes and increased wealth is allowing people to make choices, potentiating the other factors by shifting agriculture towards less efficient foods to aggravate the food-supply problems. Similar forces are affecting the supply of natural fibres produced directly or indirectly through agriculture.

There is no doubt that methods of crop protection have been a major contributor in our current ability to produce a relatively abundant and good-quality food and fibre supply. One report from a study of several major crops clearly demonstrates that crop protection has improved yields (Oerke *et al*. 1994). As an example, for rice it was estimated that 65% of crop yield is saved by crop protection. The data also suggest that superior technology could improve yields still further.

These factors together indicate that we will require more high-quality food in the future and superior crop-protection methods to allow us to produce and protect this food.

A number of elements influence our ability to produce high-quality crops in good yield. These include inputs such as crop protection, fertilizers, irrigation, and seed improved through breeding or genetic manipulation. Equally important are less controllable determinants such as soil erosion, limitations on the use of pesticides through regulations, and pest resistance. Although I will focus in this paper on the two elements of pest resistance and crop protection, it is difficult to separate these from the many other important influences, some of which were mentioned above. Therefore, this discussion will include a variety of other factors that are important to the invention process for new crop-protection methods.

There is no doubt that pest resistance can have devastating implications for a grower's ability to produce a crop, in some instances leading to complete crop loss. Such instances of crop loss occurred in cotton during 1995, one of the worst years in history for the control of tobacco budworm (*Heliothis virescens*), in the southeast United States. In some areas, owing to the presence of resistant individuals, no insecticide or combination of insecticides could control damage. Two years earlier, a study from Louisiana State University of eight field-collected populations of tobacco budworm showed high levels of tolerance to four different insecticides representing three different modes of action (Graves *et al.* 1993).

Fortunately, in the two subsequent growing seasons, tobacco budworm pressure has been lower. Furthermore, we now have access to several new tools with which to fight this pest. Notable in this regard was the introduction of Bollgard® cotton. This technology confers crop resistance to some cotton pests via the expression of the Bt (*Bacillus thuringiensis*) endotoxin in the exposed parts of the cotton plant. Since its introduction, Bollgard® cotton appears to have provided excellent control of tobacco budworm in practical applications under light to moderate insect pressure in most growing areas.

Nevertheless, the Bt endotoxin is intrinsically less potent against the related cotton bollworm (*Heliothis zea*) and this appears to have led to some instances of bollworm damage in cotton not otherwise treated. This example illustrates a key point. No single insecticide or other technology is the sole answer to the grower's insect-control problems. This was confirmed in large-field plots where overspraying Bt Cotton with the synthetic pyrethroid insecticide λ-cyhalothrin provided significant yield increases and consequent economic benefits to the growers (Mink *et al.* 1997). Although firm evidence regarding the origin of the observed yield improvements is lacking, one can reasonably speculate that these arose as a result of factors such as control of pests that were not susceptible to the Bt endotoxin or repellency to bollworm moths.

A second study carried out in the laboratory demonstrated that insects that survived exposure to sprayable Bt products at generally sub-lethal doses were more susceptible to λ-cyhalothrin than unexposed pests (Harris *et al.* 1998). This study concluded that oversprays of λ-cyhalothrin on Bt Cotton would tend to reduce the level of surviving lepidopteran pests to very low levels, vastly enhancing the dilution effect offered by the influx of susceptible individuals from refugia, and would thus work in concert to offer a sustainable resistance-management strategy.

It is reasonable to infer from these examples that use of λ-cyhalothrin sprays on Bt Cotton will prolong the life of this valuable technology by slowing the onset of resistance. It is further reasonable to conclude that new technology, be it chemical- or gene-based, will decrease the grower's dependence on established insect-control methods, therefore allowing their use to play to their strengths. Finally, one hypothesis that will be developed further in this paper is that the ideal target for any research programme directed at new insect-control agents will seek effects that are not subject to known mechanisms of resistance.

3. THE INVENTION PROCESS

Invention begins with targets. Companies use targets to focus their invention resources into those areas that will have the most beneficial impact on their business.

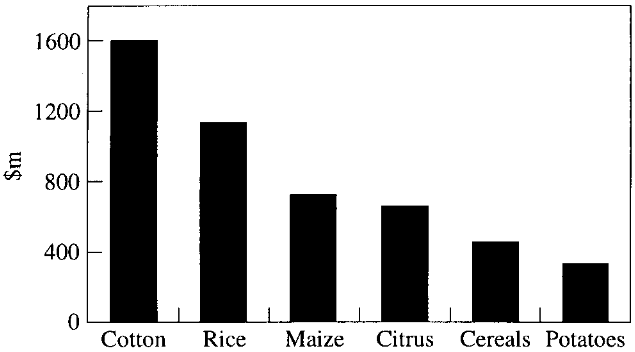


Figure 1. The major insecticide markets, 1996.

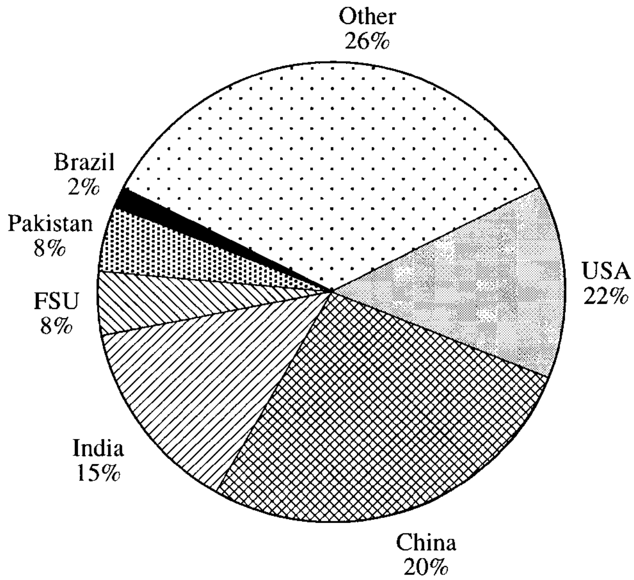


Figure 2. Cotton production, 1996.

Although methods and response to business climates vary from company to company, the targets tend to be similar.

Figure 1 provides some statistics taken from Wood MacKenzie for the largest insecticide markets (Wood MacKenzie 1997). These data considered in isolation would suggest that there is a great deal of value to be found for a new product in a number of markets. However, each of these markets is comprised of a number of pest species over a large range of territories. Furthermore, as alluded to earlier, targets for an individual company will depend on the current products in their range, the fit with products in other market sectors and the company's strategy, among other factors.

To use cotton as an example, although over 60% of pesticide usage is directed at insect control worldwide, the crop is grown on significant acreage in many parts of the world as shown in figure 2. A consequence of this geographical distribution is that the pest spectrum varies considerably from area to area as suggested in table 1.

Although different species, the two most important pests in both the USA and Asia are moths, and the only clear stand-alone target in most areas is for control of heliothine moths. Therefore, when targeting hits on the screens that do not control these key pests, a careful evaluation must be made of the value of the individual

Table 1. *Major cotton pests in the USA and Asia*

USA	
	tobacco budworm (*Heliothis virescens*)
	cotton bollworm (*Heliothis zea*)
	boll weevil (*Anthonomus grandis*)
	various bugs (e.g. lygus bugs and stink bugs)
Asia	
	cotton bollworm (*Helicoverpa armigera*)
	Earias spp.
	whitefly (e.g. *Bemisia tobaci*)
	jassids, thrips, aphids and mites

segments of the market and the pest spectrum of the particular chemical area at hand. Obviously, combining control of heliothine moths with a larger spectrum of pest control will improve the potential value of the new area of chemistry. Most companies have threshold market values where the minimum size and profitability of the potential market for the new chemical must justify the cost of bringing the chemistry to market.

Figure 3 provides a representation of a generic invention process as might typically be operated in most agrochemical companies. The bars represent increasingly difficult hurdles for an individual project or area of chemistry to surmount. The names provided are a guide to the nature of activities at each stage and example activities are included. This is by no means an exhaustive list of the many scientific inputs required at the various stages and should be considered as indicative only. The process suggested here would cover all of a company's activities, and work directed at insect control might encompass only one-third of the compounds suggested at the later stages. The process is described briefly as follows.

Screening. Most major agrochemical companies screen somewhere between 10 000 and 100 000 compounds a year from various sources. Insecticide screens will typically be run on between three and ten species that are deemed representative of the most important groups of pests selected on the basis of targets. Relatively few compounds show activity on the screens and active compounds are assessed for potency. This screening, combined with assessment of the spectrum of activity, comparison to known classes, possibly the synthesis of a few analogues, and ownership by relevant individuals in the organization, will typically be sufficient to allow progression to the second stage.

Confirmation. Between 10 and 50 analogues will often be prepared during confirmation to determine the chemical scope of the area. To be considered for further progression, an area of chemistry will usually need to demonstrate some breadth of the activity signal (spectrum) and a suggestion that potency can be improved. Alongside this activity will be a thorough glasshouse and laboratory characterization of the better analogues. In addition, preliminary thinking, data collection, and possibly some limited laboratory work around issues relevant to toxicology, ecotoxicology and mode of action will take place. Finally, a preliminary assessment of the potential business case will be made.

The confirmation stage will often last between six months and one year for most areas of chemistry.

Clarification. Relatively few new series pass the next hurdle. At this stage a large, multidisciplinary project will be formed with the hopeful outcome that the research team will be able to identify a single compound for development. Many diverse scientific inputs are required to understand the potential of a new area. For insecticide candidates, an attempt will be made to determine the mode of action and assess the potential for cross-resistance or resistance development.

Development. It will usually require an investment of US $25–100 million to bring a new insecticide to the market. The breadth of the required activities and functional skills is enormous. A few are mentioned in figure 3.

Several key questions relative to resistance need to be posed at the decision points in this process.

1. *Does the prospective area of chemistry rely on a known mode of action?* If so, does the lead have the potential to deliver second-generation performance, as evidenced by better potency, increased spectrum, etc.? If second-generation performance is lacking, it often will not be profitable for an agrochemical company to further develop such a lead, as insufficient market share is likely to be captured to justify the development costs. When second-generation performance is present, factors must be assessed which will differentiate the chemistry from known compounds sharing the mode of action. These include the potential for altered binding at the active site or possible differences in susceptibility to metabolic inactivation mechanisms. The recently introduced sodium-channel binder, indoxacarb, illustrates this point. Although targeted at *Heliothis* control and sharing a general mode of action with the pyrethroids, indoxacarb has been shown to bind the receptor differently than pyrethroids (Wing *et al*. 1998).
2. *Are products that share an established mode of action used in the contemplated market?* Imidacloprid, a nicotinic agonist, brought a novel mode of action to the sucking-pest market when introduced in the early 1990s. Although exhibiting a considerable spectrum of activity, imidacloprid lacked potent activity on certain important classes of insects such as Lepidoptera pests of cotton. Only now are we beginning to see the emergence of the next generation of nitromethylene compounds, the chemical class encompassing imidacloprid, which have a much broader spectrum of activity. Although resistance could develop to imidacloprid in those markets that were established early, it is not unreasonable to expect that market opportunities on new pests will be unaffected by any such resistance.
3. *When the mode of action appears to be novel, is it possible to realistically assess the potential for resistance development?* Limited tools exist to assess some general mechanisms, e.g. whole organisms with elevated ability to metabolize xenobiotics. The ability of these methods to predict the potential impact of metabolic mechanisms on new classes of chemistry requires assumptions based on model systems. Furthermore, few options are currently available to assess the potential for resistance to develop or to predict its extent, evolution and expansion for novel modes of action, even when the site of action is known. However, great promise appears to exist in emerging science.

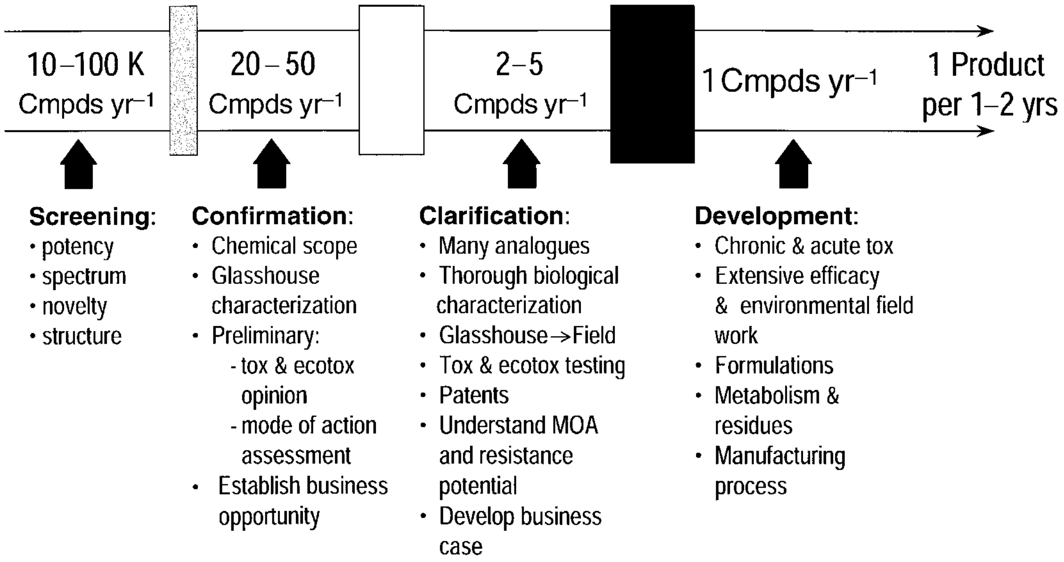


Figure 3. The generic invention process.

Emerging genomic science offers new opportunities to use organisms such as *Drosophila melanogaster* or *Caenorhabditis elegans* to pursue studies of modes of action and to explore the potential for resistance development. The mapping and sequencing of genomes in these species is becoming well advanced. A variety of techniques can be applied to study genetic effects in response to the application of a specific chemical to whole organisms. Panels of organisms that have characterized, genetically based modifications exist in some species (e.g. yeast). Although developments in these areas have been largely driven by medicinal applications, applications to the study of agrochemicals are being increasingly recognized and pursued.

Determining the mode of action of insecticides has important implications for other aspects of agrochemical development. This information can be very useful to secure product registrations and for product stewardship, for example by answering questions such as: is the mode of action unique to insects; and is the mode of toxicity the same as the mode of insecticidal activity?

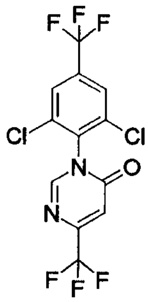
Although not exhaustive, this treatment should provide the reader with a view of the type of questions that can be posed and studied to facilitate decision-making around invention of new insecticides. The remaining discussion will focus on the two key points raised earlier, which are important in assessing the commercial potential of a new area of insecticidal chemistry: (i) an ability to characterize new chemistry against known modes of action; and (ii) an ability to understand the potential for resistance development with novel or unknown modes of action. These points will be discussed, again referring to the generic invention process represented in figure 3 and by highlighting where an understanding of resistance will come into play.

It is clear that understanding the mode of action of an insecticide can be beneficial very early in invention. When a lead is first screened, it is likely that information that is relevant to the mode of action will be secured from data such as symptoms in affected animals or *in vitro* information collected as a routine part of the screening programme. Most companies have a large data bank of these *in vitro* screens, which are run either as a routine part of the screen or once a hit is identified. These are frequently run on an exclusive basis, that is, to exclude certain known modes of action from further consideration.

Known susceptibility to resistance development can be an important factor in the decision to put a screen in place. For example, for many companies the knowledge that a new area of chemistry had the same mode of action as the cyclodiene insecticides would be a potential fatal negative. Widespread site insensitivity can be found to the cyclodiene chemistry in many species, and is particularly acute in public-health pests. It is therefore likely that cross-resistance would develop in some species to any new area of chemistry that was commercialized with this effect. An example in an area of aryl heterocycles worked on in my company a few years ago (Whittle *et al.* 1995). These compounds, shown in figure 4, were particularly effective on public-health pests. Although taken to a fairly advanced stage, the area was ultimately abandoned, primarily because of growing concerns over resistance to these 'GABAergic' insecticides.

Nonetheless, the recent highly successful introduction of fipronil can be contrasted with this example. Although the threat of cyclodiene cross-resistance is present, clearly the strength of fipronil on non-public-health pests has made this compound a huge commercial success in agricultural applications.

In vitro screens can also be used at an early stage to identify specific desirable modes of action. These screens may be used routinely to complement information generated from whole-organism assays or they may be used to follow up hits from these screens. The philosophy here seems to differ substantially between companies, with some believing that only *in vivo* hits are worth



	Activity (× permethrin)
Musca domestica	11
Blattela germanica	3

Figure 4. GABAergic aryl heterocycles.

following up, and others putting substantial investments into identifying and validating novel modes of action, developing *in vitro* screens based on these modes of action and putting large numbers of compounds across these screens.

Many factors will go into defining the value of *in vitro* mode of action screens, but one will be the potential for resistance development. These screens will often be based on a novel mode of action that may or may not be well characterized. For instance, they could be based on the mode of action of an insecticidal natural product or a protein toxin. Regardless, it is important to understand the potential for resistance development to the mode of action. However, this can be an immensely difficult subject, and it is aggravated when dealing with poorly characterized modes of action.

Genomic science seems to be delivering potential tools to meet the challenges here. Characterization of the genomes of relevant species is becoming well advanced and functional genomics are likely to soon provide indicative arrays of all the genes in an insect genome in model systems. For instance, it is possible to imagine arrays on plates where all the genes in *Drosophila* are expressed in *Escherichia coli* and provide a colorimetric response when functioning normally. Application of an insecticidal natural product would stop the colorimetric response of affected genes and possibly associated genes in, for example, a metabolic pathway. Such arrays are currently available for yeast.

In the longer-term, we may be able to use these genetic approaches to group mutants containing specific lesions relevant to important general mechanisms of resistance, such as metabolic mechanisms and reduced uptake or increased excretion. Regarding the ability of an organism to develop site insensitivity, which is arguably the most important resistance mechanism, there seems to be the potential to use genomic science to assess the inclination of the specific target site to adapt to effectors of the active site through a variety of techniques, such as assessing the natural abundance of variation in model systems.

Clearly, many considerations relate to the mode of action of a new area of chemistry and the potential of pests to develop resistance to that mode of action can have an important influence on choices made at the early stages of the invention process. As indicated in figure 3, there are many decisions to be made at these early stages and only a small fraction of potential leads can be developed, given the cost of resources in the subsequent activities.

Resistance will be equally influential on decision-making once new chemistry has progressed through the confirmation/clarification boundary. At this point, one will generally know whether the new insecticidal chemistry has (i) a known mode of action; (ii) an unknown mode of action that is partly characterized (e.g. it is known that active compounds interfere with insect development); or (iii) an unknown and likely novel mode of action that may be difficult to characterize. This information will probably form a critical success factor for the project. A critical success factor can be defined as a factor upon which progression to the next stage of the process is strictly dependent.

When the mode of action is known, a key critical success factor will be to understand the potential for cross-resistance before taking a decision to initiate expensive development activities such as chronic toxicology. Characterizing the potential for cross-resistance can be a challenge. For example, significant resistance to established products that share the mode of action may not have been observed yet in the field. Such resistance may, however, develop during the four to eight years required to bring the new chemistry to the market. In addition, even when resistance has been observed in the field it may be difficult to reproduce measurable effects in the laboratory with field-collected populations, for example because of the influence of environmental factors.

If some evidence of the basis of the mode of action exists, but it is not well understood (e.g. the new chemistry affects a metabolic pathway but the exact site of action is not known), an important critical success factor for the project is likely to be a determination of the exact site of action and understanding of the implications for resistance development.

Surprisingly frequent, however, will be the case where there are few clues regarding the mode of action at this stage. An example of this point is the natural product lepicidin, the basis of the new Lepidoptera-specific insecticide TRACER®. This product appears to have been progressed with no clear understanding of the mode of action and the assumption of novelty. Some information has recently been published in this area (Salgado *et al.* 1997).

In these cases of poorly understood modes of action, a sensible way for projects to proceed centres on collecting empirically derived data in key areas that will raise concerns during development and registration. These include acute testing on birds and *Daphnia*, indicative toxicology screens to supplement the typical acute toxicology required to distribute samples, and an assessment of soil mobility and persistence to determine if any groundwater issues are likely to arise. Such studies will highlight areas of concern. In the meantime, the project will be able to continue to pursue reasonable studies with the objectives of determining the mode of action and the potential for resistance development.

4. CONCLUSIONS

This discussion has focused on what is carried out in the agrochemical industry to use information on mode of action and resistance to facilitate the process of inventing new insecticides. However, most of the science practised involves the application of basic research from universities and private research institutions. Companies heavily rely on these fundamental studies as key sources of essential knowledge in areas such as mode of action, site of action, receptors, enzymes, metabolism, rational design, new receptor screens and genetics at a molecular level, just to mention a few areas that are important to the invention process. This fundamental work has immense importance in ensuring our continued ability to secure the new products required by agriculture.

It is also important, at this point, to put the main arguments made in this paper in context. Readers should understand that the potential for cross-resistance and the desire to introduce new modes of action do not dominate an industry's thinking in assessing the commercial potential of a new insecticide candidate. In fact, other factors—does it work? will it make money? is it safe?—will have an overriding importance and will encompass an evaluation of the possibility of cross-resistance. It is also important to remember that no product has totally lost its usefulness solely as a result of resistance development.

Nevertheless, the future will undoubtedly see a better balance of transgenic, biological and chemical means of insect control which reduces the selection pressure against any one product. Although industry has taken substantial steps towards managing resistance development through techniques such as reserving the use of the best broad-spectrum products for the periods of peak pest infestation, a superior understanding of the nature of resistance will certainly allow science to develop in managing resistance. Agriculture is heading toward a future state of integrated crop management where the best available products based on chemical, biotechnological and biological control are selectively and precisely brought to bear through superior monitoring and information techniques to fight infestations in an optimal way. In this regard, the earlier points regarding Bollgard® cotton are important. Not only will this Bt-based transgenic technology prove significant in prolonging the life of established chemical products, but the judicious use of chemicals in combination with Bt crops are likely to extend the effectiveness of this new technology.

New insect-control technology is difficult to find and costly to bring to market. When introduced, it is welcomed and quickly embraced by growers in numerous market segments. In many markets, a primary driving force is the belief that development of resistance is typically inevitable. Therefore, the fundamental studies undertaken at universities and research institutions, which underpin the efforts of the agrochemical industry, are critical in the invention and development of new insecticides.

REFERENCES

Graves, J. B., Leonard, B. R., Micinski, S., Burris, E. & Martin, S. H. 1993 Monitoring insecticide resistance in tobacco budworm and bollworm in Louisiana. In *Proceedings of the Beltwide Cotton Production Conference*, pp. 788–790. Memphis, TN: National Cotton Council.

Harris, J. G. 1998 The usage of Karate® (A-cyhalothrin) oversprays in combination with refugia, as a viable and sustainable resistance management strategy for Bt cotton. In *Proceedings of the Beltwide Cotton Production Conference*, pp. 1217–1221. Memphis, TN: National Cotton Council.

Mink, J., Harrison & Martin, S. 1997 Performance and benefits of Karate® insecticide on Bollgard® cotton. In *Proceedings of the Beltwide Cotton Production Conference*, pp. 898–899. Memphis, TN: National Cotton Council.

Oerke, E. C., Dehne, H. W., Schoenbeck, F. & Weber, A. 1994 *Crop production and crop protection: estimated losses in major food and cash crops*. Amsterdam and New York: Elsevier.

Salgado, V. L., Watson, G. B. & Sheets, J. J. 1997 Studies on the mode of action of spinosad, the active ingredient in tracer insect control. In *Proceedings of the Beltwide Cotton Production Conference*, pp. 1082–1084. Memphis, TN: National Cotton Council.

Whittle, A. J., Fitzjohn, S., Mullier, G., Pearson, D. P. J., Perrior, T. R., Taylor, R. & Salmon, R. 1995 The use of computer-generated electrostatic surface maps for the design of new 'GABA-ergic' insecticide. *Pestic. Sci.* **44**, 29–31.

Wing, K. D., Schnee, M. E., Sacher, M. & Connair, M. 1998 A novel oxadiazine insecticide is bioactivated in Lepidopteran larvae. *Arch. Insect Biochem. Physiol.* **37**, 91–103.

Wood MacKenzie 1977 Update of products section. Reference volume of the agrochemical service, pp. 36–49.