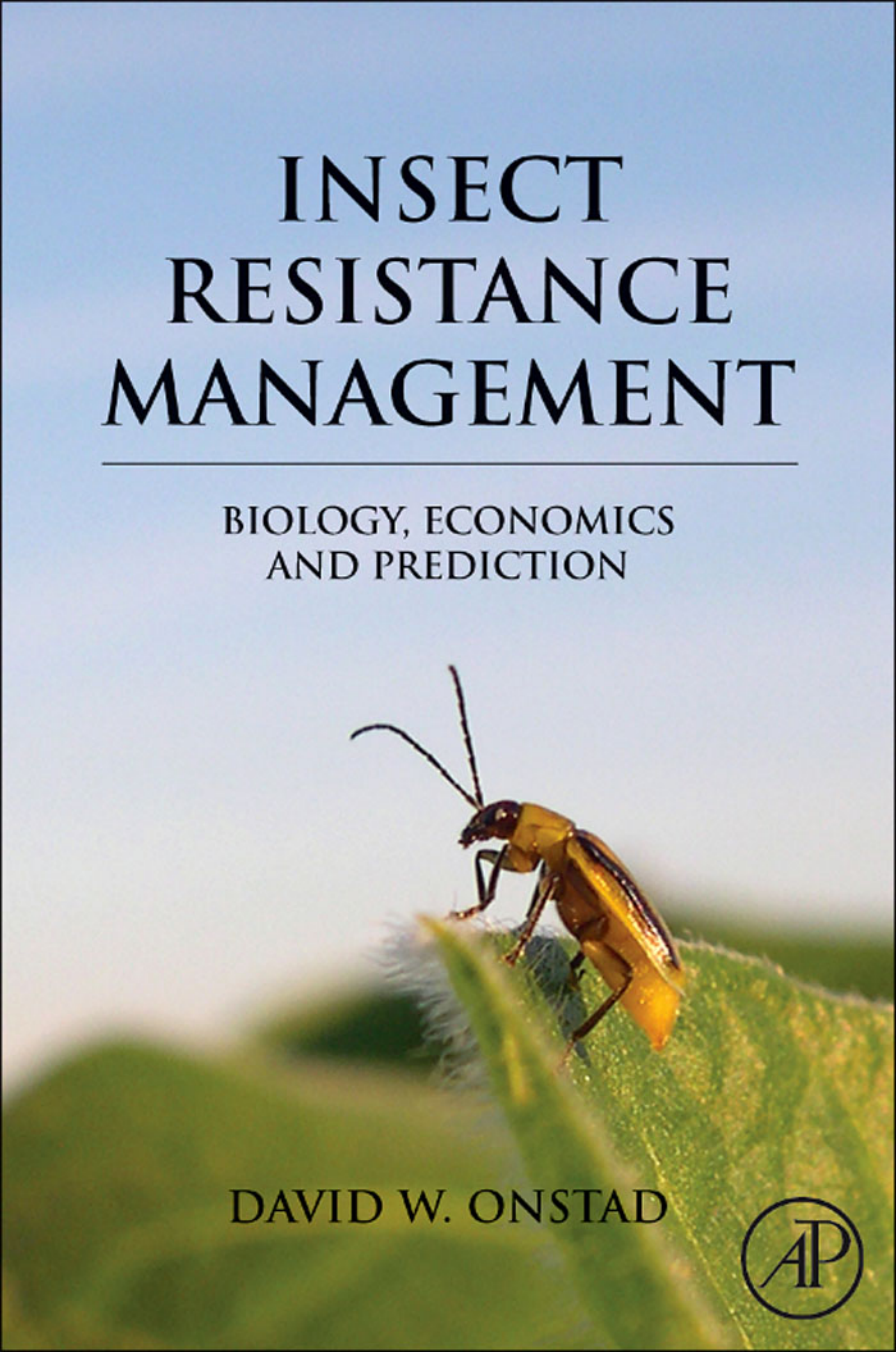
INSECT RESISTANCE MANAGEMENT

BIOLOGY, ECONOMICS AND PREDICTION



DAVID W. ONSTAD

Insect Resistance Management: Biology, Economics and Prediction

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Insect Resistance Management: Biology, Economics and Prediction

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To
Dawn Dockter, the best,
and
Nora and Emma, the best for the future

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Contents

Preface

I produced this book so that readers would be able to discover and gain much of the current knowledge about insect resistance in one volume. The study of resistance is a dynamic process that never ceases to surprise scholars. I hope that this book encourages readers to actively study this subject with curiosity and an open mind. As scientific editor, I asked all authors to accomplish three goals with each chapter. First, produce chapters that describe all major concepts, not just those derived from the author's own work. Second, provide important advice and conclusions for readers. And third, relate contents of a chapter to several themes expressed throughout the book. These themes are highlighted in Chapters 1 and 14. I believe that the book demonstrates our joint commitment to these goals.

Professors Al Gutowsky (Economics), Harvey Reissig (Entomology), and Christine Shoemaker (Engineering) were my mentors at C.S.U.S. and Cornell University. Some of my better ideas were developed under their guidance. Fred Gould hosted me during my sabbatical visit to North Carolina State University in 1994 and helped start my work in insect resistance management.

I thank both the authors and others who contributed to the development of this book. Andy Richford of Elsevier promoted the concept for the book at Academic Press. The following colleagues read portions or early drafts of several chapters: Dawn Dockter (Chapters 1, 9, 10, and 14), Casey Hoy (Chapters 1 and 9), Terry Hurley (Chapter 2), Jack Juvik (Chapter 9), and Ralf Nauen (Chapter 7). Lisa Knolhoff created figures for Chapters 2, 4, and 10. Christine Minihane, Cindy Minor, and Sunita Sundarajan of Elsevier guided me through the stages of production. Bruce Stanley and I thank Stephen Irving for use of his resistance-monitoring example in Chapter 13. Barry Pittendrigh and I thank Scott Charlesworth for creating Figures 6.1, 6.3, 6.4, and 6.5. The writing of Chapter 10 was facilitated by a Cooperative Agreement with USDA-ARS, "Contributions to a Framework for Managing Insect Resistance to Transgenic Crops." The ideas and conclusions may not represent those of the USDA or USEPA.

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The cover photograph of a female western corn rootworm beetle (*Diabrotica virgifera virgifera* LeConte, (Coleoptera: Chrysomelidae)) perched on soybean foliage in an Urbana, Illinois, USA rotated soybean field was used with permission from Joseph Spencer (© 2007).

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Chapter 1

Major Issues in Insect Resistance Management

David W. Onstad

Philosophy and History

Nature is exciting because it is dynamic, and the management of nature can be equally exciting and certainly challenging. This book presents a story about some of the most challenging aspects of pest management: the dynamics of society's competition and struggles with arthropods over evolutionary time. In this case, evolutionary time is not the millions of years required for macro-evolution and speciation, but the tens of years that are required for pest populations to evolve the ability to withstand or overcome control.

Entomologists, acarologists, and practitioners of integrated pest management (IPM) know that arthropods can evolve resistance to chemicals, host-plant defenses, and cultural practices such as crop rotation. Insect resistance is a general term representing heritable traits selected by management. These traits typically permit an arthropod to overcome pest management due to changes in behavior, maturation, or biochemical processes. Insect resistance is similar to the term host-plant resistance, which means that the plant has defenses against and is resistant to an arthropod. Throughout the book, the term insect resistance will be used, even though other arthropods, such as mites and ticks, are also frequent targets of pest management.

The greater the effectiveness and success of arthropod pest management, the greater the likelihood of the pest evolving resistance to that management tactic. This is particularly true when the goal of pest management is to reduce the pest population and maintain it at a very low level. The probability of resistance evolution will be lower when goals emphasize the prevention of damage and disease, such as the promotion of crop tolerance, which sometimes can be accomplished without harming most of the pest population. Nevertheless, if our goals or tactics involve significant pest population reduction, we likely will need to manage the evolution of resistance to the management tactics that we wish to be so effective. Insect resistance management (IRM) is the scientific approach to managing pests over the long run so that resistance does not interfere with our ability to accomplish our goals.

A common attitude in the practice of pest management is to expect that effective pesticides and other tactics will always be available in the future as each current

treatment fails due to resistance. This is not a sophisticated strategy, and it often is a wasteful and inefficient one. Of course, this requires the farmer and public health official to do nothing other than hope for the best. As each failure is observed, stakeholders search for a cure.

In the past, IRM has often emphasized this reactive approach to sequential failures of insecticides. Each insecticide is used for several years until it no longer adequately controls the pest population. Population monitoring may help identify problems before regional failure occurs. Under the best circumstances of reactive IRM, a new class of toxin with a different mode of action (physiological mechanism that kills the pest, Pittendrigh *et al*., Chapter 3; Head and Savinelli, Chapter 5) is introduced to manage the pest again with pesticides. This reliance on sequential use of tactics for control is the hallmark of reactive, some call it curative, IRM. This approach requires an optimistic view of science and industry's capabilities to produce new tactics and chemicals for future use in pest management.

The alternative approach is preventative IRM. In preventative IRM, resistance management plans are implemented when an IPM tactic is first introduced. In industry, this is called product stewardship. These plans alter the design and control of the management system so that the tactic (insecticide, crop rotation, host-plant resistance) can make a significant contribution to IPM for a period that otherwise would not have been possible. This approach is based on a pessimistic view of nature and industry: when we are careless, pests evolve faster than science and industry can develop new solutions. On the other hand, if we are careful and delay the evolution of resistance, we give our best scientists and technologists time to focus on a much wider range of management tools for the entire system. This approach does place a greater burden on practitioners and end users (ranchers, farmers, public health officials, citizens). However, since practitioners do not "own" pest susceptibility to management tactics, they should never believe and act as though elimination of susceptibility is simply an externality of their business activities (Mitchell and Onstad, Chapter 2).

The purpose of this book is to promote scientific, predictive, and preventative IRM. The book is written for those scientists, regulators, and consultants who wish to participate in the difficult but valuable efforts to (1) incorporate IRM into IPM, (2) develop economical IRM plans, and (3) design IRM plans for local environmental and social conditions.

History and Current Status of Resistance to Pesticides

Georghiou and Lagunes-Tejeda (1991) documented the history of field observations of resistance to pesticides. They stated that the first report of resistance was published by Melander (1914), who described the resistance of orchard pests to sulfur-lime, a compound typical of the inorganic chemicals used for pest management one hundred years ago. By 1989, Georghiou and Lagunes-Tejeda (1991) had counted over 500 arthropod species with strains evolving resistance in the field to toxins used against them. Within this total, 23 beneficial species were included. The resistant pests are categorized as crop pests (59%) and medical or veterinary pests (41%). By 1989, chemicals selecting for resistance included not only the modern classes of organic chemicals (cyclodiene, DDT, organophosphate, carbamate,

pyrethroid) but also inorganic and elemental chemicals (e.g., arsenicals, sulfur) commonly used before 1940.

The best source for up-to-date information about resistance by arthropods to pesticides around the world is the Arthropods Resistant to Pesticides Database, ARPD (http://www.pesticideresistance.org/), sponsored by Michigan State University, the Insecticide Resistance Action Committee (IRAC), and the United States Department of Agriculture. The database contains reports of resistance cases from 1914 to the present, including all of those reported by Georghiou and Lagunes-Tejeda (1991). Each case is defined by the time and location at which the resistance is first discovered. Mota-Sanchez *et al*. (2002) provide a detailed description of the database and an analysis of its contents. Forty-four percent of the cases involve organophosphate pesticides, while organochlorine pesticides are involved in 32% of the cases of resistance. As of 2006, the database contained over 7,400 cases involving 550 species (see also Table 5.1 of Head and Savinelli). The public can search the database for information, and authorized experts can submit new cases.

Major Themes

IRM is often considered the management of the evolution of resistance in an arthropod species. However, this is a very narrow and restricted view of the interacting ecological and socio-economic systems that not only are affected by resistance but determine whether resistance will evolve. Just as IPM does not simply focus on killing pests, IRM should not be limited to restraining the dynamics of genes. In this section, I introduce several major themes that are expressed throughout the book.

Integrated Pest Management

IPM was conceptualized during the 1950s when insecticide resistance, non-target effects, and economic waste were clearly apparent (Stern *et al*., 1959). Practitioners understood the consequences for the larger environment and the longer term, but implementation of IPM emphasized short-term economic efficiency and integration of cultural, biological, and chemical control measures. For example, by including natural enemies as biological control agents in the management of pests, IPM practitioners knew that more specific and less harmful chemicals would need to be used over the long run (Hoy, 1990; Hull *et al*., 1997).

IRM must be considered a part of IPM (Croft, 1990; McGaughey and Whalon, 1992; Glaser and Matten, 2003). Certainly after 50 years of effort to implement rational and socially beneficial IPM, most people would agree that IRM must at least account for the consequences for IPM of managing the evolution of resistance genes. McGaughey and Whalon (1992) stated that IRM within the context of IPM is based on four factors: (1) diversification of causes of mortality so that a pest is not selected by a single mechanism, (2) reduction of selection pressure for each mortality mechanism, (3) maintenance of a refuge or immigration to promote mixing of susceptible and resistant individuals, and (4) prediction using monitoring and models.

Formal representation of long-term management within the IPM paradigm is all that is needed to bring IRM and IPM together. By combining population genetics

(from IRM) with the focus on economic efficiency and environmental stewardship (from IPM) and formally considering a multi-year time period, all aspects of IPM and IRM can be combined. Chapters 5–10 provide a variety of case studies that highlight the interactions between IRM and IPM.

In essence, the linking of IRM and IPM provides a management perspective the same as long-term, area-wide, pest management. Area-wide pest management was first promoted for the eradication of a few pests by collective efforts (Myers *et al*., 1998; Smith, 1998; Bowman, 2006). Some also recognized that if regionally coordinated IPM could occur, even annual management of a constant pest could be made more efficient (Faust and Chandler, 1998). Recently, more attempts have been made to coordinate efforts for regional IPM (Pereira, 2003; Sexson and Wyman, 2005).

Siegfried *et al*. (1998) recognized the relationship between area-wide pest management and IRM when they warned proponents of area-wide pest management about the increased risk of resistance evolution in area-wide projects because of the reliance on uniform exposure of pest populations. They believed that some attributes of area-wide management are incompatible with many conventional IRM techniques, but suggested that use of biologically based control tactics, such as behavior-disrupting chemicals, may contribute to both area-wide pest management and IRM. Siegfried *et al*. (1998) concluded that both area-wide pest management and IRM require a high degree of grower compliance.

IRM requires an approach that considers not only the long term, but also the spatial dynamics of the pest and its management over a large region. Thus, all IRM should be area-wide pest management. (Note though that both IPM and IRM perspectives are generally in opposition to pest eradication activities.) The term "integrated" in IPM can also refer to the integration of management across space and over time. Thus, IPM implies that area-wide and long-term approaches can be valuable. Because this type of approach is the basis for IRM, I view IRM as an important part of future IPM strategies.

Coordination

In a few cases, IRM may be strictly a private matter for one company that both produces the livestock or crop as well as provides the tools for managing the pest. This company would likely be interested in product stewardship if an insecticidal plant or compound is used. Nevertheless, in most cases, IRM requires coordinated behavior by many individuals and businesses.

Coordinated behavior is necessary to provide the area-wide pest management described above. If the actions taken by individuals are not clearly beneficial to them, especially if their individual goals differ from those held by leaders mandating the coordination, then some kind of persuasion or coercion will be necessary. Even in the case of a unique, synthetic toxin patented by a company, any product stewardship will require obtaining the cooperation of most farmers, ranchers, pet owners, or public health departments using the product.

Keiding (1986) described the coordination and cooperation involved in the management of resistance to insecticides by *Musca domestica* in livestock barns in Denmark. Keiding stated that collaboration and exchange of information must

be maintained between the agro-chemical industry, users of the insecticides, those who advise them (e.g., extension services or farm organizations), and research institutes. He suggested that coordination could be organized by an international agency, such as the Food and Agriculture Organization (FAO) or the World Health Organization (WHO), or by a national or state institution. In the same book, Brent (1986) also promoted the coordination of public and private sectors in managing resistance. In fact, the United States National Research Council's (NRC) Committee on Strategies for the Management of Pesticide Resistant Pest Populations recommended that working groups involving all stakeholders should prioritize IRM efforts based on economic, environmental and social factors (NRC 1986, p. 275).

Forrester (1990) provides a good summary of the coordination and critical activities required to make an IRM plan succeed. He states that preventative IRM is preferable to curative IRM, because curative approaches are more restrictive and have a lower chance of long-term success. Negotiations that involve compromise and consensus amongst stakeholders are important; although, he suggests that centrally planned and regulated IRM strategies can be some of the most successful. In either case, compliance with the plan is a critical factor. Forrester (1990) emphasized the need to make IRM strategies match the local conditions for the pest, environment, and community (Head and Savinelli, Chapter 5).

From an economic perspective, coordination can be valuable but only under certain conditions. Miranowski and Carlson (1986) stated that voluntary IRM cooperation amongst farmers will likely occur only when pests can move from farm to farm, when benefits and costs of a farmer's participation are proportional to the level of participation, that free-riders receive minor benefits, and that coordination costs are low.

Resistance management strategies are only successful at the landscape level, which requires coordination of all producers in a given area. How do we (1) convince producers that resistance management is essential to maintaining effective arthropod control measures and (2) devise IRM strategies that are in the economic interest of producers? Producers are likely to recognize the threat that resistance poses to pest management, but if preventing resistance becomes too burdensome, in terms of either time or money, they will not adopt IRM techniques. Maintaining a refuge of susceptible plants (and alleles) is a common strategy, but if the pest population causes significant damage to refuge plants, producers are less likely to comply (Hurley and Mitchell, Chapter 11).

Pest Behavior

It should not be surprising that mortality is easier to measure than any type of arthropod behavior. One consequence of this is that pest behavior and behavioral resistance have traditionally not been investigated sufficiently during studies of population genetics and evolution. One objective of this book is to promote the study of pest behavior to improve IRM. Many of the cases of resistance and its management throughout the book demonstrate the importance of behavioral studies.

Toxicological resistance to pesticides has been the focus of the vast majority of IRM studies. However, evidence has been accumulating that demonstrates the importance of behavioral resistance (Lockwood *et al.*, 1984; Gould, 1991;

Hoy *et al*., 1998). Toxicological resistance is the evolution of a mechanism that reduces or prevents the intoxication of an individual once the toxin contacts or enters the body (Chapter 3). To avoid confusion, I do not use the term "physiological" resistance, because behavior can be considered an observable consequence of physiological mechanisms (Georghiou, 1972). Behavioral resistance is the evolution of any behavioral change that permits a population to avoid or overcome management tactics. Behaviors that may be important include movement of immature stages, adult dispersal, oviposition, feeding, or any social or non-social interaction in a population. Gould (1984) investigated the management of behavioral resistance using a mathematical model. Lockwood *et al*. (1984) reviewed early cases and described the shift in perspective that was needed to appreciate behavioral resistance. They recognized a connection between behavior and toxicological resistance and emphasized that the two may occur simultaneously. Gould (1991) related behavioral resistance to the evolutionary biology of plant–herbivore interactions. He encouraged the study of the behavioral responses of herbivorous arthropods in natural plant communities to discover clues to the evolution of resistance to pesticides. Hoy *et al*. (1998) emphasized the role of spatial heterogeneity in the evolution of behavioral responses to toxins, and the role of these behavioral responses in the evolution of toxicological resistance. Because natural and synthetic toxins are heterogeneously distributed in plants and across the landscapes containing plant communities, evolution of behavioral responses should be expected. Since the review of Hoy *et al*. (1998), dozens of publications have reported on observed or potential behavioral resistance to not only insecticides but also natural enemies, transgenic insecticidal plants, sterile-insect releases, and diatomaceous earth.

The diamondback moth, *Plutella xylostella*, seems to be one of the best model organisms for studying behavioral resistance. It is easy to rear, has many generations per year, and can be investigated under laboratory, greenhouse or field conditions. Therefore, the studies of behavioral responses by *P. xylostella* and their influence on toxicological resistance are summarized below. As you read, note the consequences of pest behavior, how behavior influences pest survival in a treated environment, and how it influences the toxin dose acquired by an insect and, therefore, the selection pressure for toxicological tolerance. These issues have traditionally not been investigated sufficiently during studies of population genetics and evolution.

Head *et al*. (1995a) investigated the genetic basis of toxicological and behavioral responses to a pyrethroid in populations of *P. xylostella* with different average levels of tolerance for the toxin. Heritabilities for behavioral avoidance of the pyrethroid were low and significant in only one population, although additive genetic variances were similar to those observed for the toxicological responses. The phenotypic and genetic correlations between the two traits varied among the populations. All correlations were negative and significant correlations occurred in populations with relatively high levels of additive variation for both traits. Individuals with low tolerance for the toxin fed more on leaves having low concentrations of the toxin. In a subsequent study, Head *et al*. (1995b) demonstrated that two elements of behavior were affected by selection in laboratory populations: general larval activity increased with behavioral selection and larvae displayed a greater tendency to avoid the pyrethroid. Note however, for *Leptinotarsa decemlineata* feeding on toxic potato plants, Hoy and Head (1995) observed a positive

correlation between larval movement away from high toxin concentrations and tolerance for the toxin. Head *et al.* (1995a, b), Hoy and Head (1995), Hoy *et al.* (1998), Jallow and Hoy (2007), suggested that some IRM plans could take advantage of behavioral evolution by selecting for susceptibility in landscapes with heterogeneous spatial distributions of toxins.

In a series of laboratory and greenhouse experiments, Jallow and Hoy (2005, 2006, 2007) investigated the simultaneous evolution of behavioral responsiveness and toxicological resistance in *P. xylostella*. Jallow and Hoy (2005) first measured phenotypic variation in behavioral response and toxicological tolerance to permethrin in one field and one laboratory population of *P. xylostella*. In laboratory bioassays, females from both populations were less likely to oviposit on cabbage leaf disks and seedlings treated with permethrin, and this oviposition deterrence was correlated with permethrin concentration. The laboratory population was more behaviorally responsive to the insecticide and showed a greater avoidance than the field population. They measured the toxicological response of each population with feeding bioassays, and the laboratory population was more susceptible to the permethrin. Thus, there was a negative correlation between avoidance and detoxification.

Jallow and Hoy (2006) extended their study to include the genetic basis of adult behavioral response and larval toxicological tolerance to permethrin within the two populations of *P. xylostella.* The adult behavioral response was again measured as oviposition site preference. They discovered that a high proportion of phenotypic variation for adult behavioral response to permethrin was heritable genetic variation. The larval toxicological response was measured with a topical application bioassay. Significant additive genetic variances and heritabilities for toxicological tolerance to permethrin were detected in both populations. The genetic correlations between adult behavioral response and larval toxicological tolerance to permethrin were negative, but significant only in the field population (Jallow and Hoy, 2006).

In their greenhouse study of the field population of *P. xylostella*, Jallow and Hoy (2007) investigated the changes in behavioral response and toxicological tolerance of *P. xylostella* to homogeneous and heterogeneous distribution of the toxin permethrin. They utilized three selection regimes: uniform high concentration hypothesized to result in increased toxicological tolerance, heterogeneous low concentration hypothesized to result in increased susceptibility to the toxin through indirect selection on behavior, and a control with no exposure to permethrin. All life stages of the moth were exposed to the selection regimes. The insects were observed in $1\,m^3$ cages in a greenhouse for thirty-three generations. Each successive generation was started with a random selection of pupae from the previous generation. Cohorts selected with uniform high concentrations evolved high levels of resistance to permethrin by the seventeenth generation. For generations 1–20, cohorts selected with heterogeneous low concentrations were similar to the unselected control, but in generations 21–33, those selected with the heterogeneous low concentration were more susceptible than those of the control. Jallow and Hoy (2007) concluded that low heterogeneous doses could lead to increased susceptibility to permethrin by selecting indirectly on behavior.

The work of Jallow and Hoy (2006) demonstrated that female moths that are more behaviorally responsive to permethrin produce offspring that are more susceptible

to the same insecticide. Jallow and Hoy (2007) concluded that selection on this behavioral response can result in greater susceptibility compared to scenarios with very high uniform concentrations or no toxin in the environment. The adult behavioral response can lower the exposure of larvae to the insecticide, lowering selection pressure for toxicological resistance in larvae. Thus, this behavioral response and associated larval survival could help preserve susceptible alleles in the population, which would contribute to the success of IRM.

Based on all of the evidence presented above, we can conclude that accounting for arthropod behavior is important for the prediction of the evolution and management of resistance. Behavioral resistance may evolve or behaviors may vary from environment to environment and may influence evolution of toxicological resistance. Other evidence for the important role of behavior can be found throughout this book. This does not mean that behavioral resistance will always be observed, as the case evaluated by Hawthorne (1999) indicates. Nevertheless, the evidence does support the claim that more resources should be allocated for behavioral studies during the preparation of IRM plans.

Variability and Complexity of Management Strategies

The most common IRM strategies are briefly described below to provide some background upon which I can draw another theme for the book. The challenge for all readers, as well as for all workers in the field of IRM, is not to rely completely on tradition when developing strategies for new pests and new pest-management systems. A full appreciation of strategies for managing insect resistance to any pest-management tactic requires an understanding of economics (Chapter 2), population genetics (Chapter 4), and other information about nature and society.

Denholm and Rowland (1992), Denholm *et al*. (1992), McGaughey and Whalon (1992), McKenzie (1996), Roush (1989), Roush and Tabashnik (1990), and Tabashnik (1989) provide good overviews of the strategies commonly considered when arthropods may evolve resistance to insecticides. The focus is on preventative IRM strategies for managing susceptibility before resistance genes increase in frequency in the population. Variations or even completely different plans will be needed once resistance is observable and measurable in the field (Forrester, 1990).

Kill Fewer Susceptibles

Selection pressure can be reduced by lowering the selection intensity of each treatment or by decreasing the number of treatments applied against a pest species over time. When treatments are reduced by eliminating treatments experienced by the vulnerable life stages of the pest in certain generations, the evolution of resistance can be delayed. However, resistance-allele frequency will continue to rise over many generations unless the resistant individuals have a lower relative fitness than the susceptibles (fitness cost) in the absence of treatments.

Another approach provides a spatial refuge that allows susceptible individuals a place to escape selection by the treatment. Refuges are deployed so that adequate mixing of the subpopulations occurs. Susceptible individuals can then mate with any resistant individuals, lowering the proportion of homozygous resistant genotypes in the population.

The treatment effect can be reduced to lower selection pressure by decreasing the concentration of the insecticide or other treatment. This approach is effective if it allows more susceptible individuals to escape mortality; thus, increasing the relative fitness of the susceptibles in the population. McGaughey and Whalon (1992) noted that low-dose strategies for IRM would only work when they become a significant part of an IPM program. In that case, the reduced concentration of insecticide would promote the efficacy of natural enemies of the targeted pest. All of the tactics, particularly the insecticide, would need to maintain the pest density below the economic threshold, otherwise farmers and similar stakeholders would not accept the greater damage by the pest.

Without the support of an effective IPM program, attempts to reduce the selection on susceptibles might simply lead to more damage by the pest with subsequent reduction in compliance by stakeholders. Gray (2000) has suggested that transgenic insecticidal crops be planted only with permission from an independent agent, similar to the need for prescriptions from physicians for medicines.

Kill All the Heterozygotes

If there are very few resistant homozygotes in the population, then an effective strategy may be to increase the concentration or efficacy of the treatment so that all heterozygotes are killed. This lowers the dominance of the resistant individuals relative to susceptibles. More recently with the use of transgenic insecticidal crops, this has been called the high-dose strategy (Onstad and Knolhoff, Chapter 9). A refuge for susceptibles is often included to prevent evolution of resistance by promoting the mating of homozygous susceptibles with any rare homozygous resistant individuals; heterozygote offspring in treated areas will all die in the next generation.

When the concentration of the toxin is either decreased or increased, the effect on evolution of resistance depends on the population dynamics and environment of the targeted pest and its natural enemies. Thus, information on interactions and complexities in the system should be gathered before predicting the long-term effectiveness of a strategy.

Use Two Treatments

When two or more treatments have different effects on the arthropods (e.g., different modes of actions by toxins), then it may be possible to use them either in mixtures or rotations to delay the evolution of resistance. A mixture is the simultaneous application of two treatments to the same individuals in a population. Both parts of the mixture must remain effective for the same period of time over the same region of the landscape. A refuge may be needed, as described above, to provide a source of susceptibles that can mate with any rare homozygous resistant individuals. With mixtures we expect each treatment to kill any individuals resistant to the other treatment. When multiple genes for pest control are incorporated together in a crop, this mixture is called a pyramid. Difficulties encountered when implementing an IRM strategy with mixtures include ensuring the equal persistence of both treatments and the possibility that resistance genes will interact in ways that reduce the effectiveness of the mixture (Onstad and Guse, Chapter 4). Roush (1994, 1998) explains some of the advantages and limitations of insecticide mixtures and transgenic insecticidal

crops with pyramided traits, and Gould *et al*. (2006) demonstrate that fitness costs due to resistance can also be important in IRM with pyramided crops or mixtures.

A rotation involves alternating the use of multiple treatments across generations of the targeted pest. In essence, treatments are applied to the same space at different times. In this approach, we assume individuals resistant to one treatment will be killed by the next treatment in the rotation. When large fitness costs are associated with resistance, rotations may be especially effective. Curtis *et al*. (1993), however, review experimental evidence demonstrating that rotations are not always superior to sequential treatments (reactive IRM). It is generally not recommended to alternate insecticides within a single pest generation (Roush, 1989).

A mosaic of treatments is the simultaneous application of tactics, each to a different area infested by the pest population. This is the opposite of the rotation strategy. In general, a spatial mosaic should not be considered for IRM, because it is the least likely to succeed; no refuge is provided for susceptibles for either treatment and there is simultaneous selection for resistance to both toxins in the total population.

Scientists must always be skeptical about claims that two chemicals have such different modes of action that an insect cannot evolve resistance to both simultaneously. Certainly within a given class of chemicals, cross-resistance is a common phenomenon observed in the field when resistance to one chemical is followed by rapid, if not immediate, evolution of resistance to the second chemical used in the sequence. Unless resistant populations already exist in laboratories, cross-resistance is difficult if not impossible to evaluate. When these laboratory colonies do exist, they may not contain the rare mutants with cross-resistance genes. Thus, it is very difficult to experimentally provide evidence demonstrating lack of cross-resistance in a real population. Perhaps, this means that future work should emphasize strategies that use two treatments, only one of which is a chemical. The other treatment would be cultural control, biological control, or environmental manipulation. This does not guarantee lack of cross-resistance, but broadening our scope forces stakeholders and developers to face the complexity of pest management and perhaps take advantage of it.

The Future Is Not the Past

The complexities and dynamics of nature and its management will likely require IRM strategies that do not fit easily into these three categories. Spencer and Levine (Chapter 8) describe resistance to crop rotation: a different kind of problem with a variety of IRM solutions. Pittendrigh *et al*. (Chapter 6) explain how negative cross-resistance can be used as an effective IRM strategy. In these and other cases, scientists are focusing their attention on IRM strategies that are not simple extensions of traditional approaches.

The success of any strategy depends on coordination of treatments over time and space, particularly within a region inhabited by a pest that can disperse from one field to another. For example, mixtures require coordination to avoid simultaneous use of single components of the mixture that would lead to sequential evolution of resistance first to the single component and then to the other component encountered in areas with mixtures. Rotations require coordination to avoid the creation of a spatial mosaic in a region.

One of the most difficult problems in IRM is the design and implementation of a strategy for multiple pests (McGaughey and Whalon, 1992; Gould, 1994; Wearing and Hokkanen, 1994). This especially is true when the simplest approach for each pest interacts with and affects the other. The timing of the pests may be different, the mortality caused by a toxin may be different, and the pests may generally have behaviors that differ over time and space. Tabashnik and Croft (1982) stated, "Even when the conditions are appropriate for using a high-dose strategy to delay resistance in one pest species, this strategy may greatly accelerate the rate of resistance development of other pests in the species complex." Furthermore, when the pests infest multiple crops in a landscape and are selected by multiple control tactics, IRM becomes even more complicated. New ideas and much hard work will be needed to deal with these issues in the future.

Arthropods Can Become Resistant to Any Pest Management Practice

We should expect many kinds of effective pest management to cause the evolution of insect resistance. Therefore, IRM strategies will be needed for all kinds of resistance. The chapters in this book describe cases of resistance by arthropods to pesticides, crops, and crop rotation. Pests can also evolve resistance to natural enemies, such as pathogens and parasitoids (Shelton and Roush, 2000; Kraaijeveld, 2004; Carton *et al*., 2005). Note, however, that determining the genetic basis for resistance to parasitic natural enemies can be complicated by the possibility of host populations carrying symbiotic microbes that protect them (Oliver *et al*., 2005). The following is a summary of the evidence concerning arthropod resistance to microbial control.

Although resistance by arthropods to infectious pathogens causing contagious diseases has not been considered a serious issue and is rarely observed outside the laboratory, scientists should understand the potential for resistance evolution for several reasons. First, microbial insecticides consisting of viruses, fungi, or bacteria, other than insecticidal *Bacillus thuringiensis,* are increasingly being studied and developed (Moscardi, 1999; Lacey and Kaya, 2000; Butt *et al*., 2001). Second, as more microbial insecticides are used, selection pressure may increase resulting in higher probability of resistance evolution. Third, management of domesticated beneficial insects (silkworm, *Bombyx mori*, and bees such as *Apis mellifera*) may require populations that are resistant to natural pathogens (Briese, 1981; Stephen and Fichter, 1990).

Briese (1981) was one of the first to summarize the state of knowledge concerning insect resistance to viruses, bacteria, fungi, microsporidia, and nematodes. Most of the cases were identified in laboratory colonies, bee hives, or silkworm populations. Several additional studies published since 1981 exemplify the ability of insects to evolve resistance to infectious pathogens (Milner, 1982; Ignoffo *et al*., 1985; Stephen and Fichter, 1990). For example, Briese and Mende (1983) observed a 140-fold increase in LD_{50} after serial exposure of a field-collected population of *Phthorimaea operculella* to granulosis virus over six generations in the laboratory.

More recently, Fuxa (2004) reviewed the cases of insect resistance to nucleopolyhedroviruses. Fuxa (2004) discussed cross-resistance to several pathogens. He also stated that there are several similarities between arthropod resistance to

viruses and resistance to chemical pesticides. However, he noted that there can be important differences in mechanisms and that the potential for co-evolution exists between insects and pathogens.

It is likely that arthropods can evolve behavioral resistance to pathogens. American foulbrood is the bacterial disease caused by *Bacillus larvae* infecting *A. mellifera*. Incidence of the disease in hives is determined by behaviors such as the speed with which diseased bee larvae are detected and removed by bees. Rothenbuhler (1964a, b) demonstrated that these behaviors are genetically controlled. DeJong (1976) observed a similar scenario with behavior by *A. mellifera* and the fungal disease caused by *Ascosphaera apis* (chalkbrood). In a population of *Anopheles* mosquitoes, Woodard and Fukuda (1977) found that larvae from a strain selected for resistance to nematodes were much more active and defended themselves against attacking nematodes. Although no genetics were evaluated, Inglis *et al*. (1996), Villani *et al*. (2002), and Thompson and Brandenburg (2005) observed behaviors by insects that allowed individuals to reduce or prevent infection by fungi. These insect behaviors included thermoregulation to change body temperature as well as movements and tunneling behavior to avoid contact with the pathogen.

Additional Ideas

As you read this book, keep these major themes in the back of your mind. In some sections, the issues will be addressed explicitly, while in other parts you will simply remember that they are important. For some cases, the importance is clear because the coordination of a plan was lacking, pest behavior was ignored, predictions were not made, or risks were not assessed. The relationship between IPM and IRM can often be determined from an inadequate strategy as well as from a good plan; you may realize that effective IPM can make IRM both simpler and more effective. Overall, you will notice how and to what extent the general strategies described above influenced the IRM plans that may, or may not, have been implemented. The concluding chapter will return to these major themes and express these and other important issues as a set of rules for IRM practitioners. As the themes presented above indicate, IRM is certainly more than just the study of insect evolution. Both theoretical and practical IRM require the study and appreciation of socio-economic factors that contribute to coordination, goal setting, and risk aversion (Mitchell and Onstad, Chapter 2). Because these are also important issues in modern IPM, students and scholars must take the time to learn other disciplines and other perspectives.

An openness to the great diversity and complexity of populations and individual behavior is necessary to develop the skills needed to confront, if not prevent, the evolution of resistance in arthropod pests. Populations and their environments are dynamic, and those of us investigating and managing them must be dynamic, too.

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Chapter 2

Valuing Pest Susceptibility to Control

Paul D. Mitchell and David W. Onstad

Resistance management involves understanding both the evolution of arthropods and the value of the evolving pest population. Most chapters in this book describe prediction of the evolution of resistance as well as its management. This chapter, however, focuses entirely on the issues of preference and value, of which most biologists have only a vague understanding. Understanding the valuation of natural resources such as pest susceptibility, particularly from the perspective of economics, is an important foundation for the management of insect resistance.

Management implies that decision makers have goals and that resources and labor will be allocated to achieve these goals. Hence, as noted in the first chapter, insect resistance management (IRM) must be based on the goals of the decision makers. These goals not only require focus on particular resources and their values, but also on the time horizons over which these goals will be achieved and how to address the uncertainty of knowledge. For example, if stakeholders place a high value on low frequencies of resistance-alleles, then one goal could be to minimize the expected resistance-allele frequency after a certain number of years, within the constraints of the decision maker. We often take for granted our values and express them implicitly when stating our goals. Thus, IRM goals are usually the starting point for studies of resource values and economics, with the values implicit in the stated goals.

This chapter separates the discussion into five sections. First we provide a general overview of the classification of goods from an economic perspective, focusing on those types that pertain to IRM and IPM. Second, we discuss the valuation of pest density and of pest susceptibility at a single point in time. These attributes of pest quantity and quality are the primary factors in most discussions of integrated pest management (IPM) and IRM, as well as the critical variables in most economic models. We realize that a complete evaluation of the costs and benefits of IRM and IPM should consider impacts beyond pest quantity and quality to address factors such as environmental quality and human health, but this additional evaluation would extend our efforts beyond the intended scope of the book. Third, we extend this accounting of values to consider time preferences by discussing the use of discounting of future values. Given that IRM requires management over multiple years, we must quantitatively compare values from different times to evaluate different strategies. Fourth, we extend this value accounting to consider

risk preferences by discussing methods commonly used to incorporate uncertainty into the decision-making process. Fifth, we develop simple illustrations of how these methods have been applied with a brief overview of economic IRM models. Finally, we draw some conclusions and suggest future work.

Goods and Values

Goods

To better understand the management of natural resources and environmental goods, economists classify goods based on the properties of rivalry and excludability. Rivalry describes how one person's consumption of the good changes the availability of the good for others, while excludability describes the extent to which others can be prevented from consuming the good. A private good is excludable and rival – one person's use excludes all others and when consumed, the good no longer has value. A simple example is an apple purchased from a vendor – the buyer owns it and decides its use and once consumed, the apple is gone. At the other extreme is a pure public good, which is non-excludable and non-rival – all people consume the good (none are excluded) and each person's consumption does not reduce the good available for others. The air we breathe is a simple, but not quite perfect example (one person's use of air can lessen (pollute) its availability for another's use). However, the definition is a useful theoretical construct, though identifying real world goods that are absolutely non-rival and non-excludable is difficult. Most public goods are, in some sense, not completely non-rival and non-excludable.

Several types of impure public goods exist (OECD 2001a; Cornes and Sandler, 1996). In terms of managing pests and resistance, open access and common property resources may occur. An open access resource is non-excludable and rival – anyone who wants can obtain the good and once consumed it is gone, which leads to the classical "tragedy of the commons" problem (Hardin, 1968). Fishing stocks in the open ocean are probably the most well-known example. Common property resources are also rival goods, but are excludable to outsiders, but with open access to those in the commons. Typical examples are aquifers and commonly held pastures (Bromley and Cernea, 1989). Before providing examples of these different types of private and public goods resulting from pest and resistance management, we discuss economic valuation.

Values

Deciding how to manage pests and their resistance to control requires placing a value on the pests, on the damage they cause, and on the parts and processes in the ecosystem affected by their management. For this discussion of IRM, we focus primarily on pest control and pest susceptibility as goods to be managed, noting that these goods are commonly measured with the pest population density and the frequency of susceptibility (or resistance) alleles among this population. These goods do not capture all the values that IRM can consider, but they serve as a convenient and important sub-set to illustrate the methods and issues. Incorporating other values would not change the general methodology illustrated here, but would extend the discussion beyond our intended scope. Let it suffice to say that we are

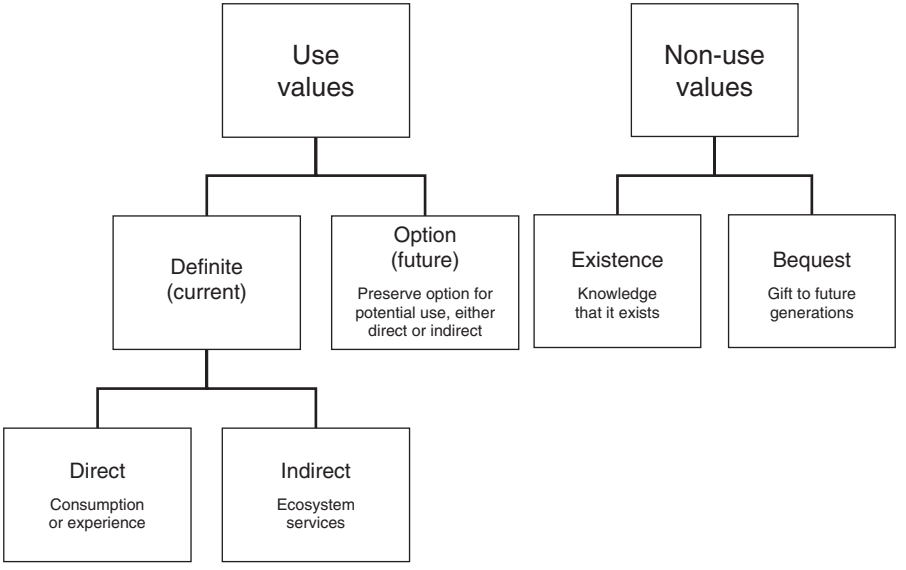

Figure 2.1 Diagram of economic values of goods. Analysis of insect resistance management focuses on use values.

not forgetting or ignoring such values, but rather not explicitly including them here for convenience.

Economists define two types of value for goods: use value and non-use value (Figure 2.1). As the name implies, use value is the value a good possesses because it can be used or consumed by a person. Use values for a good or resource can include its direct use value for consumption or experience and/or its indirect use (or functional) value as a supplier of ecosystem services (Barbier, 1991, 2000; Young, 1992; Heal, 2000). For example, honeybees provide both honey (direct use value) and pollination (indirect use value), so that the use value of a honeybee population includes the sum of the value of the honey and pollination it produces. In addition, use value includes the value of the option to potentially use the good or resource in the future, which can be either a direct or indirect use value. Such option values are often used to argue for the preservation of species biodiversity or ecosystems, since some species will likely be directly and/or indirectly useful for future problems or needs (Pearce and Moran, 1994; OECD 2001b). Finally, a good can also have non-use values that arise from the value attached to the existence of the good (existence value) and the possibility of maintaining the good for future generations to use (bequest value) (OECD 2001a).

The primary source of value identified in this book will be of the use type. For example, the value of pest density is related to the damage that the pest causes either to (a) some other good such as a crop, which can be consumed directly by humans or (b) an ecosystem service, which benefits humans indirectly. Furthermore, some stakeholders likely place a positive option value on pest susceptibility that might be taken advantage of in the future, while others attach existence and bequest values

for pest susceptibility as well. However, with the exception of Wesseler's (2003) option value approach, the predominant value of pest susceptibility emphasized in discussions (e.g., Hueth and Regev, 1974; Mangel and Plant, 1983; Regev *et al*., 1983; Plant *et al*., 1985; Onstad and Guse, 1999; Hurley *et al*., 2001, 2002; Onstad *et al*., 2003; Livingston *et al*., 2004; Hurley, 2005) is the direct use value of susceptibility for managing pest damage, now and in the future. Nevertheless, we note that, because pest populations and their genetic composition have bequest and existence values to individuals and societies, as well as indirect use values, pest control and susceptibility also have some public good qualities. Empirical estimates of these values vary widely and consensus has yet to emerge, so that such values have not been incorporated into a quantitative IRM analysis.

Pest mobility and ecology together with social institutions and the environment determine the level of excludability in pest control and susceptibility. Control of a highly localized and genetically isolated pest population can be treated as a private good, with the benefits of control and resistance management largely captured by the local land owner. Scale insects (Hemiptera: Coccoidea) are an example of such pests, due to the low mobility many of the species exhibit in all life stages and their preference for long-lived hosts (Banks and Denno, 1994).

As a pest species becomes more mobile and has greater genetic exchange, pest density and susceptibility are more like common property resources than open access resources. The distinction between common property and open access pest populations is often whether or not the "owners" of the pest population can potentially organize or not. If organized management is possible, then the population is a common property resource, if not, then it is an open access resource. Pest ecology, the natural environment, and social institutions must converge for successful common property management of a pest (Knipling, 1979; Gray, 1995; Kogan, 1998).

In some locations, pest mobility and natural barriers together create pest populations that are common property resources (Regev *et al*., 1976). For example, the surrounding desert and mountains in parts of the western US create barriers that reduce entry of some pests, so that residents of the area can potentially commonly manage pest control and susceptibility, as for example, proposed by Carrière *et al*. (2006) for *Lygus hesperus* in Arizona. Social institutions can also create such barriers for common property management of some pests, as the successful boll weevil (*Anthonomous grandis*) and screwworm (*Cochliomyia hominivorax*) eradication programs illustrate (Myers *et al*., 1998). For some pests, mobility and/or the lack of barriers do not permit area-wide management, as seems to be the case for *Diabrotica virgifera virgifera* in the US Corn Belt (Gray, 1995, Spencer and Levine, Chapter 8). As a result, the density and susceptibility of *D. virgifera virgifera* are managed as open access resources, which has contributed to (but is not the sole cause of) the pest developing resistance to various insecticides and crop rotation (Metcalf, 1983; Levine and Oloumi-Sadeghi, 1996; Meinke *et al*., 1998; Wright *et al*., 2000; Levine *et al*., 2002, Spencer and Levine, Chapter 8). Clark and Carlson (1990) also find empirical support, based on analysis of the demand for insecticides, that farmers manage insect pests as common property resources without group coordination. Numerous insect pests have developed resistance to control (CAST 2004), providing additional evidence that pest susceptibility is used as an open access resource without coordinated management.

Valuation of Pests

Valuation of Pest Population Densities and Damage

In IPM, entomologists have traditionally focused on the economic damage that a population causes at a particular place and time. The concept of the economic threshold (Stern *et al*., 1959; Onstad, 1987) shows that pest density must be considered in the economic context in which it occurs. For instance, a low pest density is acceptable as long as the damage that it could cause in the future does not exceed the cost of preventing that damage. At the farm/field level, most economic analyses of pest management focus on the effect of a pest population on the value of a crop. Usually, crop loss from a pest is some combination of a reduction in usable crop biomass (yield) and/or in crop quality, which imply a decrease in the market value of the harvested crop. Typically, this crop loss is conceptualized as some function of the pest population density (a damage function), while pest control reduces this population density or the damage it causes (a control function) (e.g., Mitchell *et al*., 2004). The net value of pest control to the owner/manager is the value of the prevented crop loss from pest damage, minus the cost of pest control.

Two types of data are generally available for economic analysis of the value of pest damage and management – either observational data of actual (or aggregate) farm use of insecticides or experimental data consisting of measures of pest population density, crop damage or yield loss, and the efficacy of different control methods. Observational data of farmer behavior suffer what economists call an endogeneity problem and so should not be used to directly estimate pest damage functions or pest control functions as in the early analyses of Headley (1968) and Carlson (1977). Rather, because both the input (pesticide) and output (crop yield) are endogenous to the farmer's decision, the decision-making process must be explicitly modeled, with most analyses assuming profit maximization or cost minimization behavior by farmers. Lichtenberg and Zilberman's (1986) seminal paper effectively argued that for damage control inputs such as insecticides, standard econometric methods must explicitly specify the damage function (technically they express the model in terms of damage prevented, but the implication is the same). Their paper generated several responses and evaluations of their methodology (e.g., Babcock *et al*., 1992; Blackwell and Pagoulatos, 1992; Carrasco-Tauber and Moffit, 1992; Fox and Weersink, 1995; Saha *et al*., 1997; Carpentier and Weaver, 1997; Hennessy, 1998), but in general, their econometric method has been accepted as the proper method for using observational data for estimating the productivity of pest control inputs.

Experimental data avoids the endogenity problem because pest management is controlled independent of yield or pest pressure, and so the damage function can be directly estimated using more traditional regression techniques. Several empirical applications extend the literature to account for pest population dynamics (Shoemaker, 1973; Talpaz and Borosh, 1974; Talpaz *et al*., 1978), uncertainty (Feder, 1979; Moffit *et al*., 1984), interactions with secondary pests and other inputs (Harper and Zilberman, 1989), global concavity of the production function (Hennessy, 1998), management of insects as virus vectors (Marsh *et al*., 2000), and separate identification of experimental errors and damage variability (Mitchell *et al*., 2004; Yang *et al*., 2006).

Usually such analyses ignore any positive or negative externalities that farm/field level pest control generates, such as the value that pest control on one farm has on the pest population and crop losses on other farms or the cost of human health impacts and environmental damages from pest control. Exceptions to this generalization exist. Harper and Zilberman (1989) examine on-farm externalities from input interactions and secondary pests and Regev *et al*. (1976) incorporate population effects on other farms. Theoretically, incorporating the effect of these and similar externalities in pest control decisions is straight forward using taxes or subsidies, command and control policies, or other policy instruments so that on-farm decisions account for the actual human health and environmental costs of their pest control decisions (Baumol and Oates, 1988; Cornes and Sandler, 1996). However, practical application is problematic because individual farm contributions are difficult to measure and to value (Knight and Norton, 1989).

The difficulty measuring farm-specific contributions to environmental pollution implies that pest control inputs usually become non-point source pollution, which has remained notoriously difficult to regulate using policy tools based on economic theory (Ribaudo *et al*., 1999). Even if accurate measurement of individual contributions to environmental pollution and human exposure were available, valuing the cost of these contributions is not clear. Tremendous advances in market and non-market valuation methods for estimating such costs has occurred (Vatn and Bromley, 1995; Willis and Corkindale, 1995; Bazerman *et al*., 1997; Champ *et al*., 2003; Freeman, 2003; Maler and Vincent, 2005), but practical application to pest control externalities remains to be established. On a more positive note, some farmers earn price premiums for their products due to some consumers' willingness to pay more for (eco-)labeled products (e.g., organic, pesticide-free, or IPM), which would seem to be a method for farmers to internalize the full cost/benefit of their pest control decisions (see Wessells *et al*. (2001) for a review of the economics of ecolabeling). However, it remains to be established whether these price premiums compensate farmers an amount equal to the actual value of the environmental damage they do not cause (Dosi and Moretto, 1998), nor would these premiums necessarily lead to optimal supply of non-use values from pest populations due to the public nature of these goods (Cornes and Sandler, 1996).

Valuation of Pest Susceptibility

The various stakeholders in pest susceptibility likely have different values for pest susceptibility. Those concerned with environmental damages and indirect use-value goods often want to protect pest susceptibility separately from concern for pest density. Companies selling toxins and transgenic insecticidal plants want to maintain susceptibility to valuable products to maintain their sales at least until patents expire. Insecticide users are generally less concerned about a particular product and tend to relate the value of pest susceptibility to pest control and damage. The US Environmental Protection Agency (EPA), which regulates commercialization of insecticides, has not made maintenance of pest susceptibility a requirement for registration of insecticides, with the exception of transgenic insecticidal crops.

Defining and valuing pest susceptibility may depend on the control tactic. Should the susceptibility of a pest population to a synthetic toxin developed by a

private company be considered a private good of the patent holder, or is pest susceptibility an open access resource that should be regulated for the public good? If the number of molecular or behavioral mechanisms for resistance are limited and already exist in the pest population, and if cross resistance to several toxins or IPM tactics is a real possibility, then should pest susceptibility be considered a common property resource of those developing new products and tactics? Society may hold a high option value for a lack of cross resistance among pest populations when a corporation commercializes a new and unique toxin – should it regulate the toxin differently than other commercialized toxins? If a pest develops resistance to an unpatented IPM tactic, whose resource was consumed?

From a legal perspective, at this time in the USA, property rights for ownership of pest susceptibility remain incompletely enforced. For synthetic toxins, the patent holder must register the product for commercialization under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which the EPA enforces. Thus far, the EPA has not chosen to impose resistance management requirements on those registering synthetic pest control products under FIFRA, with the exception of transgenic insecticidal crops (Bt corn, Bt sweet corn, Bt cotton). Hence, ownership of pest susceptibility to synthetic toxins (other than transgenic insecticidal toxins) rests in some legal sense with the product registrants, in the sense that companies manage resistance to their registered compounds as they see fit. However, this property right to susceptibility is not completely enforceable. For example, suppose two companies patent insecticides at the same time with similar modes of action; one company registers and markets its insecticide immediately, while the second company waits. Resistance develops to the first insecticide, including cross resistance to the second insecticide, so that the second company's product is worthless. Under current US legal precedent, the second company cannot successfully sue the first company for damages because the first company made the second company's insecticide worthless. Hence, companies do not completely own pest susceptibility.

Ownership of pest susceptibility to other control methods also remains undefined. For example, who owns pest susceptibility to crop rotation? As Spencer and Levine (Chapter 8) indicate, crop rotation was an effective pest control method for *Diabrotica virgifera virgifera* and *Diabrotica barberi* for many years, but both species evolved resistance by changing egg laying behavior and extending egg diapause respectively (Krysan *et al*., 1986; Levine *et al*., 1992; Levine and Oloumi-Sadeghi, 1996). If a pest develops resistance to biological or cultural control, whose resource was consumed? Generally it seems that pest susceptibility to IPM is an open access or common property resource with an undefined legal status, though this need not be the case. Ownership of other goods of this sort has been legally defined. For example, the federal government has auctioned the right to use different radio frequencies to private companies on two occasions (MacAfee and MacMillan, 1996; Ahrens, 2006).

Without any legal definition of property rights, susceptibility remains an open access resource without institutional barriers to its access. Economic theory and historical experience for other open access resources suggest that pest susceptibility is then subject to the tragedy of the commons problem of over exploitation and depletion. The only notable exception to this generalization is pest susceptibility to transgenic insecticidal crops. The EPA chose to require resistance management plans for

Bt crops because the insecticidal proteins were found to be "in the pubic interest," with the EPA's goal being to prevent "unreasonable adverse effects on the environment" as mandated by FIFRA (Berwald *et al*., 2006, p. 23–24). Indeed, Berwald *et al*. (2006, p. 33) go on to explain: "EPA considers pest susceptibility to Bt a common property resource, where a policy goal is to avoid depletion of this resource." Hurley (2005) reaches a similar conclusion concerning EPA policy goals for IRM.

The EPA could use the same arguments to justify requiring resistance management for all registered pesticides, not just those expressed in transgenic insecticidal plants. Why the EPA has not done so is not clear, though conjectures are possible. Perhaps the public benefits of transgenic insecticidal crops were considered greater than for conventionally delivered synthetic insecticides, or perhaps the threat of resistance evolving rapidly was perceived as greater due to the expected (and realized) rapid adoption of transgenic insecticidal crops. Another possibility is that setting a regulatory precedent for a radically new class of insecticides was politically easier than trying to change regulatory policy for the numerous conventional pesticides already registered. Regardless of EPA motives, given the long history of the evolution of resistance to many products and the potential for cross resistance and other interactions, how can regulations logically omit a large class of chemicals? Perhaps the new regulatory precedent set for transgenic insecticidal crops will lead to IRM requirements for other pesticides.

Conceptual and empirical issues remain for economists analyzing IRM. Whose objective should be modeled, that of farmers, companies, or regulators? What values should be incorporated into the analysis: direct use values, indirect use values, and/or non-use values? A quick examination of the research literature indicates that most economic analyses of IRM focus on the use value of the insecticide for controlling damaging pest populations (e.g., Hueth and Regev, 1974; Mangel and Plant, 1983; Regev *et al*., 1983; Plant *et al*., 1985; Onstad and Guse, 1999; Hurley *et al*., 2001, 2002; Onstad *et al*., 2003; Livingston *et al*., 2004; Hurley, 2005). The use value to farmers is often the focus, but some analyses focus on the use value to others as well. For example, Hueth and Regev (1974) explain how their results would change if farmers managed the pest for their common good instead of their individual good. Regev *et al*. (1983) examine the difference between decentralized decision making by farmers and centralized decision making by a planner maximizing the social benefit (the sum of consumer and producer surplus). Hurley *et al*. (2002) include changes in economically optimal insecticide use in an IRM model for insect resistance to transgenic insecticidal corn. The analyses of Morel *et al*. (2003) and Wesseler (2003) both include the social benefit and indirect use values in a conceptual (non-empirical) analysis of IRM for transgenic insecticidal crops to illustrate general effects and principles.

Alix-Garcia and Zilberman (2005) examine the effect of the pesticide market structure on the evolution of resistance. A standard theoretical and common empirical finding is that unregulated monopolists raise prices to restrict the supply of their goods and increase their profits, which reduces social welfare. In the context of pesticides, this implies that a patent-holding monopolist will sell less pesticide than socially optimal. However, because the problem for pest susceptibility as an open access resource is over exploitation, the monopolist's restriction of pesticide supply offsets this over exploitation. The issue then, as Alix-Garcia and Zilberman

(2005) point out, is whether the welfare loss due to restriction of pesticide supply exceeds the welfare gain from slower consumption of pest susceptibility. They show for reasonable parameterizations of their model that indeed, it is possible for the monopolist to delay the evolution of resistance more than socially optimal (considering only the direct use values of pesticides for agricultural production). The main policy implication for IRM is that the open access nature of pest susceptibility is not necessarily a reason to impose resistance management on pesticides, because the structure of the pesticide market also matters and can even offset distortions due to the open access problem.

Besides providing an excellent review of the pertinent economic and public health literature on resistance management, Goeschl and Swanson (2001) extend the standard economic analysis of IRM by modeling a co-evolutionary process in which a pest population evolves resistance and a research and development market creates new technologies to sell to farmers to control a pest population. They explicitly model the research and development process and incorporate economic optimality into farmer pest control decisions (i.e., farmers only treat when economically beneficial). Their results are too rich to fully summarize here, but a key insight they offer is that conceptually, evolving pests are like a competitor costlessly developing new products that erode the market share of companies developing new products, which can cause research and development to collapse.

Discounting and Valuing the Future

The previous discussion concerned the problem of valuing resources such as a pest population or pest susceptibility in the present time. However, IRM usually requires valuing these goods over long periods of time, often years or decades, which leads to the problem of how to value a resource in the future. Valuation of future benefits and costs typically uses the concept of (time) discounting, which is a method to convert the value of a future cost or benefit to its value in another time period, most commonly the present time. Discounting assumes that the value of a good in the future is different than the value of the same good in the current time and discounting provides a method for comparing these values by converting between them. For example, the current value of possessing $100 today is not the same to most people as the current value of possessing $100 ten years from now and a discount factor converts the future $100 into its present value.

The justification for time discounting arises from the common practice in financial markets and from human behavior. Financial markets use discount rates to determine the price for trading assets with future value and these discount rates define the interest charged for loans or paid for deposits. Studies of human behavior demonstrate the consistent devaluation (discounting) of future costs and benefits; people have a strong preference for immediate gratification over delayed gratification (e.g., Soman *et al*., 2005). Commonly, the psychological discounting consistent with human behavior has been implemented for valuing goods intertemporally using the same methods as financial markets (i.e., use of interest or discount rates) (Frederick *et al*., 2002).

Discounting for resource management also requires a time horizon – how far into the future do we measure the value of the resource? The time horizon is the

final point for the time discounted economic analysis. It can also be thought of as the endpoint defining the period during which a stakeholder will evaluate resource management decisions. All resource values after the time horizon are ignored as either too small (discounted too much) or irrelevant (e.g., because the farmer will have retired) so that they do not need to be considered. Alternatively, these values can be captured by the resource's "salvage value" – the value of the resource after the time horizon.

Salvage value, a concept borrowed from financial analysis, is the value of a capital investment at the end of its useful life for an investor. In resource economics, the salvage value of a resource is its value after the time horizon into the infinite future in its best possible uses (including non-use values). For example, Secchi *et al*. (2006) in an IRM analysis use a salvage value derived from the annualized net present value (NPV) of agricultural production after the time horizon, assuming the introduction of new pest control technologies to replace those that become obsolete due to the evolution of resistance.

The discount rate, which is comparable to the interest rate on a loan or a deposit, is used to derive the discount factor. Mathematically, the per-period discount rate d determines the discount factor δ that converts a future value into an equivalent present value with the following formula $\delta(t) = [1/(1 + d)]^t$, where t is the number of time periods until the time horizon (Figure 2.2). In continuous time, the discount factor $\delta(t) = \exp(-dt)$, where t is now the length of time between the

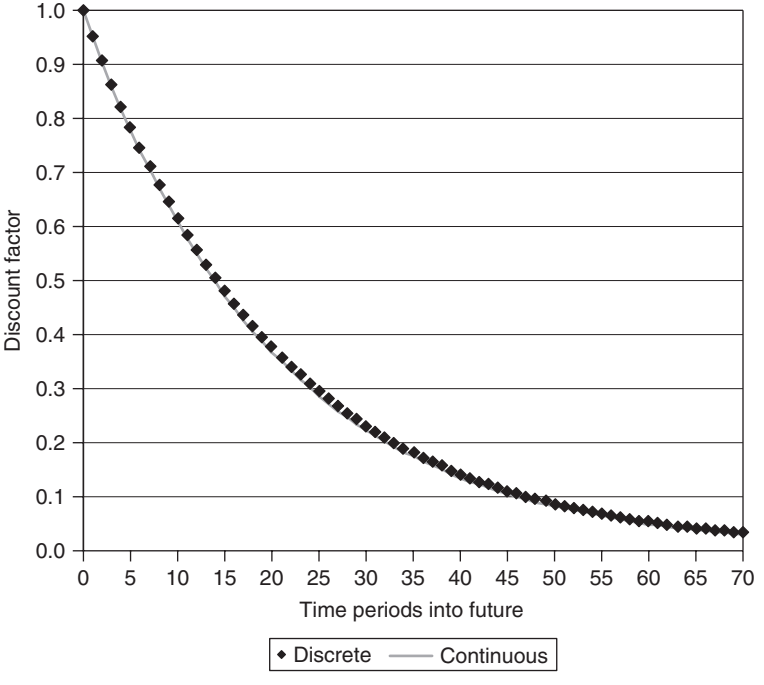

Figure 2.2 Discount factor declines over time. As the factor approaches zero, so too will the present (perceived) economic value of a good produced in the future.

present and the time horizon. With a 5% annual discount rate, the discount factor for a 10-year time horizon is $\delta = [1/(1+0.05)]^{10} = 0.4632$ with discrete time and $\delta = \exp(-0.05*10) = 0.4493$ with continuous time, so that the present value of \$100 paid 10 years in the future is \$46.32 or \$44.93 today depending on which discount formula is used. Note how the discount factors are insignificant (less than 0.03) by year 70 with a 5% discount rate (Figure 2.2). The discount factor would decline even faster with a higher discount rate. Economists and economic models place little value on goods produced or maintained beyond the time the discount factor approaches zero.

Often, an asset or activity generates a stream of income, i.e., a series of net returns over several time periods. In such cases, the entire income stream is discounted back to its present value, which is termed its NPV. For example, suppose a crop field generates a net return of π_t each year t, where π_t varies depending on the crop planted in the rotation. The NPV of the stream of returns generated by the crops from this field over a 10-year time horizon is

$$\text{NPV} = \sum_{t=1}^{10} \pi_t/(1+d)^t = \sum_{t=1}^{10} \delta(t)\pi_t \tag{2.1}$$

In some cases, the NPV of an asset or activity is converted into an annuity – the constant return each period that generates the same NPV as the varying payment. An annuity value (sometimes called an annualized NPV) is calculated as NPV/k, where k, the present-value annuity factor, equals the sum of the discount factors $\delta(t)$ from the current period to the time horizon. Continuing the crop return example, the present-value annuity factor is

$$k = \sum_{t=1}^{10} \delta(t) = \sum_{t=1}^{10} 1/(1+d)^t \tag{2.2}$$

so that the annuity equivalent NPV is

$$\text{NPV}_{\text{ae}} = \sum_{t=1}^{10} \delta(t)\pi_t / \sum_{t=1}^{10} \delta(t) = \sum_{t=1}^{10} \delta(t)\pi_t/k = \frac{1}{k}\sum_{t=1}^{10} \delta(t)\pi_t \tag{2.3}$$

For example, suppose π_t is \$100/ha for corn and \$75/ha for soybeans in a rotation, the NPV of this income over 10 years with a discount rate of 5% is \$678/ha (beginning with corn in year 1), the present-value annuity factor is 7.7217, and the annuity equivalent NPV is \$87.80/ha per year.

The debate concerning the use of discounting for valuing natural resources concerns many issues, most of which are beyond the scope of this chapter. For the purposes of this chapter, we highlight two areas. First, deriving a technical form for the discount function so that it is more consistent with human behavior is an active area of research. Frederick *et al*. (2002) review many of these areas, such as hyperbolic discounting (a discount rate r that varies with the time period t) and loss aversion (a higher discount rate for a future gain than for a future loss) (also see Gollier 2001; Groom *et al*., 2005). Second, much debate exists concerning the appropriate discount

rate to use for environmental goods. This research finds empirical support for use of lower discount rates for environmental goods and that different discount rates should be used for the different (use and non-use) values of the same resource (Henderson and Bateman 1995; Weitzman 1998; Luckert and Adamowicz 1993; Weikard and Zhu 2005). For example, Luckert and Adamowicz (1993) analyze survey data to show that empirically, people express lower discount rates for publicly managed and environmental goods relative to private goods and financial assets. In terms of IRM, this research implies that lower discount rates than are typical for private goods and hyperbolic discounting are more appropriate, which would both increase the value of maintaining pest susceptibility.

Risk

Thus far, our discussion of pest control and IRM has assumed no uncertainty in the information used to make the management decision, though typically few decisions are made under such conditions. Understanding and modeling human decision making under uncertainty is a large and active area of research beyond the scope of this chapter. The goal of this section is to explain the intuition of methods commonly used in economic models of IRM to account for uncertainty.

The traditional economic approach for incorporating uncertainty into decision making is first to convert all uncertainty into monetary outcomes with associated probabilities, which converts the uncertainty into risk – known events with known probabilities that can be expressed as a cumulative distribution function or probability density function. Next, the preferences of the decision maker in terms of monetary risk are specified. Three general types of risk preferences are recognized – risk neutral, risk averse, and risk loving, which are easiest to explain in terms of how a person responds to an uncertain outcome relative to the case with no uncertainty and the same expected (mean) outcome. Risk neutral persons are neutral to uncertainty in the sense that they are indifferent between a certain outcome and an uncertain outcome with the same mean. A risk averse person prefers the certain outcome to the uncertain outcome with the same mean, while a risk loving person prefers the uncertain outcome to the certain outcome with the same mean (Chavas 2004, pp. 31–51; Eeckhoudt *et al*., 2005, pp. 3–23). For example, in a game with a 10% chance of gaining $1,000.00 and 90% chance of losing $111.11, the mean gain is essentially zero dollars. The risk-lover would play the game, the risk-averse person would not play the game, and the risk neutral person could choose either behavior.

The empirical evidence indicates that most people exhibit risk averse behavior for most decisions, so that they value uncertain outcomes at some level less than the mean. Hence, the issue for conceptual and empirical analyses is how to incorporate into the economic analysis a cost or reduction in benefits due to uncertainty. The standard method is to assume some form of utility function to transform monetary outcomes into utility. Utility is a theoretical construct that measures the satisfaction a good gives to a consumer or user. Another term used to describe utility is preferences. Risk aversion implies that individual preferences exhibit diminishing marginal utility with respect to monetary outcomes, i.e., the more money a person receives, the smaller the increase in utility. In the case of pest management and IRM, pest control is one of the goods (Figure 2.3). By using an efficacy function

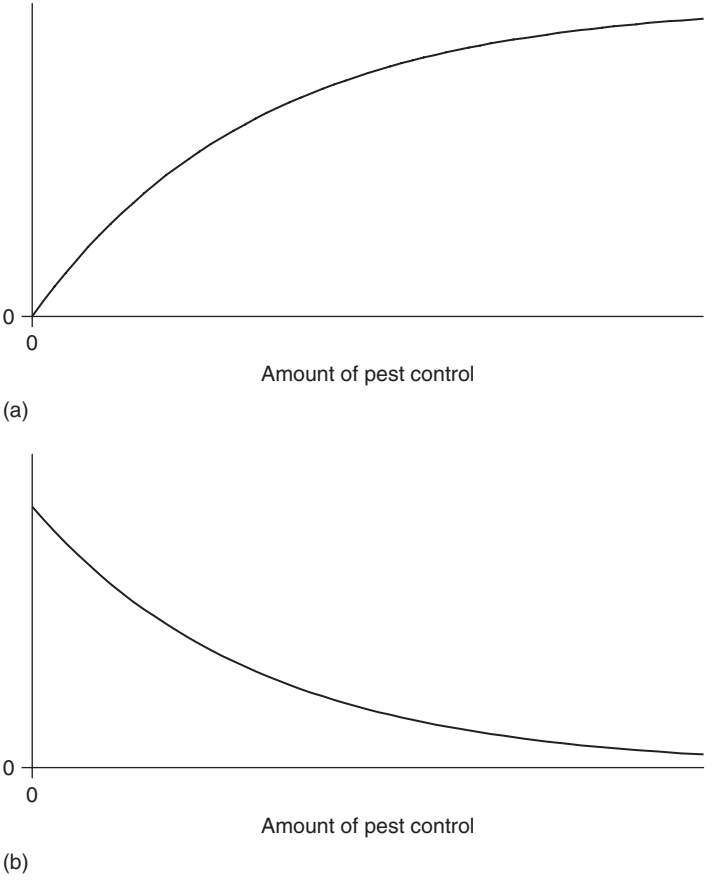

Figure 2.3 (a) Total utility increases as the level of pest control increases. (b) Marginal (incremental) utility to a farmer declines for each additional amount of pest control.

and/or a damage function, pest control is converted into monetary outcomes and then into utility. Given constant cost per unit of control, as more and more pest control is applied, total utility does increase, but each new increment of control provides a decreasing amount of marginal utility (Figure 2.3). Thus, risk aversion implies diminishing marginal utility in pest control.

Economic analyses usually focus on the expected value of utility not the expected monetary value. For decisions under uncertainty, a utility function imposes a cost to risk in much the same way that a discount function imposes a cost on a benefit not realized until the future. Combining a utility function with time discounting to model the simultaneous management of risk and inter-temporal substitution significantly complicates the optimization process. Such problems are well studied in finance and macroeconomics (see Gollier (2001) for an overview) and, to some extent, in resource and agricultural economics (e.g., Knapp and Olson, 1996; Lence, 2000; Peltola and Knapp, 2001), but are beyond the scope of this book.

This description of uncertainty and risk from an economic perspective is far from complete; the economics of risk is a large literature, even in the context of just pest management. For example, we did not discuss generalized expected utility, safety first preferences, safe minimum standards, or stochastic dominance. Chavas (2004) provides a readable introduction with several empirical examples, unfortunately none concerning insects or IRM. Gollier (2001) also provides a useful overview and summary of the economics of risk management.

Overview of Economic Models

For this section, we first define notation and then illustrate the concepts presented in previous sections of this chapter and overview recent papers. To capture the essence of the IRM problem, assume that a population-genetics model generates two key outputs that are used for economic analysis: the frequency of a single resistance allele (r_t) and the population density of a single pest (n_t) in a landscape, where the subscript t indicates the time period (or generation). This notation assumes a discrete time period; equivalent notation for a continuous time model is $r(t)$ and $n(t)$. This fairly simplified assumption abstracts from important issues to capture the essence of population-genetics models as used by economic analyses of IRM. The manager chooses ϕ_t, the proportion of the landscape to treat for the pest, or the proportion of the pest population to treat in period t. In some manner defined by the population-genetics model (but unnecessary to explain here), the frequency of the resistance allele and the pest population density both depend on the manager's choice of ϕ_t and the previous level of resistance and the pest density, which we denote as $r_t(\phi_t,r_{t-1},n_t)$ and $n_t(\phi_t,n_{t-1},r_t)$.

The manager derives different types of value from goods or services provided by the pest population and its level of resistance, such as the various direct and indirect use and non-use values as described in previous sections. In some manner defined by the economic model (but unnecessary to explain here), the monetary value of each of these goods depends on the pest population and its level of resistance, which we denote $V_{tj}(r_t(\phi_t,r_{t-1},n_t),\ n_t(\phi_t,n_{t-1},r_t))$ for value type j in period t. For example, $j = 1$ may denote all direct use values, $j = 2$ may denote all indirect use values, and $j = 3$ may denote all non-use values, but more values are possible, since each of these types of values can be further separated into subtypes.

Based on this simplified abstract model, the manager's IRM problem can be expressed as

$$\max_{\phi_t \forall t} \sum_{t=1}^{T} \sum_{j=1}^{J} \delta_{tj} V_{tj}(r_t(\cdot), n_t(\cdot)) \qquad \text{with } \delta_{tj} = [1/(1 + d_{tj})]^t \tag{2.4}$$

where δ_{tj} is the discount factor for time period t for value type j, d_{tj} is the discount rate for period t for value type j, T is the time horizon, and J is the number of value types. The population-genetics model determines how $r_t(\phi_t,r_{t-1},n_t)$ and $n_t(\phi_t,n_{t-1},r_t)$ change over time and are affected by the manager's choice of ϕ_t. Equation 2.4 represents a scenario in which the manager's IRM problem is to choose the proportion of the pest population to treat in each time period to maximize the NPV of the

discounted stream of the different types of value derived from the pest population and its level of resistance, where the underlying population-genetics model describes pest population dynamics and the evolution of resistance. Note that this general specification (Equation 2.4) uses d_{tj} to allow time-specific discount rates (such as implied by hyperbolic discounting) and value-specific discount rates (so the private and public good aspects of a pest population can be discounted at different rates).

Equation 2.4 is the most popular specification for economic analysis of IRM. Hurley *et al*. (2001) (based on the Hurley *et al*. (1997) working paper) and Onstad and Guse (1999) were among the first to analyze the economics of IRM for managing *Ostrinia nubilalis* resistance to transgenic insecticidal corn. Their analyses differ in terms of the details of the population genetics and economic models, but they both use one type of value (farmer returns) ($J = 1$), a single discount rate ($d_{tj} = d$ for all t and j), and the manager's choice variable (the proportion of conventional refuge corn to plant) does not vary across time ($\phi_t = \phi$ for all t). Other studies extend these initial analyses in different ways by relaxing key assumptions.

Secchi *et al*. (2006) also examine IRM for *O. nubilalis* and transgenic insecticidal corn. Their analysis uses the same general assumptions ($J = 1$, $d_{tj} = d$ for all t and j), but compares results for a static (time invariant) refuge and a dynamic (time varying) refuge. In addition, they add a salvage value to farmer returns derived from the annualized NPV of agricultural production, which captures the effect of allowing the introduction of new technologies to replace control methods that become obsolete due to the evolution of resistance. Livingston *et al*. (2004) examine IRM for two pests (*Heliothis verescens* and *Helicoverpa zea*) and two control methods (transgenic insecticidal cotton and conventional insecticide) using refuge either treated or untreated with an insecticide. Their analysis also uses the same general assumptions ($J = 1$, $d_{tj} = d$ for all t and j) and compares results with static and dynamic refuge. Onstad *et al*. (2003) use the same general assumptions to examine the economics of different strategies to manage *D. virgifera virgifera* resistance to crop rotation (Levine *et al*., 2002). Specifically, they use a single value (farmer income) and a single discount rate, and assume time invariant implementation of each management practice. Laxminarayan and Simpson (2002) develop and solve the same basic model ($J = 1$, $d_{tj} = d$ for all t and j, and $\phi_t = \phi$ for all t) in a continuous time framework. In a primarily conceptual analysis, Brock and Xepapadeas (2003) develop a continuous time model for the economic management of genetic diversity and pest resistance using the same basic assumptions ($J = 1$, $d_{tj} = d$ for all t and j, and $\phi_t = \phi$ for all t).

Incorporating uncertainty into the IRM problem is the next model extension. Uncertainty can arise for a variety of reasons, such as weather variability or lack of knowledge concerning biological parameters. Regardless of the source, such factors imply that the pest population density $n_t(\phi_t, n_{t-1}, r_t)$ and/or the level of resistance $r_t(\phi_t, r_{t-1}, n_t)$ are random. For this abstract model, the manager's IRM problem can be expressed as

$$\max_{\phi_t \forall t} \mathrm{E}_{r,n}\left[\sum_{t=1}^{T}\sum_{j=1}^{J} \delta_{tj} V_{tj}(r_t(\cdot), n_t(\cdot))\right] \tag{2.5}$$

where $E_{r,n}[\cdot]$ denotes the expected value over the random variables r and n. Because the order of integration and summation are interchangeable here, the problem can also be expressed as

$$\max_{\phi_t \forall t} \sum_{t=1}^{T} \sum_{j=1}^{J} \delta_{tj} E_{r,n}[V_{tj}(r_t(\cdot), n_t(\cdot))] \tag{2.6}$$

as long as the discount factor δ_{tj} does not depend on n_t or r_t. This problem is the same as for the deterministic case (Equation 2.4), except that the manager chooses the proportion of the pest population to treat in each time period to maximize the *expected* NPV of the discounted stream of the different types of value derived from the pest population and its level of resistance. In other words, the stochastic problem is the same as the previous deterministic problem, except that the value function $V_{tj}(\cdot)$ is replaced with its expected value $E_{r,n}[V_{tj}(\cdot)]$. As for Equation 2.4, the underlying population-genetics model describes pest population dynamics and the evolution of resistance.

Conceptually, the replacement of the value function with its expected value is simple, but empirical implementation is difficult because the population-genetics model outputs $n_t(\cdot)$ and $r_t(\cdot)$ and/or the value function $V_{tj}(\cdot)$ are typically highly non-linear functions of the random variable(s), so that closed-form solutions for the expected value $E_{r,n}[V_{tj}(\cdot)]$ cannot be derived analytically. As a result, empirical analysis requires use of numerical procedures such as Monte Carlo integration or quadrature (Press *et al*., 1992), so that empirical applications to IRM for insecticides or transgenic insecticidal crops are less numerous.

Hurley *et al*. (2002) develop an IRM model for *O. nubilalis* and transgenic insecticidal corn with a random annual pest population density, as well as random parameters to capture uncertainty about specific genetic factors (i.e., initial frequency of a resistance allele, heterozygote survival rate). The analysis uses two values ($J = 2$): the value of agricultural production for farmers and revenue collected by the company selling the transgenic insecticidal corn technology, which are both direct use values. However, the same discount rate is used for both values ($d_{tj} = d$ for all t and j) and the decision maker is a social planner who chooses a time invariant refuge ($\phi_t = \phi$ for all t) to maximize the expected NPV of the sum of farmer returns and company revenue. Monte Carlo integration is used to solve for expected values. The required simulations also allow calculation of the risk of resistance – the probability that the resistance allele frequency exceeds a set threshold after a set number of time periods or generations (they use a threshold of 50% and 30 generations = 15 years). Hurley (2005) uses a similar model to examine the effects of partial adoption of transgenic insecticidal corn and partial compliance with refuge requirements on the evolution of resistance. Adoption and compliance both depend on the difference between expected farmer returns with transgenic insecticidal corn and with conventional corn in each period (which depends on the current level of resistance), with equations calibrated to fit the general characteristics of observed transgenic insecticidal corn adoption and compliance data.

Incorporation of the manager's risk preferences into the IRM problem is the next key extension. However, as noted in the previous section, combining a manager's

utility function with time discounting to model the simultaneous management of risk and inter-temporal substitution significantly complicates the optimization process. We found no applications that analyzed IRM with such models, though Secchi and Babcock (2001, 2002) analyze the economics of managing bacterial resistance to antibiotics, combining a simple utility function with time discounting in a model. It is not clear how much the analysis is improved by the increased complexity and difficulty in solving such models in the context of IRM. A practical approach used in some analyses is to use measures, such as the risk of resistance, to provide some quantification of risk in stochastic IRM models maximizing the expected NPV of agricultural productivity (e.g., Hurley *et al*., 2002, Hurley, 2005).

Conclusions

We hope that we have demonstrated the importance of economics for the management of pests and pest resistance. Both IPM and IRM share a foundation in economics. Some may argue that we cannot place a monetary value on many goods and services affected by pest management and IRM. This may be true, but rational decisions still depend on the relative valuation of these goods and services in some manner. The risks of resistance evolution are not just ecological changes but also the potential losses to the health and livelihoods of millions of people who benefit from pest management. Thus, IRM must consider more than biology when practical, feasible, and effective strategies are designed and implemented. Furthermore, Onstad (Chapter 1), Head and Savinelli (Chapter 5), and Hurley and Mitchell (Chapter 11) indicate that social, regulatory, and educational factors must be considered if coordination or cooperation of stakeholders is necessary to implement successful IRM.

Valuing a resource in the present is usually feasible, but valuing future resources is often difficult. For instance, how long should susceptibility be preserved and at what cost to individuals and society? To make predictions and assess risks we must decide how far into the future we need to place values on resources. The choice of time horizon is based not only on a concern for the future but also on practical issues. Can our institutions make plans and maintain efforts over the long term? Can politicians focus on time periods beyond the next election?

Much of this chapter has been about philosophy as well as technique. Who owns susceptibility to an insecticide manufactured by a corporation? Who benefits from and controls susceptibility to crop rotation? Who should own and control these goods? How do we balance and account for the variety of values each stakeholder group places on pest density and pest susceptibility? How do we determine whether the social value of pest susceptibility justifies governmental regulation of IRM? None of these questions have easy answers, but they must be discussed and debated in academia and society.

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Chapter 3

Resistance in the Post-Genomics Age

Barry R. Pittendrigh, Venu M. Margam, Lijie Sun, and Joseph E. Huesing

Introduction

We tend to think of the word "resistance" in terms of evolutionary changes in an insect population in response to (i) a pesticide that we spray on our crops, in our homes or gardens, or on ourselves or (ii) a protein that is produced in a transgenic crop to control a pest insect. Resistance can also be defined in broader terms since insects, like all organisms, are "resistant" to many abiotic and biotic factors in their environment. Surprisingly, key features, concepts, and mechanisms developed to describe resistance development to classic pesticides may also apply to these other stressors.

Historically, unraveling these complex relationships was exceedingly difficult but fortunately the emergent "*Omics*" technologies, exemplified by research in genomics and proteomics, have provided us with the tools to better understand and discover universal commonalities in resistance development. More importantly, we can use this information to provide the basis for development of novel strategies to minimize, in a sustainable manner, the impact of insects on human health, food, and property. Indeed, when viewed through an appropriate filter these techniques may allow us the opportunity to address "resistance" in insect populations in a completely different manner than we have in the past. One such filter is evolutionary time.

If we view insect resistance within a broad evolutionary context, the first major evolutionary event we might consider is the divergence of the common ancestor of mammals and insects some 540 MYA (Grimaldi and Engel, 2005) (Figure 3.1). At this time the earliest known animals with a brain, the flatworm and acorn worm, are thought to have evolved. In fact, some consider the acorn worm to be the evolutionary link between vertebrates and invertebrates. Thus, many of the basic and common animal house-keeping functions were established at this time. Over the following several hundred million years the ancestors of modern insects evolved. At about the same time that plants began evolving flowering structures and seeds, approximately 310–390 MYA, the radiation of Class Insecta is thought to have begun. In evolutionary terms, only very recently has humankind either identified in the natural environment, or invented in the laboratory, chemistries to exploit for the control of insects. Yet each of the aforementioned evolutionary events has likely contributed unique and thus discoverable traits that enable insects to resist the variety of abiotic and biotic challenges they experience.

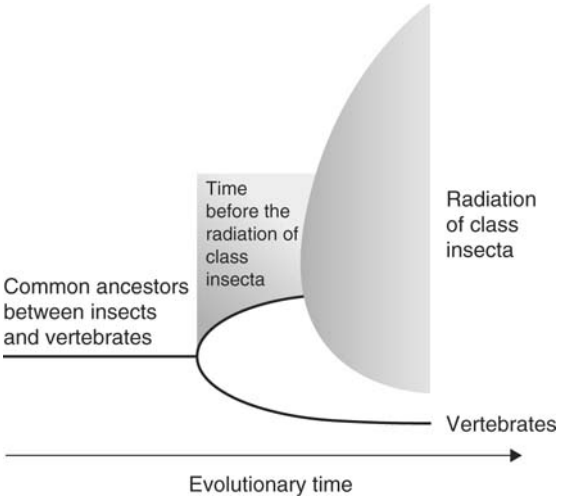

Figure 3.1 Schematic evolutionary relationship between insects and vertebrates. Common ancestors of insects and vertebrates branched into early arthropods and vertebrates approximately 540 MYA (Grimaldi and Engel, 2005). Over the following approximately several hundred million years the ancestor(s) of modern insects evolved, followed by the radiation of class Insecta.

Some of these evolutionary events have been well-studied over the past several decades. For example, many *Drosophila* researchers have focused on the conserved evolutionarily processes common to insects and mammals. This has given us important insights into the nervous system (Wang *et al.*, 2000; Li *et al.*, 2004b; Savare *et al.*, 2005), developmental biology (Fristrom, 1970; Lawrence and Morata, 1992; Bejsovec *et al.*, 2004), and human diseases (Pan *et al.*, 2004; Bilen and Bonini, 2005; Wolf *et al.*, 2006). Less well-studied are those traits that evolved in insects during the last 310–390 million years. It is these traits and specialized adaptations to their food and environment that may provide the best selective opportunities for human manipulation.

As mentioned earlier, "*Omics*" have tremendous potential to allow us to understand insect-specific responses to their environment at several sub-cellular levels in an integrated manner. This level of "systems understanding" could provide the knowledge needed to design novel pest control strategies. This is of immense practical value since the development of pesticides that interact with insect specific, target sites (e.g., the peritrophic matrix (PM) or exoskeleton, two organs almost exclusively associated with arthropods) are likely to have minimal or no direct impact on mammals.

In the last century, our options for managing insects using chemicals were restricted mostly due to our limited knowledge of the unique biochemical and molecular aspects of insects. Methodologies were also a limitation. Of major concern was that some of the insecticides also affected higher vertebrates, due to the evolutionary conservation of biological processes that are targeted by these compounds (e.g., organophosphates impact on acetylcholine esterases). At the opposite end of the spectrum has been the recent use of toxins obtained from the insect pathogenic bacterium, *Bacillus thuringiensis* (Bt), which are highly specific to particular groups of insects. These two technical extremes, one based largely on classic synthetic chemistry and the other on a fairly precise knowledge of the molecular blueprint of both host and target organisms illustrate the vision addressed in this chapter.

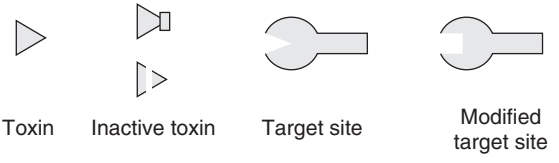

Figure 3.2 Symbols used in subsequent Figures 3.3–3.8.

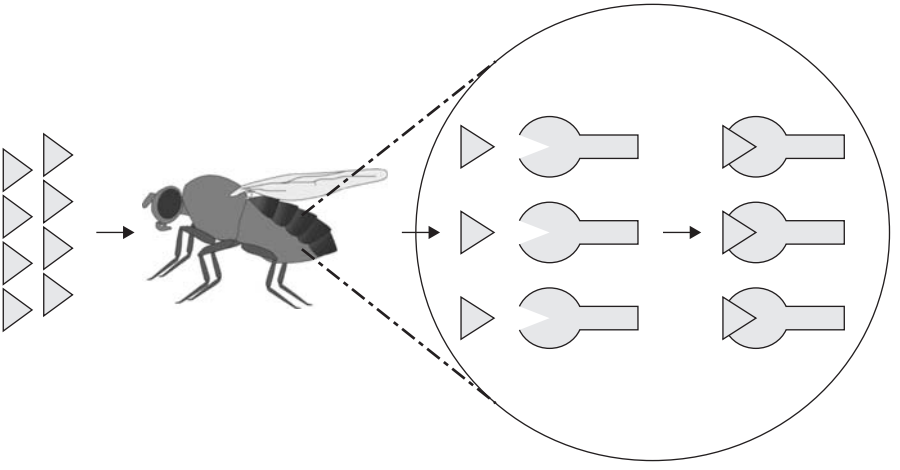

Figure 3.3 A given pesticide interacts with a target site in a pesticide susceptible insect causing death of the insect.

In this chapter, we briefly review what is known about insect resistance in a select group of classic pesticide classes and then discuss some exciting new possibilities that "*Omics*" may provide for the near future. We also discuss the potential importance of understanding the molecular mechanism by which insects resist environmental challenges. Inhibition of these other resistance mechanisms, where critical for the survival of the insect (we term these "Achilles' heel proteins"), holds the possibility for the development of novel insect control methods. We define an "Achilles' heel trait" as a target molecule that, when inhibited (or negatively impacted), reduces the ability of an organism or a population of organisms to persist in a specific environmental condition or challenge.

Resistance mechanisms in insects to classic pesticides (Figures 3.2–3.8) can be broadly classified into the following categories: (i) reduced penetration, (ii) increased sequestration or excretion or both, (iii) behavioral resistance, (iv) metabolic resistance, and (v) target site insensitivity. In the following sections each of these forms of resistance will be defined along with examples (Figure 3.2).

General Mechanisms of Resistance

Reduced Penetration

Resistance by reduced penetration occurs when insects develop a heritable mechanism(s) that reduces or prevents the entry or penetration of a toxin into the insect's body (Figure 3.4). It has been hypothesized that decreased penetration

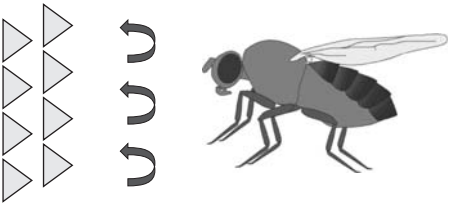

Figure 3.4 Reduced penetration: The insect population evolves a heritable mechanism/mechanisms to reduce (or prevent) the entry of the toxins into the insect's body.

can give detoxifying enzymes more time to metabolize the pesticide before it reaches its target (Plapp and Hoyer, 1968). Plapp and Hoyer (1968) observed a form of decreased penetration of dichloro-diphenyl-trichloroethane (DDT) and dieldrin in resistant *Musca domestica*. Farnham (1971, 1973) later demonstrated that the reduced penetration was due to a gene termed *pen* (penetration) located on chromosome III. A similar mechanism, and perhaps gene(s), was also observed in permethrin resistant flies (DeVries and Georghiou, 1981). In fact resistance due to decreased penetration is often observed in combination with other resistance mechanisms. For example, in pyrethroid-resistant (Learn-Pyr; LPR) *M. domestica*, resistance is due to knockdown resistance (*kdr*), over-expression of the cytochrome P450 CYP6D1, and decreased penetration (Seifert and Scott, 2002).

In combination with other resistance traits, reduced penetration, at least in the mosquito species *Culex pipiens*, appears to have a multiplicative effect on resistance (Raymond *et al*., 1989, 2001). In other words, insects that carry the reduced penetration trait, coupled with other resistance mechanisms, may be much more resistant to pesticides than if other resistance mechanisms are combined. For example, in this model, target site insensitivity combined with increased enzymatic detoxification results in merely additive resistance while the same combination used with reduced penetration results in multiplicative resistance. If this model can be shown to apply to other pest systems, then from a resistance management prospective, reduced penetration as a resistance mechanism could have important implications for resistance management. Unfortunately, we do not have the degree of understanding of the molecular mechanisms of reduced penetration as we do for other resistance mechanisms, notably target site insensitivity and metabolic resistance.

Beyond the important issue of pesticide resistance, understanding the molecular mechanisms by which insects reduce penetration of harmful compounds through their exoskeletons and digestive systems may also provide new opportunities to develop novel strategies to compromise that ability. For example, the peritrophic matrix (PM; peritrophic membrane) of insects is a complex digestive system tissue composed of proteins, glycoaminoglycans, and chitin. The PM not only serves as an integral part of the controlled enzymatic degradation and absorption of food but also serves as an exclusionary barrier to bacteria, viruses, and damaging mechanical materials (Lehane, 1997; Tellam *et al*., 1999). The PM thus minimizes the impact of both negative biotic and abiotic factors in the insect diet. This interaction of the tissues of the digestive tract, including, presumably, the PM, as well as the associated gut bacteria, appears to be a key dynamic in the success of Bt toxins as insecticidal agents (Broderick *et al*., 2006). Inhibition of production of some or all of the components of the PM would undoubtedly be an additional and highly effective and selective way to control a pest insect.

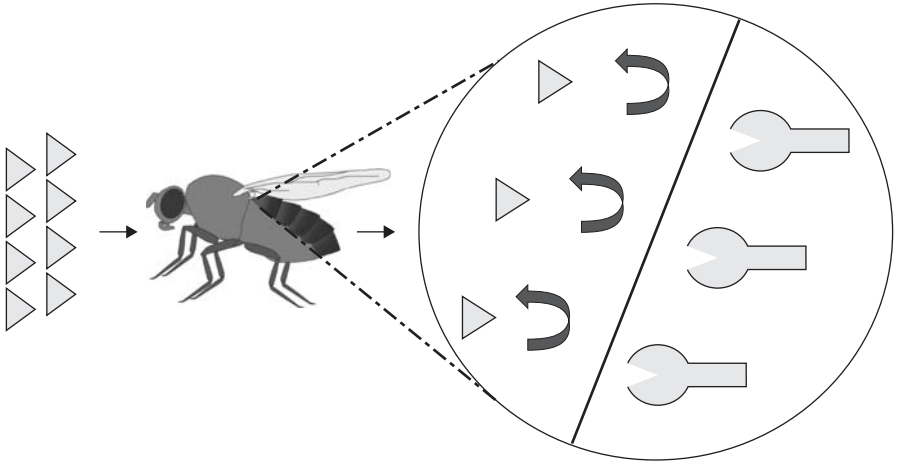

Figure 3.5 Sequestration: After entry of the pesticide into the insect's body, enzymes or proteins bind to the toxin and transfer them away from the target site to various organelles such as fat body and hemolymph for safe storage.

Increased Sequestration or Excretion

Increased sequestration occurs when enzymes or proteins in an insect's body bind to pesticide molecules and subsequently transfer them away from the target site to various organelles such as the fat body and hemolymph for safe storage (Lee and Clark, 1998; Nicholson *et al*., 2006) (Figure 3.5). Sequestering of toxins may have arisen early in the evolution of insects and was perhaps strongly influenced by their interactions with flowering plants many of which contain noxious secondary compounds (Fraenkel, 1959). A well-studied example of this is the relationship of the *Danaus plexippus* (Lepidoptera: Danainae) with otherwise poisonous milkweed plants (*Asclepias spp.*). Milkweed plants produce noxious cardenolide (cardiac glycosides) molecules. *Danaus plexippus* larvae feeding on milkweed plants sequester these molecules in their bodies, which in turn makes the insect unpalatable. In this case, an insect herbivore developed a mechanism to both sequester a poison and then to use that poison as a defense against predation (Nishida, 2002). The ability to sequester plant toxins seems to be particularly prevalent in the Lepidoptera. Thus, "resistance management" may not be a purely human activity since plants also respond evolutionarily to the insect circumvention of their defenses by evolving new forms of the toxin as evidenced in the tobacco hornworm (*Manduca sexta*) tobacco (*Nicotiana* spp.) interaction (Huesing and Jones, 1988).

Many resistant insects sequester pesticides and the esterase enzymes frequently mediate this process. Esterase-based resistance can be classified into two types: (i) increased levels of insecticide sequestration, which involves a rapid binding of the insecticide resulting in broad spectrum resistance and (ii) changes in substrate specificity due to point mutations, wherein a group of insecticides with a common ester bond are metabolized into less toxic forms, which typically confers narrow spectrum resistance.

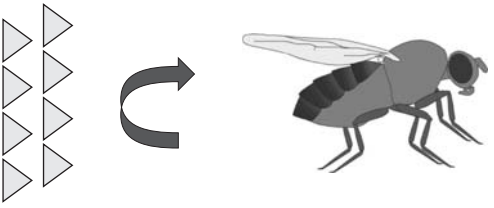

Figure 3.6 Behavioral resistance: A given insect population evolves a heritable mechanism/mechanisms to avoid the toxin molecules by changing their behavior.

Examples of esterase-mediated sequestering include two aphid species, *Myzus persicae* and *Myzus nicotianae*, as well as a mosquito species (*C. pipiens*). Overexpression of carboxylesterases has been associated with binding to, but not necessarily the metabolism of, insecticides (e.g., organophosphates and carbamates) (Field *et al.*, 1988, 1994; Raymond *et al.*, 1998). Other examples where esterases play a role in sequestration include *Nilaparvata lugens*, *Leptinotarsa decemlineata*, *C. quinquefasciatus*, *C. pipiens*, *C. tarsalis*, and *C. tritaeniorhynchus* (Lee and Clark, 1998; Karunaratne and Hemingway, 2000; Small and Hemingway, 2000). Additionally, in the case of *C. tritaeniorhynchus*, the carboxylesterase gene CtrEstbeta1 is involved in the sequestration of organophosphates (Karunaratne and Hemingway, 2000). In *Tenebrio molitor*, glutathione S-transferases (GSTs) may be involved in sequestering pyrethroids (Kostaropoulos *et al*., 2001).

Behavioral Resistance

Any behavior, such as avoidance, that results in an increased chance of an insect's or its offsprings' survival can be defined as behavioral resistance (Figure 3.6). For example, in *Plutella xylostella* behavioral changes in oviposition have been observed as an avoidance behavior against pesticides. Sarfraz *et al.* (2005) observed that when laboratory raised *P. xylostella* were given a choice to lay eggs on insecticide treated host plants, the moths preferentially laid more eggs closer to the soil rather than on the stem and foliage.

Aversion behavior has been observed in *Blatella germanica* to food ingredients contained in gel baits (Wang *et al*., 2004); fructose, maltose, and sucrose, are typically phagostimulants to non-averse (susceptible) laboratory strains of German cockroaches. However, the "Cincy strain" of *B. germanica* avoided all of these compounds when they were incorporated into an agar diet substrate. The aversion trait appears to be weakly sex linked with females showing a higher degree of the aversion trait (Wang *et al*., 2006). However, there also appeared to be a cost to resistance, as the Cincy strain produced fewer progeny than their non-averse counterparts (Wang *et al*., 2004).

Metabolic Resistance

Metabolic resistance refers to the general situation where organisms increase the rate of metabolism of a given toxin. This can occur (i) by increasing the levels of given enzymes that "breakdown" or alter the pesticide to a less toxic form or (ii) by a structural change in an enzyme that allows it to more easily process the pesticide substrate (Figure 3.7).

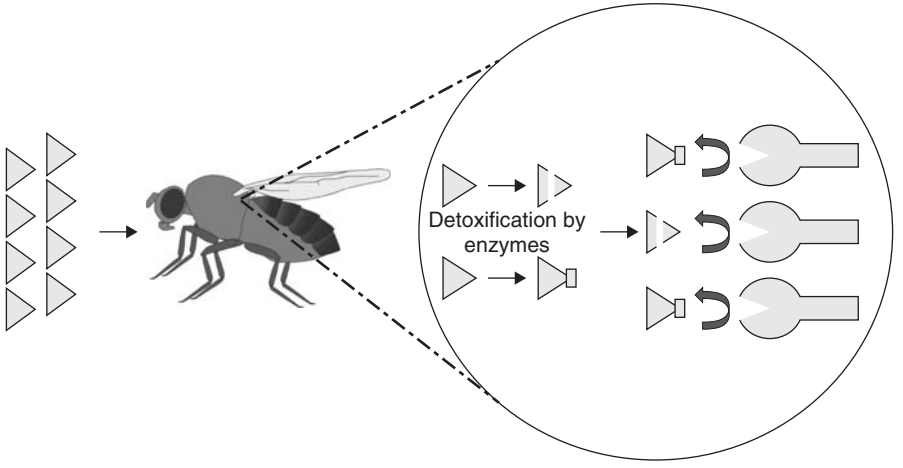

Figure 3.7 Metabolic resistance: After the toxin enters the insect's body, enzymes in the insect alter the toxin such that it no longer binds to its intended target site, thereby allowing the insect to survive the given dose of the toxin.

The literature on metabolic resistance is vast and it is not possible in this chapter to cover all of the different examples; see Ishaaya (1993), Feyereisen (1995), Keseru (1998), Scott (1999), and Li *et al*. (2007) for reviews on the topic of metabolic resistance. We focus instead on a few select examples to illustrate some generalities associated with metabolic resistance. Most studies to date have focused on the role of cytochrome P450s, GSTs, or esterases in metabolic resistance.

Cytochrome P450s are a class of enzymes found in most organisms, including bacteria, plants, fungi, insects, and mammals. They comprise a superfamily of heme-thiolate proteins, which act on both endogenous compounds such as steroid hormones as well as exogenous toxic xenobiotic compounds that insects encounter in their environment. P450s metabolize pesticides by N—, O—, and S-alkyl hydroxylation, aromatic hydroxylation, aliphatic hydroxylation and expoxidation, ester oxidation, as well as thioether and nitrogen oxidation.

GSTs are a family of enzymes that play a variety of biological roles in the cell including detoxification of xenobiotics such as pesticides, carcinogens, and drugs. All eukaryotic species have cytosolic and membrane GSTs. In some cases the expression levels of given GSTs are directly related to the tolerance of the organism to the toxic chemicals (Hayes and Pulford, 1995). GSTs are involved in the resistance of insects to organophosphate, organochlorines, DDT, and pyrethroids (Ranson *et al*., 2001; Ranson and Hemingway, 2005; Li *et al*., 2007).

An esterase, e.g., acetylcholinesterase (AChE), is a hydrolase enzyme that cleaves the ester bonds in pesticides to yield an acid and an alcohol. There are many kinds of esterases that differ in their substrate specificity, their protein structure, and their biological function. Esterases have been associated with resistance of insects to organophosphates, carbamates, and pyrethroids (Li *et al*., 2007).

Metabolic resistance to pesticides is probably the most common mechanism by which a diverse array of insects, including lepidopteran, coleopteran, and dipteran

species, evolve resistance (Hemingway *et al*., 1991; Ottea *et al*., 1995; Rose *et al*., 1997; Chandre *et al*., 1998; Kasai *et al*., 1998; Stuart *et al*., 1998; Feyereisen, 1999; Kasai *et al*., 2000; Li *et al*., 2000; Scharf *et al*., 2000). Metabolic pesticide resistance in insects is typically polygenic and is often associated with over-transcription of the aforementioned detoxification enzymes cytochrome P450s and GSTs (Houpt *et al*., 1988; Heckel *et al*., 1998; Maitra *et al*., 2000; Tang *et al*., 2000; Kranthi *et al*., 2001; Rajurkar *et al*., 2003). The cloning of numerous P450 and GST genes that are over-transcribed in resistant insects but which fail to map back to a major resistance locus has led workers in the field to hypothesize that there is a *trans*-regulatory gene that controls the expression of these detoxification enzymes (Grant and Hammock, 1992; Liu and Scott, 1997; Dombrowski *et al*., 1998) and in susceptible insects a repressor acts to reduce the expression levels of detoxification enzymes associated with resistance. It has also been hypothesized that a mutation in this regulatory gene no longer allows it to suppress expression of these genes, which results in over-transcription of cytochrome P450s, and in turn results in resistance (Grant and Hammock, 1992; Carino *et al*., 1994; Maitra *et al*., 1996; Liu and Scott, 1997; Dombrowski *et al*., 1998; Kasai *et al*., 1998). To date, no such trans-acting repressor has been identified in insects but the search continues. Additionally, in bacterial systems this repressor-mechanism hypothesis has been challenged (Shaw *et al.*, 1998).

There is considerable evolutionary sequence divergence among cytochrome P450s and GSTs in the Class Insecta even among relatively closely related species such as *Drosophila melanogaster* and *Anopheles gambiae* (Ranson *et al*., 2002). However, it remains to be determined if the regulation of P450s and GSTs across insect taxa are more conserved than the actual genes themselves (Handschin *et al*., 2004).

Although the role of P450s in insecticide resistance is often associated with over-transcription of these enzymes, structural changes in the P450 can also lead to pesticide resistance. For example, Amichot *et al*. (2004) observed that three mutations in *Cyp6a2*, from *D. melanogaster*, increased the mutant CYP6A2's ability to metabolize DDT. Their work is in keeping with observations in humans, where P450 polymorphisms have been shown to be associated with drug and pesticide metabolism (Guengerich *et al*., 1999; Eaton, 2000). Such structural changes in P450s, leading to changes in pesticide resistance, have also been observed in fungi (Lamb *et al*., 1997; Delye *et al*., 1998).

Although much of the research to date on metabolic pesticide resistance has focused on P450s, GSTs, and esterases, it remains to be determined if other genes or pathways are critical for pesticide resistance. For example, changes in glucose utilization have been associated with DDT exposure and metabolic resistance in a variety of organisms including marine microorganisms, insects such as *D. melanogaster* and mammals (Ela *et al*., 1970; Plapp, 1970; Maltseva and Golovleva, 1982; Bauer and Capone, 1985; Ahuja *et al*., 2001; Ahuja and Kumar, 2003; Okazaki and Katayama, 2003; Pedra *et al*., 2004, 2005). Additionally, a genome-wide comparison of two metabolically DDT resistant strains of *Drosophila* (*Rst(2)DDT*^{Wisconsin} and *Rst(2)DDT*$^{91\text{-}R}$), as compared to one susceptible strain (Canton-S), revealed dozens of putatively differentially over-transcribed genes in the resistant strains. These over-transcribed genes included P450s, GSTs, oxidoreductases, as well as UPD-glucuronosyltransferases (UGTs), diazepam binding inhibitor, other lipid

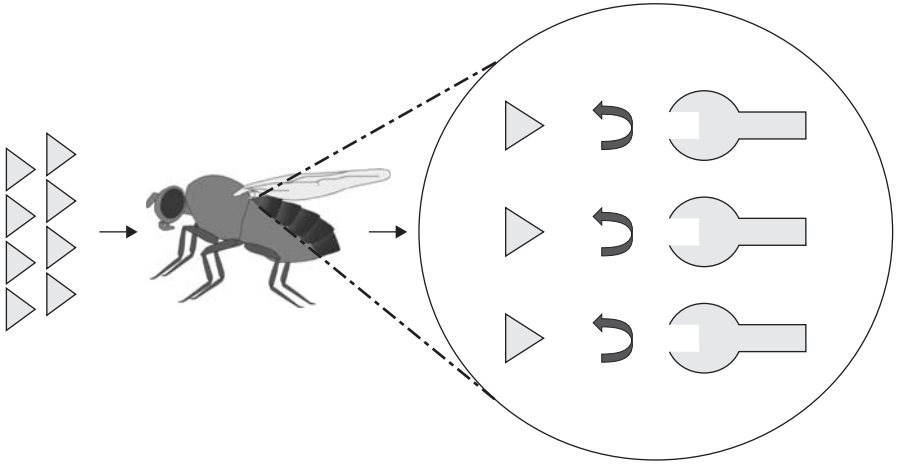

Figure 3.8 Target site insensitivity: Due to conformational changes in the target site of the toxin, the pesticide becomes less toxic to the insect. Such changes in the target site may reduce the ability of the toxin to bind to the target site or it may change the target site's response to the toxin.

metabolism genes, peptidases, immunity/defense proteins, as well as other gene categories (Pedra *et al*., 2004). It has been demonstrated in rats that dietary DDT increases enzymatic activity of certain hepatic UGTs (Okazaki and Katayama, 2003). However, for many of the differentially expressed genes observed by Pedra *et al*. (2004) it is still not clear what role, if any, they may actually play in metabolic resistance.

It is important to remember that differential expression does not mean that the gene and its resultant protein product are actually conferring resistance. First, any given differentially expressed gene could be regulated under a common mechanism with another gene whose protein product is actually involved in resistance. Additionally, even if the gene is over-transcribed it does not necessarily mean more protein is produced to perform a given detoxification process. Thus, caution is warranted in equating over-transcription of a specific gene with a role in metabolic pesticide resistance (Pedra *et al*., 2004).

Target Site Insensitivity

Target site insensitivity refers to a scenario where there is an alteration of the target molecule(s) that directly interacts with the pesticide, which results in the toxin being less toxic to the target pest (Figure 3.8). Target site insensitivity has been observed in a variety of insect species in response to a diversity of pesticides. Some of these are outlined below.

Resistance to Classes of Insecticides

Resistance to DDT and Pyrethroids

Resistance to DDT was first reported in 1947, only a few years after its introduction into the marketplace (Brown, 1986). Crow (1954) demonstrated that resistance in

D. melanogaster to DDT was polygenic. Subsequent mapping studies demonstrated that several major loci contributed to metabolic DDT resistance in *D. melanogaster*, the best studied being the *Rst(2)DDT* locus (loci) on the second chromosome (Dapkus and Merrell, 1977; Dapkus, 1992). Work by Daborn *et al.* (2002) and Brandt *et al.* (2002) suggest that the *Rst(2)DDT* locus (or closely linked loci) may be due to over-transcription of one (*Cyp6g1*) or two (*Cyp6g1* and *Cyp12d1*) P450 genes. Over-transcription of *Cyp6g1* appears to be commonly found across a diversity of DDT-resistant strains of *D. melanogaster* (Daborn *et al*., 2002) and *Cyp12d1* appears to be inducible (in some *D. melanogaster* strains) in the presence of DDT (Brandt *et al*., 2002; Festucci-Buselli *et al*., 2005). Metabolic resistance to DDT in *D. melanogaster* is associated with one of the major metabolites having an OH group added to the DDT molecule. In mosquitoes, DDT resistance has also been associated with elevated GST levels.

Target siteinsensitivity in the voltage-gated sodium channel confers resistance to both pyrethroids and to DDT. For pyrethroids and DDT the major target site is thought to be the α-subunit of the voltage-sensitive sodium channel (VSSC) (also known as the voltage-gated sodium channel) and in *D. melanogaster* VSSC is encoded by the *para* gene (Williamson *et al*., 1996; Pittendrigh *et al*., 1997). Pyrethroids and DDT are thought to cause prolonged opening of the VSSC by both stabilizing the open configuration of the channel and prolonging the open state. Amino acid changes in the VSSC have been shown to confer both pyrethroid resistance and DDT resistance in a variety of insect species.

A leucine to phenylalanine amino acid substitution in the hydrophobic IIS6 transmembrane segment of an *M. domestica* VSSC resulted in moderate increases in resistance to DDT and certain pyrethroids; this is known as *knockdown resistance* (*kdr*). Coupled with a second methionine to threonine substitution in the intracellular S4–S5 linker domain II (intracellular IIS4–S5 loop) conferred high levels of resistance known as *super-kdr* (Williamson *et al*., 1996). Subsequent work by Pittendrigh *et al*. (1997) showed amino acid changes in IIIS6 in the temperature sensitive $para^{74}$ *D. melanogaster* strain conferred moderate DDT resistance. The *para* temperature sensitive lines $para^{t1}/para^{ts2}$, and $para^{DN7}$ had amino acid changes, respectively in intracellular IS4–S5 and IIIS4–S5 loops; all three strains were DDT resistant. Heterozygous $para^{74}/para^{DN7}$ flies, carrying *kdr-like* and *super-kdr-like* alleles in *trans*, showed elevated levels of DDT resistance.

The most prevalent resistance-associated mutation in *kdr* insects, results from a leucine-to-phenylalanine substitution in the S6 hydrophobic segment of VSSC domain II (Williamson *et al.*, 1996; Dong, 1997; Jamroz *et al.*, 1998; Martinez-Torres *et al.*, 1998, 1999b). Alternative substitutions at this position also confer resistance to DDT and/or pyrethroids: a leucine–histidine substitution is associated with pyrethroid resistance in *Heliothis virescens* (Fabricius) (Park and Taylor, 1997) and a leucine–serine substitution confers DDT resistance and low levels of permethrin resistance in a strain of *C. pipiens* from China (Martinez-Torres *et al.*, 1999a). An additional methionine–threonine replacement is found in strains of housefly and horn flies showing very high levels of pyrethroid resistance (*super-kdr* phenotype) (Williamson *et al.*, 1996; Jamroz *et al.*, 1998). A list of sodium channel mutations, across multiple insect species, conferring resistance to pyrethroids are summarized in Table 3.1.

Table 3.1 Species with voltage-sensitive sodium channel mutations associated with pyrethroids and DDT resistance

Species	Amino acid change[a]	Reference
kdr* and *kdr-like		
Musca domestica	L to F	Williamson *et al*. (1996), Miyazaki *et al*. (1996)
Blattella germanica	L to F	Miyazaki *et al*. (1996), Dong (1997), Dong *et al*. (1998)
Plutella xylostella	L to F	Schuler *et al*. (1998)
Myzus persicae	L to F	Martinez-Torres *et al*. (1999b)
Anopheles gambiae	L to F	Martinez-Torres *et al*. (1998), Ranson *et al*. (2000)
Culex pipiens	L to F	Martinez-Torres *et al*. (1999a)
Culex quinquefasciatus	L to F	Xu *et al*. (2005)
Haematobia irritans	L to F	Guerrero *et al*. (1997)
Leptinotarsa decemlineata	L to F	Lee *et al*. (1999)
Frankliniella occidentalis	L to F	Forcioli *et al*. (2002)
Cydia pomonella	L to F	Brun-Barale *et al*. (2005)
Ctenocephalides felis	L to F	Bass *et al*. (2004)
Culex pipiens	L to S	Martinez-Torres *et al*. (1999a)
Anopheles gambiae	L to S	Ranson *et al*. (2000)
Heliothis virescens	L to H	Park and Taylor (1997)
***Super-kdr* (and *Super-kdr*-like)**		
Musca domestica	M to T and L to F	Williamson *et al*. (1996), Miyazaki *et al.* (1996)
Haematobia irritans	M to T and L to F	Guerrero *et al.* (1997)
Drosophila melanogaster	V to M	Zhao *et al.* (2000) Pittendrigh *et al*. (1997)[b]

[a]L: leucine; F: phenyl Alanine; S: serine; H: histidine; M: methionine; T: threonine; V: valine.
[b]Temperature sensitive strains, with VSSC mutations, that also showed resistance to pyrethroids and DDT. ($para^{DN7}$, $para^{ts1}/para^{ts2}$, $para^{74}$ $para^{DTS2}/para^{DN43}$)

Resistance to Organophosphates and Carbamates

The function of AChE is to degrade the neurotransmitter acetylcholine (Ach) in synapses of animals including insects. Mutations in the AChE-encoding locus, known as *Ace* in *D. melanogaster*, have been shown to confer target site insensitivity to organophosphate and carbamate insecticides, which primarily target AChE. A range of other amino acid substitutions in *M. domestica* and *D. melanogaster* AChE confer pesticide resistance and these mutations typically reside near to or within the active-site of the enzyme (Feyereisen, 1995). Such AChE mutations, associated with pesticide resistance, have also been observed in other species, including *L. decemlineata* (Zhu and Clark, 1997), *Bactrocera oleae* (Vontas *et al*., 2002), *Aedes aegypti* (Vaughan *et al*., 1998), *Aphis gossypii* (Li and Han 2004), *Helicoverpa armigera* (Ren *et al.*, 2002), *C. quinquefasciatus* (Liu *et al.*, 2005), *Cydia pomonella* (Cassanelli *et al.*, 2006), *B. dorsalis* (Hsu *et al.*, 2006), and *C. pipiens* (Alout *et al.*, 2007). Additionally, Mazzarri and Georghiou (1995) observed that oxidase and non-specific esterase enzymes were also involved in organophosphate and carbamate resistance in *A. aegypti* populations from Venezuela.

Resistance to Dieldrin

In *D. melanogaster* the *Resistance to dieldrin* (*Rdl*) gene encodes the γ-aminobutyric acid (GABA) receptor subunit RDL (ffrench-Constant *et al*., 1998). The *Rdl* gene was cloned from a mutant line of *D. melanogaster* that was both resistant to picrotoxin (PTX) and cyclodiene insecticides (ffrench-Constant *et al*., 1991). PTXs were previously known to be vertebrate $GABA_A$ receptor antagonists. Dieldrin resistant populations of *D. melanogaster*, collected from a variety of locations around the world, all shared the same alanine to serine substitution (A302S) (ffrench-Constant *et al*., 1993a). This amino acid change results in the RDL subunit becoming insensitive to both dieldrin and PTX. In *D. simulans* (ffrench-Constant *et al*., 1993b) and the aphid *M. persicae* (Anthony *et al*., 1998) there is a serine to glycine substitution in the resistant insects. More recently, Le Goff *et al*. (2005) have observed two amino acid substitutions, namely, an alanine to glycine (A301G) and a threonine to methionine (T350M), in the RDL GABA receptor, which conferred around 20,000-fold resistance to the insecticide fipronil in the resistant *D. simulans* line. In *P. xylostella*, an alanine 302 to serine amino acid change in the GABA receptor (*PxRdl*) has also been associated with the fipronil resistance phenotype (Li *et al*., 2006).

Resistance to Imidacloprid

Imidacloprid is a member of the neonicotinoid class of insecticides (chloronicotinyls) (Nauen *et al*., 2002) and is a known nicotinic acetylcholine receptor (nAChR) agonist. Acetylcholine (ACh) is an endogenous agonist as well as being an excitatory neurotransmitter of the cholinergic nervous system. Resistance mechanisms to imidacloprid have been observed across multiple insect species, including *N. lugens* (Liu *et al*. 2005), *Ctenocephalides felis* (Rust, 2005), *Bemisia tabaci* (El Kady *et al*., 2003; Prabhaker *et al*., 2005), and *L. decemlineata* (Alyokhin *et al*., 2007) with different forms of resistance evolving in these different species.

B. tabaci that are imidacloprid susceptible typically do not metabolize ^{14}C-imidacloprid into P450-mediated metabolites (Rauch and Nauen, 2003). The imidacloprid/neonicotinoid resistance of the Q- and B-type *B. tabaci* strains does not appear to be based on target site insensitivity (Rauch and Nauen, 2003). The resistance appears to be associated with mono-oxygenase-mediated activity, with 5-hydroxy-imidacloprid being the only resultant metabolite after topical application of imidacloprid (Rauch and Nauen, 2003).

Imidacloprid binds to nAChR with high affinity in both *B. tabaci* and *M. domestica*, whereas the mono-hydroxy metabolite exhibits a much lower affinity (Nauen *et al*., 1998; Rauch and Nauen, 2003). *M. domestica* produce significant amounts of the mono-hydroxy and olefin derivatives of imidacloprid and it is likely that detoxification of imidacloprid by *M. domestica* cytochrome P450s may account for the lower toxicity of the insecticide toward this insect as compared with the pesticide susceptible strains of *B. tabaci* (Byrne *et al*., 2003; Nishiwaki *et al*., 2004).

Cytochrome P450-mediated resistance to imidacloprid is not limited to insects. A study of the enzymatic basis of imidacloprid metabolism in humans showed that the human cytochrome P450, CYP3A4, oxidizes and reduces imidacloprid at the imidazolidine and nitroimine moieties, respectively (Schulz-Jander and Casida, 2002).

To date, target site insensitivity to imidacloprid has only been observed in *N. lugens* (Liu *et al*., 2005). Resistance was conferred by a single-point mutation at Tyrosine151Serine (Y151S) in the alpha subunit of nAChR and a correlation was observed between the frequency of the point mutation and imidacloprid resistance (Liu *et al*., 2005). Furthermore, it has been shown that the Y151S mutation is responsible for a substantial reduction in specific [^{3}H] imidacloprid binding (Liu *et al*., 2005). These aforementioned studies demonstrate that divergent resistance mechanisms to imidacloprid have evolved across species, supporting the possibility that multiple resistance mechanisms may be selected within a species.

Resistance to Toxins of Bt

A large body of literature exists in the rapidly developing field of Bt resistance mechanisms and their implications for resistance management (Gould, 1998; Carrière and Tabashnik, 2001; Tabashnik, 1997; Tabashnik *et al*., 2003, 2004, 2005, 2006; Griffitts and Aroian, 2005; Carrière *et al*., 2006). Thus, we will be unable in this chapter to cover all aspects of Bt resistance but we will focus on resistance issues associated with the Bt Crystal (Cry) δ-endotoxins.

In their native state Bt Cry δ-endotoxins are encoded by genes carried on plasmids within the spore-forming bacterium Bt. The δ-endotoxin(s) reside in a parasporal crystal comprised of Cry protein and DNA (Clairmont *et al*., 1998). This crystalline material is the form of the δ-endotoxin used in commercial spray formulations. In some cases more than one particular Bt Cry α-endotoxin can occur in the same crystal. Other non-Cry δ-endotoxin proteins, e.g., Cyt (cytosolic) toxins, may also be present in the crystal. Bt Cry δ-endotoxins used in commercial transgenic insecticidal crops do not occur in the crystalline form found in *B. thuringiensis*. *B. thuringiensis* also produces other toxins, which are not associated with the crystal such as the Vegetative Insecticidal Proteins (Vip) and the broad spectrum beta-exotoxins.

Both bacterially and plant produced Bt Cry δ-endotoxins must be ingested by insects to cause insect mortality. The crystals of the bacterial formulation dissolve in the insect gut and liberate the Cry δ-endotoxin while Cry δ-endotoxins expressed *in planta* appear to exist as protein in the ingested food. Most Cry δ-endotoxins undergo some level of proteolytic processing for optimal activation. For those δ-endotoxins produced *in planta* there is the opportunity to design the gene so that minimal or no processing need take place. Once activated, the toxin binds with high affinity to specific receptors in the midgut epithelium. Following binding, the δ-endotoxin inserts into the gut membrane to create pores. The resulting pores lead to a loss of homeostasis and ion balance followed by bacterial septicemia (Broderick *et al*., 2006). The Bt δ-endotoxins are highly specific to particular insect orders e.g., Cry1A toxins target lepidopteran insects. Since humans appear to lack these receptors Bt has no toxic effect on them.

Insecticides containing Bt have been registered for use in the United States since 1961. In 1996, the first broadly successful commercial transgenic insecticidal crop, Bollgard cotton producing a δ-endotoxin Cry1Ac, was marketed in the United States. Sprayable forms of Bt have also been extensively used to control vector species, including mosquitoes. To date, the only two insect species reported to have developed resistance in the field to Bt sprays (Bt strain kurstaki) have been

P. xylostella (Ferre and Van Rie, 2002) and *Trichoplusia ni* (Janmaat and Myers, 2003). Bt resistant strains of other species of insects have been observed in the laboratory, including, but not limited to, mosquitoes (Wirth *et al*., 2005), *H. virescens* (Gahan *et al*., 2001), and *Pectinophora gossypiella* (Tabashnik *et al.*, 2002).

Resistance to Bt δ-endotoxins can in principle occur through several mechanisms such as: (i) a deficiency in the midgut proteases needed to activate the protoxin (Oppert *et al*., 1994); (ii) production of proteases that could rapidly degrade the δ-endotoxin, (iii) a specific change in the receptor moiety to which the δ-endo toxin binds, e.g., cadherins, aminopeptidase N (APN), or glycolipids; or, (iv) to loss of the moiety altogether (Griffitts *et al*., 2005). In the case of resistance to select Bt δ-endotoxins, mutations at single loci have been shown to confer resistance (Tabashnik *et al*., 1997; Gahan *et al*., 2001; Morin *et al*., 2003; Baxter *et al*., 2005; Herrero *et al*., 2005; Li *et al*., 2005; Xu *et al*., 2005). It has been suggested that invertebrate immune responses may play a role in Bt resistance (Griffitts and Aroian, 2005). Cross-resistance in invertebrates to different Bts is not typically associated with insect strains that have high-levels of Bt resistance, but is more commonly associated with strains of insects that display moderate levels of resistance to Bts (Griffitts and Aroian, 2005).

Cry1Ab and Cry1Ac represent two of the most important Bts in commercial use today. When insects develop resistance to Cry1Ab, the insects may also become cross-resistant to Cry1Ac. There are currently at least three competing models for the receptor mediated process involved in Cry toxicity (Pigott and Ellar, 2007). One model, discussed here, involves APN and cadherin-like proteins acting as co-receptors, with monomeric Cry1Ab binding a cadherin-like protein that induces proteolytic processing of the Cry1Ab which results in toxin oligomerization. The Cry1Ab oligomers can then bind APN, which drives pore formation (Bravo *et al*., 2004).

Resistance in some insects to Cry1Ab and Cry1Ac toxins is due to an early stop codon in the APN gene or the cadherin gene. This early stop codon results in a truncated protein receptor product. The size of the truncated product is fairly consistent across species of insects where resistance has been observed (Griffitts and Aroian, 2005). Thus, two genes and their sequences consistently associated with Cry1Ab and Cry1Ac resistances have been identified in some insect populations. It is important to note that to date no insect strain with putative resistance to either Cry1Ab or Cry1Ac had been shown capable of surviving on a commercial transgenic insecticidal plant (Tabashnik *et al*., 2004).

D. melanogaster is normally unaffected by Cry1Ac since it does not have an endogenous Cy1Ac receptor. Gill and Ellar (2002) recently showed that a Bt APN receptor gene isolated from *Manduca sexta* could be expressed in transgenic *D. melanogaster.* They demonstrated that the Cry1Ac bound to the introduced receptor resulting in death to the transgenic *D. melanogaster*. These experiments provide strong evidence supporting the role of the APN protein in Cry1Ac toxicity.

Resistance to Spinosad

Spinosad, a pesticide derived from a soil fungus, is thought to target nAChRs in insects (Narahashi, 2002). Resistance to spinosad has been documented in several

insect species. In *D. melanogaster* a knock-out mutation of *Dalpha6*, a gene encoding a nAChR subunit, resulted in a 1181-fold increase in resistance to spinosad (Perry *et al*., 2007). In *M. domestica*, spinosad resistance is recessive and has been mapped to autosome 1. Widespread resistance to spinosad has been noted in *P. xylostella* in Hawaii and Thailand (Zhao *et al*., 2002). Insects taken from the fields in Hawaii and further selected in the laboratory displayed incompletely recessive resistance.

Resistance to Indoxacarb

Indoxacarb (DPX-JW062) is an oxadiazine insecticide useful in killing a wide variety of insect pests. Insects use an esterase/amidase to decarbomethoxylate indoxacarb to N-decarbomethoxylate JW062 (DCJW). Both indoxacarb and DCJW are sodium channel blockers (Shono *et al*., 2004). Some *M. domestica* strains appear to be partially resistant due to an increased P450 activity (Shono *et al*., 2004). Differential sensitivity to indoxacarb in cockroach sodium channels is due to amino acid changes which influence voltage dependence of slow and fast inactivation, as well as channel sensitivity to DCJW (Song *et al*., 2006).

Other Pesticides

As we can not review resistance associated with every class of pesticide, we instead recommend the following key publications and reviews: (i) Clark *et al*. (1995) for avermectins and milbemycins insecticides; (ii) Mordue and Blackwell (1993) for azadirachtin/neem; (iii) Arena (1963) for rotenone and ryania; (iv) Sattelle *et al*. (1985) for nereistoxin analogues; and, (v) Ashok *et al*. (1998) and Dhadialla *et al*. (1998) for juvenile hormone mimics.

Genomics, and Proteomics

Use of Genomics and Proteomics to Understand Pesticide-Resistance Genes

Genomics holds tremendous opportunities for us to understand both (i) how insects become “resistant” to human-made or natural poisons and (ii) how insects become “resistant” to other biotic and abiotic challenges they experience in their environments.

First, by analogy, we can think of the recent genomics and proteomics revolution as “Henry Ford and mass production meet molecular biology”. Prior to the development of many current genomics techniques most researchers investigated a single or a limited number of genes and their potential role in a given biological process. With genomics and proteomics, large-scale genome or proteome-wide comparisons are routinely performed between susceptible and resistant organisms or challenged and unchallenged organisms (Pedra *et al.*, 2004, 2005). For example, the expression levels of thousands or tens of thousands of genes can be determined in a single experiment. The differential expression of proteins can also be determined in a given treatment or tissue. These technologies enable researchers to rapidly discover genes and their associated proteins that play a critical role in an organism’s response to challenges in their environment.

Specifically, this approach has and will continue to allow researchers to investigate differences between susceptible and resistant insects without the need for *a priori* knowledge of the potential genes involved in resistance. Using whole-genome oligoarray gene chips, investigators recently determined the specific genes that are differentially expressed between DDT susceptible and metabolically resistant strains of fruit flies (Pedra *et al*., 2004). Some of these genes were previously determined to be associated with metabolic pesticide resistance, such as cytochrome P450s (e.g., *Cyp12d1*/*Cyp12d2*, *Cyp6g1*, and *Cyp6a2*, and *Cyp6a8*), GSTs, and oxidoreductases (Daborn *et al*., 2001, 2002; Pedra *et al*., 2004). However, genes previously not known to be associated with resistance were also observed (e.g., diazepam binding inhibitor). As previously mentioned, care needs to be exercised not to equate differential expression with actual direct, or even indirect, involvement in resistance. Genes can be differentially expressed because of several factors: (i) genetic hitchhiking, (ii) the genes may also be under the control of the same regulatory process as the genes that code for the proteins that actually confer resistance, and hence are also up- or down-regulated, or (iii) thegenes may be differentially expressed as a response to cellular or organismal changes that occur due to increases or decreases in the expression of the resistance genes. Nonetheless, these techniques may very well reveal heretofore unknown components of resistance mechanisms.

It is important to note that oligoarrays and cDNA spotted arrays are used to detect changes in transcription (mRNA expression); however, changes in transcription do not necessarily mean there are changes in translation (protein expression). Since the protein is typically the critical molecule involved in resistance, a determination must be made of any real and meaningful differences in protein levels. This is especially true where resistance is thought to be associated with differential expression of metabolic enzymes.

Two example strategies can be taken to illustrate the role of protein expression in resistance. First, where there is a known gene and protein product, western blots can be used to determine differences in expression. Second, to screen the proteome for differences, 2-dimensional gel electrophoresis (2-DGE) coupled with matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF) can be used to identify proteins that may not have previously been known to be associated with resistance. As all techniques have limitations it is wise to investigate systems with a variety of approaches. For example, 2-DGE may not allow for the detection of all the proteins that are differentially expressed between resistant and susceptible strains. Festucci-Buselli *et al*. (2005) used western blots to demonstrate differences in protein expression (CYP6G1 and CYP12D1) between DDT susceptible and resistant insects, but when Pedra *et al*. (2005) used 2-DGE to investigate differences between strains, neither CYP6G1 and CYP12D1 were observed, however, proteins associated with metabolic rates were more highly expressed in the DDT resistant strains.

New advances in proteomics techniques may provide researchers the opportunity to determine (i) if other groups of proteins are also differentially expressed (or modified) in resistant versus susceptible insects and (ii) if these differences are consistent across resistant strains. Additionally, transgenic insects (Daborn *et al*., 2002) and RNAi can be used to verify the roles that a given gene and its resultant protein(s) may play in a specific biological process.

Genomics and Proteomics for Discovery of Resistance Mechanisms for Abiotic and Biotic Challenges

The advent of the staggering array of "*Omics*" technologies now allows us to develop comprehensive descriptions of virtually all components and interactions in an organism (Joyce and Palsson, 2006). Broadly speaking, "*Omics*" is a neologism that describes the sub-disciplines of biology and engineering involved in the compilation and analysis of biological information associated with "omes," e.g., genomes, proteomes and metabolomes (*Omics*.org, 2007). While there is not consensus on what necessarily constitutes a "ome," at least in the sense as the term is used in "*Omics*," there are common features of these art areas (Fields and Johnston, 2002). Most importantly, "*Omics*" uses a systems approach and is heavily dependent on informatics and computational technologies (Nature *Omics*Gateway, 2007). Indeed, the wealth of information is so great that the challenge now becomes how to integrate and extract useable information from these amassed datasets.

Genomic and proteomic techniques have provided researchers with the tools to more rapidly discover how insects evolved resistance to a wide array of abiotic and biotic factors. In the case of pest insects, such "resistance mechanisms" may have the potential to be used as target sites for the development of novel pest control agents. The genes or proteins that confer the "resistance mechanism" to a given stressor can now become the target site for compounds that alter (e.g., inhibit) the protein's function. These biotic and abiotic targets may include plant defensive compounds contained in the insects' diet, oxidative stress, temperature, desiccation, or other stressors experienced in the insect's life history.

An example of the kind of environmental challenge that we wish to emphasize is an environmental stress that all organisms experience, namely, the alteration of cellular redox homoeostasis that can lead to oxidative stress. Such an imbalance can be due to (i) a lack of antioxidants in the cell or (ii) an excess of reactive oxygen species (ROS). Excessive ROS can lead to irreversible cellular damage and death through damage of cellular molecules including proteins and even DNA. Oxidative stress occurs when cells detoxify ROS, allowing the cell to survive.

Oxidative stress can be due to either exogenous effects or endogenous reactions. For example, energetic radiation such as ultraviolet (UV) rays, can lead to hydroxyl radicals leading to an increase ROS in the cell. Additionally, exogenous oxidants (e.g., peroxides), redox recycling agents (e.g., quinone compounds), hormones, and endotoxins all can lead to increased intracellular ROS production. Compounds such as hydrogen peroxide can lead to ROS by uncoupling cytochrome P450 reactions. In mammals, physiological signaling, including immune system responses, contribute to intracellular ROS production. Conversely, low levels of intracellular antioxidants can also lead to the accumulation of ROS. For example (i) if glutathione production is reduced, (ii) if there are fewer antioxidant vitamins in the cell, or (iii) if ROS-scavenging enzymes are inhibited, or (iv) a combination of these factors, then the levels of ROS will in increase in the cell.

It is critical that cells are able to neutralize oxidative stress, since reactive oxidative chemicals can prolong cell cycles causing arrested development of the overall organism (Wiese *et al*., 1995). Cells use multiple systems to protect themselves against ROS, including glutathione production, which acts as an intracellular

antioxidant buffer system in the cell. The thiol-containing moiety on the cysteine residue of glutathione has reducing power (supplies electrons) that nullifies the oxidative potential of the ROS. Glutathione homeostasis by the balance between glutathione (GSH) and glutathione disulfide (oxidized glutathione; GSSG): GSH is oxidized by glutathione peroxidase to generate GSSG, which in turn can be reduced back to GSH by glutathione reductase. However, the rate-limiting step in the production of GSH is the enzyme γ-glutamylcystein synthase, which converts N-acetylecysteine into GSH. The ratio of GSH to GSSG in the cell is typically 100:1, which means that the oxidation of GSH can dramatically influence the redox status in the cell. Fluorochrome probes (e.g., 2′, 7′-dichlorofluorescein) can be used to assay this change in the oxidative status of the cell. Cells also employ proteins and vitamins to reduce oxidative stress, including superoxide dismutase, catalase, quinone reductase (detoxifies quinone compounds), metallothionein (traps heavy metal cations), and vitamins such as E and C that trap free radicals.

Oxidative stress influences the regulation of gene expression, causing both induction of some genes and repression of others. For example, ROS are known to induce the expression of antioxidant proteins as well as the enzymes that the cell uses to regenerate these proteins (e.g., Trx and glutathione reductases). Conversely, ROS at the same time repress such genes as α-actin, troponin I, some cytochrome P450s, as well as genes that code for proteins in mammals associated with sugar regulation (e.g., insulin) and the immune system (IL-2) (Barker *et al*., 1994; Beiqing *et al*., 1996; Matsuoka *et al*., 1997). These aforementioned genes represent only a subset of the total genes differentially expressed due to oxidative stress. For example, it has also been demonstrated that UV-B radiation strongly inhibits mitochondrial transcription, which results in a repression of mitochondrial function; the mitochondria, which is a major generator of ROS, is very susceptible to oxidative stress (Vogt *et al*., 1997).

Recent work in *D. melanogaster* and *Spodoptera littoralis* has shown that there is evolutionary conservation between mammals and insects in some of the mechanisms by which both groups of organisms deal with oxidative stress (e.g., superoxide dismutase, catalase, ascorbate peroxidase and GST peroxidase) (Krishnan and Kodrík, 2006; Magwere *et al*., 2006). Additionally, Krishnan and Kodrík (2006) demonstrated that, in *S. littoralis*, these aforementioned enzymes are associated with the digestive system, suggesting that they potentially play a role in dealing with oxidative radicals associated with their food.

In addition to the conserved mechanisms for combating oxidative stress there is growing evidence of insect-unique systems for neutralizing oxidative stress. Dubuisson *et al*. (2004) recently observed that luciferin (which is involved in bioluminescence) is a scavenger for the oxidant peroxynitrite. Their observations are in keeping with hypotheses proposed for marine organisms, suggesting that bioluminescence may have initially evolved as an antioxidant mechanism and secondarily as a light-producing system. These findings suggest that if insect-unique antioxidant systems occur in other insect species, then synthetic inhibitors targeting these insect-specific antioxidant systems may be used to selectively interfere with the ability of insects to protect themselves from the effects of oxidative stress in their environment.

Understanding oxidative stress certainly has more immediate implications for issues concerning pesticide resistance. For example, *A. gambiae* mosquitoes that

are DDT resistant, via GST activity, appear to also be more responsive/resistant to oxidative stress (Enayati *et al*., 2005; Ranson and Hemingway, 2005). Vontas *et al.* (2001) also demonstrated that pyrethroids-induced oxidative stress responses in *N. lugens*. Thus, it is possible that insect strains that live in environments where they experience more oxidative stress may be pre-disposed to being more resistant to pesticides (a hypothesis that remains to be tested). Alternatively, since P450s are typically down-regulated during times of oxidative stress, it would be logical that over-expression of P450s may, in some circumstances, be a means to inhibiting an insect's ability to mitigate the effects of oxidative stress in their environment. It remains to be determined if insects that are more pesticide-resistant, due to increased P450 expression/activity, show increased susceptibility to oxidative stress.

More selective inhibition of systems that allow insects to respond to biotic and abiotic stressors may lead to practical insect control methodologies in the future (Figure 3.9). Ultimately, we envision the use of "*Omics*" as a tool to gain a molecular understanding of the diversity of insect responses to stress, as well as how these responses operate in a systems manner. In this way, we can more selectively

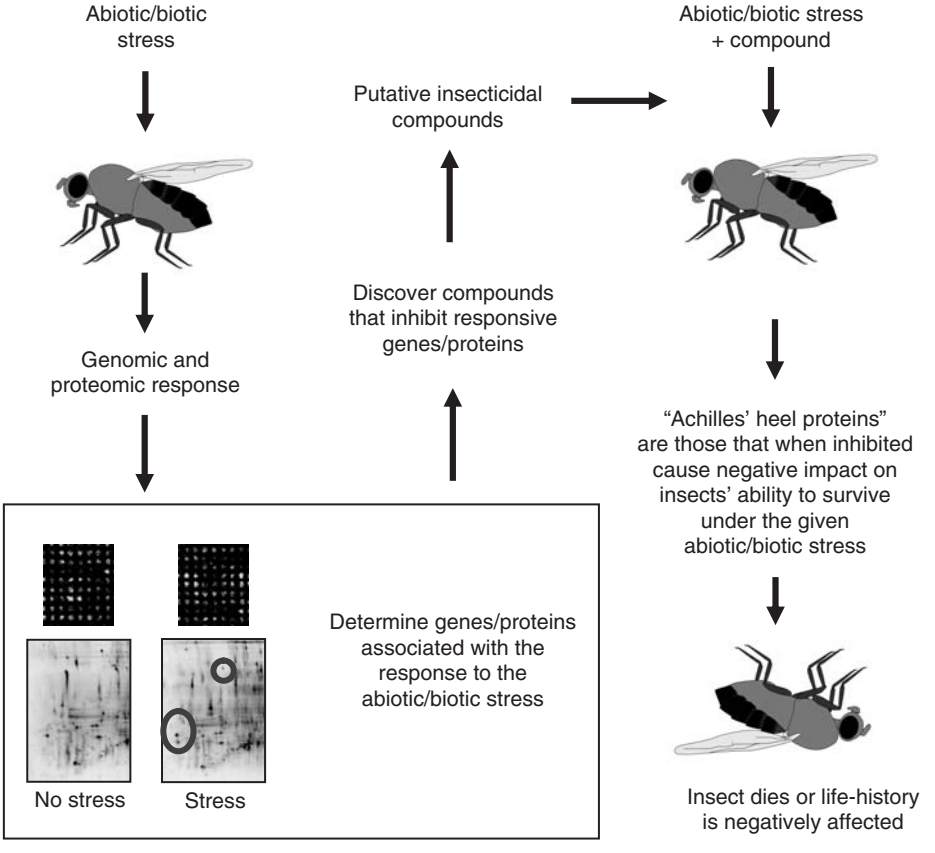

Figure 3.9 Potential discovery strategy for Achilles' heel proteins.

manipulate control strategies and technologies to maximize the costs of resistance alleles to the pest. This approach could extend the life of safe and proven control technologies and also help foster other novel approaches.

Conclusions

In this chapter, we have reviewed the current status of the mechanisms of action of select insecticidal agents and known forms of resistance to them. We have used examples to illustrate that insects use evolutionarily conserved resistance mechanisms common to all animals (e.g., some aspects of oxidative stress) as well as those that are particular to insects (e.g., the peritrophic membrane in the digestive system of insect). Finally, we have illustrated how the "*Omics*" revolution is just beginning to reveal more in depth knowledge of the system-wide bases of these mechanisms (e.g., metabolic pesticide resistance). Perhaps not surprising, but none the less exciting, are emerging examples of the involvement of hitherto unidentified genes and mechanisms involved in resistance. These findings should allow us to identify novel and safe pesticides as well as better design resistance management strategies to ensure their long-term utility.

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Chapter 4

Concepts and Complexities of Population Genetics

David W. Onstad and Charles A. Guse

An understanding of population genetics is needed to both explain past cases of resistance as well as predict the evolution of insect resistance in the future. In this chapter, we discuss simple, yet important, concepts and introduce the reader to diverse research that either complicates heuristic traditions or corrects misconceptions or both.

Without Natural Selection

For most of the chapter the focus is on diploid, sexually reproducing arthropods with discrete generations. Unless otherwise indicated, we assume that the population size is very large so that random genetic drift does not lead to significant changes in resistance allele frequency. The models we describe are monogenic (a single autosomal locus). We assume that mutations to the gene do not occur during the period of selection.

The Hardy–Weinberg principle describes the equilibrium frequencies (proportions) of genotypes when mating is random. For p the proportion of allele s, and q the proportion of allele r, $p + q = 1$ and $(p + q)$ males mate with $(q + p)$ females. Thus, the frequency of ss in the population is p^2, the frequency of rr is q^2, and the frequency of rs is $2pq$. The frequencies of p and q remain constant over many generations in a population in which mixing with other populations and natural selection do not occur.

These relationships under Hardy–Weinberg conditions also demonstrate that the heterozygote, rs, will be the genotype that carries the most r alleles in the population as long as $q < 0.5$. Homozygous resistant individuals, rr, will be rare compared to heterozygotes at low values of the r allele frequency, q.

Evolution Due to Natural Selection

Major Implicit Assumptions
1. Random mating within entire population.
2. Fitness does not depend on location.
3. Survival and reproduction do not change over generations.
4. No density-dependent survival, reproduction, or behavior.

Natural selection causes evolution when the environment (or pest management) acts upon the genetic variation in a population. Without multiple alleles at a single locus or multiple genes with variable response to the environment, selection cannot occur. Essentially, selection creates differential fitness amongst genotypes and changes the average fitness of the population. Fitness determines the number of offspring and alleles that an individual contributes to the next generation. Thus, fitness depends on survival, reproduction, and any other behaviors that influence the ecological and genetic "success" of an organism.

Given a single locus with two alleles, r for resistance and s for susceptibility, the generational change in allele frequency is

$$q(t+1) = [q(t) \times p(t) \times \mathrm{Wrs}(t) + q^2(t) \times \mathrm{Wrr}(t)]/\overline{W}(t) \tag{4.1}$$

where Wii is the fitness of genotype ii, and t is the index for generation. The first term on the right-hand side is derived from $2pq\mathrm{Wrs}/2$ and represents the r alleles provided by the heterozygotes. The average fitness of the entire population, $\overline{W}$, is the weighted average based on Hardy–Weinberg proportions for each genotype:

$$\overline{W} = p^2\mathrm{Wss} + 2pq\mathrm{Wrs} + q^2\mathrm{Wrr} \tag{4.2}$$

$\overline{W}$ also determines the weighted sum of all allele frequencies. Note that, because of selection, p, q, and $\overline{W}$ vary over generations t. Equations (4.1) and (4.2) and any underlying models are calculated iteratively to evaluate the dynamics of allele frequencies changing over a given number of generations or until a constant q is found.

Table 4.1 provides an overview of the dynamics simulated with the equation (4.1). Typically, fitness values are standardized to the highest value of genotypic survival. But any genotypic survival values can be used, provided all three genotypes are measured on the same scale (per generation, per year, proportions surviving, etc.)

When the homozygous resistant individuals are most fit and the fitness of the heterozygotes is intermediate, the resistance allele frequency, q, eventually reaches 1.0. The opposite occurs when homozygous resistant individuals are least fit of the

Table 4.1 The evolutionary outcomes calculated by equation (4.1) for the four basic relationships for genotypic fitness values in a diploid species

Relationship	Evolutionary outcomes
$\mathrm{Wss} < \mathrm{Wrs} < \mathrm{Wrr}$	q fixes at 1.00
$\mathrm{Wss} > \mathrm{Wrs} > \mathrm{Wrr}$	q fixes at 0.00
$\mathrm{Wss} < \mathrm{Wrs} > \mathrm{Wrr}$	Reaches an equilibrium q_e between 0.00 and 1.00: $\mathrm{Wss} = \mathrm{Wrr}$: $q_e = 0.5$ $\mathrm{Wss} < \mathrm{Wrr}$: $0.5 < q_e < 1.0$ $\mathrm{Wss} > \mathrm{Wrr}$: $0.0 < q_e < 0.5$
$\mathrm{Wss} > \mathrm{Wrs} < \mathrm{Wrr}$	A threshold exists, q_T, such that when: $q_0 < q_T$: q fixes at 0 $q_0 > q_T$: q fixes at 1 $q_0 = q_T$: q remains at equilibrium

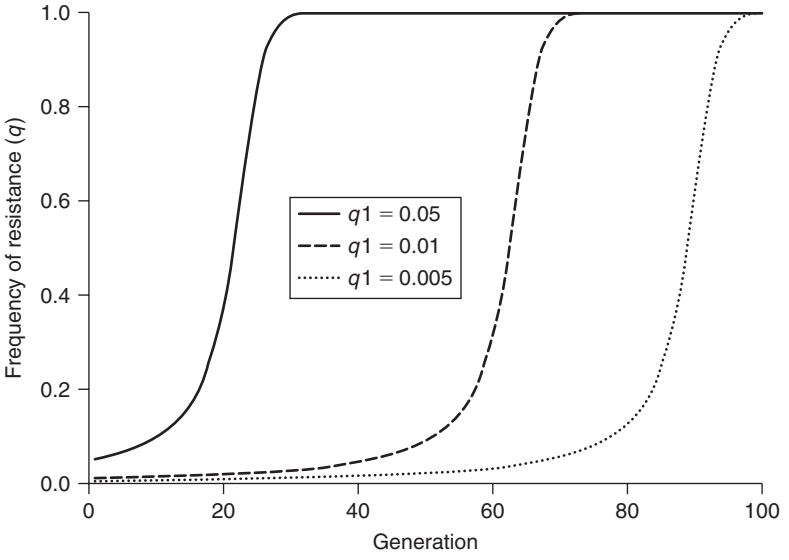

Figure 4.1 The change in resistance allele frequency over time for a population under selection beginning with three different initial allele frequencies. Genotypic fitness is held constant at Wss = 0.5, Wrs = 0.51, and Wrr = 1.0 with resistance expression close to recessive.

three genotypes. Figure 4.1 shows how the initial frequency of the resistance allele affects the number of generations until resistance evolves in the population when Wss < Wrs < Wrr. The initial allele frequency has an inverse relationship to the number of generations required to pass through the lag phase. Regardless of the initial allele frequency (q_0), q eventually approaches 1.

Both the relative fitness of the heterozygote as well as the overall difference in relative fitness between homozygotes determine the evolution of resistance. Fitness values are often reported in the range from 0.00 to 1.00. The range is a convenience that defines the genotype with the maximum fitness as the standard and the fitness of the other genotypes as proportions relative to that standard. Figure 4.2 shows how changes in intermediate heterozygote fitness influence the evolution of resistance. The curve on the far right represents Wrs = Wss, while the curve on the far left represents the scenario with Wrs = Wrr. Minor differences in fitness of the heterozygote can dramatically influence how quickly resistance evolves. Figure 4.3 presents the change in resistance allele frequency over time for four scenarios with additive expression of resistance and Wrr = 1.0. As the relative difference between Wss and Wrr decreases (going from left to right on the figure), the rate of the evolution of resistance is slower.

The outcomes are more complicated when the fitness of the heterozygote is either the maximum or the minimum of the three genotypic fitnesses (Table 4.1). Evolutionary theory indicates that an allele frequency changes to maximize fitness

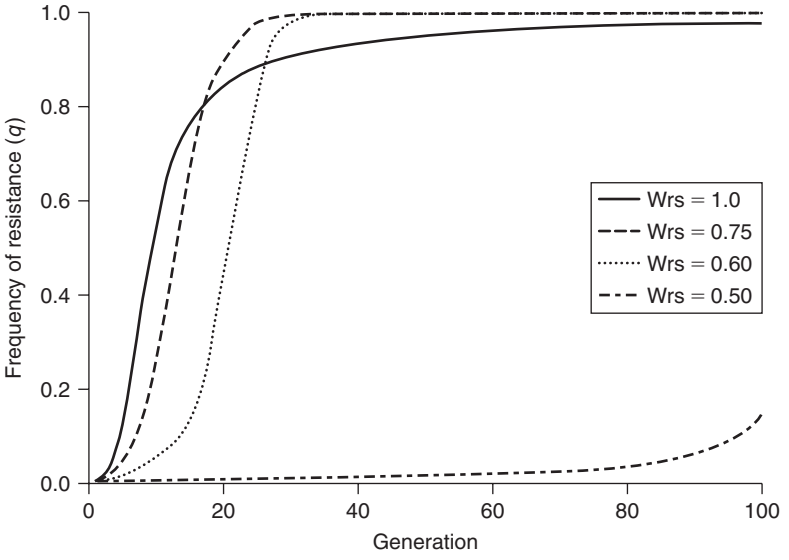

Figure 4.2 The change in resistance allele frequency over time with constant initial $q = 0.01$, Wss = 0.5 and Wrr = 1.0, and incremental increases in fitness of the heterozygote, Wrs.

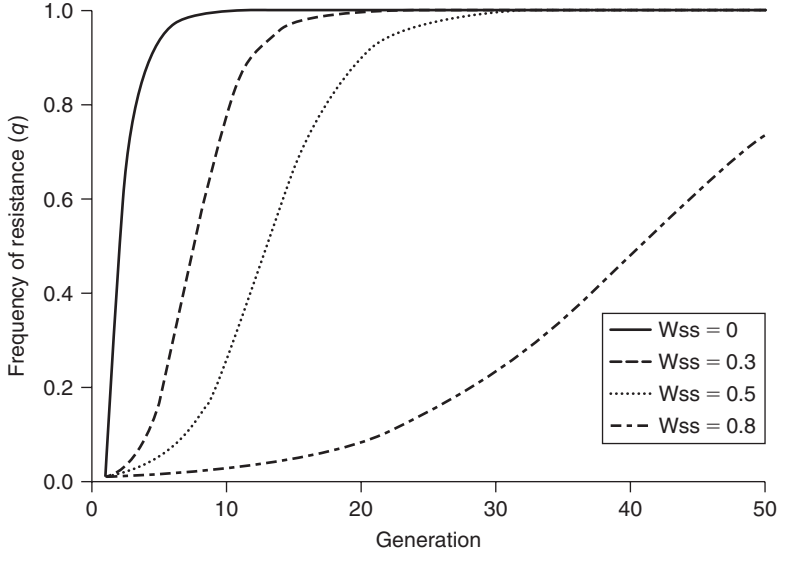

Figure 4.3 The change in resistance allele frequency over time with constant initial $q = 0.01$, Wrr = 1.0, additive expression of resistance Wrs = (Wss + Wrr)/2, and incremental increases in fitness for the susceptible homozygote, Wss.

of the population (Spiess, 1977). An equilibrium allele frequency is established when the heterozygote has the highest fitness (overdominance): Wss < Wrs > Wrr. When Wrr = Wss the equilibrium resistance allele frequency is $q_e = 0.5$.

When Wrr > Wss; $0.5 < q_e < 1.0$ as above. When Wrr < Wss; $0.0 < q_e < 0.5$. In general, the equilibrium allele frequency according to Spiess (1977) is:

$$q_e = \frac{\text{Wss} - \text{Wrs}}{[(\text{Wss} - \text{Wrs}) + (\text{Wrr} - \text{Wrs})]} \tag{4.3}$$

Equation (4.3) can also be used to find the threshold, q_T, which is an unstable equilibrium point (Table 4.1) when the heterozygote has the minimum fitness (underdominance). When Wrr = Wss the threshold allele frequency $q_T = 0.5$. When Wrr > Wss; $0.0 < q_T < 0.5$ as above. When Wrr < Wss; $0.5 < q_T < 1.0$. When the initial allele frequency, q_0, is below the threshold, selection is against resistance. When q_0 is above the threshold, selection is toward resistance.

Although it is mathematically possible to have an equilibrium allele frequency, it is difficult to maintain the allele frequency at equilibrium in the real world. Random fluctuations in the population and its environment such as from mutation or random genetic drift (see section below) will cause the population allele frequency to shift and the selection pressure will push the population toward one extreme or the other.

Natural Selection in Patchy Landscapes

Fitnesses Constant Over Time

Major Implicit Assumptions
1. Random mating within entire population from all patches.
2. Uniform distribution of offspring in landscape.
3. Survival and reproduction do not change over generations.
4. No density-dependent survival, reproduction, or behavior.

Much of population genetics and insect resistance management (IRM) emphasizes evolution of a population inhabiting two or more patches that produce differential fitness in the genotypes. An obvious example is a set of treated fields (patch 1) and untreated refuges for susceptible pests (patch 0). Equation (4.1) can be used if random mating and uniform oviposition across the landscape still occur within the entire population from all the patches. We assume that genotypic fitness is approximated as the survival of each genotype in each patch, S0 and S1, multiplied by the fecundity in that type of landscape, F0 and F1, and weighted by the proportional area of the patch, P0 and P1. Thus, for genotype 'ii' in the treated patch, $\text{W1ii} = \text{S1ii} \times \text{F1ii} \times \text{P1}$. For a landscape where fecundity is equal in both patches, genotypic fitness in the whole population across the entire landscape is calculated as: $\text{Wii} = \text{S1ii} \times \text{P1} + \text{S0ii} \times \text{P0}$.

Figure 4.4 demonstrates how evolution occurs over time in a landscape with two patches. Reproductive capacity is equal in both patches and therefore effectively 1.0. There are no fitness costs; therefore, S0ii = 1 for all genotypes in the refuge. S1rr = 1, S1rs = 0.5, and S1ss = 0.1 in the treated patch. As the proportion of treated fields increases, the overall fitness of the susceptibles, Wss, decreases and the differential fitness between genotypes becomes more important, resulting in a shift of the curves to the left in the figure.

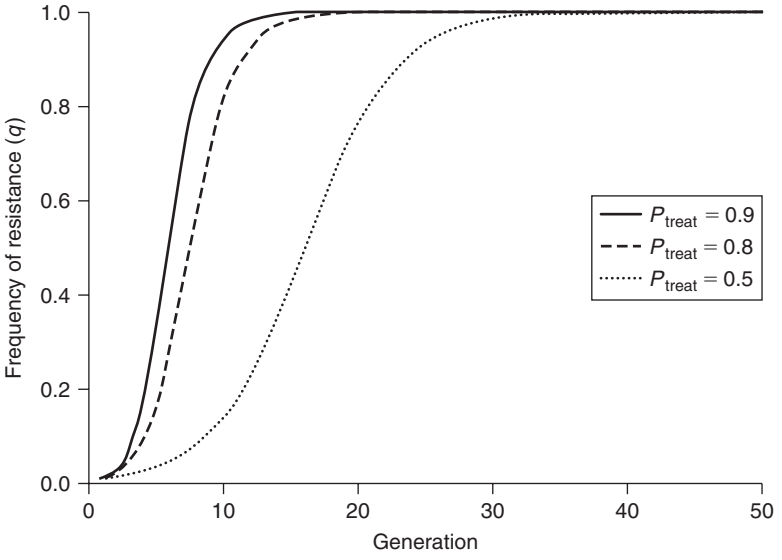

Figure 4.4 The change in resistance allele frequency over time with constant initial allele frequency and constant genotypic fitness within treated patch and refuge patch in the landscape. Evolution is changed by altering the proportion of treated patch in the landscape.

Variable Fitness Over Time and Space

Major Implicit Assumptions
1. Random mating within entire population from all patches.
2. Uniform distribution of offspring in landscape.

Note that equation (4.1) contains no terms for population abundance. However, fitness is likely to change over time and space as arthropod density changes. Given that density-dependent processes such as survival, reproduction, and dispersal are common phenomena in arthropod populations, we should expect fitness to vary over time and space. Stakeholders and economists want to predict population density to better predict consequences of IRM for pest damage and economic losses (Mitchell and Onstad, Chapter 2).

As Chapters 8–12 indicate, there are many ways to combine models of gene frequency with models of pest density. Modelers must make decisions about what aspects of population dynamics to include with the population genetics. The most important decisions are concerned with the interactions of phenotype density and behavior. Many of the first, simple models were created by Comins (1977), Roughgarden (1979), Taylor and Georghiou (1979), Tabashnik and Croft (1982), and Alstad and Andow (1995).

Gene Flow and Population Structure

Gene flow is the process acting on and creating genetic sub-populations. The flows occur over landscapes and amongst populations that are distributed in space with varying distances of separation. Whether space is simple or complex in a model,

we need to carefully define gene flow and population structure and deal with both concepts simultaneously. The genetic structure of the population in a landscape is determined by gene flow, and the movement of genotypes and genes is dependent on the spatial structure of the population.

Background on the population-genetic issues related to gene flow can be obtained from several publications (Hedrick, 2006). Mallet (2001) provides one of the best recent overviews of gene flow. He clarifies a number of issues that often confuse non-experts, emphasizing the actual movement of genes and genotypes in his analysis. Felsenstein (1976) reviews research concerning models of island populations and dispersal. This traditional work in population genetics still has relevance to current problems regarding patches of transgenic insecticidal crops and refuges of conventional crops. In particular, Felsenstein (1976) summarizes the relatively old studies by stating that the alleles found in island populations will depend on the amount of dispersal amongst the islands, with threshold levels of dispersal possibly determining the final outcome. Several authors have studied gene flow in heterogeneous landscapes (Caprio and Tabashnik, 1992; Caprio, 2001; Ives and Andow 2002; Sisterson *et al.*, 2005).

Care must be taken when using the term gene flow or claiming some consequence of gene flow for IRM. Gene flow depends on dispersal. To understand these complex processes, several factors must be identified for each resistance gene. First, does the dispersal occur before or after mating, or both? Second, do males and females have different dispersal rates and behaviors? Third, is gene flow unidirectional or multi-directional? If flow occurs in several directions, such as to and from a particular crop or refuge, are the flows equal or unequal? Are they equal in terms of proportion of gene or insect population or in terms of numbers of alleles or insects? Fourth, is dispersal a constant over time and space or does it interact with (a) other processes, (b) insect density, or (c) environmental conditions? Thus, when someone claims to know how gene flow affects evolution of resistance, be prepared to ask a series of questions.

Mating

The mixing of a population for the purposes of mating is a critical process in the evolution of resistance. Mating involves a complex set of behaviors. For the purposes of IRM, details of these behaviors may need to be measured empirically and included in simulation models. Trimble *et al.* (2004) studied the effects of sublethal residues of azinphosmethyl on pheromone production, calling, female attractiveness and the ability of males to locate sources of pheromone. They compared the performances of susceptible and resistant *Choristoneura rosaceana* and found effects that depended on the phenotype and treatment. Some studies have shown a fitness cost in mating due to insecticide resistance. This was observed in males competing for mates (Doherty and Hales, 2002; Berticat *et al.*, 2002a) and mating rate and fertility (Boivin *et al.*, 2001). In other cases, the resistant individuals have an advantage. Arnaud and Haubruge (2002) evaluated susceptible and malathion-resistant male *Tribolium* beetles in mating competition for susceptible females, and in most cases, found that resistant males had a greater reproductive success rate than susceptible males. Genetics and natural selection can also be important

when females mate with more than one male (Haubruge *et al.*, 1997; Alyokhin and Ferro, 1999a, b; Baker *et al.*, 2005).

Traditional, simple models assume that panmixia or random mating occurs in populations. However, when density-dependent effects and heterogeneous landscapes are investigated, variable dispersal (and adult emergence) can lead to non-random mating. For instance, in models of patchy landscapes, mating can be modeled as random within a patch but non-random for the entire population. In modeling studies, Caprio and Hoy (1995) and Caprio (2001) evaluate random and non-random mating under various dispersal scenarios. They concluded that dispersal for mating and mating biases (assortative mating) must be considered along with dispersal that distributes offspring when making predictions about IRM. Guse *et al.* (2002) simulated mating that is influenced by irrigation of the cropland and by dispersal of males between habitats and found significant differences in the evolution of resistance.

Random Genetic Drift and Demographic Allee Effects

In the analysis of small or sparse populations, two factors can make predictions based on typical models of large populations inaccurate. One process is random genetic drift, which occurs when chance and stochastic processes cause the frequency of genes to increase or decrease independently from the effects of natural selection. The greatest concern about random genetic drift is that rare alleles can either be lost or become fixed at 100% unexpectedly in a small population solely by chance. Most IRM models do not include random drift, because they are deterministic (lack random processes) and assume that the population densities will always be very large. However, densities of arthropods can be driven to low levels in pest management. Stochastic models that allow chance to influence the changes in variables simulate random genetic drift (Caprio and Tabashnik, 1992; Caprio, 1994; Caprio and Hoy, 1994; Storer *et al.*, 2003).

The Allee effect is a demographic effect that has been recognized in ecology for many decades (Berec *et al.*, 2006). The effect occurs when the fitness of an individual in a small or sparse population decreases as the population density declines. Even though the Allee effect can be modeled deterministically, a stochastic model would also be reasonable because some conditions and events producing the effect are stochastic, such as the inability to find a mate at low density.

The number of homozygous resistant (RR) genotypes are expected to be very rare and at extremely low densities when selection of the population begins. For example, in fields of transgenic insecticidal crops, arthropod densities are expected to be very low and most surviving genotypes are expected to be RR. At initial allele frequencies of 0.00001–0.001, there will be one RR individual for every million to 10 billion insects in a population. In the case of *Diabrotica virgifera virgifera*, densities range from 1 to 100 million/ha in cornfields depending on the life stage (Onstad *et al.*, 2006). Thus, for this insect, there can be fewer than 100 RR individuals per ha at the start of selection. In this scenario, random drift and the Allee effect may influence the evolution of resistance depending on how populations mix and how long the densities remain low.

Gene Interactions

Arthropod gene interactions can be important in IRM. Epistasis is the positive (synergistic) or negative (antagonistic) interaction of genes. Gould (1986a, b) explored the antagonistic effect of resistance-gene interactions on insect fitness. He used data on *Mayetiola destructor* epistasis to calibrate an IRM model (Chapter 9). Epistasis can be measured in laboratory studies on susceptible and resistant colonies, but without these data, it is generally impossible to predict whether or not it will occur when two genes are being evaluated. Chemical insecticides are often formulated with synergists to increase toxicity.

Linkage indicates that genes are on the same chromosome. When recombination of the arthropod genome does not completely happen, non-random association of the genes during inheritance occurs (Groeters and Tabashnik, 2000). This is called linkage disequilibrium or gametic phase disequilibrium. Gould (1986b) explored the effects of linkage on *Mayetiola destructor* IRM. Assumptions about epistasis and linkage may be important when modeling the evolution of multiple genes in arthropod populations.

Selection Intensity and Resistance Genes

Should insect resistance be modeled as monogenic (trait controlled by a major gene at a single locus) or polygenic (trait controlled by several genes)? A major gene has significant effects on resistance by itself, whereas a minor gene contributes much less to tolerance or a behavioral change. Reference to a polygenic scenario or a quantitative-genetic analysis usually means that minor genes are involved. Roush and McKenzie (1987) and Roush and Daly (1990) argue that field resistance to pesticides by arthropods only evolves when one or two major genes are the basis for resistance. McKenzie and Batterham (1998) use mutagenesis to demonstrate that major genes are the primary cause of resistance in a variety of cases. A different perspective is taken by Via (1986) who discusses quantitative genetics and the polygenic model of resistance to pesticides. Certainly, scientists have observed both qualitative and quantitative resistance involving multiple genes (Crow, 1954; Pittendrigh *et al.*, Chapter 3). Scientists will likely find evidence supporting either hypothesis in different laboratory and field studies.

Several modeling studies have demonstrated that resistance evolves faster when a major resistance gene is involved compared to a scenario with several minor genes. Plapp *et al.* (1979) simulated a simple model with six loci (genes) each with two alleles. They found that when low toxin doses are investigated, resistance evolves faster with major genes conferring high tolerance than with several minor genes. Gardner *et al.* (1998) compared a rotation of low and medium doses of the same toxin with a constant application of a dose intermediate between the two using a simulation model. They found that both strategies cause resistance to evolve quickly with either a single major gene or polygenic quantitative resistance. However, the rotating of the doses delayed resistance evolution when resistance is due to quantitative resistance.

In an attempt to determine the genetic basis of field-evolved resistance by arthropods to pesticides, Groeters and Tabashnik (2000) related published data on

pesticide selection intensity to an analysis of a stochastic model. They concluded that measurements of selection intensities for nine species varied widely and that field and laboratory selection intensities are generally similar. They evaluated the roles of six unlinked loci (genes) with their stochastic model. Because the initial allele frequencies were inversely proportional to their effects on resistance, major genes had the lowest initial frequencies (highest fitness costs). Results indicated that resistance alleles with major effects dominated responses to selection no matter what the selection intensity. Resistance evolved faster in models with major genes than in models without them. The most intense selection tended to prohibit minor genes from contributing. Groeters and Tabashnik (2000) concluded that knowing the intensity of selection is crucial for IRM predictions, but knowledge of the number of loci and their relative contributions to resistance is not. Thus, models simulating a few major genes (one or two loci) should be satisfactory for modeling and predicting the consequences of IRM decisions.

Tabashnik (1990) studied the influence of gene amplification at a single locus on the evolution of insecticide resistance. Gene amplification increases the number of copies of a gene per haploid genome above normal levels. When a resistance mechanism depends on the amount of biochemical products of gene expression, gene amplification will lead to greater tolerance to a toxin. Tabashnik (1990) compared simulations of a conventional two-allele model to simulations of three- and four-allele models in which additional alleles are derived from existing alleles at a rate greater than assumed mutation rates. Each subsequent allele produces more mRNA and protein to increase tolerance. Results were similar for the models when insecticide concentration was low or moderate. In contrast, when 10% of the population was not exposed in a refuge, high insecticide concentrations slowed resistance evolution in the two-allele model, but caused rapid evolution of resistance in the three- and four-allele models even at very low initial allele frequencies. Tabashnik (1990) concluded that IRM strategies based on use of a high dose of toxin are not likely to succeed, if gene amplification or other mechanisms generate alleles that confer high levels of resistance.

Dominance

For a single gene, one allele is dominant over a second allele when the expression of the first allele determines the response of the heterozygote to its environment. Thus, when susceptibility to a toxin is dominant, Sr, the phenotype is vulnerable to the toxin, but when the susceptibility is recessive, sR, the phenotype is resistant. In general, dominance is the term used to describe how heterozygotic phenotypes respond in comparison with homozygotic phenotypes.

Typically, dominant resistance occurs through a gain of function; the organism can now do something it could not before. For example, detoxification enzymes are now expressed at a higher level, thereby allowing more of the toxin to be detoxified. One allele causing a gain in function may, therefore, provide resistance. On the other hand, recessive resistance is often associated with a loss of function. For example, a change in a target site means that the toxin can no longer bind to the given receptor. However, in the heterozygous state, the interactions between the wild-type protein, still produced by the single S allele, and the toxin are enough

to cause mortality. Additive expression of resistance causes the phenotype to respond to the environment at a level intermediate between the responses of the two homozygotes.

For cases with intermediate heterozygote fitness, the simplest way to model relative fitness for heterozygotes is with a function similar to

$$\text{Wrs} = (1 - h) \times (1 - \text{SC}_{\text{ss}}) + h \times (1 - \text{SC}_{\text{rr}}) \tag{4.4}$$

where $0 \leqslant h \leqslant 1$ is the dominance level for resistance to the pest control treatment and $0 \leqslant \text{SC} \leqslant 1$ is the selection coefficient. In this function, $h = 0$ represents recessive resistance, $h = 1$ represents dominant resistance, and $h = 0.5$ implies additive expression. A selection coefficient typically equals the mortality due to the pest control treatment. A simplistic conceptual model of gene expression can lead to problems, however, because dominance is as much about environment as it is about genetics.

Although dominance is often heuristically described as a constant genetic property, this is not true for real situations (Bourguet *et al.*, 2000). The dominance of resistance depends on the environment experienced by the arthropods, including the dose of toxin (Roush and Daly, 1990). For example, several bioassays indicate that the dominance of resistance to toxins decreases as toxin concentration increases (Tabashnik *et al.*, 2004). Figure 4.5 shows how the toxin dose can alter the survival of heterozygotes (the phenotypic response), and therefore, the identification of the dominance of resistance to the toxin. Because toxin concentration in pesticide residues and even in transgenic insecticidal crops can vary over time, dominance, and selection on the targeted pest are dynamic conditions. Onstad and Gould (1998) postulated that crop senescence and reallocation of plant nitrogen from toxin to grain could cause toxin titer to decline during much of the growing season. Furthermore,

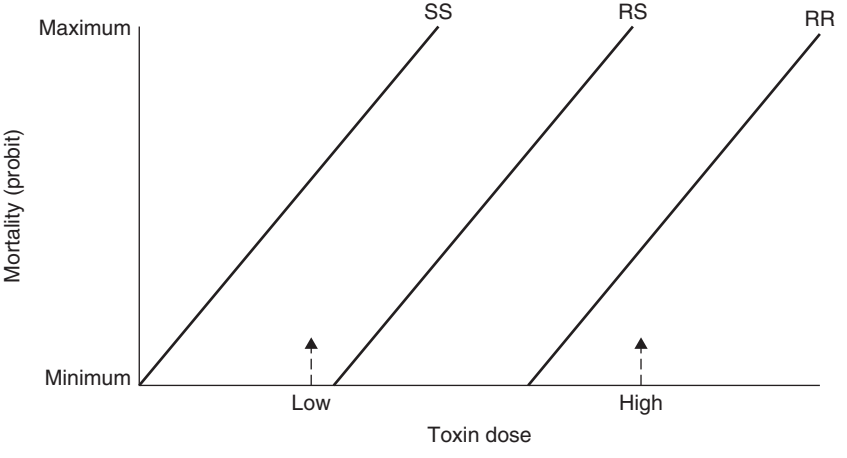

Figure 4.5 Effect of toxin concentration encountered by pest on mortality of three genotypes. Most of susceptibles (SS) and none of the heterozygotes are killed by a low dose. Resistance is identified as dominant. At a high dose, all SS and RS plus some RR individuals are killed; resistance is considered recessive.

since each life stage will respond differently to its environment and toxin, then logically dominance of resistance can also vary with the life stage of the pest that is evaluated (Bouvier *et al.*, 2002). For all of these reasons, claims based on a single level of dominance in a complex IRM scenario should be met with skepticism.

When selection pressure and the evolution of resistance begin, resistance alleles are rare, and the alleles occur mostly in heterozygotes. Homozygous resistant individuals are extremely rare. Therefore, the selection of heterozygotes, which is dependent on dominance, determines the early progress of evolution of resistance. Dominance also determines how heterozygotes respond to conditions without a toxin. Therefore, fitness costs are influenced by dominance. For example, the fitness of heterozygotes relative to homozygous susceptibles in refuges is an important factor in the evolution of resistance to transgenic insecticidal crops.

Fitness Costs

A fitness cost for resistant phenotypes is the reduction in relative fitness occurring when and where the selective agent is absent. In other words, the difference between the fitnesses of a resistant phenotype and a homozygous susceptible individual when a treatment is removed is the fitness cost of resistance. Fitness costs can be measured in fecundity, survival, behavior, and any other way that fitness is measured.

Roush and McKenzie (1987) concluded that fitness costs tend to be small in resistant arthropods. Gould *et al*. (2006) included small fitness costs for arthropods into a model of a pyramided, two-toxin crop and observed significant sensitivity of resistance evolution to the fitness costs. Significant fitness costs have been observed in arthropods evolving resistance to *Bacillus thuringiensis* (Bird and Akhurst, 2005; Carrière *et al.*, 2005; Higginson *et al.*, 2005; Janmaat and Myers, 2006) and other toxins (Bourguet *et al.*, 2004). Fitness costs may be influenced by environmental stress and host plant quality (Raymond *et al.*, 2007).

Costs of resistance to toxins are usually associated with particular molecular mechanisms of resistance. In addition, fitness costs are often described in terms of antagonistic pleiotropy in which the resistant gene causes another distinct phenotypic effect on the resistant individuals (McKenzie, 1996). Stable resistance is associated with fitness costs that are very small, whereas resistance instability over time is produced by high fitness costs.

Fitness costs may affect symbionts or be affected by symbionts in the pest's body. Berticat *et al.* (2002b) demonstrated that *Wolbachia* density is altered by the presence of insecticide-resistant genes in the mosquito, *Culex pipiens*. *Wolbachia* are responsible for various alterations in host reproduction. Mosquito strains with genes conferring resistance were more infected by *Wolbachia* than a susceptible strain. Berticat *et al.* (2002b) showed that this interaction also operates in natural populations. They suggested that mosquitoes may control *Wolbachia* density less efficiently when they carry an insecticide-resistant gene and suffer a fitness cost.

Haplo-diploidy

The population genetics models described above all represent diploid species of arthropods. However, it is not uncommon to find species that are haplo-diploid, meaning the males have one set of functioning chromosomes (haploid)

and females have two sets. In these species, males either (1) develop from unfertilized eggs or (2) experience paternal genome loss in fertilized eggs when the paternal chromosomes are inactivated or eliminated during the early development of males. (*Mayetiola destructor* has a different kind of paternal genome loss and is not haplo-diploid.) Carrière (2003) relates the evolution of haplo-diploid species to type of resistance mechanism. Pesticide resistance can result from a gain or loss of function. For example, detoxification of pesticides by enzymes is based on a gain of function: greater enzyme production reduces toxin concentration. Other examples of gain of functions involve reduced pesticide penetration or enhanced sequestration or excretion. An example of a loss of function is reduced sensitivity of target sites in the arthropod body. According to Carrière (2003) when resistance involves a loss of function, R males and RR females should be equally tolerant of the pesticide, but with a gain of function RR females should have greater tolerance than R males. Carrière (2003) found support for his hypothesis that, in most species, haploid males should be less tolerant to pesticides than diploid females, by reviewing cases of sex-linked resistance in *Musca domestica*, *Ceratitis capitata*, *Drosophila melanogaster*, and *Haematobia irritans*. In these diploid species, an allele associated with the female chromosome is not present or expressed on related male chromosomes.

In an associated empirical analysis, Carrière (2003) tested his hypothesis that tolerance to pesticides is lower in males than in females in haplo-diploid systems, by comparing the relative tolerance of males and females between haplo-diploid and diploid arthropods. He reviewed 16 reports pertaining to 10 haplo-diploid species involving 56 cases of pesticide tolerance observed in both males and females. He also obtained 85 cases of tolerance in both sexes from 33 reports on diploid species. Carrière (2003) found that the ratio of male to female tolerance is much smaller in haplo-diploid than in diploid arthropods (Figure 4.6), indicating that resistance alleles are not strongly up-regulated (with gain of function) in haploid males. He then assessed whether factors other than ploidy affect male tolerance and discovered that males were generally less tolerant than females in both haplo-diploid and diploid arthropods. Carrière (2003) concluded that sexual size dimorphism and sex-dependent selection may account for the lower tolerance in males than in females. Therefore, the lower tolerance of males, particularly in haplo-diploid species, must be considered when developing model predictions and IRM strategies (Caprio and Hoy, 1995; Crowder *et al.*, 2006).

Resistance Evolution and Pest Generation Time

Two empirical studies have discovered an apparent influence of generation time on the rate of evolution of arthropod resistance. Both Georghiou (1980) and Tabashnik and Croft (1985) found that the shorter the generation time (greater number of arthropod generations per year) the faster the evolution of resistance. Georghiou (1980) analyzed data for seven species of soil-dwelling crop pests selected by cyclodiene insecticides, while Tabashnik and Croft (1985) evaluated data for 24 arthropod species selected by an organophosphate pesticide in apple orchards. In a third investigation, Rosenheim and Tabashnik (1991) also identified a similar relationship in their empirical analysis of 56 pests of apple and pear.

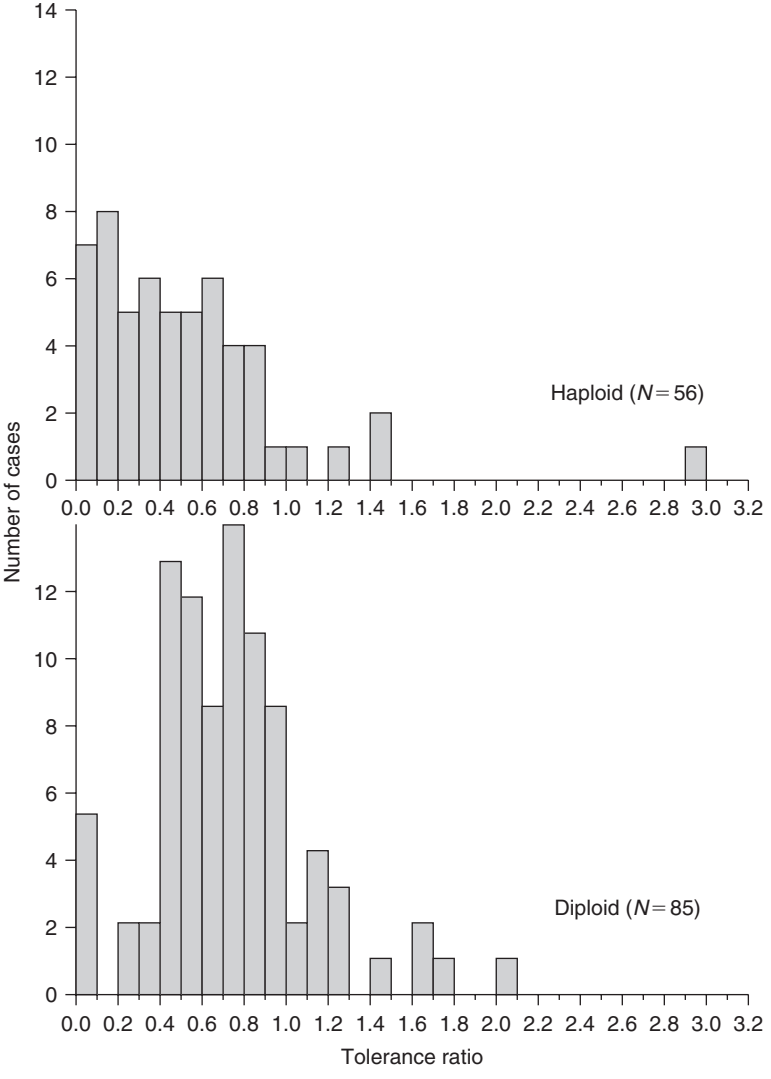

Figure 4.6 Distribution of the ratio of male to female pesticide tolerance in haplo-diploid and diploid arthropods (from Carrière, (2003) with permission from Entomological Society of America).

However, in their overall analysis of 682 arthropod species in North America, Rosenheim and Tabashnik (1991) found little influence of generation time on the evolution of resistance. The highest rates of evolution were observed for arthropods with 3.5–10.5 generations per year. Thus, pests with a medium number of generations per year, including the 56 pome-fruit species (all with less than 13 generations per year), may be more likely to evolve resistance to a pesticide more quickly than those with shorter or longer generation times. Nevertheless, Rosenheim and Tabashnik (1991) provide several reasons why generation time cannot be directly

influencing the rate of resistance evolution. First, secondary pests not targeted by pesticide applications will have varying numbers of applications applied during each of their generations. Thus, secondary pests may incur zero to many pesticide applications in a given generation. Second, although generation time significantly influences the intrinsic rate of increase of a population, the realized rate of population growth for many pests will vary over time and space to obscure any effects of generation time. Third, not all pest generations are the same from a management perspective. For example, crops may be vulnerable to damage and only need protection during a short period coinciding with one or two pest generations. Generations of the pest occurring at other periods may be insignificant and not receive any pesticide applications. Fourth, pests that have long generation times and also damage a crop or livestock for much of the time during each generation will likely be treated several times per generation with a pesticide; the opposite scenario can occur with pests passing through several generations without incurring even one treatment. Therefore, Rosenheim and Tabashnik (1991) concluded that there should be no direct relationship expected between pest generation time and the rate of resistance evolution.

In a modeling study, Rosenheim and Tabashnik (1990) also drew the same conclusion, but noted that complex interactions between generation time and genetic, demographic, and management factors could result in some significant influence of generation time on the predicted number of years for resistance to evolve. It will be interesting to see how the use of high-dose IRM strategies and transgenic insecticidal crops will possibly change some of these empirical relationships.

Temporal and Spatial Scales in Hypotheses

A number of ecologists have expressed concern about the lack of temporal and spatial scales in ecological hypotheses (Levandowsky and White, 1977; Allen and Starr, 1982; O'Neill *et al.*, 1986; Peters, 1988; Roughgarden *et al.*, 1989; Weins, 1989). Vagueness and the lack of operational definitions are indications of immature theories (Loehle, 1987; Murray, 2001; Krebs, 2006) and prevent us from evaluating predictions about evolution over time and space. Often claims are made and conclusions drawn about the conditions that promote or inhibit the evolution of arthropod resistance without the operational temporal and spatial scales being specified. Without scales for example, we do not know whether a given concept pertains to a square meter and a day or to a million square kilometers and a year. If hypotheses in population genetics are to be tested and implemented, we need to strive for more precise concepts that include general temporal and spatial scales for which the concepts are valid. Onstad (1992) evaluated this problem in epidemiology and proposed criteria for identifying appropriate scales and definitions of important terms.

Criteria for identifying temporal and spatial scales should be based on consistency of observation and ecological validity. Scales must account for behavior and longevity of all phenotypes. Units must be effective for both discrete and overlapping generations. Model computation or analysis may require small units of time and space to ensure proper calculation of functions and stable results, but these computational units are not the conceptual units of interest here.

Temporal and spatial units must correspond for logical reasons. The minimum time unit for analysis of evolution is clearly the generation time for the targeted

arthropod. Onstad (1992) discusses the various ways to measure generation time for arthropods with discrete or overlapping generations. The minimum spatial unit should be the two- or three-dimensional space that is traversed on average by the targeted arthropod during a generation. The ecologically proper spatial scale depends on a species' behavior, and therefore, must be large enough to encompass all normal movement by the average or median individuals in the phenotypes (Weins, 1976; Addicott *et al.*, 1987; Caprio, 2001).

The maximum spatial and temporal scales should also be declared in any hypothesis. This issue is similar to the choice of time horizon, which is important for management of resistance (Mitchell and Onstad, Chapter 2). Ecological and genetic conditions underlying the hypothesis may not be valid after a certain number of arthropod generations or when a very large number of minimum spatial units are considered together. Obviously the maximum spatial scale should not exceed the existing area inhabited by a species, and even areas that are inhabited that have never and will never be treated outside a core area may not be properly included in hypotheses.

These minimum and maximum scales are used by scientists and IRM practitioners to test or implement the hypotheses. Samples should not be taken at units smaller than the minima and not taken in times or areas beyond the boundaries defined by the maxima. For example, should resistance allele frequency be measured as the mean for a minimum spatial unit, for an area logically selected as the maximum, or for a region encompassing a species' entire geographic range? The latter effort would likely indicate that resistance on average is very low, while at a smaller unit the resistance is increasing at an observable rate (Chapter 13).

Conclusions

Natural selection of arthropod populations occurs in heterogeneous landscapes where management tactics are applied in a variable manner over time and space. The number and intensity of selective treatments are the most important operational factors determining how quickly resistance evolves. Dominance is important, and its variability must not be underestimated. The greater the dominance of resistance in heterozygotes in all life stages under treated conditions, the faster resistance evolution occurs. On the other hand, the greater the fitness costs, the slower evolution of resistance occurs.

Other conditions and processes highlighted above represent part of the complexity of real systems in which we attempt to manage resistance. Epistasis and linkage of genes likely will have unpredictable effects on IRM. Random genetic drift may slow the evolution of resistance during its early phases. Gene flow, dispersal, and mating behaviors must be carefully evaluated and measured before making predictions about how they will influence IRM. Subsequent chapters in this book describe how many scientists and IRM practitioners have interpreted and conceptualized population genetics within the context of each system's complexity.

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Chapter 5

Adapting Insect Resistance Management Programs to Local Needs

Graham Head and Caydee Savinelli

Introduction

In the past 50 years, the increasing use of insecticides in agricultural and urban settings has brought with it increasing selection for insecticide resistance. More than 600 cases of insecticide resistance have been detected in that time, with some instances of resistance to all major insecticidal classes (Georghiou, 1987; Whalon *et al*., 2006). As a consequence, insect resistance management (IRM) has become an essential part of product stewardship for insecticides. For IRM to be effective, the nature of the pest system and the insecticides involved must be considered, and appropriate IRM tactics devised. This involves analyses of local conditions and capabilities to understand the risk of resistance in a particular system and how it may be mitigated. However, these scientific assessments of resistance risk and appropriate management strategies are only the first step in creating effective IRM programs. The design of IRM programs must consider constraints imposed by the nature of different agricultural systems, human behavior, and economics (Forrester, 1990); programs must be easy for farmers to understand and implement, they must be practical and flexible, and they must be cost-effective. Once designed, IRM programs then must be implemented broadly and appropriately by fitting resistance management practices into existing pest management programs and educating key stakeholders on their roles and responsibilities. Developing countries in which agriculture is dominated by smallholders represent a special challenge because of the large number of people that must be educated and because their individual resources are very limited.

This chapter focuses on the practical considerations involved in implementing IRM plans, how they vary among countries and cropping systems, and what can be learnt from global attempts to manage resistance in a number of cosmopolitan pests that have a history of evolving resistance to many classes of insecticides. In particular, lessons are drawn from IRM experiences in developed and developing countries in relation to three major insect pests: the sweetpotato whitefly *Bemisia tabaci* in cotton; the diamondback moth *Plutella xylostella* in cruciferous vegetable crops; and the disease-vectoring anopheline mosquitoes (*Anopheles* spp.). In each case, the challenges posed by the need to adapt resistance management programs

to the resources and capabilities of different regions are explored. The recurrent themes of this chapter are that successful resistance management programs enroll a broad set of stakeholders through carefully targeted educational programs and employ a range of tactics that are chosen to complement pest management practices already in place.

Creating Effective IRM Programs

The Challenge in Designing IRM Programs

In simplest terms, IRM programs work by reducing the selection pressure related to any given insecticide so that the target pest species evolve resistance to the insecticide more slowly, if at all. The ways in which this can be done are necessarily limited and all involve either:

(1) designing products with inherently lower resistance risks, including products with highly effective novel modes of insecticidal action or combining multiple modes of action within a single product (known as tank mixes for conventional insecticides or pyramids for transgenic insecticidal crops) or
(2) controlling the way in which insecticides are applied so that not all target pests are exposed to the insecticide and those that are exposed encounter a lethal dose (Georghiou, 1987). Possible strategies include temporal rotations of different products, the use of refuges where no insecticide is applied to allow some susceptible insects to survive, and the use of ultra-high rates. For example, farmers planting transgenic insecticidal crops (known as Bt crops because they contain insecticidal genes derived from the bacterium *Bacillus thuringiensis*) in the US are required to plant spatial refuges usually consisting of conventional crop varieties on their farms.

Whatever strategies are involved, IRM programs must be technically effective, feasible, and economical in order to be successful. To be technically effective, aspects of pest biology, and the genetics of potential resistance must be considered in designing the program. For example, insecticide rotations must consider the mode of action of the insecticides to be rotated to avoid possible cross-resistance (Table 5.1). However, even a carefully designed program can fail if it is not properly implemented. Because IRM programs generally rely on farmers, farmer behavior becomes the key to successful implementation. Farmers will be more likely to adopt a new program if it fits into existing agricultural practices, and if the actions required of farmers are not prohibitively costly or time consuming.

Pests with a High Risk of Resistance Evolution

Pest species vary greatly in their propensity to evolve insecticide resistance; certain arthropod pests have evolved resistance to a large number of insecticidal active ingredients from a variety of different classes (Table 5.2), while other pests have little or no history of insecticide resistance. Pests that have evolved resistance to many different insecticides include important crop pests, parasites of livestock, common urban pests, and disease vectors. However, most of these pest species

Table 5.1 Mode of Action Classification of Insecticides (excerpted from IRAC, 2005)

Group no*	Primary site of action	Examples
1	Acetylcholine esterase inhibitors	Carbamates, organophosphates
2	GABA-gated chloride channel antagonists	Organochlorines, fiproles
3	Sodium channel modulators	Pyrethroids, pyrethrins
4 & 5	Nicotinic acetylcholine receptor agonists	Neonicotinoids, spinosyns
6	Chloride channel activators	Avermectins
7	Juvenile hormone mimics	Juvenile hormone analogues
10	Mite growth inhibitors	Clofentezine
11	Microbial disruptors of insect midguts	*Bacillus* crystalline proteins
12	Inhibitors of ATP synthase	Diafenthiuron, propargite
13	Uncouplers of oxidative phosphorylation	Chlorfenapyr
15 & 16	Inhibitors of chitin biosynthesis	Benzoylureas, buprofezin
17 & 18	Molting disruptors	Cyromazine, azadirachtin
19	Octopaminergic agonists	Amitraz
20 & 21 & 24	Mitochondrial electron transport inhibitors	Hydramethylnon, rotenone, cyanide
22	Voltage-dependent sodium channel blockers	Indoxacarb
23	Inhibitors of lipid synthesis	Tetronic acid derivatives
26	Aconitase inhibitors	Fluoroacetate
27	Synergists	Esterase inhibitors

*Classification scheme represents current expert consensus. Not all known mode of action groupings are shown. A full list can be found at: http://www.irac-online.org/documents/moa/MoAv5_1.doc.

Table 5.2 Top 16 resistant arthropods, based on the number of unique active ingredients for which resistance has been reported and the number of cases reported (Whalon *et al*., 2006)

Species	Order: Family	Pest type	No. active ingredients	Cases
Tetranychus urticae	Acari: Tetranychidae	Crop	79	325
Plutella xylostella	Lepidoptera: Plutellidae	Crop	76	278
Myzus persicae	Hemiptera: Aphididae	Crop	68	293
Leptinotarsa decemlineata	Coleoptera: Chrysomelidae	Crop	48	183
Musca domestica	Diptera: Muscidae	Urban	44	183
Boophilus microplus	Acari: Ixodidae	Livestock	43	127
Blatella germanica	Blattodea: Blattellidae	Urban	42	213
Bemisia tabaci	Hemiptera: Aleyrodidae	Crop	39	169
Panonychus ulmi	Acari: Tetranychidae	Crop	38	178
Aphis gossypii	Hemiptera: Aphididae	Crop	37	103
Culex pipiens pipiens	Diptera: Culicidae	Urban	34	119
Helicoverpa armigera	Lepidoptera: Noctuidae	Crop	33	434
Heliothis virescens	Lepidoptera: Noctuidae	Crop	33	106
Culex quinquefasciatus	Diptera: Culicidae	Urban	30	229
Spodoptera littoralis	Lepidoptera: Noctuidae	Crop	30	50
Anopheles albimanus	Diptera: Culicidae	Urban	21	72

belong to a relatively small number of families of arthropods (i.e., the mite family Tetranychidae, the mosquito family Culicidae, the moth family Noctuidae, and the aphid family Aphididae).

A number of biological factors help to explain why the species in Table 5.2 have repeatedly evolved insecticide resistance. First and foremost, these species are

under intense selection for resistance. All of these pests are major targets of insecticide use because of their significant economic, and in some cases human health, impact. For example, many of the herbivorous species in Table 5.2 are pests of cotton (*B. tabaci*, *A. gossypii*, *H. armigera*, and *H. virescens*) which is a crop for which only low levels of damage are tolerated and insecticide use is particularly high. Similarly, diamondback moth is a pest of cruciferous vegetables which are sold primarily damage-free, requiring significant insecticide use. In the case of the disease vectoring species (particularly the mosquito species), the diseases they are associated with demand that control measures be intensively applied. Therefore, high insecticide use partially explains why resistance has evolved relatively often with these species. In addition, many of the herbivorous pests in this group, such as the heliothines and the sucking pests, are pests of several major crops, resulting in their being exposed to the same or similar insecticides on different crops. From a logistical perspective, IRM is particularly difficult for these species because of the need to coordinate actions across crops and regions.

Other aspects of pest biology also give the species in Table 5.2 a high capacity for evolving resistance and make resistance, once established, more likely to spread. These species are biochemically pre-adapted to evolve insecticide resistance. The herbivorous species are polyphagous and evolved to deal with a variety of plant defensive chemicals, particularly alkaloids (e.g., *L. decemlineata* and the cotton pests), and therefore had mechanisms available to de-toxify and excrete novel toxins. In addition, several of the species are capable of asexual reproduction (mites, aphids, and whiteflies), which can speed the rate of adaptation to insecticides. Furthermore, these species are typified by high rates of dispersal, with the adults being highly mobile and/or human activities contributing to their long distance movement (e.g., *B. tabaci* and *P. xylostella* may be moved on host plants that have been grown in one area to be sold in another, and many of the pest species may be found inside ships or airplanes). Many of these life history characteristics are common to related species, which helps to explain why multiple species within particular arthropod families appear to have a strong propensity to evolve insecticide resistance (Table 5.2).

Collectively, the factors described above make the species in Table 5.2 consistently high risks for insecticide resistance evolution. IRM programs for these pests must recognize these factors if they are to be successful. But the mobility and adaptability of these species also poses another challenge. Many of these species have become established globally, meaning that effective IRM programs are needed for a wide variety of agricultural and social systems. The cosmopolitan distribution of these pests presents an additional set of logistical problems. Different countries, and even different crops within a single country, often require different IRM strategies because of differences in farming practices and in pest biology. The greatest logistical challenges arise in practicing IRM in regions dominated by smallholders such as much of Africa, Asia and Central America. In these regions, agriculture is characterized by a large number of small landholders who rely on indigenous pest management and are often lacking in basic biological and ecological information about the insect pests (Abate *et al*., 2000). To illustrate the nature of these problems, and some possible solutions, the next section describes IRM efforts directed against three of these globally important pests: *B. tabaci*, *P. xylostella*, and the anopheline mosquitoes (*Anopheles* spp.).

IRM Programs for High-Risk Global Pests

Plutella xylostella

Pest Status

The diamondback moth *Plutella xylostella* feeds on plants in the family Cruciferae, including almost all cruciferous vegetable crops. It is the most destructive pest of crucifers in the world. Plant damage is caused by larval feeding. Individual larvae can only cause limited damage but many larvae may infest a single plant and many of the cruciferous crops that *P. xylostella* infests can only be marketed if they are essentially undamaged. *P. xylostella* probably arose in Europe, but has since spread to Asia and the Americas and occurs wherever crucifers are grown. It is a highly mobile species, capable of long migratory flights, and also is dispersed through human movement of infested crucifer seedlings and vegetables (Talekar and Shelton, 1993).

P. xylostella has only become a significant pest relatively recently, with major problems observed in the 1970s apparently caused at least in part by the evolution of insecticide resistance (Talekar and Shelton, 1993). This suggests that intensive insecticide use in some agricultural systems may have created an environment free of natural enemies in which pests capable of rapidly evolving insecticide resistance could thrive. Pyrethroid resistance was detected in the 1980s, and resistance to other classes of insecticides was observed soon after. A large number of modes of action are available for the control of susceptible *P. xylostella* but resistance has been observed to all but the newest modes of action in one or more regions (Table 5.3). This species has the distinction of being the only known pest species to evolve resistance to *Bacillus thuringiensis* (Bt) insecticidal proteins used under field conditions, and has done so in multiple world areas (Tabashnik *et al*., 2003). Not surprisingly, resistance issues in *P. xylostella* have been greatest in cropping areas with intensive insecticide use and benign climates, such as Southeast Asia, the Indian subcontinent, tropical Africa, and the Hawaiian Islands. Under these conditions, *P. xylostella* can infest crops year-round and farmers may apply insecticides weekly throughout the year.

P. xylostella Management in Developed Countries

In developed countries, farmers generally have access to, and can afford, a number of different insecticides when it comes to controlling any particular pest or pest

Table 5.3 Regions and mode of action groupings for which resistance has been observed for three high resistance risk species (Whalon *et al*., 2006)

Species	Regions[a]	Modes of action[b]
Plutella xylostella	Afr, Asia, Aust, NAm, SAm	1, 2, 3, 4, 5, 6, 11, 15, 18, 22
Bemisia tabaci	Asia, Eur, NAm, SAm	1, 2, 3, 4, 7, 9, 16
Anopheles spp.	Afr, Asia, Eur, NAm, SAm	1, 2, 3

[a] Afr: Africa; Aust: Australia; Eur: Europe; Nam: North America; Sam: South America.
[b] Mode of action groupings are as defined in Table 5.1.

complex. Therefore, alternation of insecticides from different mode of action groupings (Table 5.1) is one effective and widely used way of preserving and/or regaining susceptibility to different insecticidal modes of action i.e., as a proactive or reactive IRM strategy. This approach has been used to combat insecticide resistance in *P. xylostella* in the United States (Mau and Gusukuma-Minuto, 2004) and Australia (Vickers *et al*., 2001), with different groups of insecticides being used at different times of the year. These programs are known as Window Programs. For example, on several of the Hawaiian Islands, *P. xylostella* was observed to rapidly evolve resistance to spinosad soon after its introduction and spinosad had to be removed from the market. After a window program was implemented by University of Hawaii extension personnel and farmers using insect growth regulators and other modes of action, susceptibility to spinosad was recovered and the product could be reintroduced (Zhao *et al*., 2002; Mau and Gusukuma-Minuto, 2004). However, this success still may be short-lived if the window program is not maintained with a high level of farmer adoption; the intense selection for insecticide resistance that occurs with *P. xylostella* populations in the Hawaiian Islands, together with the isolated nature of these island populations, makes the risk of resistance evolution very high if strict IRM programs are not followed.

To facilitate programs of this sort, and to help farmers make good insecticidal choices more generally, the private sector, in the form of the Insecticide Resistance Action Committee (IRAC-US and IRAC-International; see http://www.irac-online.org), has worked with regulatory agencies in North America, the European Union and Australia to introduce mode of action information onto insecticidal product labels. This mode of action labeling indicates the grouping to which a particular insecticide belongs (Table 5.1) and recommends that farmers utilize multiple insecticidal modes of action where possible in controlling any given pest species.

However, effective IRM programs do not depend upon a single approach. Alternation of different insecticidal modes of action is just one component of IRM. In addition, IRM for any pest requires consistent scouting and resistance monitoring so that timely and appropriate decisions on insecticide applications can be made. In particular, effective reactive IRM programs depend on early detection of resistance and accurate characterization of the spatial distribution of resistance through monitoring (Stanley Chapter 13). For example, a coordinated resistance monitoring program was an integral part of the Hawaiian program described above (Zhao *et al*., 2002). Monitoring programs of this sort require the development of suitable assay systems for assessing pest susceptibility to different insecticides. The assay systems must be sensitive, but also simple and cost-effective, so that accurate results can be obtained quickly on a large number of insects. IRAC has helped to develop many such systems (see http://www.irac-online.org). In addition, effective resistance monitoring involves regular sampling of field populations at a sufficient frequency and intensity to be able to determine whether pest susceptibility is changing. The technical capacity to carry out monitoring work of this sort routinely exists in most countries (including many developing countries) and has been an essential part of successful IRM efforts.

Furthermore, diversified Integrated Pest Management (IPM) programs as a whole contribute to IRM by reducing pest pressure and reducing the selection pressure for resistance to any single pest control tool. For example, use of more

selective chemistries, and various types of habitat management, can better preserve natural enemy communities and thereby help to control *P. xylostella* populations. As a consequence, the number of insecticide applications needed for *P. xylostella* can be reduced (Talekar and Shelton, 1993; Vickers *et al*., 2001). Similarly, cultural controls can be important in reducing pest populations, and in slowing the spread of resistance. For example, ensuring that crucifer seedlings grown in one area of the United States for transplant to other areas are free of potentially resistant *P. xylostella* helps to slow the spread of insecticide resistance.

P. xylostella Management in Developing Countries

While the same IRM strategies used in developed countries theoretically could be implemented in developing countries, several major barriers exist. First, the tools themselves may not exist. Farmers in developing countries typically are more limited in their resources than their counterparts in developed countries and are less able to afford complex insecticidal rotation programs. In addition, a larger percentage of the insecticides available in developing countries tend to be of poor quality, or are spurious. Spurious insecticides are so common in countries like India that specific laws have been passed in an effort to stem their sale (e.g., see http://agricoop.nic.in/announce.htm#AMENDMENTS%20TO%20INSECTICIDES%20ACT, 1968). Government action of this sort, in cooperation with the private sector, can help to improve the quality of pest control tools available to farmers, and thereby broadly improve IRM across cropping systems and pests.

Second, and even more important, the IRM and IPM knowledge of smallholder farmers in developing countries tends to be much more limited than in developed countries (Pontius *et al*., 2002). Smallholder farmers usually have less access to educational material on pest management and IRM, and do not have the support of consultants and extension services that are routinely present in developed countries. As a consequence, the primary focus for IRM in developing countries must be on teaching farmers to make good pest management decisions, particularly with respect to the frequency and timing of insecticide applications.

Fortunately, a variety of regional and international organizations (some public sector, some private sector, some government-funded, some non-governmental organizations) have played major roles in developing suitable educational material and educational programs on pest management. In particular, FAO (the Food and Agricultural Organization of the United Nations; www.fao.org) plays a large part in the education and implementation of IPM and IRM programs in developing countries, particularly through Farmer Field Schools (FFS). FFS were started in 1989 in Indonesia to reduce farmer reliance on pesticides in rice and have expanded since then to vegetables, cotton, and other crops throughout Asia and other world areas. At present, FFS programs are being conducted in over 30 countries. The overall aim of FFS is make farmers become experts through hands-on training. Farmers are taught the following principles: (1) grow a healthy crop; (2) conserve natural enemies; and (3) observe crops regularly to make appropriate and timely decisions for pest control (Gallagher, 2003). A study by Williamson *et al*. (2003) explored pest management practices, decision tools and sources of pest control information of vegetable growers in Kenya. They compared FFS-trained growers with untrained growers from the same area. The FFS-trained growers had more

access to sources of pest control information, had more confidence in their pest management decision-making, and used fewer pesticide applications. This result is common to FFS programs in other countries and crops (Pontius *et al*., 2002). FFS-trained growers used regular field observations to make decisions around pesticide use, though they did not use specific insect economic thresholds. The untrained growers made pest management decisions based on dealer advice or their own experience. In some cases, the untrained grower would inspect the field shortly after the initial insecticide application and, if pests were still present, an additional application was subsequently made. Collectively, the practices taught by FFS can dramatically reduce the risk of insecticide resistance evolving in these systems by helping to ensure that insecticides are used effectively while simultaneously reducing insecticide use.

Other programs that more specifically focus on *P. xylostella* IPM and IRM also operate in developing countries, and complement the broader educational efforts of FFS and the like. In particular, substantial effort has gone into characterizing the natural enemies of *P. xylostella*, educating farmers on the benefits of preserving these biological control agents, and supplementing natural biological control through inundative releases (Sarfraz *et al*., 2005). Given that the appearance of *P. xylostella* as a globally important pest was due, at least in part, to the elimination of natural enemies through intensive insecticide use, these are logical pest management approaches to pursue. In addition, resistance monitoring work with *P. xylostella* is carried out by scientists from agricultural research institutes in many developing countries, as evidenced by the reports captured in the Michigan State University Resistance Database (Whalon *et al*., 2006). This work has resulted in a variety of recommendations for alternation (e.g., Wu *et al*., 2005a) or mixing (e.g., Attique *et al*., 2006) of multiple insecticidal modes of action for use against *P. xylostella* in countries where certain insecticides are failing. However, the means for broadly implementing (or even widely disseminating) these recommendations to vegetable farmers in affected areas do not necessarily exist in developing countries.

Bemisia tabaci

Pest Status

The sweetpotato whitefly *Bemisia tabaci* (also known as the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring) is a significant crop pest with a broad host range, attacking plants from 63 families (Mound and Halsey, 1978). In recent years, *B. tabaci* has become an important pest on cotton and vegetables. It causes three types of damage: (1) direct damage through piercing and sucking sap from plant foliage, (2) indirect damage caused by the accumulation of honeydew produced by *B. tabaci* which leads to mold growth, and (3) transmission of viruses such as cotton leaf-curl virus (CLCV).

As a consequence of extensive exposure to insecticides, *B. tabaci* has developed resistance to a wide range of insecticides. Resistance to organophosphates, carbamates, and pyrethroids is well established and involves mechanisms based on enhanced detoxification of insecticides and/or modifications to their target sites within insects (Table 5.3; Whalon *et al*., 2006). The introduction of new insecticidal

modes of action, such as the neonicotinoid insecticides that target the acetylcholine receptor in the insect nervous system, insect growth regulators that inhibit chitin biosynthesis, and a juvenile hormone mimic, offer additional means of control of *B. tabaci* in cotton.

B. tabaci Management in Developed Countries

In developed countries such as Australia and the United States, cotton production is highly mechanized and depends on efficient management of resources and maximum utilization of new technology. Cotton crop consultants play a key role in crop scouting and insect control recommendations. IPM and IRM programs and technical information are available from a number of sources including extension publications, field demonstrations, educational meetings, and the Internet. These programs employ a variety of strategies and are broadly implemented through stakeholder coalitions. In addition, mode of action labeling is being utilized on product labels (see Table 5.1; IRAC, 2005).

For example, in Australia, cotton IPM focuses on four key principles: (1) conservation and utilization of beneficial insects; (2) preferential use of selective insecticides; (3) emphasis on both profitability and sustainability; and (4) integration of all farm management activities throughout the annual cycle of production, not just the cotton season (Fitt *et al*., 2004). In the case of *B. tabaci,* there are three separate thresholds for (a) early season suppression, (b) the use of insect growth regulators, and (c) for knockdown late in the season. Thresholds are based on rates of population increase relative to the accumulation of degree days and crop development. A decision support matrix has been developed to assist in the interpretation of population monitoring data by showing how *B. tabaci* thresholds change with cumulative degree days, plant growth stage, and the type of control needed (Farrell, 2006). In addition to the use of chemical control, parasites and beneficial insects are an important part of the management. It is recommended that the use of early season broad spectrum insecticides such as synthetic pyrethroids and organophosphates be avoided to conserve the beneficial insects (Farrell, 2006). These programs reflect the input of multiple stakeholders, but particularly the National Cotton Extension Team and the Australian Cotton Cooperative Research Centre. The Extension Team utilizes a number of ways of communicating IPM messages, including field days, IPM core groups, newsletters, and Cotton Pest Management Guides. In addition, there are Best Management Practices that provide a framework that Australian growers can use to evaluate their own performance against industry standards. There also are a number of computer-based decision support systems that can be used for the analysis of pest and crop performance (Fitt *et al*. 2004).

Comparable approaches to IRM have been used in the United States for *B. tabaci*. Since the early 1990s, *B. tabaci* has displayed resistance to the pyrethroids and pyrethroid and organophosphate combinations in cotton in Arizona and California (Ellsworth and Martinez-Carrillo, 2001; Palumbo *et al*., 2001). In Arizona, the IPM program consists of a multi-level, multi-component pyramid which organizes all of the *B. tabaci* management tactics into three major "keys": sampling, effective chemical use, and avoidance (Ellsworth and Martinez-Carrillo, 2001; Palumbo *et al*., 2003; Figure 5.1). Effective chemical use is underpinned by three components: *B. tabaci* action thresholds, effective and selective chemistry,

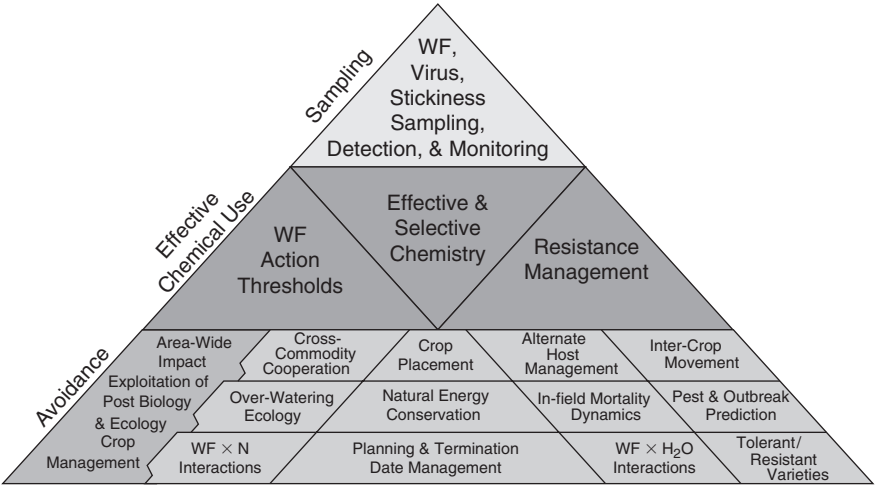

Figure 5.1 Successful management of *B. tabaci* depends upon a range of tactics that include effective chemical use and IRM, and requires coordination across crops (reprinted from Ellsworth and Martinez-Carrillo (2001) with permission from *Crop Protection* and Elsevier).

and resistance management. The *B. tabaci* action threshold in Arizona allows the cotton grower or consultant to decide when to make insecticide applications that will protect the crop against yield loss and minimize the risk of sticky cotton. Selective chemistries such as the insect growth regulators and the neonicotinoids preserve biological control agents while providing effective *B. tabaci* control. Successful adoption of the pyramid approach has depended upon organized education efforts through the University of Arizona Extension Service, including grower and/or crop consultant training and literature. Some of the success of this program also can be attributed to simultaneous improvements in IPM for other cotton pests. Since its initial introduction in 1996, transgenic insecticidal cotton has been widely adopted to control *Pectinophora gossypiella* and other lepidopteran pests in Arizona, replacing more broad-spectrum insecticides (Cattaneo *et al*., 2006). Like the selective chemistries used for *B. tabaci* control, transgenic insecticidal cotton is highly selective in its effects and thereby helps to conserve natural enemies in cotton systems. Obviously the positive interactions between the IPM programs for *B. tabaci* and *P. gossypiella* are not specific to this system; wherever possible, IPM and IRM programs should be coordinated across the major pests of a given crop.

IPM and IRM programs for *B. tabaci* often need to involve multiple crops because of the broad host range of this pest. For example, the University of Arizona, in cooperation with the National Cotton Council, has developed and implemented a cross-commodity *B. tabaci* management program with the objective to manage *B. tabaci* and harmonize insecticide use in multi-crop systems (Palumbo *et al*., 2003; Figure 5.1). The guiding principles behind this program are to maximize the efficacy and longevity of all insecticidal modes of action for all crops in Arizona attacked by *B. tabaci*. The program centers around a series of guidelines for insecticide use based upon the crop and the cropping system; recommendations are provided for insecticide use on cotton, melons, and vegetables that vary depending

Table 5.4 Recommended maximum number of neonicotinoid insecticide applications per crop season in three different sorts of cropping systems in Arizona (Palumbo *et al*., 2003)

Cropping system	Cotton	Melons	Vegetables
Multi-crop	0	1[a]	1[b]
Cotton/melon	1	1[a]	–
Intensive cotton	1	–	–

[a]Soil applications only.
[b]Soil or foliar applications.

upon whether the cropping system is cotton-intensive, cotton and melons, or a multi-crop system. Table 5.4 shows the maximum recommended number of neonicotinoid applications for cotton, melons, and vegetables in these different cropping systems. For example, in a cotton/melon system, one neonicotinoid application can be made on both cotton and melons, while in a multi-crop system, one application can be made on both melons and vegetables but no neonicotinoid applications should be made on cotton. These guidelines are intended to avoid sequential exposure of multiple generations of *B. tabaci* to insecticides with the same mode of action, with particular emphasis on controlling the total number of neonicotinoid applications (i.e., no more than two neonicotinoid applications should be made per season in any cropping system).

In addition to the University Extension service providing guidance on resistance management for *B. tabaci* in cotton in the United States, there are federal government sponsored agencies such as the Center for IPM (CIPM). The CIPM is the management entity for the National Information System of the USDA Regional IPM Centers, which coordinate information regarding pesticide use and resistance management programs. The CIPM also maintains databases for the National Agricultural Service (NASS) and the US EPA Pesticide Label System (Stinner, 2004). This coordination of IPM Centers and database systems is needed because of the vast amount of resistance management information that is generated in the United States. IRAC-US supports these types of efforts and has worked with the Southern Integrated Pest Management Center to develop literature on insecticide mode of action and IRM for cotton insect pests. The IRAC website (www.irac-online.org) has a number of publications on *Bemisia* control in both cotton and vegetables.

B. tabaci Management in Developing Countries

In developing countries, the challenges for *B. tabaci* IRM are similar to those described for *P. xylostella*; insecticide resistance is common, farmer awareness of IPM and IRM principles is limited, and spurious and low quality insecticides are rampant. The broad host range of *B. tabaci* reduces the risk of insecticide resistance evolution to some degree by ensuring that some proportion of the *B. tabaci* population remains unexposed to the insecticides used in cotton (as with other polyphagous cotton pests like *Helicoverpa armigera*; see Wu *et al*., 2002; Ravi *et al*., 2005). IRM efforts necessarily focus on field-based training in pest management, with the aim of rationalizing insecticide use.

In India, over half of the total insecticides used are applied on cotton, even though cotton only occupies 5% of the cropped area. *B. tabaci* is one of the major pests of cotton in India, and a survey carried out in 1998 revealed high levels of pyrethroid resistance in *B. tabaci* (Kranthi *et al*., 2002). However, the same survey indicated that *B. tabaci* remains susceptible to cyclodiene organochlorines, and organophosphates. In India, IPM and IRM-related activities are implemented through 26 Central Integrated Pest Management Centers (Russell *et al*., 2000; Krishna *et al*., 2003). The major activities of these Centers include conducting FFS, monitoring for pest levels, and production and release of biocontrol agents.

Similarly, *B. tabaci* is a key pest of cotton, and many vegetables, in China. Bioassay results with *B. tabaci* collected around Urumqi, the capital of Xinjiang, demonstrated that there were high levels of resistance to pyrethroids. The samples collected were less susceptible to imidacloprid than the reference susceptible strain, but the numbers were not high enough to compromise field activity (Ma *et al*., 2007). Although insecticides are currently the primary control measure for insects in cotton, IPM programs are being developed and implemented through Chinese agricultural research institutes and extension agencies. Field demonstrations are the primary tool for disseminating IPM principles to Chinese cotton growers. A measure of success has been the reduction in the number of insecticide applications from as many as 25 to as few as 10 per growing season. As with the case of Arizona, a part of this success is attributable to the simultaneous introduction of transgenic insecticidal cotton for the control of lepidopteran pests like *H. armigera*. Transgenic insecticidal cotton has been widely adopted in large parts of China. The selectivity of transgenic insecticidal cotton has helped to preserve natural enemy populations in cotton and has broadly contributed to a resurgence in cotton pest susceptibility to conventional insecticides such as pyrethroids (Wu and Guo, 2005; Wu *et al*., 2005b). Another key to the success of IPM and IRM programs for *B. tabaci* in China has been the training of cotton farmers, and one of the challenges is to reach the more than ten million cotton farmers in China (Wu and Guo, 2005).

During the early 1990s, cotton production in Pakistan declined as a consequence of heavy pressure from *B. tabaci* and the concomitant high levels of CLCV. In response to the increase of CLCV, the use of insecticides against *B. tabaci* increased significantly, which in turn led to *B. tabaci* control failures. Results from bioassays conducted at IRAC-Rothamsted and the Central Cotton Research Institute in Multan, Pakistan, and from a monitoring program established by IRAC at the University of Faisalabad, confirmed strong resistance to organophosphates and pyrethroids, and a growing threat to the effectiveness of endosulfan. Although *B. tabaci* (and CLCV) remains an important pest for cotton farmers in Pakistan, the severity has declined due to a number of IRM tactics adopted in response to the CLCV crisis. These tactics include (1) extensive education to reduce and rationalize insecticide use on cotton provided by national advisory services and the regional IRAC organization; (2) avoiding the use of broad-spectrum insecticides early in the cotton season; (3) a switch to novel and more specific products for controlling *B. tabaci* when necessary, particularly towards the end of the cotton season; and (4) identification and widespread introduction of CLCV-tolerant cotton cultivars (I. Denholm, Rothamsted, personal communication).

Anopheline Mosquitoes

Pest Status

The mosquito genus *Anopheles* contains approximately 400 species of which 30–40 are capable of transmitting malaria, with the best known being *Anopheles gambiae* in Africa and *Anopheles albimanus* in the Americas. They are important disease vectors on all continents except Australia. Anopheline mosquitoes are highly mobile and also are dispersed through human activities such as the movement of used car tires.

Mosquito control programs use a variety of physical, chemical, mechanical, cultural, biological, and educational measures. Larval control can be achieved through water management and the use of larvicides. Insecticides are also widely used to control adult mosquitoes but only four different classes of insecticides are available: organochlorines (which are now banned in most countries), organophosphates, carbamates, and pyrethroids (Zaim and Guillet, 2002). These four classes all target the insect central nervous system, which makes them fast-acting. However, their mode of action involves interference with only a single physiological process: cholinergic nerve transmission. Organophosphates and carbamates both inhibit acetylcholinesterase (Eto, 1974), while synthetic pyrethroids and DDT (organochlorines) affect voltage-gated sodium channels (Khambay, 2002). Therefore, the choices in insecticidal mode of action for the control of adult mosquitoes are much more limited than for agricultural pests like *P. xylostella* and *B. tabaci* (Nauen, 2007). Even the newest class of insecticides in this respect, the synthetic pyrethroids, was introduced more than 30 years ago. Pyrethroids are the most commonly used insecticide, and are used both as indoor residual sprays and insecticide treated bed-nets (Zaim and Guillet, 2002). In both cases, mosquitoes may be killed if they land on a treated surface or they may be repelled.

Insecticide resistance has been observed to varying extents in all regions where anophelines are present and has arisen to all four classes of chemistry used against adult anophelines (Table 5.3; Whalon *et al*., 2006). Resistance issues are greatest in tropical regions where mosquito generation times are shorter, mosquito populations are higher, and insecticidal control programs generally are less effective.

Anopheline Mosquito Management in Developed Countries

In developed countries, mosquito control programs are multifaceted and use a mixture of larval and adult control through cultural, chemical, and biological means. Larval treatments with insect growth regulators or bacterial endotoxins are widely used, as well as a variety of cultural means to reduce habitats that could potentially support mosquito larvae. Using diverse pest management approaches dramatically reduces the risk of insecticide resistance evolving.

Where the risk of resistance evolution to adulticides is significant, insecticidal rotations can be used, though the alternatives are limited as described above (Nauen, 2007). The preferred strategy is to rotate insecticides with entirely different modes of action (Table 5.1), rather than merely alternating members of one chemical class or different chemical classes that affect the same target site. For example, knockdown resistance (kdr) renders DDT and pyrethroids less effective, but carbamates or organophosphates still can be used. Furthermore, in the absence

of modified acetylcholinesterase resistance (MACE), which would affect all of these chemistries, carbamates and organophosphates could be used in rotation (Nauen, 2007).

Biochemical and molecular methods are available for detecting insecticide resistance in adult mosquitoes, and for determining the nature of that resistance. For example, pyrethroid resistance in mosquitoes may be conferred by kdr or elevated levels of microsomal monooxygenases (cytochrome P-450s), while resistance to carbamates and organophosphates can be produced by elevated levels of esterases or by MACE (Hemingway *et al*., 2004). Techniques exist to distinguish these different types of resistance, including biochemical assays of enzymatic activity and a variety of highly sensitive molecular methods (e.g., Benting *et al*., 2004). However, the capacity to carry out the newer molecular methods is limited, even in developed countries.

Anopheline Mosquito Management in Developing Countries

In developing countries, IRM for anopheline mosquitoes is challenging because of widespread resistance, limited alternative tools, and misuse of the available tools. Larval treatments are relatively ineffective because of the difficulty in identifying and treating a high enough proportion of the larval habitat, so there is increased reliance on treating for adult mosquitoes. DDT may be the best tool for use in indoor residual sprays, but is not approved for use in many countries. The value and impact of bed-nets on IRM is unclear. On the one hand, the widespread use of pyrethroid-impregnated bed-nets might be expected to increase the selection pressure for pyrethroid resistance. However, the repellent effect of insecticide treated bed-nets actually may reduce selection for physiological tolerance to pyrethroids if these two responses are negatively correlated, as has been shown in other species such as *P. xylostella* (Hoy *et al*., 1998). Regardless, additional education is needed to increase the use of insecticide treated bed-nets where they are available.

A number of international organizations have substantial educational programs focused on anopheline control and management, including the World Health Organization (WHO) and the Bill & Melinda Gates Foundation. Some of these same organizations also support resistance monitoring programs. For example, WHO has published a number of monographs on methods for monitoring resistance to various insecticides, including diagnostic dose bioassays for mosquitoes (see www.who.org), and IRAC has sponsored a long-term project on monitoring and management of mosquito resistance in Mexico (Hemingway *et al*., 1997; Penilla *et al*., 2006). In addition, IRAC has produced a manual on IRM for insect disease vectors (IRAC, 2007). However, more broad-based programs are needed for the control of anophelines and other disease vectors in developing countries. These programs need to combine resources from the public and private sector to increase the number of effective tools available and to effectively implement control strategies. A model for such programs may be a new initiative supported by the Bill & Melinda Gates Foundation, which has led to the formation of the Innovative Vector Control Consortium. The Consortium brings together academia and industry to improve the portfolio of chemical and technological tools available for combating insect-vectored diseases (Hemingway *et al*., 2006).

Conclusions

IRM is not easy, particularly in developing countries, but experience indicates that it can be highly successful in prolonging the durability of insecticides even when dealing with pests with a high risk of resistance evolution. Proactive resistance management in the form of more effective product design and constraining product use is most effective where feasible, but reactive IRM programs also have been successful in regaining susceptibility to insecticides where resistance had already evolved. Where the target pests are polyphagous, effective IRM programs have coordinated farmer practices and insecticide use patterns across different host crops. In all cases, resistance monitoring and intensive education also are essential components of successful IRM programs. Substantial coordinated efforts, and prominent successes, can be pointed to in the United States and Australia.

However, focusing on the IRM successes in developed countries can be misleading when it comes to implementing programs in the developing world. In countries like the United States and Australia, the amount of access that farmers have to educational material and technical advisors such as extension personnel is vastly greater than in developing countries. It is no coincidence that, even within developed countries, the most effective IRM efforts have occurred in cropping systems and regions where farmers are highly aware of resistance as an issue and where they receive strong consistent support from local experts (e.g. cotton systems in Australia and Arizona). In these situations, farmers recognize the need to practice IRM, they have access to large amounts of IRM information, and they have confidence in their technical advisors and the recommendations they provide. Successful resistance management programs enroll a broad set of stakeholders through carefully targeted educational programs and employ a range of tactics that are chosen to complement pest management practices already in place (Forrester, 1990). Additionally, growers and/or crop consultants must be able to understand the program and its benefits, both in terms of pest management and economics. Programs that are too complex and/or expensive will not be adopted, whatever their potential benefits might be (Hurley and Mitchell Chapter 11).

In developing countries, these challenges are greater because of the scale of the systems and the limited resources, but the potential rewards also are greater because of the high levels of insecticide use and relatively low yields in many countries. In developing countries, IRM must focus more on basic education of farmers, particularly around rational insecticide use, because that is an essential building block for all IPM and IRM programs, and because the overall level of knowledge of insecticides and IPM tends to be low (Pontius *et al*., 2002). Additional public and private sponsored IRM programs are needed that focus on practical approaches to IPM and IRM, particularly in Asia and Africa. Given the limited resources and technical capacity in most of the countries in these regions, many of these programs will need to be driven by international and regional organizations.

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Chapter 6

Negative Cross-Resistance: Past, Present, and Future Potential

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Introduction

Two of the more important scientific events of the 20th century have arguably been (i) the discovery, development, and large-scale use of antibiotics and (ii) the green revolution. Antibiotics have dramatically reduced the mortality rates in the human population that occur as a result of bacterial diseases. Antimicrobial compounds have also been used to reduce mortality rates in livestock, thereby allowing for greater levels of production and in some cases a reduction in cost for sources of human dietary proteins. Additionally, the green revolution, with its large-scale use of insecticides, has increased both the quantity and quality of food for this expanded human population. However, the Achilles' heel of these scientific advances has been the evolution of resistance, both in microbes and in insects.

Although efforts have been made to slow the development of resistance to antibiotics and insecticides, the evolution of resistance is considered to be inevitable. Once widespread resistance develops, costly mitigation measures are implemented. Thus, the focus of the academic and industrial research community has often been to identify and deploy novel antibiotics and insecticides with different modes of action. One alternative to this use-and-discard approach involves negative cross-resistance (NCR) strategies to control organisms containing resistance alleles (Ogita, 1961a, b, c; Chapman and Penman, 1979; Cilek *et al*., 1995; Pittendrigh *et al*., 2000; Khambay *et al*., 2001). NCR occurs when a mutant allele confers resistance to one toxic chemical and hyper-susceptibility to another. Thus, in practical terms, a NCR toxin is a compound that can be used to preferentially kill insects that are resistant to another insecticide (Figure 6.1).

The concept of NCR is not new, with examples dating back to the early 1960s (Ogita, 1961a, b, c). As well, NCR occurs across a wide array of toxins and organisms, including insects (Peiris and Hemingway, 1990; Hemingway *et al*., 1993), weeds (Oettmeier *et al*., 1991; Gadamski *et al*., 2000; Poston *et al*., 2002), and fungi (Josepovits *et al*., 1992; Vanden Bossche, 1997; Hollomon *et al*., 1998; Leroux *et al*., 2000) (Table 6.1). However, few commercial examples of NCR exist for insect control (Yamamoto *et al*., 1993; Hoy, 1998; Kamidi and Kamidi, 2005). Why then has NCR played such a limited role in pesticide management?

Traditionally, much of the effort in the industrial entomology community has focused on two areas: (i) identifying toxins with novel modes of action and

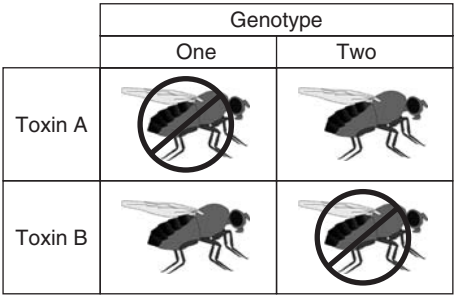

Figure 6.1 NCR refers to a situation where (i) toxin "A" causes higher mortality rates to a genotype carrying "allele one", than a genotype carrying "allele two" and (ii) toxin "B" causes higher mortality rates to a genotype carrying "allele two", than a genotype carrying "allele one".

Table 6.1 Examples of organisms where toxin pairs have been observed to cause negatively-correlated or negative cross-resistance

Organism	Toxin pair(s)	References
Insects		
Drosophila melanogaster	DDT and deltamethrin[a]	Pedra *et al*. (2004)
	DDT and phenylthiourea[b]	Ogita (1961a, b, c)
Plodia interpunctella	*Bacillus thuringiensis* (Bt) toxins	Van Rie *et al*. (1990)
Helicoverpa zea	Bt toxins Cry1Ac and Cry1F	Marcus (2005)
Helicoverpa armigera	Bt toxins	Liang *et al*. (2000)
Pectinophora gossypiella	Bt toxin and gossypol	Carrière *et al*. (2004)
Musca domestica	Pyrethroids and dihydropyrazoles[c]	Khambay *et al*. (2001)
	Pyrethroids and amides[d]	Elliott *et al*. (1986)
Heliothis virescens	AaIT and pyrethroids[e]	McCutchen *et al*. (1997)
Haematobia irritans	Pyrethroids and diazinon[f]	Sheppard and Marchiondo (1987)
Nephotettix cincticeps	*N*-propylcarbamate and *N*-methylcarbamate[g]	Yamamoto *et al*. (1993)
Tetranychus urticae	Organo-phosphates and synthetic pyrethroids	Chapman and Penman (1979)
Plants		
Conyza Canadensis and *Echinochloa crus-galli*	Atrazine and triazine	Gadamski *et al*. (2000)
Amaranthus hybridus	Pyrithiobac and imazethapyr	Poston *et al*. (2002)
Fungi		
Ustilago maydis	Benzimidazoles and diethofencarb[h]	Ziogas and Girgis (1993)
Botrytis cinerea, *Venturia nashicola* and *Venturia inaequalis*	Benzimidazole and *N*-phenylanilines	Josepovits *et al*. (1992)
Botrytis cinerea	Benzimidazoles and phenylcarbamates[i]	Hollomon *et al*. (1998)
Mycosphaerella graminicola	Triazoles and pyrimidine derivatives/triflumizole[j]	Leroux *et al*. (2000)

The following alleles, genes, or loci are associated with the respective toxin pairs given above: [a]*para*$^{ts-1}$ (voltage sensitive sodium channel); [b]*Rst(2)DDT* (differential expression of one or more cytochrome 450s); [c]*super-kdr* (super-knock-down resistance); [d,e]*kdr* (knock-down resistance); [f]cytochrome P450; [g]AChE(acetylcholinesterases); [h,i]β-tubulin (single amino acid changes); and [j]P450 sterol 14!-demethylase.

(ii) improving the efficacy and spectrum of toxins that have already been discovered (Broadhurst, 1998). Screening for novel toxins typically involves automated systems where tens of thousands of compounds are tested against multiple insect species at once (Broadhurst, 1998). In the agricultural and pharmaceutical industries, high throughput (HTS) and ultra high throughput screening (UHTS) are used to evaluate in excess of 100,000 compounds a year (Kniaz, 2000; Curtis *et al*., 2004). In contrast, the systemic investigation of NCR compounds has been restricted to academic laboratories and involved testing a few dozen compounds (Oettmeier *et al*., 1991; Palmer *et al*., 1991; De Prado *et al*., 1992; Tabashnik *et al*., 1996; Hedley *et al*., 1998; Pedra *et al*., 2004). It is not known to what extent industry has used these systems to develop NCR factors, however, no NCR-based products have been forthcoming. In part, this may be due to logical business models that necessarily develop new products based on market needs and value capture. In this regard, NCR products would only be developed in response to verified resistance to currently marketed products. However, in principle the same large-scale screening processes used to discover current insecticidal compounds can be used for NCR discovery. In fact, advances in molecular cloning and expression of peptides in display technologies could allow for the rapid develop of NCR products in a reactionary manner.

In this chapter we will (i) explore the current status of NCR in the peer-reviewed literature, (ii) examine discovery strategies in more detail, (iii) discuss how to deploy the resulting NCR compounds, and (iv) address potential limitations and possible future opportunities for such an approach in resistance management.

Existing Examples of NCR

NCR has been shown to occur in pairs of toxins active against a wide variety of insects, including *Plutella xylostella* (Chen *et al*., 1993), *Blattella germanica* (Hemingway *et al*., 1993), mosquitoes (Peiris and Hemingway, 1990), *Tetranychus urticae* (Hatano *et al*., 1992), *Cydia pomonella* (Dunley and Welter, 2000), and *Haematobia irritans* (Table 6.1 and Cilek *et al*., 1995).

In some cases, NCR has been associated with a single amino acid change in the targeted allele. For example, NCR between the fungicides benzimidazoles and diethofencarb in *Ustilago maydis* was due to a mutation at a single locus (Ziogas and Girgis, 1993). This single locus NCR scenario has also been observed in *Drosophila melanogaster*, where Pedra *et al*. (2004) observed that a DDT-resistant strain, known as $para^{ts-1}$, was highly susceptible to deltamethrin.

One of the best studied areas for NCR is pyrethroid resistance associated with voltage-sensitive sodium channel (VSSC). The VSSC has been well documented as the target site for both DDT and pyrethroid insecticides (Van den Bercken and Vijverberg, 1980; Narahashi and Lund, 1980; Vijverberg *et al*., 1982; Pittendrigh *et al*., 1997; Lee *et al*., 1999). There are several mutations that can occur in the VSSC, which result in pyrethroid resistance, and also confer NCR to other pesticides.

In *D. melanogaster* the $para^{ts-1}$ allele has a mutation (and an alternative splice form) in the α-subunit of the VSSC. The $para^{ts-1}$ *D. melanogaster* strain is so named because when the fly line is heated to 37ºC the flies become paralyzed. When the flies are returned to room temperature they are no longer paralyzed. The $para^{ts-1}$ allele also confers DDT resistance (Pittendrigh *et al*., 1997). In a small-scale screen

of pyrethroids, Pedra *et al*. (2004) were able to discover a NCR toxin (deltamethrin) for the $para^{ts-1}$ allele. Subsequently, population selection experiments were performed to demonstrate that DDT and deltamethrin could be respectively used to increase and decrease the frequency of $para^{ts-1}$ in a population of *D. melanogaster* containing both the $para^{ts-1}$ and wild-type alleles (Figure 6.2). The allelic frequency of $para^{ts-1}$ was calculated by heating males in the population to 37ºC and determining the number that became paralyzed; $para^{ts-1}$ is sex-linked and recessive, thus obvious in males, which only have one X chromosome.

Another example of a possible VSSC NCR scenario involves use of an insect-selective neurotoxic peptide (AaIT), which has been transformed into baculovirus. AaIT is more toxic to *Musca domestica* and *Heliothis virescens* that are tolerant of pyrethroids (via *knock-down resistance*, *kdr*) than are pyrethroid susceptible strains (Elliott *et al*., 1986; McCutchen *et al*., 1997). The *kdr* phenotype is caused by a single amino acid change in the VSSC and represents a major mechanism of resistance to pyrethroids. Deployment of AaIT against the *kdr* phenotype may be useful in reducing the allelic frequency of the *kdr* alleles in the population (McCutchen *et al*., 1997). The usefulness of AaIT for minimizing resistance in the field will depend on (i) its selective toxicity to the various alleles of *kdr*-type resistance that may occur in field populations of pest insects and (ii) whether scientifically acceptable and economically feasible delivery strategies can be developed.

NCR target site insensitivity has also been observed in *super-knock-down resistance* (*super-kdr*) and pesticide susceptible houseflies (Khambay *et al*., 2001). The *super-kdr* phenotype is caused by two amino acid changes in the VSSC where one of these amino acids changes is analogous to the $para^{ts-1}$ mutation (although they are in

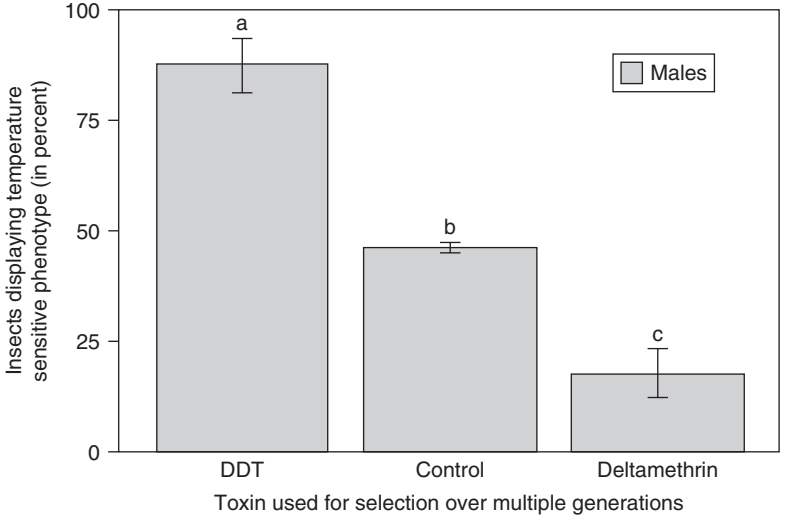

Figure 6.2 Bar graphs showing the number of males displaying the temperature sensitive phenotype after five generations of selection with: (a) DDT, (b) no selection, or (c) deltamethrin. The starting frequency of the wild type and $para^{ts-1}$ alleles were each 50%. In males, the temperature sensitive phenotype is a direct measure of the allelic frequency of $para^{ts-1}$ in the population (reprinted from Pedra *et al*., 2004, with permission from *Pesticide Biochemistry and Physiology* and Elsevier).

different domains of the channel) (Williamson *et al*., 1996; Pittendrigh *et al*., 1997). The *super-kdr* houseflies exhibit very high levels of resistance to pyrethroids, but appear to be more sensitive to *N*-alkylamides than the pyrethroid susceptible strains.

Since there are several mutations that can occur in the VSSC, which result in pyrethroid resistance, it is not known if any one NCR compound would be effective against all these mutations. For example, in *D. melanogaster*, $para^{ts}$ alleles other than $para^{ts-1}$ conferred DDT resistance but did not show NCR with deltamethrin (Pittendrigh *et al*., 1997).

D. melanogaster has also been used as a model system to understand the molecular mechanisms of metabolic pesticide resistance and NCR factors associated with tolerance to it. Ogita (1961a–c) observed that *D. melanogaster* strains metabolically resistant to DDT were more susceptible to phenylthiourea (PTU) in their diets than the DDT susceptible strains. The DDT-resistant strains are thought to metabolize PTU into the more toxic phenylurea, thus causing greater toxicity in the resistant insects. The DDT susceptible insects are less capable of metabolizing PTU into phenylurea, thus allowing the susceptible strains to better survive on the media containing PTU.

Metabolic NCR has also been observed in several populations of pyrethroid resistant *H. irritans*, which are in turn highly susceptible to diazinon (Sheppard and Marchiondo, 1987; Crosby *et al*., 1991; Barros *et al*., 2002). The increased resistance to pyrethroids and susceptibility to diazinon is thought to be due to increased cytochrome P450 activity in the resistant flies (Cilek *et al*., 1995).

Several practical applications of NCR exist in the literature. For example, *N*-propylcarbamate and *N*-methylcarbamate have been used to control *Nephotettix cincticeps* populations containing mutant and wild-type acetylcholinesterases (Yamamoto *et al*., 1993). The use of *N*-methylcarbamate on the *N. cincticeps* population selected for a population more susceptible to *N*-propylcarbamate and vice versa. Yamamoto *et al*. (1993) were able to shift the resistance level back and forth by alternating between using the two aforementioned carbamates. Additionally, Chapman and Penman (1979) observed that some mite populations resistant to organophosphates in the field were also hyper-susceptible to synthetic pyrethroids.

Recently, Kamidi and Kamidi (2005) used what they proposed was an NCR strategy to reduce tick infestation of a Kenyan dairy herd. Using two commercially available acaracides, chlorfenvinphos and amitraz, they effectively managed resistance and population size in the tick population to help reduce the incidence of diseases in cattle associated with the ticks. According to R. Kamidi (personal communications) they still have not recorded any tick borne diseases on the farms being tested, except in one instance where pesticide applications had been missed. Other groups in Kenya have met with similar success using this strategy.

NCR versus Just Negatively Correlated Resistance

One critical point to remember is that NCR refers to a scenario where the locus causing increased levels of resistance to "toxin A" is the same locus causing hyper-susceptibility to "toxin B" (Figure 6.3a). Theoretically it is possible that an insect strain displays negatively correlated resistance without actually being negatively cross-resistant. For example, insects may be highly resistant to "toxin A" due to "locus 1" but may be hyper-susceptible to "toxin B" due to "locus 2"

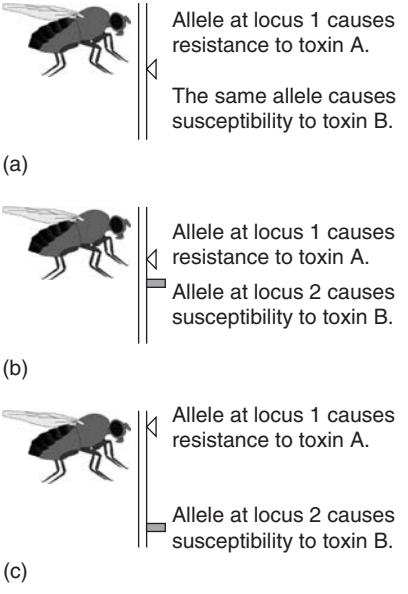

Figure 6.3 NCR versus strictly negatively correlated resistance. In (a) a given allele (or alleles) from a single locus confers resistance to one toxin and hyper-susceptibility to a second toxin (NCR). In (b) one locus confers resistance to one toxin and another tightly linked loci confers hyper-susceptibility to a second toxin (negatively correlated resistance). In (c) one locus confers resistance to one toxin and another distant locus that confers hyper-susceptibility to a second toxin (negatively correlated resistance).

(Figure 6.3b&c). For negatively correlated resistance, where two separate loci are respectively involved in resistance and hyper-susceptibility, the linkage between such loci will also be important in how effective "toxin A" will be in reducing the allelic frequency of resistance to the "toxin B" (and vice versa). The more tightly linked the loci, the more likely they can be selected back and forth in a NCR strategy (Figure 6.3b). However, the less tightly linked these loci are the less effective such a paired compound strategy will be in managing resistance (Figure 6.3c).

Screening and Development of NCR Toxins

Based on a lock-and-key understanding of how toxins impact target systems, we can begin to design (or selectively screen) toxins useful in an NCR strategy. Two such examples exist in the literature. First, Oettmeier *et al*. (1991) were able to demonstrate that specific amino acid changes in the photosystem II D-1 protein confers resistance to a triazinone herbicide. They were also able to determine the position and substituted groups in the herbicide that conferred NCR to the protein coded for by this mutant allele, defining a lock-and-key relationship between the pesticides and the NCR mechanism.

Second, Hedley *et al*. (1998) were able to selectively screen compounds that provided NCR in insecticide-resistant *Myzus persicae*, based on the knowledge that the mechanism of resistance in this insect was through increased esterase activity. Thus, they tested compounds that were bioactivated by esterase activity, so that aphids with higher esterase activity were more sensitive to their effects than the wild-type insects. Unfortunately, the most potent NCR factor observed, monofuoroacetic acid, would not be practically applicable.

Both aforementioned studies provide evidence that compounds can be designed or selectively screened based on *a priori* knowledge of the target site, to provide for NCR to metabolic resistance (Hedley *et al*., 1998) as well as target site insensitivity

(Oettmeier *et al*., 1991). Structure-based (rational) design of NCR compounds could be employed where we have an in-depth understanding of the molecular mechanisms by which insects have developed resistance to an initial class of pesticides. Industrial laboratories have already used structure-based (rational) design to develop herbicides, fungicides, and insecticides (Walter, 2002). As our understanding of insecticidal molecules, their respective target sites, and the molecular mechanism of resistance in pest species increases (Schnepf *et al*., 1998) the scientific community will be in a better position to determine the feasibility of developing NCR toxins for specific forms of pesticide resistance.

However, several challenges to the discovery of NCR compounds exist for insect control. If the approach is based on a whole animal screen separate homozygous susceptible and resistant insect populations must be maintained since it will be critical that NCR compounds are screened against heterozygous insects. Initial screens for NCR compounds will involve bioassays with the homozygous susceptible and resistant insect populations (Pittendrigh and Gaffney, 2001). The NCR compounds discovered from these screens would then have to be tested against heterozygotes (crosses between the homozygous susceptible and resistant insects) in order to determine which putative NCR compounds would also be effective in killing heterozygous insects (Pittendrigh and Gaffney, 2001). Rearing resistant strains and performing crosses to maintain them are a cost that will have to be factored into the approach and may prove to be prohibitively expensive. However, live animal screens have a distinct advantage in so far as effects observed in the laboratory give very high confidence of utility in the field.

Screening strategies based on either the specific target site receptor, e.g., *Bacillus thuringiensis* (Bt) toxins, or on a specific metabolic resistance mechanism, e.g., esterases, are very conducive to HTS approaches routinely used in industrial laboratories. Since the screening and development of NCR compounds is likely to be reactionary in nature given that *a priori* knowledge of the nature of the resistance is usually lacking, HTS approaches will probably be necessary for rapid development of NCR compounds. The development of transgenic insect lines like those developed in *D. melanogaster* may provide a means of capturing the best attributes of both the live animal and biochemical screening approaches.

Another strategy involves insect transformation with resistance alleles. Crystal delta-endotoxins (Cry proteins) are a class of insecticidal proteins found in the soil dwelling bacterium Bt. Cry proteins kill insects through a receptor mediated process. Cry1Ac is a Bt protein with a high degree of specificity against lepidopteran insects such as *Manduca sexta*. *D. melanogaster* lack a midgut receptor for Cry1Ac and so are not negatively affected by it (Figure 6.4a). Gill and Ellar (2002) transformed *D. melanogaster* with a gene that encodes a Cry1Ac-binding aminopeptidase N receptor (APN) obtained from *M. sexta*. The APN protein was expressed in the digestive system of *D. melanogaster* and the flies became susceptible to Cry1Ac (Figure 6.4b).

Others have also isolated or identified additional Bt receptors from other insects (Morin *et al*., 2003; Rajagopal *et al*., 2002; Xie *et al*., 2004; Flannagan *et al*., 2005; Griffitts *et al*., 2005). In cases where resistance is associated with a Bt Cry receptor, or similar receptor mediated process, this approach, could be used to transform *D. melanogaster* with resistance alleles isolated from resistant pest insects and the resulting transgenic flies could be screened to identify NCR Bt Cry variants (or other classes of compounds for NCR).

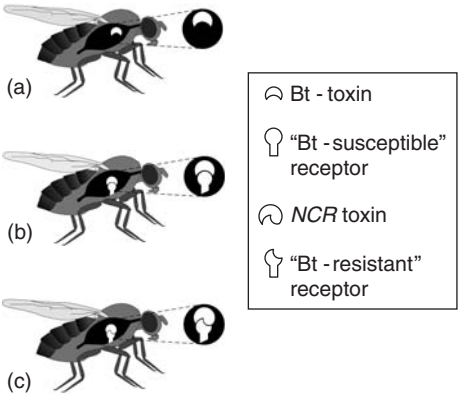

Figure 6.4 Hypothetical example of use of transgenic *D. melanogaster* to discover NCR toxins. In (a) *D. melanogaster* does not have the receptor that would make it susceptible to a given toxin (e.g., non-transgenic *D. melanogaster* and Bt-Cry1Ac; Gill and Ellar (2002)). (b) When Gill and Ellar (2002) made transgenic *D. melanogaster* with an aminopeptidase *N* (APN) receptor from *Manduca sexta*, the transgenic flies were now susceptible to Bt-Cry1Ac. (c) Transgenic *D. melanogaster* could also be created with alleles that confer resistance to the first toxin, and the resultant strains could be used to discover or test putative NCR toxins useful in pest control.

The utility of combining a biochemical screen with a live animal assay is possible in part because *D. melanogaster* are very easy and inexpensive to rear and maintain (Figure 6.4c). Additionally, separate fly strains could be developed for each novel resistance allele discovered in the field. Transgenic *D. melanogaster* strains can also be produced that express both the susceptible and resistant forms of receptor, so that the putative NCR compounds identified in initial screens could subsequently be tested for their potential toxicity against the heterozygous insects. Thus, most or all of the allelic forms of resistance for any given trait could be screened for NCR using a set of transgenic *D. melanogaster* strains.

Future studies will need to be performed to determine the feasibility of such an approach, however, all the tools necessary to develop this strategy are currently available for *D. melanogaster*. Regardless of the success of using *D. melanogaster* in such a strategy, the fact that we are continually gaining a better understanding of the molecular mechanisms by which insects develop resistance to pesticides means that *in vivo* or *in vitro* screening strategies for NCR compounds are now feasible. Additionally, other emergent technologies hold out possibilities for the discovery and development of peptides or proteins useful in NCR.

An example of a purely biochemical approach to screening is affinity selection using phage display technologies (Sidhu *et al*., 2000). Phage display has been employed as a process to rapidly screen very large peptide libraries to select peptides for high affinity binding to a given target. In fact, current molecular techniques can easily facilitate production of variant peptides even to the point of saturation mutagenesis of every amino acid residue in a peptide target. The premise is that specific changes in the primary structure that enhance binding affinity will also enhance biological activity. For example, Marzari *et al*. (1997) used phage display to identify Cry1Aa toxin regions implicated in receptor binding, Similarly, Koiwa *et al*. (1998) used a similar approach to identify plant cysteine proteinase inhibitor variants (cystatins) for use in control of *Callosobruchus maculates*, a Coleopteran pest of cowpeas. Thus, where the target molecule is known, phage display can be used as a strategy to discover molecules that are more effective in killing pest insects (Koiwa *et al*., 1998; Koiwa *et al*., 2001).

Where the pesticide-target protein and NCR allele (or alleles) of the respective gene is (are) known, phage display could be used to identify NCR toxins. The protein that

results from the resistance allele, could be used as the selective agent in phage display. This approach may be useful in identifying NCR toxin variants or chimeras built from multiple unrelated sources (Figure 6.5). Other approaches, such as combinatorial chemistry, may also be useful especially for non-peptide chemistries for development of NCR compounds useful in insect control.

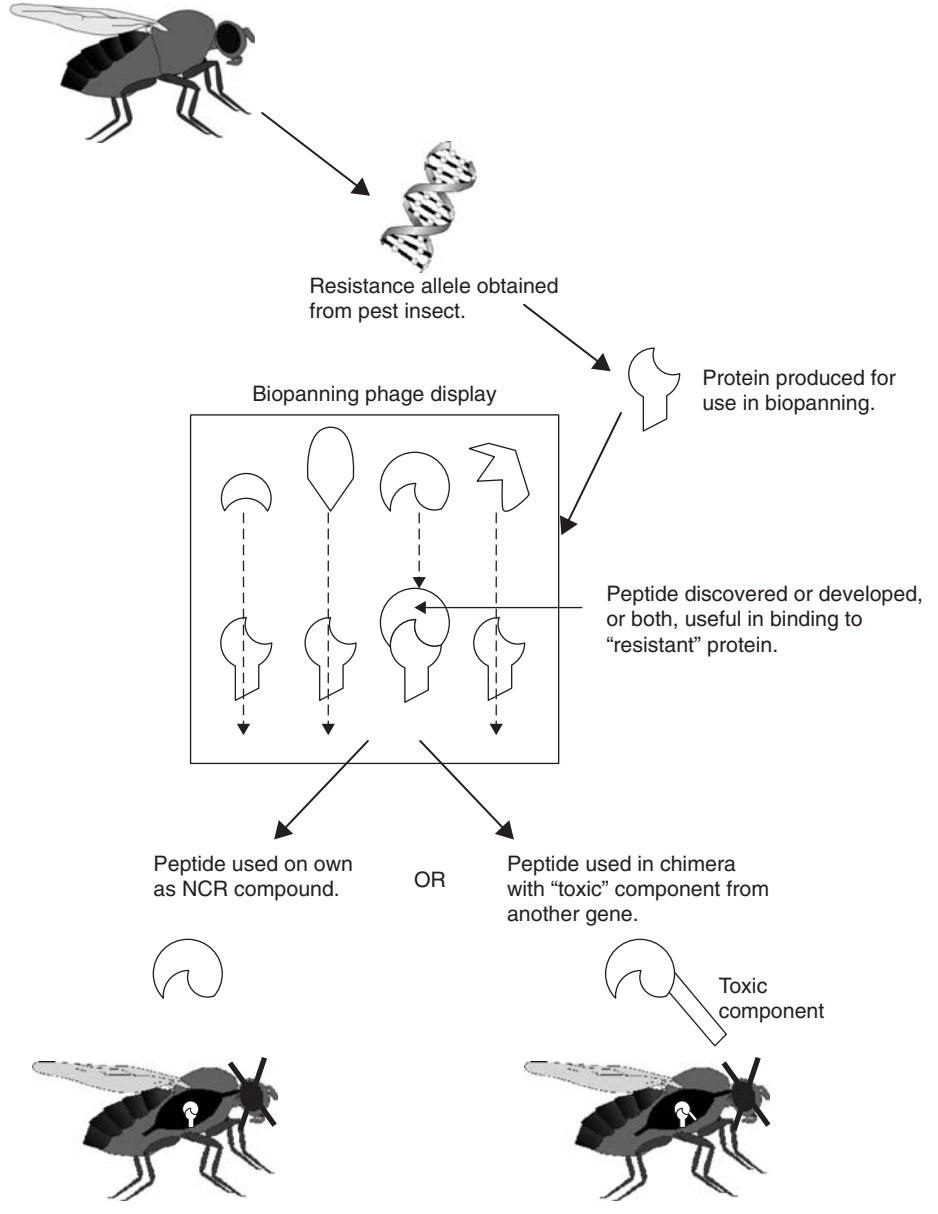

Figure 6.5 Phage display bioscanning could be used to discover polypeptides that selectively interact with proteins that are coded for by resistance genes.

Deployment Strategies

A variety of NCR deployment strategies have been suggested, including: (i) rotation of NCR compounds; (ii) periodic pyramiding of the NCR compounds with a separate group of pesticides (with different modes of action) to both concurrently minimize the pest population and use the NCR compound to minimize the resistance alleles in the insect population, and (iii) continuous pyramiding of the NCR compounds with a separate group of pesticides (Pittendrigh *et al*., 2000). However, some of these strategies might require constant monitoring of resistance levels in the insect populations, something which may not be economically feasible. Additionally, the use of multiple compounds or traits in a cropping system could present significant challenges to industry both in terms of discovery of those compounds as well as in breeding and deployment.

One approach that might prove feasible is the use of an "active refuge" (Pittendrigh *et al*., 2004). This NCR strategy would take advantage of the high-dose refuge strategy currently employed for the management of resistance in transgenic plants expressing insecticidal toxins (Figure 6.6a). The "active refuge" strategy involves reactive deployment of the NCR toxin in the refuge (Pittendrigh *et al*., 2004), where the NCR toxin acts as a "filter" to keep the resistance alleles out of the insect population (Figure 6.6b). The active refuge approach is particularly attractive since modeling experiments suggest that the NCR toxins do not have to be particularly effective in killing the homozygous (RR) and heterozygous (RS) resistant insects in order to control resistance in the insect population for many generations (Figure 6.7). In fact, even with a small refuge size (e.g., 4%) an NCR compound deployed in the refuge that killed only about 40% of the heterozygous (RS) insects was highly effective in keeping the resistance allele at a low frequency for many generations beyond the currently used "passive refuge".

This fact may prove to be particularly attractive since the discovery of moderately effective compounds is far easier than the discovery of "block-buster" products.

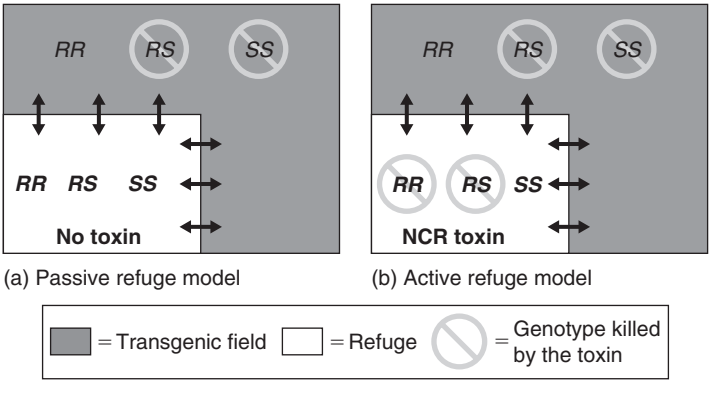

Figure 6.6 Passive versus active refuges. (a) No genotype-specific mortality occurs in the passive refuge. (b) Both the resistant homozygous (RR) and heterozygous (RS) resistant insects are killed by the NCR toxin in the active refuge. In both the passive and active refuges the primary plant-protectant gene in the "transgenic field" selectively kills the *RS* and *SS* individuals (reprinted from Pittendrigh *et al*. (2004), with permission from *Journal of Theoretical Biology* and Elsevier).

Indeed, the focus in large-scale commercial screening is often the discovery of compounds that produce high mortality among heterozygous (RS) insects, or mass-kill toxins. Thus potentially useful NCR compounds might have been deprioritized since they alone would not be highly effective in controlling the size of the insect population and would thus not be commercially viable.

That a NCR approach is feasible with the current array of biotechnology-derived crops is supported by several lines of evidence. One of the first reported cases of NCR with Bt toxins was in *Plodia interpunctella* (Van Rie *et al*., 1990). NCR (or negatively correlated resistance) has also been documented in *Helicoverpa zea* between the Bt toxins Cry1Ac and Cry1F (Marcus, 2005) and with other Bt toxin combinations in *Helicoverpa armigera* in China (Liang *et al*., 2000). These cases hold out the possibility that NCR factors may be discovered or developed with the potential for use in an active refuge strategy for resistance management.

Recent work by Carrière *et al*. (2004) raises another possibility for management of Bt resistance alleles using NCR and natural products. Carrière *et al*. (2004) observed that Bt resistant *Pectinophora gossypiella* had higher fitness costs in the presence of gossypol in their diet as compared to the Bt susceptible counterparts. Gossypol is a plant secondary compound found in cotton and when incorporated into artificial diet caused some Bt-resistant *P. gossypiella* strains to display greater delays in developmental time and decreased pupal weights as compared to the Bt-susceptible strains.

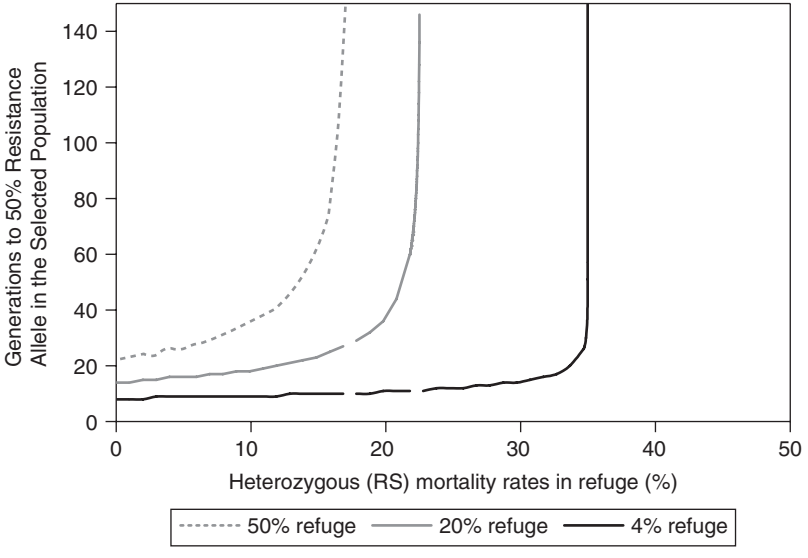

Figure 6.7 Effects of varying refuge size (*G*) and increasing heterozygous, *RS*, mortality rates, in the refuge, on delaying the development of resistance in the insect population when an active refuge is used (i.e., an NCR compound in deployed in the refuge). Details of the assumed conditions are given in Figure 3 of Pittendrigh *et al*. (2004). This figure demonstrates that NCR compounds deployed in the refuge only needs to kill a small number of the heterozygous (RS) individuals (less than 40%) in order to dramatically delay the time it takes for the resistance allele to become common (50% frequency) in the insect population. Low toxicity NCR compounds should be easier to discover and develop than high toxicity NCR compounds (reprinted from Pittendrigh *et al*. (2004), with permission from *Journal of Theoretical Biology* and Elsevier).

Additionally, recent work by Gassmann *et al*. (2006) demonstrated that entomopathogenic nematodes selectively increased fitness costs for *Cry1Ac* Bt-resistant *P. gossypiella*. Their work centered on the interaction of entomopathogenic nematodes and *Cry1Ac* resistant *P. gossypiella*. This interaction is complex, involving the resistant insect as well as a highly pathogenic bacterial symbiont of the genus *Xenorhabdus*, which is in turn closely related to bacteria of the genus *Photorhabdus*. Previous work has shown that *Photorhabdus* and *Xenorhabdus* bacteria produce extremely toxic insecticidal proteins (Forst *et al*., 1997; Bowen *et al*., 1998; Brown *et al*., 2004). While it is not clear if the nematode or the insecticidal proteins or an interactive combination of the two are responsible for the NCR effect, the work by Gassmann *et al*. (2006) does demonstrate that NCR mechanisms (increased fitness cost for the resistant insects) operate in naturally occurring biological systems. This work holds out the possibility that naturally occurring host plant resistance factors, or life history tradeoffs, may have the potential for managing resistance through a NCR-type or ecological NCR strategy.

The fact that naturally occurring host plant resistance factors may play a role in NCR (Carrière *et al*., 2004) raises an interesting question that has not been sufficiently addressed in the literature. Are there sets of NCR compounds that are found in different classes of plants? If so, how have these compounds shaped food choices and the evolutionary history of different insect species? For example, if an insect evolves the ability to detoxify compounds from one host plant, does this in turn make the insects more susceptible to another group or class of host plants? Therefore, suppose an insect population contained a novel allele ("Allele 2") that allowed the population to preferentially survive on host plant "A", but reduced the insect's fitness on its original host plant (host plant "B"). Insects with "Allele 1" would likely remain on their original host plants (host plants "A"), but those insects carrying "Allele 2" would preferentially survive on host plant "B". This is an area of research that needs further investigation, to determine if such phenomena exist and, if so, what role does NCR play (if any) in the evolution of plant-insect interactions.

Additional Issues

The Third Allele

Regardless of the deployment strategy used, a key question to address will be the likelihood of the development of resistance to a NCR toxin pair. First, let us assume that a screening strategy for NCR compounds reveals a series of putative compounds useful for development for a practical NCR deployment strategy. The next question is which of these compounds should proceed to commercial development. Many factors will influence this decision but obviously it would be in a company's best interest to develop an NCR compound that has the longest commercial life. To that end, a factor to consider is the probability that a third allele arises in the insect population that confers resistance to both the first toxin and the NCR toxin. Pittendrigh and Gaffney (2001) outlined a screening strategy to address this issue (Figure 6.8).

Briefly, toxin-pairs could be applied to insects that have been mutagenized to screen for alleles capable of surviving both toxins at once. For toxin pairs where

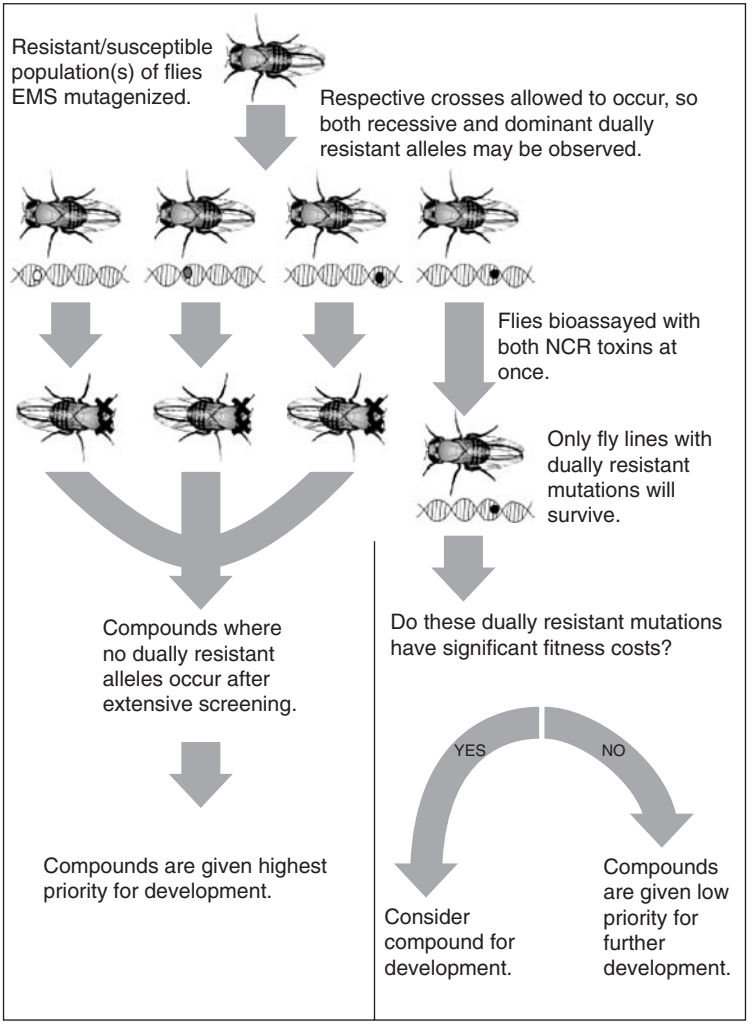

Figure 6.8 A hypothetical screening strategy to test for the existence of a putative third allele (dually resistant allele) that confers resistance to a pair of NCR toxins. (reprinted from Pittendrigh and Gaffney (2001), with permission from Elsevier). (Flies presented in this figure are reproduced with the permission and copyright of Exploratorium, www.exploratorium.edu).

dually resistant mutants are not observed, or are observed at a lower frequency than other toxin pairs (NCR compound plus first toxin), then the given NCR compound should be given higher priority for further development (Figure 6.8, left-hand side). Those toxins pairs (the first toxin plus the NCR toxin) in which resistance alleles arise more frequently would be given lower priority for development of the NCR compound (Figure 6.8, bottom right-hand side). Such a strategy may help prioritize those NCR compounds for development for practical uses.

Economic Factors

Development and deployment of NCR compounds in the field will ultimately depend on multiple scientific and economic factors. The use of an NCR strategy may not work in all situations. For example, if multiple forms of resistance occur in a pest species to a particular pesticide (Hemingway *et al*., 1993), it may be difficult or even impossible to identify a single compound that can provide generalized NCR. In the case of chlorpyrifos and propoxur resistance in cockroaches of the 14 lines of *B. germanica* surveyed for resistance, 10 lines showed esterase-like resistance and 2 lines showed NCR (or negatively correlated resistance) (Hemingway *et al*., 1993). The development of multiple NCR compounds to deal with this diversity of resistance may be too costly. In contrast, target site insensitivity to dieldrin is due to amino acid changes that are highly conserved across divergent taxonomic groups (ffrench-Constant *et al*., 1998) suggesting that commercial development of a single NCR toxin to combat resistance may be feasible.

Economics and competing commercial interests will also influence the decision to develop NCR as an approach to resistance management. Resistance to pesticides that have little commercial value, such as an insecticide useful in a niche market, may not justify the costs of developing an NCR factor. Alternatively, resistance to high value pesticides may even warrant the development of multiple NCR factors effective against diverse forms of resistance. The ability to rapidly respond to the emergence of resistant alleles via high throughout screening capabilities as outlined here greatly enhances the utility of NCR approaches.

Conclusions

NCR has been observed across a variety of species and chemical classes. However, to date, it has not typically been used in wide-scale insect resistance management (IRM). The lack of forthcoming NCR products may be due to logical business models that necessitate the cost effective development of new products due to the needs of the marketplace. In this regard, NCR products will likely only be developed in response to verified resistance to currently marketed high-value products. An additional reason for the lack of NCR compounds may have been the practical limitations in the methodologies needed to efficiently discover them. Development of NCR compounds can be achieved through a variety of methods, for example, use of large-scale screening processes modified from those currently used for screening for novel pesticides. Such screening approaches could involve field-resistant insects, or in some specific cases transgenic *D. melanogaster* expressing the resistance trait. Additionally, advances in molecular cloning and expression of peptides in display technologies could allow for the rapid development of NCR products as soon as resistance occurs in insect populations in the field. Rational design of traditional chemistries as well as proteins is also well established. Thus, field-resistant insects, high throughput transgenic live insect systems, phage display technologies, and rational design approaches, or any combination of these, could be used to assess a wide array of receptor/toxin combinations to model a best fit for NCR toxins useful in the field.

Deployment strategies have already been presented in order to optimize the usefulness of such NCR toxins in resistance management. In the case of transgenic

plants expressing insecticidal toxins, such at Bt toxins, an "active refuge model", where the NCR toxins are deployed in the refuge, could be used to keep resistance to a minimum in the pest population.

Because of the long history of resistance evolving in insects to insecticidal agents, integrated pest management (IPM) and, in the case of biotechnology derived crops, IRM, have been developed to slow or prevent resistance evolution. The NCR approach described here adds a new dimension to those strategies.

Additionally, although NCR has been demonstrated across classes of synthetic pesticides, we know little about the existence of NCR (or lack thereof) with host-plant defensive molecules and the insect populations that feed on these host plants. If NCR is a common ecological phenomenon, or even exists in some cases, the role that it plays (or has played) in plant-insect interactions remains to be elucidated.

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Chapter 7

Resistance by Ectoparasites

Lisa M. Knolhoff and David W. Onstad

Control of ectoparasites is important because of the inherent value of the animal host. Evolution of resistance to insecticides and acaricides in these pests is a major concern, but economic or ethical limitations leave few insect resistance management (IRM) options available. The purpose of this chapter is to highlight the similarities between the resistance problems for the variety of pests infesting humans, livestock, pets, and domesticated bees. There has been much work on resistance mechanisms, subsequent monitoring, and even predictions of resistance, but less has been done to actively and effectively manage resistance. The economics or value of the host precludes certain steps from being taken. One cannot ethically put a value on human life, which severely limits control and IRM options for both mosquitoes and lice. For livestock and apiary pests, the case studies demonstrate that improved IRM will depend on the implementation of more sophisticated integrated pest management (IPM).

Definitions

A few descriptions of commonly used insecticides and resistance mechanisms are provided here because certain terms are used throughout the chapter. The purpose here is not to address resistance *per se*, but rather to inform the reader of mode of action and resistance to insecticides as they would relate to resistance management. Table 7.1 is not meant to be a comprehensive explanation of mode of action; subtle differences may occur which are not noted here. Likewise, the resistance mechanisms listed may not be the only means of adapting to a chemical control. A more complete summary of chemical modes of action can be found in Ware and Whitacre (2004), which is available online at http://ipmworld.umn.edu/chapters/ware.htm. A valuable resource of information about mode of action and how it relates to resistance management can be found at www.irac-online.org. The Insecticide Resistance Action Committee (IRAC) is an international organization dedicated to implementing appropriate resistance management strategies in agriculture and public health. Head and Savinelli (Chapter 5) present a modification of the IRAC classification system for mode of action of insecticides.

Pyrethroids (and previously, the organochlorine dichloro-diphenyl-trichloroethane (DDT)) are commonly used for control of medical or veterinary pests because of their relatively low mammalian toxicity. They are synaptic nerve poisons;

Table 7.1 Some commonly used classes of pesticides for control of ectoparasites

Class	Example	Mode of action	Common resistance mechanisms (if applicable)
Pyrethroids	Permethrin, fluvalinate	Na^+ leakage in neurons	Target site, metabolic detoxification, or behavior modification
Organochlorines-1	DDT	Na^+ leakage in neurons	Target site, metabolic detoxification, or behavior modification
Organochlorines-2 (cyclodienes)	Dieldrin	Blocks GABA-gated chloride channels	Target site
Organophosphates	Malathion, coumaphos	AChE inhibition	Metabolic detoxification, target site
Carbamates	Carbaryl	AChE inhibition	Metabolic detoxification, target site
Formamidines	Amitraz	Binds to octopamine receptors	
Spinosyns	Spinosad	Nicotinic ACh receptor agonist*	
Neonicotinoids	Imidacloprid	Nicotinic ACh receptor agonist*	
Phenylpyrazoles	Fipronil	Blocks GABA-gated chloride channels	
Avermectin	Ivermectin	Activates chloride channels	

AChE: acetylcholinesterase; GABA: gamma-aminobutyric acid.
*Although the spinosyns and neonicotinoids have the same mode of action, the binding site of each to the ACh receptor is different.

they cause sodium ion leakage from voltage-gated channels. Resistance to these compounds can occur through target site mutations in sodium channels or through metabolic detoxification. Pyrethroid insecticides are known for their rapid insecticidal effects; the allele for target site resistance is called *kdr* for "knock-down resistance". If resistance is characteristically similar to *kdr* mutants, but the allele has not yet been identified in a particular insect, it may be referred to as "*kdr*-like". Finally, behavioral resistance may also occur because these compounds have an irritant effect.

The other major classes of insecticides have different modes of action. Organophosphate and carbamate insecticides inhibit acetylcholinesterase (AChE), leading to a buildup of acetylcholine in synapses. Resistance to these compounds usually occurs through metabolic detoxification or insensitive AChE. Cyclodiene organochlorines inhibit gamma-aminobutyric acid (GABA) receptors in neurons and therefore prevent chloride ion uptake. Target site resistance is most common; one example is the *Rdl* allele conferring resistance to dieldrin.

Mosquitoes

Mosquitoes (Diptera: Culicidae) are unrivaled in their vector capability and are responsible for the transmission of a number of diseases of medical importance. Vector control is the main component of disease control programs because of its relative ease and lowered cost with respect to pathogen control. The fact that

mosquitoes have different habitats at different life stages allows for more control options. Only adult females feed on blood; larvae are aquatic and non-parasitic. Preference for human hosts, anthrophily, or other animals, zoophily, also plays a role in vector control, disease transmission, and in IRM strategies.

The short life cycle and high reproductive potential of mosquitoes predispose populations to evolving resistance. Resistance to at least one class of traditional synthetic insecticides is common in the major mosquito genera *Aedes*, *Anopheles*, and *Culex* that vector human disease (Hemingway and Ranson, 2000). For example, mosquitoes are resistant to organochlorines, organophosphates, and pyrethroids in China (Cui *et al.*, 2006). Use of DDT and especially pyrethroids for control of *An. gambiae s.s.* vectors of malaria has led to widespread resistance in Africa (WHO, 2005a). Resistance to organophosphates is found in many parts of the world in *Culex pipiens* (Labbe *et al.*, 2005).

The study of resistance to organophosphates in *C. pipiens* mosquitoes has allowed certain aspects of selection and migration to be examined. In southern France, there is a cline of frequencies of resistance alleles for organophosphate insecticides, suggesting that mutations arose once and spread by migration of the insect (Chevillon *et al.*, 1999; Lenormand and Raymond, 2000). However, there are multiple mutations favoring resistance in *C. pipiens*, even at one locus (Labbe *et al.*, 2005). Certain alleles are favored in specific local environments depending on both selection pressure with insecticides and natural climate/landscape conditions (Labbe *et al.*, 2005). Understanding the spread of resistance will certainly help us to mitigate it in the future.

Insecticide resistance in mosquitoes is of great concern in areas of the world where malaria is present. Malaria (*Plasmodium* spp.) is a serious problem in many developing countries and is vectored by *Anopheles* mosquitoes, the most important of which is *An. gambiae* (Hemingway and Ranson, 2000). The widespread use of DDT (an organochlorine) to control malaria-vectoring populations in Africa and southern Asia has selected for resistance and caused the World Health Organization (WHO) to shift its goal from malaria eradication to malaria control (Hemingway and Ranson, 2000). WHO advocates the use of insecticide-treated bednets (ITNs) in an effort to curb malaria (WHO, 2006a). This is likely to be a better IRM strategy in that control is used only where it is most needed and reduces the need for indiscriminate sprays of insecticide.

The focus of *Anopheles* and malaria control is on female mosquitoes, because they, unlike males, require a blood meal. Most females coming in contact with an ITN searching for a host are mated (Curtis *et al.*, 1993). Host-seeking females tend to first land on the top part of the net because of the concentration of heat and carbon dioxide there and then search downwards on the net to find a human host (Guillet *et al.*, 2001).

Insecticide-Treated Bednets

ITNs covering sleeping people act like baited traps, in that host-seeking mosquitoes are either killed or repelled by the insecticide (WHO, 2006a). Irritant insecticides on ITNs are most effective in preventing bites because the mosquito will be repelled before encountering and biting a person. Some bites may occur, however,

either through entry in a hole in a torn net or if the person is sleeping against the net and a mosquito bites through it. Besides the use of ITNs for mosquito control, outdoor spraying and indoor residual spraying are commonly used. We will focus on the use of ITNs as an IRM strategy but will address these other methods later in this section.

Pyrethroids are the only class of insecticides approved for use with ITNs (Hemingway and Bates, 2003). They are known for both excitorepellency, and increased tendency to take off and fly, and rapid knock-down effect causing mortality of mosquitoes (Pates and Curtis, 2005). Mosquitoes have been known to evolve behavioral resistance such as exophily (Pates and Curtis, 2005), a behavior causing mosquitoes to avoid internal walls treated with insecticide. There is no evidence yet, however, of behavioral resistance by the avoidance of ITNs (Pates and Curtis, 2005). Pyrethroid resistance in anopheline mosquitoes has most likely not been selected by ITNs (Curtis *et al.*, 1998; Takken, 2002); it is not seen in areas without extensive pyrethroid use in agricultural areas (Vulule *et al.*, 1996). Pyrethroid-treated ITNs seem to remain effective where mosquitoes are resistant to pyrethroids via *kdr*, presumably because mosquitoes are not repelled before receiving a lethal dose (Chandre *et al.*, 2000). Where mosquitoes are resistant to pyrethroids via both *kdr* and metabolic mechanisms, control could be compromised (Enayati and Hemingway, 2006).

If using a single insecticide on an ITN, Curtis *et al.* (1998) noted that, because of the excitorepellency of pyrethroids, a low-dose strategy is better for IRM. With low doses, resistant heterozygotes leave ITNs before being killed by insecticide, reducing the selection pressure. With higher concentrations of insecticides, the heterozygotes incur higher mortality. In the initial stages of evolution of resistance, it is the frequency and fitness of heterozygotes that is most critical.

Curtis (1985) modeled possible IRM strategies for mosquito control, and found that the use of mixtures of insecticides were the best at delaying resistance when alleles were at least partly recessive. Mixtures refer to the simultaneous use of two insecticides; it is thought that if resistance alleles are rare, then resistance to two insecticides should be especially rare. Curtis *et al.* (1993) found that mixtures of insecticides on an ITN would be more effective than other strategies at delaying the evolution of resistance because it is assumed only mated females are exposed to the toxins in the ITN when searching for a host. Studies are currently underway to create ITNs that function as a mixture of insecticides, in that a mosquito comes in contact with two insecticides during a single attempt to feed. Guillet *et al.* (2001) tested one approach involving the use of ITNs that have been treated on the top half with a non-irritant insecticide and on the bottom half with a pyrethroid (Figure 7.1). This takes advantage of mosquito host-searching behavior; they tend to land on the top of the net and then travel down, making the ITN effectively a mixture, rather than a mosaic, of insecticide treatment (Guillet *et al.*, 2001). Guillet *et al.* (2001) found the best control when ITNs are treated with a carbamate on the top half and a pyrethroid on the bottom half. Placing the non-pyrethroid insecticide on the top of the net, farther from human contact, is advantageous because other classes of insecticides tend to have higher mammalian toxicity. This strategy, however, has not been tested yet for sustainability.

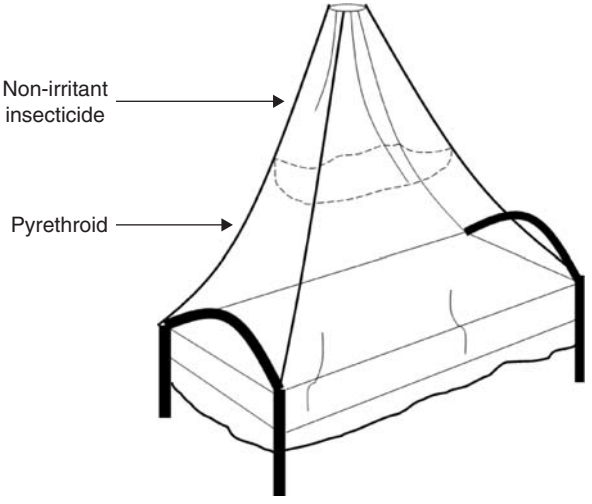

Figure 7.1 Idealized diagram of an ITN functioning as an insecticidal mixture (concept from Guillet *et al.*, 2001). Host-seeking mosquitoes land on the top part of the net and receive a dose of a non-irritant insecticide (e.g., carbamate). As the mosquitoes travel down the net to locate the host, they receive a dose of pyrethroid insecticide.

Sustainability of ITNs (and other control methods) can be enhanced through the use of alternate hosts of mosquitoes, such as cattle, which may act both as a refuge for IRM and as a dead-end host of malaria. Kawaguchi *et al.* (2003) suggested that evolution of resistance can be delayed in zoophilic mosquitoes with the incorporation of cattle close to, but not within, human dwellings. Their mathematical model indicated that evolution of resistance is delayed with an increase in the number of cattle and with insecticide sprays used only in areas occupied by humans.

ITNs, like any other IRM strategy, require the cooperation of all parties involved. They provide a common means of attaining the goals of both IRM and public health (the reduction of malaria transmission), thus aiding in their implementation. Malaria is endemic in many rural areas of Africa, where it is especially difficult for poor communities to pay for and distribute ITNs without help from developed countries (Curtis *et al.*, 2003). Retreatment of ITNs is essential for their long-term efficacy, but it is difficult to implement. Many ITNs are never retreated; in one field study, only about a third had enough insecticide to be effective in preventing bites (Erlanger *et al.*, 2004). As a consequence, long-lasting nets have been manufactured that are more durable, both structurally and with respect to insecticide effectiveness (Kroeger *et al.*, 2004; Tami *et al.*, 2004; Lindblade *et al.*, 2005). Education is also important in implementing ITNs, in that knowledge of malaria increases their use (Nganda *et al.*, 2004). WHO recommends the establishment of national guidelines and increasing communication and publication of information relating to insecticide resistance (WHO, 2003).

Indoor Residual Spraying

There has been an effort to obtain experimental data on resistance management using indoor residual spray (IRS) regimes. A field trial was set up in southern Mexico to test the effectiveness of certain IRM strategies on DDT resistance in *An. albimanus* mosquitoes (Hemingway *et al.*, 1997). Indoor spraying regimes tested

included (1) exclusive use of a pyrethroid or DDT, (2) annual rotation of three classes of insecticides (organophosphate, pyrethroid, and carbamate), and (3) spatial mosaic (within a village) of an organophosphate and pyrethroid. Metabolic detoxification by glutathione S-transferase (GST) was previously shown to be the major resistance mechanism to DDT in the mosquitoes in this area (Hemingway *et al.*, 1997; Penilla *et al.*, 1998). Levels of GST were measured after 3 years of treatment, and there was a mean decrease relative to that found in susceptible strains in every treatment except the exclusive use of DDT (Penilla *et al.*, 2006). However, there was not a clear correlation between DDT resistance and mean GST levels in their study, so they plan to retroactively test for target site (*kdr*) resistance. Once the data on both mechanisms of resistance are combined, this study will provide valuable insight into the most effective IRM strategies based on experimental field data from IRS.

In addition, this group conducted a survey of villagers to determine perceived effects of the spray programs (Rodríguez *et al.*, 2006). They found that most people found lower numbers of mosquito bites under all treatment regimes, but that few actually associated this with a reduction in malaria incidence. It is interesting to note that most of people associating reduced incidence of malaria were in the rotation regime, which had the additional perceived benefit of reducing cockroaches in the home.

This field study highlights the need for increased education about mosquito and malaria control, as well as the dynamics of resistance under certain IRS regimes. This is important because in 2006 WHO endorsed the use of IRS programs in combination with ITNs to combat malaria (WHO, 2006b). Of particular interest is their support for the use of DDT "where indicated", meaning where *Anopheles* vectors have not already evolved resistance. Previously, WHO supported the reduced reliance on DDT, and this insecticide was only allowed under special circumstances in public health. WHO recognizes the potential of the evolution of resistance in vector mosquitoes but notes in their position statement that, because DDT is not used in agricultural settings, that the chance of resistance evolving in the public health sector is diminished (WHO, 2006b). However, this is a contentious issue: some welcome DDT as another control method and others bemoan its shortsightedness. One thing is agreed upon: that IRS with DDT should be used judiciously with effective IRM practices (Berenbaum, 2005), but the only evidence existing on its operation for effective resistance management is the study in southern Mexico documented above. The results of Penilla *et al.* (2006) indicate that mixtures, rotations, and mosaics may mitigate resistance to DDT, but operational factors could make them difficult to apply.

There has been much effort in monitoring resistance, especially in Africa; IRS with DDT may only be effective in areas where malaria transmission is unstable and the *Anopheles* vector is susceptible, particularly in the highlands and fringes (Figure 7.2; WHO, 2005b). In light of the danger malaria poses to public health, resistance may be an acceptable risk to WHO. In 2003, WHO guidelines seemed to indicate that public health IRM simply involves monitoring for resistance, so that another product can subsequently be used when it is detected (WHO, 2003). This is one case where the costs to the host outweigh the costs associated with resistance management.

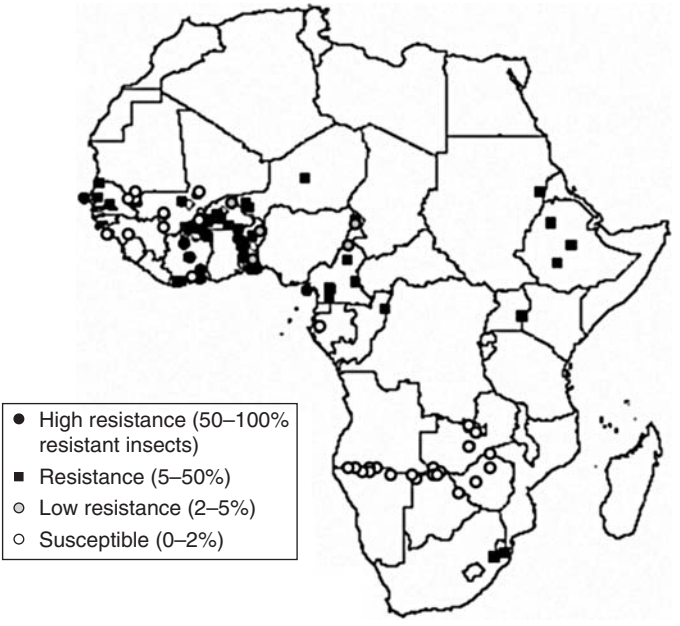

Figure 7.2 Distribution of DDT resistance in *An. gambiae s.s.* and *s.l.*, and *A. arabiensis* in Africa (modified from WHO, 2005b).

Human Head Lice

Another pest with a history of resistance problems is the human head louse, *Pediculus humanus capitis* (Phthiraptera: Pediculidae). These insects feed on human blood and spend their entire life cycle on the scalp. *P. h. capitis* lice provide different problems for IRM because of their host specificity. They are most often considered a pest of young children because of the close proximity of students to each other in elementary school classrooms. *P. h. capitis* are most often spread by physical contact because they cannot survive for long off of a host (Burgess, 2004). There is a great need to further investigate the basic biology of lice to understand resistance and their spread to another host (Burgess, 2004). The public's use of large amounts of over-the-counter treatments with questionable effectiveness has led to large numbers of *P. h. capitis* infestations (Mumcuoglu, 1996).

P. h. capitis has evolved resistance to insecticides worldwide, namely natural pyrethrins and synthetic pyrethroids. The vast majority of treatments for *P. h. capitis* contain permethrin because pyrethroids have low mammalian toxicity (Mumcuoglu, 1996; Yoon *et al.*, 2004). Probable reasons for outbreaks of resistance include the almost exclusive use of permethrin for control and cross-resistance to DDT, which was previously used to control *P. h. capitis* (Mumcuoglu, 1996). In addition, many of these control products have relatively long residual activity, which is expected to select for resistance (Mumcuoglu, 1996). Overdiagnosis of *P. h. capitis* and subsequent unnecessary treatment is also a probable factor leading to resistance; Pollack *et al.* (2000) found that most cases of infestations are

misdiagnosed. Only 59% of submissions to their laboratory contained lice specimens, and of these, slightly over half contained live lice or viable eggs. They concluded that subjects without active infestations were more likely to receive treatments than those with active infestations.

Resistance to other insecticides besides the most commonly used pyrethroids can still be present in some populations. Insecticides available only by a prescription from physicians in the United States include lindane, an organochlorine, and malathion, an organophosphate (Yoon *et al.*, 2004). Yoon *et al.* (2004) did not detect resistance to lindane in lice from the United States, but an earlier study by Meinking *et al.* (2002) did find resistance using different methodology. Malathion resistance was detected in lice in the United States by Yoon *et al.* (2004) and in the United Kingdom by Downs *et al.* (1999). Resistance to carbaryl, a carbamate, has evolved in some populations in the United Kingdom, but it is not yet widespread (Downs *et al.*, 2002). Ivermectin, an avermectin, is a possible treatment for control of resistant head lice, but it is not yet approved for use by the public (Mumcuoglu *et al.*, 1990; Yoon *et al.*, 2004).

The main options for IRM in *P. h. capitis* are mixtures of insecticides (Yoon *et al.*, 2003) and using one insecticide after another has lost its effectiveness. However, as Curtis *et al.* (1993) noted, the latter is not a strategy *per se*, in that this is what would be done normally in absence of any real plan for IRM. Mixtures of treatments are possible to implement, and may cause significant mortality, but their effectiveness for IRM has yet to be studied. Many studies examine cross-resistance to pyrethroids because of their common use and control failure. Fortunately, there does not seem to be any cross-resistance to carbaryl, a carbamate, from permethrin-resistant *P. h. capitis* (Picollo *et al.*, 2000). Aliphatic alcohols may cause significant control of head lice, and there is no cross-resistance from permethrin-resistant lice (Mougabure Cueto *et al.*, 2002). Yoon *et al.* (2003) found that an insecticidal lotion without its active ingredient, malathion, still caused some mortality; this would make a mixture easier to create. A high dose strategy is not likely to be effective IRM; mortality was not affected by very high doses of two pyrethroids and it was only moderately increased with very high doses of malathion (Downs *et al.*, 2002).

Resistance management and control of *P. h. capitis* requires much education and communication between parents, teachers, and health workers. Schools that are proactive in educating parents about head lice and regular head combing may lead to fewer incidents of head lice in children (Downs *et al.*, 2002). Another study indicated that keeping parents informed about head lice did not reduce the incidence, but did decrease the level of infestation in numbers of lice (Downs *et al.*, 2000). Downs *et al.* (1999) suggested that insecticidal treatments for lice be available only by prescription to slow the development of resistance. However, antibacterial medications are also only available by prescription, and this has not completely prevented the evolution of resistance to antibiotics.

Fleas of Cats and Dogs

The most important ectoparasite of cats and dogs is the flea *Ctenocephalides felis* (Siphonaptera: Pulicidae). Besides causing discomfort to pets (and owners), fleas may cause allergic dermatitis in highly sensitive animals, and they may transmit

pathogens or endoparasites. Adult fleas infest pets; females lay eggs on pet fur. The eggs soon drop from the fur, normally where the pet rests. Larvae are non-parasitic and feed on adult feces, which consists of partially digested blood.

Control of *C. felis* has relied on chemical application of both the pet and places where it rests (Rust and Dryden, 1997). Reports of resistance in *C. felis* have led to the use of newer treatments like insect growth regulators, IGRs, and botanical compounds (Rust and Dryden, 1997). Other compounds such as fipronil, a phenylpyrazole, and imidacloprid, a neonicotinoid, are now commonly used as well (Rust and Dryden, 1997).

Resistance has been documented to the major classes of insecticides, but tests of resistance have used variable methods of detection (Bossard *et al.*, 1998). Because resistance patterns are generally unknown, the treatment decision by pet owners is usually based on price and simplicity (Rust and Dryden, 1997). Although resistance is commonly cited as the reason for control failure, this is not always certain. Some reports of resistance may actually be due to incomplete treatment (Rust and Dryden, 1997) or inherent variability of susceptibility in flea populations or variability in the tests themselves (Bossard *et al.*, 1998). For example, Bossard *et al.* (2002) found high levels of variability in susceptibility within some of the strains they tested.

A simpler test for resistance will clarify these issues; Rust *et al.* (2002) recently developed a larval assay. Because the assay begins with flea eggs, many environmental effects (Bossard *et al.*, 1998) are minimized, and there is also no need to rear fleas until they reach adulthood. The authors determined a threshold of 3 ppm of imidacloprid at which to administer further tests to diagnose resistance; subsequent tests validated this concentration (Rust *et al.*, 2005).

Genetic and molecular studies on resistance in *C. felis* have only recently been conducted. A PCR (polymerase chain reaction) assay for both *kdr* mutants (Bass *et al.*, 2004a) and *Rdl* mutants (Bass *et al.*, 2004b; Daborn *et al.*, 2004) has been developed. Both of these alleles were common in the laboratory populations tested from the United Kingdom and the United States (Bass *et al.*, 2004a, b). It is interesting to note that most of their samples were from fleas that had been in laboratory colonies for a few years without any selection pressure. The *Rdl* allele also seems to confer cross-resistance to fipronil, a phenylpyrazole (Bass *et al.*, 2004b; Daborn *et al.*, 2004). When using these assays to test field-collected strains from United States of America and Europe both alleles were common, but frequencies were highly variable among the populations (Bass *et al.*, 2004a, b). Some studies are underway to predict molecular mechanisms of resistance before they occur. Bass *et al.* (2006) found protein subunits of the target site of imidacloprid, a neonicotinoid, that are likely to be involved in resistance if it evolves.

A history of resistance obliges people to take an integrated approach for control. Carlotti and Jacobs (2000) outlined the major flea control methods. To prevent resistance, they suggested using chemicals with different modes of action and integrating cultural controls such as animal grooming and vacuuming of carpets (IPM). Simply leaving an untreated refuge is generally not acceptable for medical/veterinary pests; there is a low threshold, if any, of tolerance for fleas. Temporal refuges may be implemented by only treating acute infestations, but this is probably not acceptable to the pet or its owner (Bossard *et al.*, 1998).

Mites on Bees

The mite *Varroa destructor* (Acari: Varroidae) is one of the most serious pests of honey bees (*Apis mellifera*) worldwide. Mites feed on the hemolymph of bees, preferentially of the drone brood, leaving them severely deformed. They may also transmit viral pathogens (Sammataro *et al.*, 2000). If left unchecked, *V. destructor* infestations can destroy a honey bee colony in a few years.

The life history of *V. destructor* probably makes resistance easier to evolve in this species. A female mite enters a brood cell right before it is capped for the bee to pupate. She soon starts laying eggs, the first of which is a male, followed by female eggs. Nymphs feed and develop on the pupal bee, and when mature, the single male mates with all of the sisters in the cell. Haplodiploidy and sibling mating in *V. destructor* greatly increase the chance of fixation of new mutations (Cornuet *et al.*, 2006).

Typical control of *V. destructor* involves the use of fluvalinate, a pyrethroid, treated strips placed in the hive during times of no honey production. Intensive use of these strips has selected for resistance in some parts of Europe (Troullier, 1998), the United States (Elzen *et al.*, 1998; Macedo *et al.*, 2002), Israel (Mozes-Koch *et al.*, 2000), and Mexico (Rodríguez-Dehaibes *et al.*, 2005). The spread of pyrethroid resistance in Europe roughly follows that of the initial spread of the mite according to bee movement, suggesting that resistance evolved once and spread thereafter (Martin, 2004). Coumaphos, an organophosphate insecticide, was soon introduced for emergency use after control problems with fluvalinate, but resistance to coumaphos is now present in Florida (Elzen and Westervelt, 2002) and northern Italy (Spreafico *et al.*, 2001). Resistance to both pyrethroids and amitraz, an amidine, has been reported in the United States in Minnesota (Elzen *et al.*, 2000) and in Mexico (Rodríguez-Dehaibes *et al.*, 2005). Amitraz was previously used for mite control (Sammataro *et al.*, 2000).

Determining the mechanism of resistance may make monitoring for it easier. Some resistance to fluvalinate seems to be partly due to detoxification via monooxygenases (Hillesheim *et al.*, 1996; Mozes-Koch *et al.*, 2000), but sodium channel target site (*kdr*-like) resistance is also possible because it is the most common means of pyrethroid resistance. A sodium channel gene has now been sequenced for *V. destructor* (Wang *et al.*, 2003); this has implications in using a PCR-based assay to monitor for resistance.

Most suggestions for IRM involve either an acaricide rotation or an additional IPM method. Milani (1999) suggested the rotation of chemical and non-chemical means of control in different seasons, combined with breeding for resistant bees (Martin *et al.*, 1997), to delay resistance. In Minnesota, mites that were resistant to fluvalinate were also resistant to amitraz, but not to coumaphos (Elzen *et al.*, 2000). This group suggested a rotation involving the use of coumaphos for 2 years followed by 1 year of fluvalinate (Elzen *et al.*, 2001) for IRM, but this strategy has not yet been tested for sustainability.

Other IPM-like methods for managing resistance incorporate trapping or heat-killing *V. destructor* mites. Webster *et al.* (2000) designed a trap in the floor of the hive preventing live fallen mites from returning to the comb. Even though their *V. destructor* colony was resistant to fluvalinate, they found larger amounts of mitefall

during fluvalinate treatment than without. They hypothesized that live mites falling in their traps had experienced sublethal doses of insecticide, and that a possible IRM strategy is to use either chemical means plus their floor trap or to use less effective essential oils in addition to the floor trap. One other method to delay resistance includes possible use of heat to kill *V. destructor*. The male drone brood is preferred by mites, but drones contribute little to the bee colony. Therefore some of the drone brood can be sacrificed for *V. destructor* control. Removing some of the drone-brood comb is done in some small apiaries in Europe, but it is labor intensive (Huang, 2001). Huang (2001) extended this idea to develop special frames for the bees to make drone-brood comb. These frames have internal heating elements that can be activated to kill mites and associated drone brood.

Ticks of Cattle

The tick *Boophilus microplus* (Acari: Ixodidae) is a pest of cattle in Latin America and in Australia; three *Boophilus* species, including *B. microplus*, are pests in sub-Saharan Africa (Estrada-Peña *et al.*, 2006). *B. microplus* has been eradicated from the United States since the 1940s, and great care is taken to prevent its reintroduction through cattle crossing the border from Mexico. There is concern for the welfare and comfort of cattle with respect to ticks, but vector capability is also of great importance, as they may transmit protozoan or bacterial diseases. De Castro (1997) estimated an annual cost of between US$13.9 and 18.7 billion for damage and control of ticks and their diseases on cattle throughout the world.

Ticks may be categorized by the number of hosts they require to complete their life cycle. Cattle ticks are single host ticks, meaning that they complete parasitic stages on one host. These ticks are free-living only from the time the engorged female drops from the host to lay eggs until the newly hatched larvae locate another host. There are likely to be a mixture of developmental stages on the host at any one time.

Most cattle producers rely solely on acaricides for tick control, which may be done in the form of dips, sprays, or pour-on formulas (George, 2000). Amitraz (an amidine), coumaphos (an organophosphate), and cypermethrin (a pyrethroid) are the main acaricides used (Jonsson *et al.*, 2001; Rodríguez-Vivas *et al.*, 2006). Many tick control schemes involve regular prophylactic dipping, which has led to some cases of resistance (Sangster, 2001). Also not surprisingly, an increase in number of acaricide treatments leads to higher incidence of resistance (Jonsson *et al.*, 2000; Rodríguez-Vivas *et al.*, 2006).

Because of intensive use of acaricides, resistance has evolved in many parts of the world. Some areas in Kenya have experienced resistance to amitraz (Kamidi and Kamidi, 2005). In Mexico, resistance is commonly seen with more than one class of acaricide (Foil *et al.*, 2004), and multiclass resistance is widespread in Brazil (Graf *et al.*, 2004). In Australia, Sutherst *et al.* (1979) estimated that widespread resistance to an acaricide occurs in 4–7 years after a new product is introduced; Foil *et al.* (2004) gave an average of 12 years for evolution of resistance to amitraz.

Chemical companies involved in tick control have an interest in IRM. Graf *et al.* (2004) suggested that resistance has possibly arisen due to rotation of trade names, rather than rotation of chemical classes. They highlighted the need for resistance management and claim that chemical controls should not be viewed as a renewable

resource. High investment risks are present for chemical companies (Witty, 1999), especially because the market for livestock pests tends to be shrinking in comparison to that of companion animal pests (Graf *et al.*, 2004). Regulatory procedures are one factor that may be preventing effective control products from entering the market. Exact thresholds vary among nations, but many require an average mortality in the target pest of 95–98% for introduction of a control product (Graf *et al.*, 2004). These authors applauded the recent changes to regulatory guidelines in Australia, allowing lower efficacy products on the market that "aid in the control of" target pests.

Because of a history of resistance problems in Australia, there has been an effort to take an integrated approach in tick control, including methods of dipping of cattle in acaricides, pasture spelling/rotation, tick vaccines, and raising tick-resistant cattle (Angus, 1996). Pasture spelling is the practice of removing cattle from pastureland for a period of time to prevent ticks from locating a host until most ticks die. Graf *et al.* (2004) suggested a combination of pasture rotation with acaricidal treatment to delay evolution of resistance. Sutherst *et al.* (1979) found that the combination of the use of tick-resistant breeds of cattle and pasture spelling offered the best sustainable control of *B. microplus* in a mathematical model. Concerns about organic beef and improved productivity have renewed interest in breeding for tick-resistant cattle (Frisch et al., 2000).

In Australia, efforts were made to minimize the risk of evolution of resistance to pyrethroids. The likelihood of cross-resistance to DDT that was previously used as an acaricide was recognized, which led to the registration of newer pyrethoids with a slightly different chemical structure (George *et al.*, 2004). The second tactic was the use of organophosphates to synergize the toxicity of the pyrethoids (George *et al.*, 2004). This not only probably delayed resistance, but also provided a more economical use of pyrethroids, which tend to be more expensive (George *et al.*, 2004). George *et al.* (2004) pointed out that a possible added benefit to the use of these insecticide mixtures is control of horn fly (*Haematobia irritans*), but they noted that there is still a lack of evidence to its utility in IRM. In Brazil widespread resistance to multiple classes of insecticide would probably yield this type of treatment ineffective (Graf *et al.*, 2004).

Some programs are not aimed at prevention or delay of evolution of resistance, but rather at the control of ticks that have already evolved some level of resistance. Kamidi and Kamidi (2005) tested the rapid rotation of amitraz and chlorfenvinphos (an organophosphate), with each being used once a week, as an IRM strategy for ticks showing resistance to each of these acaricides at different times. It should be noted, however, that they measured incidence of tick-borne diseases, rather than any data on the ticks themselves. Disease incidence drastically decreased after deployment of the rapid rotation of acaricides, and the authors claimed that this strategy was still effective after 2.5 years. Davey *et al.* (2004) found that three intervals of coumaphos, an organophosphate, treatment did not completely kill all organophosphate-resistant ticks, but it did lower the reproductive potential sufficiently that they felt resurgence was not likely to occur. A subsequent study by this group revealed that a repeated treatment of a high dose of organophosphate insecticide effectively controlled resistant ticks (Miller *et al.*, 2005). They concluded that this was probably an effective measure to prevent reintroduction of *B. microplus* into the United States from cattle being traded from Mexico.

George *et al.* (2004) noted that even though integrated tick control measures have been suggested (Sutherst *et al.*, 1979; Norton *et al.*, 1983), little has been done to help producers implement them. They advocate means of educating both producers and regulatory groups of the benefits and problems of tick control and resistance management. However, evidence is severely lacking as to which plan would be best for resistance management (Willadsen, 2006).

Blow Fly in Sheep

Cutaneous myiasis in sheep is caused by the Australian sheep blow fly, *Lucilia cuprina* (Diptera: Calliphoridae). *L. cuprina* is not a constant menace, but "flywaves" may be anticipated according to weather predictions and monitoring of populations (Levot, 1995). Females oviposit in wet fleece, namely around the backside of the animal, and often when a bacterial infection (called fleece rot) is already present. Rainy weather predisposes the sheep to *L. cuprina* infestation and associated fleece rot.

Larvae feed on the flesh of sheep and can cause painful wounds. They drop off the host to pupate in the soil. Prevention of *L. cuprina* infestations can be done through chemical or cultural means. Cultural controls include mulesing and tail docking. Mulesing is the process of cutting off large patches of skin on the backside of the animal to remove the skin folds there that are conducive to *L. cuprina* infestations. Research is underway to develop either non-surgical or sheep breeding alternatives to this controversial practice (James, 2005). Dipping or spraying sheep with insecticide is the most common control; IGRs are used most often because of control failures from other insecticides (Levot and Sales, 2004).

L. cuprina has a history of evolving resistance to the major classes of insecticides used against it. Organochlorines were used from 1948 to 1958 when resistance problems surfaced (Levot, 1995), and in 1962 they were withdrawn from the market (Hughes and McKenzie, 1987). Resistance to dieldrin, an organochlorine, took only 3 years to evolve, probably because of previous use of lindane, another organochlorine, for sheep lice (*Bovicola ovis*) control (Hughes and McKenzie, 1987). Organochlorines were soon replaced by organophosphates, which also later failed to control *L. cuprina*. Lack of other control options in the late 1960s and 1970s drove organophosphate resistance to fixation (Hughes and McKenzie, 1987; Levot, 1995). It took 5 years for the frequency of resistance to diazinon, an organophosphate, to reach 95% in field populations from the time that it was first detected (Levot, 1995). Despite widespread resistance, organophosphates are still used for *L. cuprina* control because they still provide quick protection for the short term (Levot, 1995). Cyromazine is an IGR that is effective for longer-term control (about 14 weeks), but it is slow acting and does not provide much protection for active infestations (Levot, 1995). Resistance has not yet evolved in the field since cyromazine was introduced to producers in 1979 (Levot, 1995). Resistance has been recorded, however, to diflubenzuron (another IGR) in some parts of Australia (Levot and Sales, 2002).

Because of this insect's relative ease in rearing and because resistance mechanisms are known, certain IRM assumptions have been examined. Strains resistant to dieldrin, diazinon (an organophosphate), or malathion (another organophosphate), respectively, created in the laboratory by artificial mutagenesis had identical mutations in resistance alleles as those found in field populations (McKenzie and

Batterham, 1998). Artificial mutagenesis was also used to study and predict possible resistance to cyromazine and four loci were found to confer resistance, all at low resistance ratios (Yen *et al.*, 1996). A resistance ratio is a comparison of the insecticide concentration required to cause mortality of resistant to susceptible insects. Only one of these alleles was likely to make a viable homozygote, so based on these results they predicted a low chance of evolution of resistance to cyromazine (Yen *et al.*, 1996). Levot and Sales (2004) also found low resistance ratios to cyromazine in a laboratory-selected population, so they too concluded that the chance of evolution of resistance is low. However, this laboratory-selected resistance may not effectively mimic that found in the field. Levot and Sales' (2004) study included other IGRs as well, and they found cross-resistance to dicyclanil in diflubenzuron-selected laboratory populations, but this cross-resistance was not found in resistant populations collected from the field. This surely complicates any extensions from laboratory work to field application. Roush and McKenzie (1987) outlined some of the problems with comparisons between laboratory-selected and field-selected resistant strains of insects, namely that polygenic resistance tends to be selected in the laboratory, and monogenic resistance tends to be selected in the field.

Establishment of resistance alleles depends both on initial frequency and concentration of selective agent, i.e. selection pressure, Scott *et al.* (2000). If selection occurs at a range outside the normal phenotypic variation of susceptible insects, then monogenic resistance is expected to occur (McKenzie, 2000). The combination of knowing the resistance gene and applying the chemical control above the LC_{100} of susceptibles allows prediction of and hopefully prevention of resistance. Because initial development of resistance is dependent on the fitness of heterozygotes, McKenzie and Batterham (1998) advocate the use of square-wave decay curves to determine a concentration lethal to resistant heterozygotes. This is analogous to a high dose strategy for IRM.

Other IRM strategies include the use of a refuge or the use of two insecticides in a mixture or rotation. In their model of dieldrin resistance, Goss and McKenzie (1996) found that even a small increase in the amount of insects in a refuge has a large impact on the time for resistance to evolve. The feasibility of a refuge strategy depends on the size of the population and if there is some level of tolerance to fly infestations. The genetic bases of resistance to two different IGRs, diflubenzuron and cyromazine, seem to be independent (Batterham *et al.*, 2006), so this has potential for use in a mixture or rotation IRM strategy.

Horn Fly on Cattle

The horn fly, *Haematobia irritans* (Diptera: Muscidae), is mainly a pest of cattle, although it may infest other types of livestock as well. Adults look similar to house flies (*Musca domestica*), but are about half the size and have piercing mouthparts to feed on the blood of cattle. Economic damage occurs in lost productivity of cattle due to blood loss and stress on the animals. Females oviposit in fresh manure; larvae may remain there to pupate or may first migrate to the soil. *H. irritans* damage to cattle in the United States is estimated to cost US$730 million annually (Drummond *et al.*, 1981).

A common method of control of *H. irritans* is the use of ear tags that have been impregnated with pyrethroid or organophosphate insecticides (Byford and

Sparks, 1987; Foil *et al.*, 2005). Resistance to pyrethroids is widespread because it can evolve in as little as 2 years with the use of ear tags on cattle (Quisenberry *et al.*, 1984). Barros *et al.* (2001) found that resistance had evolved to all ear tags with organophosphates they tested in less than 9 years. Resistance is common because of the high reproductive potential and mobility of this insect (Byford *et al.*, 1999).

Even though resistance to pyrethroids via the *kdr* mutation might confer some fitness cost (Scott *et al.*, 1997), resistance is persistent even without selection. Weinzierl *et al.* (1990) found no reversion of pyrethroid efficacy after 2 years of disuse. Likewise, Jamroz *et al.* (1998) found no decrease in *kdr* allele frequency in a wild population with no pyrethroid selection. Guglielmone *et al.* (2002) reported some decrease in frequency of resistant pyrethroid alleles (*kdr*) after cessation of selection pressure, but this decrease was not enough to restore susceptibility to cypermethrin. It is likely that a combination of resistance mechanisms to pyrethroids is present in *H. irritans* because the level of resistance to pyrethroids does not seem to be highly correlated to *kdr* frequency (Jamroz *et al.*, 1998). A combination of resistance mechanisms may contribute to the persistence of pyrethroid resistance observed in field populations.

IRM has been difficult for this insect. Insecticide-free refuges are essentially non-existent, due to the host-specific nature of the insect, widespread use of ear tags, and the high mobility of horn flies (Byford *et al.*, 1999). Because ear tags are easy for producers to implement, many studies have focused on examining mixtures or rotations of insecticides in ear tags for IRM. Barros *et al.* (2002) found high levels of susceptibility to the organophosphate diazinon in populations with a history of pyrethroid resistance. This is in contrast to the studies of Barros *et al.* (1999) and Guerrero *et al.* (2002), where resistance evolved to both insecticides in yearly rotations of pyrethroid and organophosphate ear tags. In a laboratory study, McKenzie and Byford (1993) found that mixtures and rotations of pyrethroids and organophosphates delayed and reduced the magnitude of resistance to each insecticide, but did not prevent it from evolving. In a field study by Byford *et al.* (1999), mosaic treatment of pyrethroid and organophosphate was not shown to select for resistance to either insecticide in a 3-year study. A mosaic strategy is possible within a farm, but probably not feasible across the landscape.

Because of the constant selection pressure from insecticide-impregnated ear tags, Sparks *et al.* (1985) recommended a return to control methods that deliver discrete doses of insecticide, such as sprays or dust bags, for IRM. These methods have not received much attention, however, because they are more labor intensive than the use of ear tags. The low cost and ease of insecticide-impregnated ear tags keeps them in use, even though control methods using discrete doses of insecticide would be better at managing resistance.

Musca domestica

Muscoid flies are common pests of livestock. Their similar life histories and association with production animals allow a more comprehensive study of IRM for veterinary pests. *M. domestica* flies are a worldwide nuisance pest of both humans and production animals. They lay eggs in manure or other refuse and feed in these areas as well. Although they cannot bite, adult flies may attempt to feed on moist

areas of cattle, and as such, they are sometimes implicated in disease transmission. *M. domestica* is a notorious and well-known pest with a long history of evolving resistance to chemical controls, and a large amount of research has been done on resistance mechanisms. For this reason, the approach taken in this section will be to apply what is currently known about resistance to a broader context of IRM principles.

M. domestica has become a model of the study of resistance because it has evolved resistance to every major class of insecticide used against it. Keiding (1999) provided an extensive review of resistance in *M. domestica*; highlights are provided here. Widespread resistance to DDT and other organochlorines quickly evolved in the 1940s and 1950s because of their ubiquitous use worldwide. Resistance to DDT and other organochlorines, such as lindane and dieldrin, in *M. domestica* is still present, despite the discontinued use of these products in most countries by the 1970s because of toxic effects on the environment. After the control failures of the organochlorines, organophosphates were commonly used and resistance also evolved to many of these as well. Resistance to carbamates has been documented; where it occurs, it is associated with either cross-resistance to organophosphates or intensive use of baits with methomyl, a carbamate. Resistance to pyrethroids is common in North America and Europe, again due to widespread use. It was slow to evolve in Asia because of a low initial frequency of resistance alleles, but it is now widespread.

Monitoring populations for resistance is important in determining initial frequency of alleles. The development of molecular methods to determine frequencies of resistant alleles will certainly quicken this process. Many IRM strategies rely on the assumption of low initial frequencies of these alleles. Resistance mechanisms and their respective alleles have been intensely studied in *M. domestica*, leading to molecular diagnostic assays. PCR-based assays have been developed for alleles conferring target site knock-down resistance (Huang *et al.*, 2004). These assays have the potential to be used for quicker results on initial frequencies of resistant alleles before a control method is used.

Because of the prevalence of resistance to most insecticides used, now researchers look for methods of control that do not confer cross-resistance or perhaps have negative cross-resistance. These compounds could be used in possible mixtures or rotations with commonly used insecticides. IGRs, such as diflubenzuron and cyromazine, are now commonly used for control of *M. domestica*, as are some of the newer insecticidal compounds such as fipronil (a phenylpyrazole) or spinosad (a spinosyn). Resistance has developed in some locations in the United States to cyromazine, probably due to low doses added to chicken feed (Tang *et al.*, 2002). Tang *et al.* (2002) found that resistance is probably due to three loci, and they recommend a high-dose sprayed on manure to combat it. Liu and Yue (2000) found that spinosad was effective against one pyrethroid-resistant strain. Scott (1998) came to the same conclusion, but noted the slow action of spinosad. A pyrethroid-resistant strain exhibits cross-resistance to fipronil (Wen and Scott, 1999).

The results above may present a warning in the use of these compounds for control of other muscoid flies. As previously noted, *L. cuprina* has not yet evolved resistance to cyromazine, and it may be unlikely (Yen *et al.*, 1996; Levot and Sales, 2004), but it is possible to inadvertently select for it (Tang *et al.*, 2002). Also, if fipronil is to be used for *H. irritans* control, extreme caution should be

taken because many populations are resistant to pyrethroids and could be cross-resistant to fipronil (Wen and Scott, 1999).

Widespread resistance in *M. domestica* provides lessons in IRM strategies that may be applied to other medical/veterinary pests. The tolerance threshold is quite low for ectoparasites, and so intensive use of a single compound for control quickly selects for resistance. Like most other insects in the case studies documented here, major resistance problems force people to take an IPM-like approach to combating resistance. Crespo *et al.* (2002) found that populations of *M. domestica* in poultry farms were most reduced using a combination of chemical control (cyromazine), cultural control (using lime to dry the manure), and biological control (parasitic wasps).

Discussion

Given that arthropods have evolved resistance to crops, crop rotation, and insecticides applied to crops, it is not surprising that they have evolved resistance to insecticides and acaricides used to manage pests on animals we consider very valuable: pets, livestock, and ourselves. Resistance presents a serious problem in control of ectoparasites and efforts need to be taken to maintain susceptibility. Resistance can be directly selected from control methods of ectoparasites or indirectly selected from outside sources.

Georghiou (1990) reviewed the problem of crop-protection chemicals contributing to the evolution of resistance in vectors of animal diseases. Broad-spectrum insecticides used for pest control on crops can reduce the fitness of mosquitoes infesting the same or nearby habitats. Certain fungicides and herbicides used on crops can even synergistically promote the efficacy of insecticides targeted against mosquitoes (Georghiou, 1990). Four types of evidence support the claim of a link between agricultural pesticide use and resistance in mosquitoes. One, mosquito resistance is often higher in agricultural than in non-agricultural areas. Two, mosquito resistance can be observed before insecticides have been targeted against the vectors of disease. Three, there often is a correlation between intensity of insecticide use on crops and the level of resistance in mosquitoes. Four, fluctuations in mosquito resistance have been observed in synchrony with seasonal fluctuations of crop spraying. In general, these studies demonstrate that IRM must account for selection pressure from all sources in a pest's habitat to be effective. Georghiou (1990) proposed two guidelines for mosquito IRM in agricultural areas. First, agricultural and public health agencies and industries must collaborate on mosquito IRM. Second, comprehensive IPM for crops must be supported by collaborators to reduce dependency on insecticides.

The link between public health and agricultural use of insecticides helps explain the persistence of resistance to pyrethroids and other insecticides. Resistance to DDT is common in all or most of these pests, even though it has not been used for decades. This is likely related to the prevalence of pyrethroid use for control, but resistance may remain in spite of the disuse of either compound. Persistence of pyrethroid resistance in absence of selection has been shown in *H. irritans* in field conditions and in *C. felis* in the laboratory. There is generally little or no fitness cost to resistant genotypes (Roush and McKenzie, 1987); one example is pyrethroid resistance in *V. destructor* (Martin *et al.*, 2002). Another reason for

persistence of resistance to DDT and pyrethroids is that multiple mechanisms of resistance exist. Resistance to pyrethroids in *H. irritans* may occur in the form of the *kdr* mutation (Guerrero *et al.*, 1997), metabolic detoxification (Sparks *et al.*, 1990), or behavioral mechanisms (Lockwood *et al.*, 1985; Byford and Sparks, 1987). Multiple mechanisms of resistance to pyrethroids (Brogdon *et al.*, 1999) and DDT (Penilla *et al.*, 2006) are also seen in *Anopheles* mosquitoes, *P. h. capitis* (Lee *et al.*, 2000) and *M. domestica* (Liu and Yue, 2000).

Persistent resistance to other insecticides is less common than to pyrethroids but nevertheless is still present. Resistance to organochlorines is widespread in mosquitoes, even though those compounds have not been used for decades (Hemingway and Ranson, 2000). Laboratory colonies of *C. felis* fleas had variable frequencies of *Rdl* alleles without selection by dieldrin or any other insecticidal compound (Bass *et al.*, 2004b). IRM strategies should account for persistent resistance to certain compounds and the possibility of cross-resistance to another compound that may be used.

Monitoring and prediction of resistance is recognized as an important first step in its mitigation. Resistance mechanisms have been intensely studied in *M. domestica* and mosquitoes, most notably (Hemingway, 1998). The WHO has developed bioassays for resistance in a number of pests of public health importance. Furthermore, as the cases presented here indicate, molecular methods for diagnosing resistance have been developed (or are in development) for a variety of pests. PCR-based diagnostic procedures provide a reliable estimate of initial allele frequencies, and they are much less labor intensive than bioassays.

There is much attention on predicting and monitoring resistance, but little has been done to actually manage resistance or to change practices according to predictions. This may be due to the inherent problematic nature of IRM for ectoparasites because of the value of the host. The value of the hosts of these pest arthropods – pets, livestock, and ourselves – contributes to the difficulty in researching or implementing long-term IRM solutions. The creation of untreated refuges to increase the number of susceptible individuals is common in agricultural settings. However, managed refuges are generally not acceptable for medical pests because there is usually no threshold of tolerance of these insects relating to either vector capability or comfort level. Managed refuges may not even be possible in certain arthropods spending all or most of their life cycle on or in close proximity to the host. Use of a refuge depends on whether the pest is holometabolous or hemimetabolous. A holometabolous insect is likely to only be a temporary parasite, usually (but not necessarily) during the adult stage; a refuge could consist of non-parasitic larvae. Conversely, a different control method may be used on the larval stage, essentially functioning as a rotation strategy for IRM. Hemimetabolous insects or acarines are usually either permanent parasites or spend very little time off of a host. An untreated refuge may not be possible in these situations, so other strategies such as insecticide mixtures or rotations may be useful. Finally, a pest's fidelity to a host further complicates the use of a refuge. As discussed above, some mosquitoes may feed on an alternative host, but examples such as these are probably the exception rather than the rule.

Another typical IRM strategy is the use of high doses of insecticide to ensure no heterozygotes survive treatment, but this too proves to be difficult for ectoparasites. Detriment to the host is of great concern, as exemplified by the *V. destructor*/

A. mellifera association. Because both are arthropods, they are likely to be susceptible to similar compounds. Finally, a high-dose strategy may not even yield effective control. For example, a higher dose generally does not cause higher mortality in *P. h. capitis* lice (Downs *et al.*, 2002).

Coordination and education among all affected parties is crucial in mitigating resistance; one cannot do it alone. There have been calls for more communication in almost every case study here. A concern for public health gives greater impetus to control disease vectors and manage resistance. Even though there is a need for more coordination in distribution of ITNs and educational materials about malaria, it is one example where there is some level of centralization of mosquito control. WHO has a great influence on policy-makers of different nations, and they have made great strides in education about malaria, and consequently mosquito resistance. Another organization dedicated to coordination on resistance management activities is IRAC. This international group consists of industry leaders and some academics, and they advise regulatory bodies on policy issues relating to sustainable agriculture and public health. Information on resistance and resistance management issues is available on their website (www.irac-online.org).

Other case studies show examples of difficulty in coordinating IRM efforts. For example, *B. microplus* can be easily spread between herds of cattle, and George *et al.* (2004) lament the disconnect between researchers and producers in integrated tick control. Some pests are so ubiquitous that a coordinated effort to manage resistance would be almost impossible. A great number of people own pets, and a great number of these pets will encounter fleas at some point in time. The regulation of flea control methods through veterinarians could slow the evolution of resistance. However, as mentioned above, resistance to antibiotics has not been prevented by exclusive prescription by physicians.

Finally, the role of behavior in resistance, and therefore resistance management, is one that should not be overlooked. The behavior of an ectoparasite could be exploited in its control and managing resistance; this is seen in the possible use of ITNs that are treated with two insecticides, one on the top and one on the bottom (Figure 7.1). These nets function as a mixture strategy for IRM because of mosquito host-seeking behavior. Conversely, a parasite may evolve resistance by behavioral means. Behavioral resistance has evolved in a number of the arthropods mentioned here, namely to pyrethroid insecticides (or DDT) because of their excitorepellent effect. A notable example of behavioral resistance is certain *Anopheles* mosquitoes changing their resting places in response to indoor residual spraying. Some species naturally rest indoors, but have adapted to indoor sprays by resting outdoors where insecticide is not sprayed, i.e., exophily. Behavioral avoidance of pyrethroids is also seen in *H. irritans*, where they have been noted to rest on cattle on the areas where they are farthest from the insecticide-treated ear tag, namely the underside and backside (Byford *et al.*, 1987). Resistance by a temporal change in behavior is another possibility. There is concern that mosquitoes could change preferred feeding times to hours of the day when people are not likely to be sleeping under an ITN (Pates and Curtis, 2005).

There are many difficulties in devising and implementing IRM strategies for ectoparasites. The valuable nature of the host, or even ethical reasons, may preclude certain types of strategies from being tested. Models greatly assist in the

determination of the best strategy, but they clearly need to be built on reliable biological data, which may be difficult to obtain due to host specificity of the ectoparasite. Some people may view the magnitude and severity of a disease to outweigh the very real risk of evolution of resistance by the vectors of the disease. Because of these challenges, many have adopted IPM-like methods of control to manage resistance.

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Chapter 8

Resistance to Crop Rotation

Joseph L. Spencer and Eli Levine

Background

Crop rotation is the agricultural practice of sequentially growing a series of plant species on the same land (Yates, 1954). Rotational cycles may be as short as 2 years, like the corn–soybean (*Zea mays, Glycine max*) rotation that dominates the US Corn Belt, or as long as 10–12 years for the grass-legume pasture and cash-grain rotation that is found in Argentina (Bullock, 1992).

The origins of crop rotation as an agricultural practice lie in antiquity; MacRae and Mehuys (1985) report that it was in use more than 3,000 years ago in Han Dynasty China. Historically, crop rotation was a remedy for poor soil productivity and involved a series of crops that incorporated legumes with cereal crops (Karlen *et al.*, 1994). Though the mechanism responsible for the agronomic benefit of rotation was not understood, the benefits of incorporating a leguminous crop in a cropping sequence was known during Roman times and reported by a number of contemporary historians (Karlen *et al.*, 1994). The modern concept of crop rotation goes back to the four-crop Norfolk rotation (turnip, barley, clover, and wheat) that was popular in 18th century England. In the United States, Thomas Jefferson and George Washington experimented with a variety of rotational schemes on their farms. Rotation, along with artificial addition of lime and soil minerals, became nearly universal in England by the middle of the 19th century; immigrant farmers brought these practices to the United States where many different rotational systems were developed and used extensively during the 19th century. The discovery that a mutualism between leguminous plants (e.g., alfalfa, soybean, and clover) and common soil bacterium *Rhizobium* fixed atmospheric nitrogen (converting unusable N_2 gas into biologically useful NH_3) that was then available to successive crops provided an explanation for some of the benefits of crop rotation. Corn grown in a 2-year rotation with soybean yields 5–20% more than corn grown in a continuous cultivation (Bullock, 1992). In addition to the obvious fertility benefits, soil organic matter can be increased and soil structure may be improved when crop rotation is practiced (Bullock, 1992).

However, under some circumstances, the added cost of fertilizers, pesticides, labor, and other inputs to protect yields of continuous crops may be more than offset by local demand, market forces, or premiums (i.e., for animal feed, specialty crops, seed production, and ethanol production, etc.). Where the added costs (including

yield losses due to increased plant disease in some continuously grown crops like soybean) for continuous production of one crop cannot be justified, crop rotation offers a simple, economical and environmentally benign alternative to reliance on chemical inputs.

Specialist Pests are Vulnerable to Crop Rotation

The intentional use of crop rotation as a tool for managing pests is a relatively recent development. Along with other cultural controls like alteration of planting date to disrupt pest-host synchrony or field sanitation to remove overwintering sites, crop rotation for pest management requires an understanding of pest ecology. Not all pest organisms can be managed with crop rotation. Pests that may be vulnerable to crop rotation (1) have a life stage(s) that is relatively immobile (i.e., it is incapable of moving far enough to locate a host plant if one is not present), (2) have a narrow feeding relationship requiring consumption of host plant tissue for development, and (3) cannot survive for long periods in the absence of a host plant. Soil- and root-dwelling nematodes, soil-borne pathogens, and certain weed species are examples of non-insect pests that may be controlled with rotation (Bullock, 1992).

The best example of insect pest management using crop rotation involves control of diabroticite corn rootworm beetles (Coleoptera: Chrysomelidae) in the US Corn Belt. Nationwide, 67% of cornfields are in rotation a corn–soybean rotation, 14% of fields are grown as continuous corn, and the remaining 19% are in other rotations (United States Department of Agriculture Economic Research Service (USDA ERS), 2003). Since, the first reports of corn rootworm injury to crops, entomologists have recommended crop rotation to control corn rootworms. Stephen A. Forbes (1883) suggested that because the northern corn rootworm (NCR), *Diabrotica barberi* (then known as *Diabrotica longicornis*), depended on availability of corn roots as food for the relatively immobile larvae, rotating production of corn with a plant that NCR larvae could not eat, would destroy rootworm populations by depriving newly emerged NCR larvae of the corn roots. Forbes (1883) asserted that crop rotation was such a plainly obvious solution to the problem of rootworm control that no special comment was required to explain his reasoning. Forbes' reasoning, regarding the logic of annual crop rotation, could also have been applied to the related western corn rootworm (WCR), *D. virgifera virgifera*. Though it would not become a pest until the 20th century, today the WCR arguably surpasses the NCR in its overall impact on corn production. Together, corn rootworms are likely the most economically significant pests of US corn. It has been estimated that annual losses and management expenditures related to corn rootworm exceed $1 billion (Metcalf, 1986). Despite the biological vulnerability of corn rootworms to crop rotation, there are now populations of NCR and WCR that are resistant to rotation. The history of corn rootworm management reminds us that any management practice that affects pest survival or reproduction can select for resistance, even a seemingly "unbeatable" cultural control.

US Corn Production, Corn Rootworm, and Insecticides

In the rich, fertile soils of the US Corn Belt, rotations were not commonly accepted during the early 20th century, even though higher yields were demonstrated for

rotated corn (also referred to as first-year corn). Superior yields of corn in rotation could not compensate for the poor economic return from the other crops in rotation; thus many farmers continued to grow continuous corn at the expense of depleted soil fertility. Significant corn production was also necessary to feed draft animals in the period before mechanization of agriculture. Introduction of fertilizers and pesticides, along with mechanization, enabled farmers, even those who may have practiced diversified agriculture, to simplify their operations and reduce their costs (Bullock, 1992).

An agricultural transformation began after the US Civil War in the late 19th century and bloomed in the early 20th century thanks to development of hybrid corn and improved farming techniques that dramatically altered the landscape of crop production (Allen and Rajotte, 1990). Availability of hybrid corn, fertilizers, pesticides, irrigation, and mechanization enabled corn to be produced over a vast area with increased yield. Expansion of corn cultivation to the west also brought the NCR and WCR (a once sparsely distributed leaf beetle) into contact with an abundant potential new host plant. Adoption of cultivated corn as a host plant by the NCR and the WCR and human reliance on insecticides to control the ravages of pest corn rootworms are responsible for the rise of corn rootworms as the most serious pests of US corn; *Diabrotica* are truly "man-made" pests (Metcalf, 1986).

History of *Diabrotica*

Both the NCR and WCR are species indigenous to North America (Chiang, 1973). The NCR was described by Thomas Say as *Galleruca longicornis* from specimens collected in 1820 from Colorado (Say, 1824). Branson and Krysan (1981) argue that the NCR invaded areas of the United States before the introduction of corn (*ca.* 700 AD (Galinat, 1965)) and only later switched to corn after extensive corn cultivation reached the central plains after the Civil War. The NCR was first reported to be a pest of corn in July 1880 in Stark County, Illinois by Cyrus Thomas in his Fifth Annual Report of the State Entomologist (Thomas, 1881). Even in this earliest correspondence, crop rotation was suggested as "the most feasible means of dealing with the insect". In his sixth report (Thomas, 1882), Thomas noted that C. V. Riley, State Entomologist of Missouri, had already reported the same rootworm species feeding in corn roots from Missouri in 1878 as part of his March 1879 report to the Commissioner of Agriculture. In his "First Annual Report of the State Entomologist" covering 1882, Forbes (1883) presents a detailed account of suspected NCR injury predating the Missouri records, along with a thorough treatment of NCR biology and suggestions for controlling this pest which prominently features crop rotation.

The WCR was first collected in 1867 and described by LeConte (1868) who collected two specimens from wild gourd (probably *Cucurbita foetidissima*) near Fort Wallace, Kansas. Gillette (1912) was first to report WCR as a pest of cultivated corn (sweet corn) in 1909 and 1910 near Fort Collins and Loveland, Colorado, respectively. Smith (1966) suggested that the native hosts of WCR populations in the Colorado-New Mexico-Arizona region of the United States were likely *Tripsacum* (any of approximately 15 species of perennial grass closely related to corn, *Zea mays*). Krysan *et al.* (1977) and Branson and Krysan (1981) suggested

WCR likely evolved in the same region as corn and, as a corn specialist, it followed corn as it was moved into the southwestern United States after 700 AD.

Despite being grass specialists, both NCR and WCR adults will feed compulsively on species in the Cucurbitaceae containing cucurbitacins B and E (Metcalf, 1979). This compulsion is proposed as evidence of diabroticite coevolution with cucurbitaceous (squashes) host plants prior to a host shift onto graminaceous plants after which the cucurbitacin compulsion was retained (Metcalf, 1983; Tallamy *et al.*, 2005).

Corn Rootworm Biology

Corn rootworm biology has been extensively reviewed (Chiang, 1973; Krysan, 1986; Levine and Oloumi-Sadeghi, 1991). The univoltine adults of the NCR and WCR are present in cornfields from July through frost where they feed on corn foliage, pollen, silks, and developing kernels, and in the case of the NCR, the pollen of other plants. High densities of feeding adults (usually more than five WCR per plant) may interfere with corn pollination due to silk clipping (Levine and Oloumi-Sadeghi, 1991). Oviposition has traditionally taken place almost exclusively in cornfields from late July through mid-September (Shaw *et al.*, 1978; Levine and Oloumi-Sadeghi, 1991). WCR may lay as many as 1,000 eggs, while NCR may deposit 300 eggs in their lifetime. The diapausing eggs spend the fall and winter in the soil until late spring and early summer when they hatch, and the larvae begin to feed on corn roots (Levine and Oloumi-Sadeghi, 1991). Larvae of both species can survive only on the roots of corn and a limited number of grassy weeds (Branson and Ortman, 1967, 1970; Oyediran *et al.*, 2004a, b; Ellsbury *et al.*, 2005). Larval feeding disrupts root system function, reducing the amount of water and nutrients available to developing corn plants, which can reduce grain yield (Levine and Oloumi-Sadeghi, 1991). The feeding injury also may facilitate infection by root and stalk rot fungi, resulting in further damage. Extensive root injury makes plants more susceptible to lodging (i.e., the plants fall over); yield losses may result from the difficulty in harvesting fallen corn. After completion of three larval instars, WCR pupate in the soil near the corn roots. The pupal stage lasts 5–10 days (Fisher, 1986), and adults begin to emerge in late June or early July. Male adults start emerging about 1 week before first emergence of females; thus, there is protandry in the species.

Newly emerged females begin releasing sex pheromone on the day of emergence (Hammack, 1995). The unmated, and frequently teneral, females expose a pheromone gland at the tip of their abdomen to broadcast their readiness to accept a mate. Mate-seeking males respond to pheromone and rapidly locate and mate with nearby "calling" females on or close to the plant that supported their development (Hill, 1975; Lew and Ball, 1979). Mating lasts 3–4 hours, during which the male transfers a large spermatophore to the female; most females mate only once, while males may mate multiple times (Branson *et al.*, 1977; Sherwood and Levine, 1993).

Insecticides and Corn Rootworm Control

Following World War II, a new era in pest control began, as synthetic organic insecticides became widely available (Aspelin, 2003). Soil-applied cyclodiene insecticides soon became important management tools for protecting corn roots

from corn rootworm larval feeding injury (Hill *et al.*, 1948). The first WCR control failures were reported in 1959; by 1961, central Nebraska WCR were 100-fold more resistant to cyclodiene insecticides than susceptible populations elsewhere in the state (Ball and Weekman, 1962; Metcalf, 1983). The rise of insecticide-resistant corn rootworms coincided with expansion in the WCR and NCR geographic ranges (Metcalf, 1983).

Through the 1920s–1940s, the distribution of WCR had slowly expanded eastward across the western corn-growing region (reviewed by Metcalf, 1983). An apparent acceleration in the eastward spread of WCR, coincided with the rise of widespread cyclodiene resistance in the early 1960s (Metcalf, 1982, 1983). Whereas, the WCR distribution expanded from Colorado to the Missouri River (756 km) between 1909 and 1948 at just 19 km/year, cyclodiene-resistant WCR spread from Nebraska to Wisconsin (579 km) between 1961 and 1964 at *ca.* 193 km/year. WCR had spread to most of the Corn Belt by 1979 (1,000–2,500-fold levels of cyclodiene resistance were still present in WCR at the expanding front) and reached the corn producing areas of the eastern states by the mid-1980s (Tallamy *et al.*, 2005). Metcalf (1983) hypothesized that the increased movement rate was related to the increased fitness among cyclodiene-resistant beetles and a behavioral change associated with the genes for resistance.

Resistance to Crop Rotation

NCR and Prolonged Diapause

Although crop rotation usually prevents problems with NCR, instances of rootworm injury to corn grown in rotation with other crops have been reported (Levine and Oloumi-Sadeghi, 1991). In the early 1930s, Bigger (1932) noted that growing corn after oats or sweet clover failed to control this pest. Lilly (1956) reported severe NCR injury in cornfields that were planted to oats or soybean the previous year but observed that 2 years without corn always provided control. Branson and Krysan (1981) and Hill and Mayo (1980) suggested that NCR infestations in rotated corn can be explained by oviposition in fields planted in crops other than corn the preceding season, but other studies failed to support this hypothesis. Chiang (1965a) sampled for NCR eggs in fields other than corn where adults were feeding and concluded that few, if any, eggs were laid in those sites. In Illinois, Shaw *et al.* (1978) found that oviposition in soybean fields and injury to corn the following year were negligible where soybean fields were free of volunteer corn; root injury did not reach economic levels even when corn was planted after weedy soybeans. In South Dakota, Gustin (1984) found that NCR adults laid the majority of their eggs in corn plots, regardless of the maturation stage of corn, rather than in the stubble of small grains. Boetel *et al.* (1992) studied the oviposition habits of NCR at three locations in South Dakota and concluded that while adults were often found feeding on weeds and non-corn crop plants, the adults returned to corn to lay the vast majority of their eggs.

An alternate explanation for root injury to rotated corn was that NCR eggs may undergo prolonged diapause; i.e., eggs pass through two or more winters before hatching rather than the typical 1-year pattern (Levine and Oloumi-Sadeghi, 1991) (Figure 8.1). Diapause is a state of arrested development occurring in one stage of the life cycle that allows an insect to survive seasonally recurring periods of

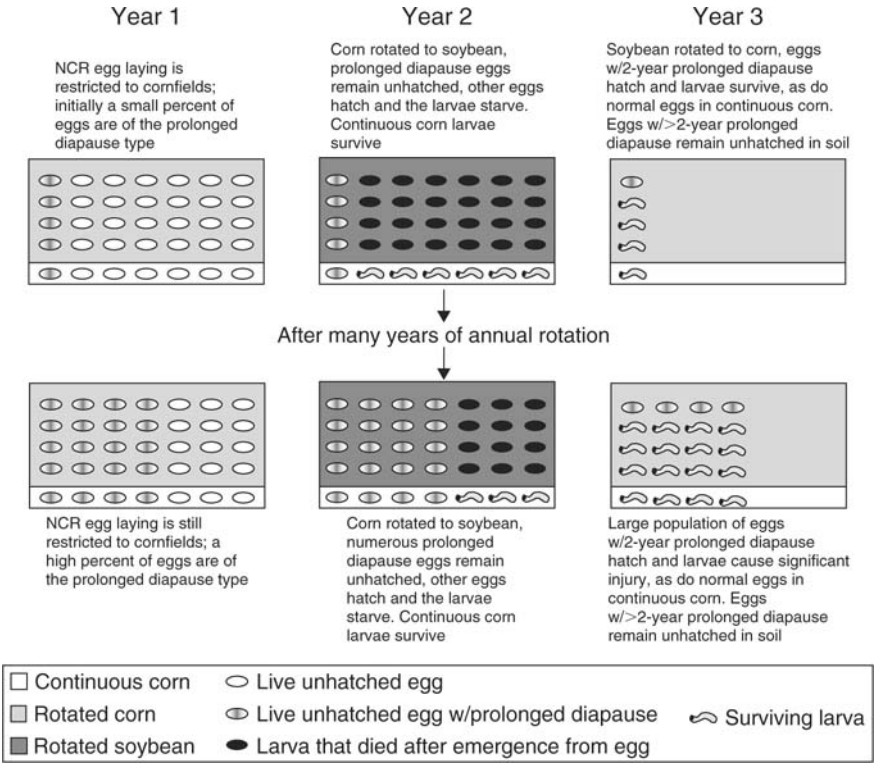

Figure 8.1 NCR prolonged diapause. Selection scenario and fate of NCR eggs (with and without prolonged diapause) deposited in continuous and rotated cornfields. Only eggs deposited in year 1 are followed. Lower section depicts outcome after years of crop rotation-imposed selection for prolonged diapause.

adverse conditions. Prolonged diapause (sometimes also referred to as extended diapause) is common among insects and it may spread emergence over as many as 12 or more years for certain species (Tauber *et al.*, 1986; Danks, 1992). Usually only a small percentage of individuals remain in diapause while the major portion of the population becomes active. Chiang (1965b) was first to document that NCR eggs (from Minnesota) could remain in diapause for more than one winter, but concluded that the percentage of eggs with this trait (0.3%) was too small to be of economic consequence. Krysan *et al.* (1984, 1986) reported, however, that about 40% of the eggs from a population of NCR collected in South Dakota in 1981 underwent prolonged diapause. Larvae from this large portion of the population could potentially cause significant injury to corn after a 1-year rotation with another crop. Indeed, reports of corn rootworm injury to corn planted after soybeans became more prevalent in the early 1980s, particularly in the northwestern region of the Corn Belt (Iowa, Minnesota, Nebraska, and South Dakota). Prolonged diapause has been confirmed for NCR eggs from South Dakota (Krysan *et al.*, 1984, 1986), Minnesota (Krysan *et al.*, 1986), Illinois (Levine *et al.*, 1992b),

Michigan (Landis *et al.*, 1992), and North Dakota (Levine and Weiss, unpublished data). A 1988 survey of 313 randomly sampled fields of corn in northwestern Iowa suggested that the prolonged diapause trait was generally distributed throughout the NCR population infesting rotated corn (Tollefson, 1988).

Levine *et al.* (1992b) showed that diapause in NCR eggs was quite variable in length, ranging from 1 to 4 years in Illinois and South Dakota populations. Levine *et al.* (1992b) collected 1,211 NCR eggs from Champaign, Illinois female beetles during the summer of 1985. Among the 777 eggs that survived to hatch during the 4-year study period, 50.6%, 41.2%, 8.0%, and 0.3% hatched after one, two, three, and four simulated winters, respectively (Levine *et al.*, 1992b). Similarly, of 311 eggs that hatched from a Madison, South Dakota population, 48.9% hatched after the first simulated winter (batches of eggs were buried in the field at a depth of 20 cm near Brookings, South Dakota), 20.6% after two winters, 20.9% after three winters, and 9.6% after four winters (Levine *et al.*, 1992b). The differences among populations may reflect adaptation to field cropping patterns at the adult collection sites. Collections from Illinois were from an area that was annually rotated between corn and soybean. The South Dakota farm utilized many different rotations during the 25 years prior to when collections were made. These data support the hypothesis that NCR are adapting to site-specific cropping practices. The less varied rotational patterns used by Illinois farmers may explain the large percentage of egg hatch that occurred following two winters; the more varied cropping patterns in South Dakota may explain the nearly equal hatch of eggs after two or three winters (Levine *et al.*, 1992b).

Levine *et al.* (1992b) also obtained eggs in August 1986 from NCR females collected in Champaign county and from rotated cornfields in four Illinois counties north of Champaign County that had experienced greater than expected rootworm injury. The percentage of eggs that hatched after prolonged diapause (>1 chill period) ranged from 13.9% in northwest Illinois to 51.3% in east-central Illinois. There was a highly significant correlation between the percentage of NCR with prolonged diapause in a given county and the percentage of rotational corn grown in that county (Levine *et al.*, 1992b). This finding is supported by reports of a greater incidence of prolonged diapause in areas of South Dakota and Minnesota where corn is rotated annually than in areas where corn is planted without rotation (Krysan *et al.*, 1986; Ostlie, 1987). In addition, significant differences in the percentage of eggs showing prolonged diapause were found between eggs laid by different females collected at a single location, a finding which suggests that there is a genetic component to prolonged diapause (Levine, unpublished data). Growing corn in an annual rotation with another crop provides intense selection pressure for NCR eggs to remain in diapause for 2 years. Larvae that emerge from eggs that pass two winters before hatching have a greater chance for survival and are therefore more likely to pass their genetic information on to their progeny under such a cropping pattern (Levine *et al.*, 1992b). The early reports by Bigger (1932) and Lilly (1956) of NCR injury to rotated corn are now best explained by our knowledge of prolonged diapause.

The NCR can be considered to have two phenotypes: univoltine (individuals with the typical one-winter egg diapause) and semi-voltine (individuals with a prolonged egg diapause). Both phenotypes live in a heterogeneous environment

with respect to their host plant. Semi-voltine beetles are at a distinct reproductive dis-advantage compared with univoltine beetles in fields where corn is planted year after year because their reproductive rate is essentially half that of univoltine beetles. On the other hand, in fields where corn is regularly rotated with another crop, univoltine beetles are at a disadvantage. Because both types of corn are often planted in the same area, beetles are subject to disruptive selection (Krafsur, 1995). Krysan (1993) suggested, as we also stated earlier, that in regions where planting practices in individual fields tend to remain the same for several years, it is possible for NCR populations to adapt to the cropping practices of *individual* growers. Local adaptation, however, could be counteracted by random intermating; prolonged diapause would only slowly increase in frequency in areas where a large number of cornfields were rotated annually with another crop (Krafsur, 1995).

WCR and Behavioral Resistance to Crop Rotation

WCR first entered northwest Illinois in 1964 (Petty, 1965) and only compounded an existing NCR problem. Management of WCR (and NCR) in continuous corn typically involves planting-time application of soil insecticides to protect corn roots from larval feeding injury or application of foliar insecticides to protect silks from adult feeding, which can interfere with pollination (Levine and Oloumi-Sadeghi, 1991). In addition to the use of insecticides, excellent management of WCR also could be achieved with crop rotation, at least in the past. Following WCR entry into the state, the use of soil-applied insecticide in cornfields increased in Illinois; through the late 1960s, $>60\%$ of all cornfields were treated for corn rootworms. Insecticide usage remained at $\geqslant 50\%$ of all cornfields until the late 1970s (Pike and Gray, 1992). Thereafter, insecticide use began a steady decline into the 1990s as educational efforts succeeded in reducing use of insecticides on rotated corn. In 1990, 80% of continuous corn and only *ca.* 13% of rotated corn in Illinois were treated with insecticide (Pike and Gray, 1992). The 1960s–1990s shift from widespread prophylactic insecticide application to reliance on cultural control was a victory for integrated pest management (IPM). Ironically, insecticide use at this level was probably still excessive. Steffey *et al.* (1992) surveyed root injury in first-year corn around Illinois from 1986–1989, and found only 1.7% of rotated cornfields experienced injury that exceeded theoretical economic injury levels. Steffey *et al.* (1992) concluded that Illinois corn producers rarely needed to apply soil insecticides to prevent rootworm injury in corn rotated with soybean. At the time, most of the economic injury reported by Steffey *et al.* (1992) was attributed to NCR prolonged diapause. Areas with high adoption of crop rotation (northeast, central, and east-central Illinois) were identified as areas most at risk from this injury.

In 1987, the first evidence of WCR behavioral adaptation to crop rotation was observed in isolated seed cornfields in Ford County, IL (Levine and Oloumi-Sadeghi, 1996); the roots of corn plants in rotated cornfields suffered serious corn rootworm larval feeding injury. More than 95% of the adults collected in the fields were WCR, suggesting the problem was not related to NCR prolonged diapause. Careful analysis of the unexpected situation and follow up studies in 1988 ruled out corn rootworm oviposition around volunteer corn or grassy weeds during the

previous years. Because these were seed cornfields, it was suspected that pyrethroid insecticides, used in seed corn production fields to control corn earworms, *Helicoverpa zea* (Boddie), had repelled adult WCR beetles into nearby soybean fields where they laid eggs (Levine and Oloumi-Sadeghi, 1996). Retrieval of WCR from soil samples in soybean fields and subsequent emergence cage data in rotated corn supported this hypothesis.

The repellency hypothesis had to be abandoned beginning in 1993; serious WCR larval injury to first-year corn (corn planted after another crop, primarily soybeans) in east-central Illinois and northwestern Indiana began to increase. In addition, the 1993–1995 "problem area" included many commercial cornfields that were far from pyrethroid-treated fields. Growers who had successfully controlled WCR with only crop rotation suffered serious crop losses (Levine *et al.*, 2002). Studies in Illinois and Ontario, Canada, ruled out a WCR prolonged diapause; only a very small percentage (0.21%) of WCR eggs were capable of prolonged diapause and could potentially injure corn following a 1-year rotation with another crop (Levine *et al.*, 1992a). None of the eggs examined from "problem area" expressed the prolonged diapause trait (Levine and Oloumi-Sadeghi, 1996).

This possibility, that WCR females deposited large numbers of eggs outside of cornfields as part of their "normal" activity, was counter to the well-understood biology of WCR. However, the events of 1995 forced scientists to reconsider what was normal for WCR. What was once an isolated curiosity only a few years earlier, had spread to nine east-central Illinois counties and 15 nearby Indiana counties where growers suffered devastating losses due to severe WCR larval feeding on the roots of rotated corn (Levine *et al.*, 2002). In response, extensive survey and sampling efforts were mounted throughout the area; all of the larvae recovered from injured corn roots were WCR, $\geqslant$90% of corn rootworm adults emerging in affected fields were WCR, and only WCR eggs were recovered from soil samples taken in soybean fields adjacent to affected cornfields. The unlikely conclusion became inescapable: a behavioral change had indeed occurred and WCR beetles in east-central Illinois and northwestern Indiana were leaving cornfields to lay their eggs in neighboring fields of soybean and other crops (e.g., alfalfa, wheat, and oats) as well as in corn.

Enthusiastic adoption of crop rotation over a broad area (e.g., 95–98% of corn in east-central Illinois is rotated, usually with soybean (Onstad *et al.*, 1999, 2003a)) combined with the great efficacy of the technique, created a strong selection that favored an existing, but presumably uncommon, WCR phenotype with reduced ovipositional (egg laying) fidelity to cornfields (Onstad *et al.*, 2001b) (Figure 8.2). The presence of some WCR expressing what we now would call "rotation-resistant behavior" may be evident as in late-1970s–1980s records of root injury for first-year corn (Shaw *et al.*, 1978; Steffey *et al.*, 1992). Unexpected injury in rotated corn was frequently attributed to the presence of volunteer corn or grassy weeds in rotated soybean fields that were known to be attractive to ovipositing WCR and NCR (Shaw *et al.*, 1978). Perhaps awareness of the potential for NCR prolonged diapause to produce injury in first-year corn delayed consideration of alternative explanations. So strong was the belief that WCR biology could not overcome crop rotation, that as late as 1993, it was suggested that "it is highly unlikely that the

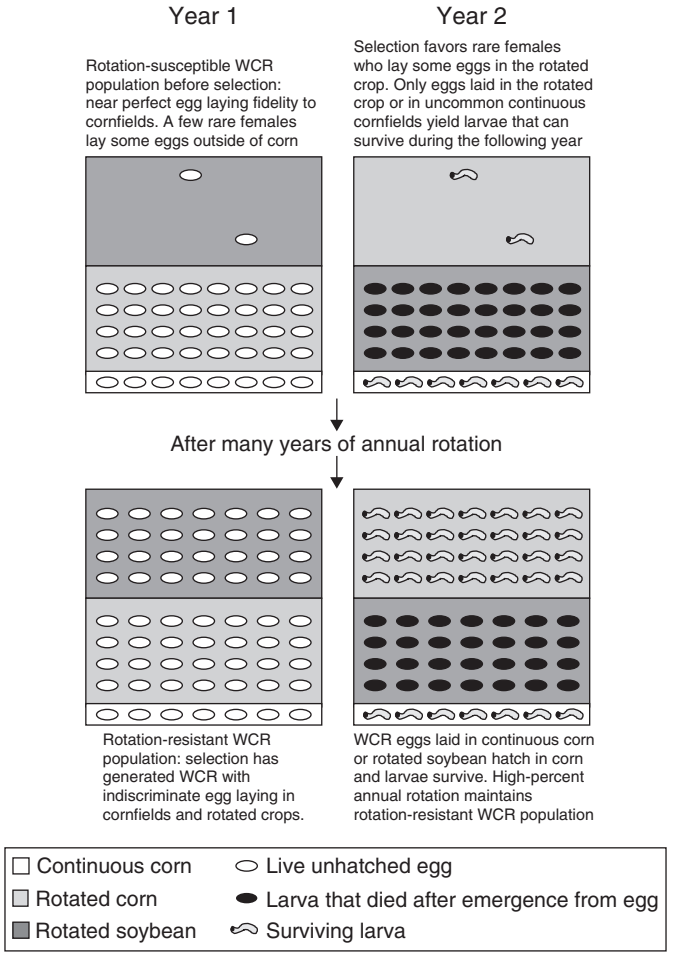

Figure 8.2 WCR rotation resistance. Selection scenario and fate of WCR eggs deposited in cornfields (rotated and continuous) and rotated crops (i.e., soybean). Only eggs deposited in year 1 are followed. Lower section depicts outcome after years of crop rotation-imposed selection for rotation resistance.

WCR could become adapted to crop rotation by oviposition in the alternate crop" (Krysan, 1993). An appreciation for the selective force that widespread crop rotation could impose on WCR was apparently lacking.

In the case of the WCR in east-central Illinois, females with reduced ovipositional fidelity to cornfields realized a reproductive advantage over females with perfect ovipositional fidelity since they lay some of their eggs in non-host fields (e.g., soybean) which are rotated to corn with high probability. Over time, the once-uncommon females that laid some of their eggs outside of cornfields gained a reproductive advantage, resulting in a significantly greater proportion of females with reduced ovipositional fidelity to cornfields. It is hypothesized that this selection

generated a WCR population in which a high proportion of females have some propensity to exit cornfields and oviposit in soybean fields (or other locations in addition to cornfields). Modeling by Onstad *et al.* (2001b) suggests that a high level of rotation in the landscape (⩾80%) is necessary for evolution of behavioral resistance; below that level of rotation, the contribution of rotation-susceptible alleles from WCR produced in continuous corn prevents rapid evolution of rotation resistance. The agricultural landscape of east-central Illinois presented conditions that strongly favored evolution of rotation resistance as envisaged by Onstad *et al.* (2001b).

Today, rotation-resistant WCR are found across large portions of Illinois, Indiana, and smaller portions of Michigan, Ohio, Wisconsin (Spencer *et al.*, 2005), and the Canadian Province of Ontario (Meloche and Hermans, 2004); rotation-resistant WCR are also likely now in eastern Iowa. The rate of gradual west- and northward expansion of rotation resistance appears to be progressing faster than predicted by Onstad *et al.* (1999). Based on survey data from 2000, over 3.6 million ha of corn were located in this portion of the Corn Belt (Doane's Market Research, 2001 cited in Alston *et al.*, 2002).

The Biology of Rotation-Resistant WCR

Frequent, season-long adult WCR movement outside of cornfields and presence in rotated crops are characteristic of rotation-resistant WCR beetle behavior (O'Neal *et al.*, 1999; Isard *et al.*, 2000, 2004; Levine *et al.*, 2002; Pierce, 2003; Rondon and Gray, 2003; Spencer *et al.*, 2005; Pierce and Gray, 2006). In the area threatened by WCR rotation resistance, WCR eggs may be recovered as commonly in the soil of soybean fields as in the soil of cornfields (Levine *et al.*, 2002; Pierce, 2003, Rondon and Gray, 2004; Pierce and Gray, 2006). Though the presence of many WCR eggs in soil indicates subsequent WCR injury is likely, recovering corn rootworm eggs from soil requires special equipment and is not a feasible monitoring technique. Measurement of adult WCR abundance in crops rotated with corn is the basis of determining subsequent risk of economic WCR larval injury to rotated corn (O'Neal *et al.*, 2001). Movement of female adults plays a key role in the phenomenon of WCR rotation resistance and in WCR biology generally.

The pre-mating and mating behavior of rotation-resistant WCR males and females appears to be similar to that historically reported for rotation-susceptible populations (Ball, 1957; Hill, 1975; Guss, 1976; Bartelt and Chiang, 1977; Branson *et al.*, 1977; Lew and Ball, 1979, 1980), though the populations have not been directly compared. Likewise, the post-mating behavior of all WCR females likely involves a period of feeding on corn silks and pollen for as long as a week before many fly out of their natal field and disperse or migrate an unknown distance to downwind cornfields (Coats *et al.*, 1986; Spencer *et al.*, 2005).

During the post-mating, pre-ovipositional dispersal period, favorable atmospheric conditions (e.g., instability due to heating of air near the ground and the passage of summertime convective storms) and light winds promote flight and favor ascent from cornfields (Witkoski *et al.*, 1975; VanWoerkom *et al.*, 1983). A diel periodicity in flight tendency is reflected in peaks of flight during early-late morning and early evening; Isard *et al.* (1999, 2000) review many of the factors that influence flight patterns. During the passage of summertime storms, some

WCR adults may be drawn into storms and carried 10's of kilometers before being washed out of the storm in rain (Grant and Seevers, 1989, 1990). When WCR-bearing storms pass over Lake Michigan, evidence of storm transport can be found in the piles of WCR beetles that wash-up along the waterline (Grant and Seevers, 1989). After dispersal, most WCR will locate a new cornfield, where they continue to feed and provision their first batch of *ca.* 100 developing eggs. It is after post-mating dispersal, that rotation-resistant individuals commence frequent interfield movement between corn and soybean fields. At this point, the behavioral difference between rotation-resistant and susceptible populations becomes plainly evident. Earlier work on WCR movement (Hill and Mayo, 1980; Lance *et al.*, 1989; Naranjo, 1991) emphasize a reluctance among WCR to leave cornfields; when interfield movement occurred, it was nonrandom and oriented toward flowering corn (Naranjo, 1994). Frequent interfield movement leading to high WCR abundance in the rotated crop is a hallmark of rotation-resistant WCR (Levine *et al.*, 2002; Spencer *et al.*, 2005). Interfield movement of rotation-resistant WCR has a strong diel periodicity (Isard *et al.*, 2000); likelihood of take-off is influenced by predictable changes in local atmospheric conditions (Isard *et al.*, 2004). Where rotation-resistant populations are present, large numbers of WCR adults become noticeable in soybean and other non-host fields adjacent to cornfields within *ca.* 1 week after adult females are first noted in cornfields; they remain abundant in rotated crops throughout the growing season (O'Neal *et al.*, 1999; Isard *et al.*, 2000; Levine *et al.*, 2002; Rondon and Gray, 2003; Pierce and Gray, 2006). Where WCR are still susceptible to crop rotation, few or only modest numbers of WCR adults are detected in rotated crops, even when there is a high abundance in adjacent corn (Figure 8.3) (Levine *et al.*, 2002; Spencer *et al.*, 2005; Pierce and Gray, 2006).

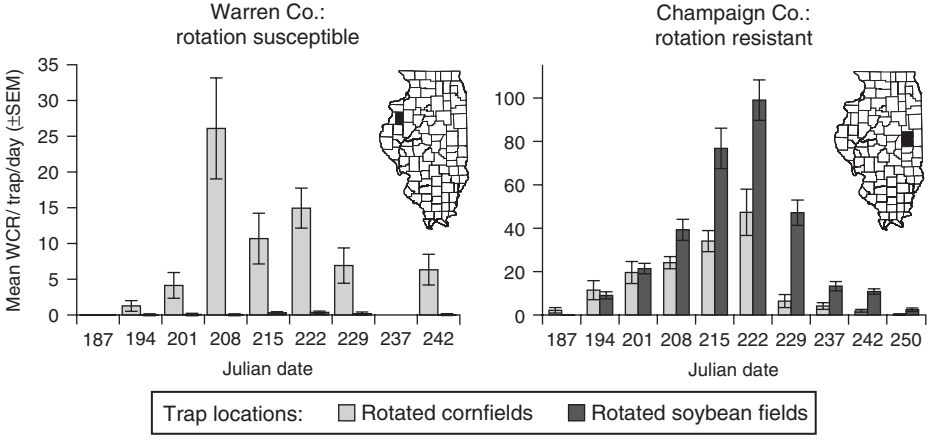

Figure 8.3 2000 WCR seasonal abundance patterns in soybean and cornfields from western (Monmouth, IL in Warren County; rotation-resistant population) and eastern (Urbana, IL in Champaign County; rotation-susceptible population) Illinois. Each bar represents a mean daily capture rate (±SEM) for cucurbitacin + insecticide-baited vial traps positioned at ear height in corn ($n = 5$) or at the top of the plant canopy in soybean ($n = 5$) at each location.

Movement and the Mechanism of Behavioral Resistance to Crop Rotation

Most (*ca.* 60%) of the rotation-resistant WCR in soybean fields or collected while moving between cornfields and other rotated crops are female (O'Neal *et al.*, 1999; Levine *et al.*, 2002; Rondon and Gray, 2003). This proportion is reminiscent of the proportion of females that was previously typical of first-year corn prior to WCR rotation resistance (Godfrey and Turpin, 1983). After rotation-resistant WCR leave a cornfield and arrive in a soybean field, many feed on soybean tissue (despite a lack of nutritive value to WCR; Mabry and Spencer, 2003). During the growing season, *ca.* 55.8% of $n = 5{,}825$ WCR females in soybean contained identifiable soybean tissue in their gut contents (JLS 1996–2001 dissection data). By late July when WCR abundance in Illinois soybean can exceed 200 beetles per 100 sweeps, 86% of females have soybean tissue in their gut contents (Spencer *et al.*, 2005). A similar proportion of WCR from soybean fields in rotation-susceptible regions also contain ingested soybean tissue, an indication that soybean herbivory is not a unique characteristic of rotation-resistant beetles. Greater abundance of rotation-resistant WCR adults in soybean makes this behavior more evident. Although readily eaten, soybean tissue does not support WCR egg development (Mabry and Spencer, 2003). In the laboratory, few field-collected WCR that eat only soybean tissue live $\geqslant$1week. However, WCR adults that eat a 50:50 mixed diet that alternates daily between corn and soybean tissue, survive and reproduce as well as WCR fed on a continual diet of corn plant tissues (Mabry *et al.*, 2004).

Gravid females or those capable of maturing some eggs account for only 20% of the females that fly into soybean fields from cornfields (Mabry and Spencer, 2003). The season-long presence of many females without mature eggs in soybean fields suggests that egg laying is not the reason most females leave corn for soybean. Clearly, the 80% of females that enter soybean fields without sufficient reserves to mature eggs must return to a cornfield to feed before they can lay eggs (Mabry *et al.*, 2004). Laboratory assays indicate that soybean herbivory significantly increases WCR activity and the likelihood of egg laying (Mabry, 2002; Mabry *et al.*, 2004). Using a behavioral assay, Knolhoff *et al.* (2006) found that female WCR from rotation-resistant populations were faster to escape an arena than rotation-susceptible populations; greater general activity levels may explain the abundance of moving WCR. A predisposition toward greater activity combined with behavioral effects of soybean herbivory may provide the proximate mechanism behind the back-and-forth movement of rotation-resistant WCR from soybean fields back into adjacent cornfields (Mabry *et al.*, 2004; Spencer *et al.*, 2005).

Although abundant rotation-resistant WCR readily visit and deposit eggs in corn, soybean, and other rotated crops (Rondon and Gray, 2003, 2004; Schroeder *et al.*, 2005), the number of eggs laid in corn may be greater (Rondon and Gray, 2004; Schroeder *et al.*, 2005) or fewer than the number of eggs laid in soybean (Pierce and Gray, 2006) or other rotated crops. Pierce and Gray (2006) measured season-long patterns of WCR adult abundance, oviposition and crop phenology in corn and soybean fields near the east-central Illinois epicenter of rotation resistance. They found that differences in relative corn phenology can lead to significantly greater numbers of WCR eggs laid in late-planted corn versus early-planted corn in east-central Illinois (Pierce and Gray, 2006). They also found that WCR were present in

soybean fields before corn was mature and that egg-laying outside of corn occurred throughout the season. O'Neal *et al.* (2002, 2004) hypothesized that early corn planting might play a critical role in the phenomenon of rotation resistance and perhaps even be the mechanism behind the phenomenon if WCR left maturing corn to oviposit in still-green soybean fields. Pierce and Gray (2006) tested whether phenology differences between corn and soybean alone could lead to WCR egg laying in soybean under field conditions in an area *without* rotation resistance. Pierce and Gray (2006) planted corn on dates that were 1 month apart to create extreme differences in corn and soybean phenology in Champaign County and in an area 220 km northwest of Champaign that was free from rotation-resistant WCR (Warren County, Illinois). The number of eggs laid by WCR in Champaign County soybean plots was uniformly high and twice as many eggs were laid in the late corn (planted May 15) than in early corn (planted April 15). In Warren County, no WCR eggs were recovered from soybean field soil, and there were no differences in the number of eggs recovered from cornfields planted on different dates. While differences in corn phenology can influence egg laying by rotation-resistant WCR in corn, a wide phenology difference was not sufficient to stimulate egg laying by WCR in soybean fields from an area where rotation-resistant WCR were not known to be present.

Interfield WCR movement rates between corn and soybean fields, or other rotated crops like wheat, suggest a possible explanation for crop-to-crop variability in WCR egg laying. Interfield movement from corn into soybean was slower than movement from corn into wheat (Spencer, 2003–2005 unpublished data). WCR abundance (measured with canopy level sweep net and above canopy aerial net samples and on Pherocon AM sticky traps) was significantly greater in soybean than wheat or wheat double-cropped with soybean. Corn in rotation with wheat escaped yield-reducing root injury from WCR larvae, while corn after soybean or wheat double-cropped with soybean suffered injury likely to reduce yields. Using the same crop treatments, Schroeder *et al.* (2005) also report the least root injury in corn after wheat, however, none of the injury in rotated corn had the potential to reduce yields. The generally hotter and drier conditions in wheat and wheat stubble (compared to the soybean canopy) may promote more rapid movement (*ca.* 6.8–7.2 m/day from corn into wheat versus 4.8–5.1 m/day into soybean) and consequently fewer insects accumulate there and gravid females have less opportunity to lay eggs in wheat fields. Average adult WCR intrafield movement rates (within cornfields) range from 4.9–9.1 (Spencer *et al.*, 2003) to 11.9 m/day (Spencer, 2003, unpublished data).

Managing Rotation-Resistant Corn Rootworms

Though crop rotation is no longer an effective pest management tool against some WCR and NCR populations, developing rootworm larvae still cannot survive on soybean roots. When eggs of either species are deposited in cornfields that are rotated to soybean, the larvae starve and die soon after emergence. Crop rotation remains the primary recommended management option where rotation-resistant WCR and NCR populations are absent. Where rotation resistance is present, application of a planting-time soil insecticide or selection of a transgenic insecticidal corn hybrid are options for producers of first-year corn.

Monitoring Rotation-Resistant WCR

The O'Neal *et al.* (2001) economic threshold for adult WCR abundance in rotated soybean fields provided growers with a science-based tool to assess their risk and guide decision-making about use of soil insecticides or planting of rootworm-protected transgenic corn to protect rotated cornfields. The monitoring protocol of O'Neal *et al.* (2001) calls for deployment of 12 widely spaced Pherocon® AM yellow sticky traps (unbaited) in a soybean field from the last week of July through the third week of August. Traps are changed weekly to count the trapped WCR and determine the average number of WCR captured per trap per day. Seasonal averages of ⩾5 WCR/trap/day indicate that economic injury to first-year corn planted in that field is likely during the following year. The cost of monitoring is *ca*. $50 per field (traps cost *ca*. $1 each).

Although the threat of economic injury due to WCR larval feeding is now high each year in both continuous *and* first-year cornfields in many Illinois counties, surveys show that economic injury does not occur in all fields (Schroeder and Ratcliffe, 2003, 2004; Steffey *et al.*, 2003, 2004). Outside of the known problem area and locally within the affected region there are areas where WCR abundance and egg laying in soybean is too low to cause economic injury to rotated corn (Gray and Steffey, 2004). However, few growers monitor WCR in soybean with traps. Gray and Steffey (2004) indicate that the trapping method is primarily relied on by growers from areas where rotation-resistant WCR populations are newly arrived. They note that in regions of Illinois where rotation-resistant WCR are well established, "Use of Pherocon® AM traps . . . is *noticeably lacking*" (Gray and Steffey, 2004). The cost of traps and labor for monitoring are difficult for growers to justify even though they are much less costly than automatically treating every field every year (soil insecticide use or transgenic corn costs *ca.* $42/ha).

Root injury surveys (Schroeder and Ratcliffe, 2003, 2004) offer strong evidence in favor of monitoring WCR rather than simply assuming the worst (or the best). The Schroeder and Ratcliffe (2003) survey covered 36 counties and included several northwestern Illinois counties (Bureau, Lee, Marshall, Ogle, and Stark) where an extant rotation resistance problem was widely suspected. In those five counties, average root injury (on the Iowa State 1–6 scale (Hills and Peters, 1971)) was ⩾3.0 in just 10 of 50 (20%) randomly sampled fields (five roots were dug from each of 10 fields/county; ratings ⩾3.0 indicate economic injury is possible). In the following year, the Schroeder and Ratcliffe (2004) survey sampled in 24 counties outside the area of established rotation resistance. They reported that 8.4% of randomly collected first-year corn roots from these counties rated ⩾3.0 (some of the injury may also be due to NCR feeding which cannot be distinguished from that of WCR). The Schroeder and Ratcliffe (2003, 2004) surveys illustrate just how risky assumptions can be. Based on the county-level "assumptions" about the status of the of rotation-resistant WCR threat, up to 80% of the 2003 fields would have been treated unnecessarily and 8.4% of the 2004 roots from the "unthreatened" counties would have suffered economic injury because high-WCR populations were not detected in time to take action. Monitoring WCR abundance in soybean fields requires a modest financial and time commitment; however, when it saves a grower from making a management error, the savings can far exceed the per field cost of monitoring.

Insecticides

Where WCR or NCR resistance to rotation is present and monitoring indicates the potential for economic injury exists, growers should consider management options. A variety of insecticide formulations are available to growers. Protection of corn roots from larval injury is typically accomplished with soil insecticides applied as granules or liquids in the furrow or in a narrow (*ca.* 18 cm wide) band over the surface at planting. Rootworm emergence from soil insecticide-treated corn may actually be higher than that from untreated. The insecticide only penetrates a short distance from the application point, however, in this zone it protects the developing root allowing it to grow to a large size. Eventually, the root growth extends outside of the protected area and can be exploited by rootworm larvae. Because a larger root is eventually produced, more larvae may develop on a root that was treated with soil insecticide (Gray *et al.*, 1992). The juxtaposition of insecticide-treated and untreated areas around every plant is hypothesized to be a reason why resistance to modern soil insecticides used against rootworm has not occurred – rootworms emerging from around a single plant will include adults that developed with and without exposure to the soil insecticide. In effect, each plant includes a built-in refuge for susceptible insects (Gray *et al.*, 1992).

Increasingly, many cultivars are sold with a seed-applied insecticide known as a "seed treatment". Seed treatments offer some advantages over insecticide application (e.g., reduced human exposure and toxicity versus traditional soil insecticides, targeted application reduces active ingredient per ha, water soluble treatments are easily absorbed by the plants, and no special equipment is needed to apply them). At low to moderate rootworm pressure, seed treatments can effectively contribute to corn pest management; their efficacy is questionable under heavy pest pressure (Gray *et al.*, 2006). Seed treatments are frequently applied to rootworm-resistant transgenic corn hybrids and marketed as providing protection against secondary insect pests that were once controlled along with the rootworm by broad-spectrum insecticides, but are not affected by the rootworm-specific toxins in the transgenic insecticidal corn.

Because of widespread rotation resistance in parts of the eastern Corn Belt, 58% and 52% of Illinois and Indiana cornfields, respectively, were treated with soil insecticide in 2003. Meanwhile in Iowa, a state without rotation-resistant WCR as of 2003, only 14% of all cornfields were treated with insecticide (USDA, 2004). In 2004, producers in 31 Illinois counties (accounting for 2 million ha of corn), faced a moderate to high risk of WCR injury in rotated corn. Management costs for these producers, based on a $42/ha cost for soil insecticide or purchase of a rootworm-resistant transgenic corn hybrid (discussed below), amounted to *ca.* $81 million.

Foliar sprays are also used for corn rootworm management. These sprays are applied to prevent silk clipping (feeding on corn silks that interferes with pollination by removing silks before ovules can be fertilized) in seed corn and commercial/hybrid corn production. If plants are too tall to permit application from in-field sprayers when pollination is occurring, foliar sprays may be applied by applicators using airplanes. Owing to rapid silk growth and abundant pollen, high rootworm densities are necessary to justify foliar sprays. Presence of other silk-feeding pests, like *Popillia japonica*, may necessitate treatment even when rootworm populations

are below threshold. Adult WCR are the primary targets for WCR suppression programs in portions of Nebraska. Use of scouting with aerial application of methyl parathion targets egg-laying females to reduce larval injury to continuous corn during the following year. In these areas, there is a growing problem with resistance to methyl parathion (organophosphate) and carbaryl (carbamate) (Meinke *et al.*, 1998).

Transgenic Insecticidal Corn

In 2003, rootworm-protected transgenic insecticidal corn hybrids became available to US corn growers. Transgenic insecticidal corn hybrids express a *Bacillus thuringiensis* (Bt) toxin in root tissue that kills rootworm larvae as they attempt to colonize the roots (Vaughn *et al.*, 2005; Storer *et al.*, 2006). Transgenic corn hybrids are an effective alternative to soil insecticides and seed treatments at a comparable price, and when used as part of an IPM program, they offer a variety of benefits compared to soil insecticides (Rice, 2003; Vaughn *et al.*, 2005). Unlike insecticide treatment, there is no risk of grower exposure to insecticide during handling and application. Also, specialized insecticide boxes and spray equipment are not needed to "apply" the protection. The high species specificity of the Bt toxin also means that most non-target species, including beneficial insects, are unharmed in the process of protecting corn from rootworms. However, like any management tool that kills pests, use carries the risk that resistance may develop. In an IPM framework, planting transgenic insecticidal corn for rootworm management should always be justified by monitoring that indicates a risk of economic injury.

The use of refuges is a key element of the insect resistance management (IRM) plans required for transgenic corn (EPA Office of Pesticide Programs, 2001). The requirement that at least 20% of a cornfield is set aside as a refuge (EPA Office of Pesticide Programs, 2003), allows a significant WCR population to develop without exposure to the specific Bt protein present in the transgenic area of a field. Use of non-transgenic refuges as part of an IRM plan is grounded on the assumption that mate-seeking refuge males will thoroughly disperse from refuges to find and mate with potentially resistant females emerging from transgenic corn. If assumptions about the movement and mating of males and females in the refuge and transgenic portions of a field are wrong, the likelihood of rapid resistance development may be greater than assumed.

Anticipatory modeling of corn rootworm resistance to transgenic insecticidal corn varieties is made difficult because gene frequencies for any Bt resistance alleles in wild populations are unknown. Uncertainty about starting allele frequencies for resistance genes and model parameters dealing with mating, dispersal, toxin expression, and cross-resistance necessitate conservative approaches to IRM.

The importance of preserving susceptibility to transgenic crops may be of special importance for regions where other types of resistance are already present. For example, when WCR larvae consumed diet treated with the same Bt toxin expressed in a transgenic insecticidal corn variety, neonate larvae of the rotation-resistant WCR had the lowest susceptibility to growth inhibition of any tested population and one of the highest rates of survival (Siegfried *et al.*, 2005). If traits associated with rotation resistance confer some advantage to WCR encountering

Bt protein it may be particularly important that transgenic insecticidal corn varieties be used judiciously in areas inhabited by rotation-resistant populations.

Gray (2000) suggested that WCR susceptibility to transgenic insecticidal corn constitutes a natural resource and proposed that transgenic insecticidal corn be used only in fields where monitoring indicates its use is justified. However, in a modeling analysis, Crowder *et al.* (2006) concluded that economic thresholds would not be valuable when transgenic insecticidal corn is very effective and has a low price premium. Prescriptive use of Bt corn would help guarantee that susceptibility to a particular Bt toxin is not squandered.

Prediction and Rootworm IRM

Evidence supporting the claim that resistance by WCR to crop rotation is based on genetic changes was published by Onstad *et al.* (1999). They clearly showed that rotation resistance has been spreading away from its initial focus in east-central Illinois in the mid-1980s. By 2001, the geographic spread of the resistant phenotype had extended into Michigan and Ohio (Onstad *et al.*, 2003a). Onstad *et al.* (1999, 2003a) created a simple model implemented with a geographic information system to describe the spread of the resistant phenotype from one county in Illinois

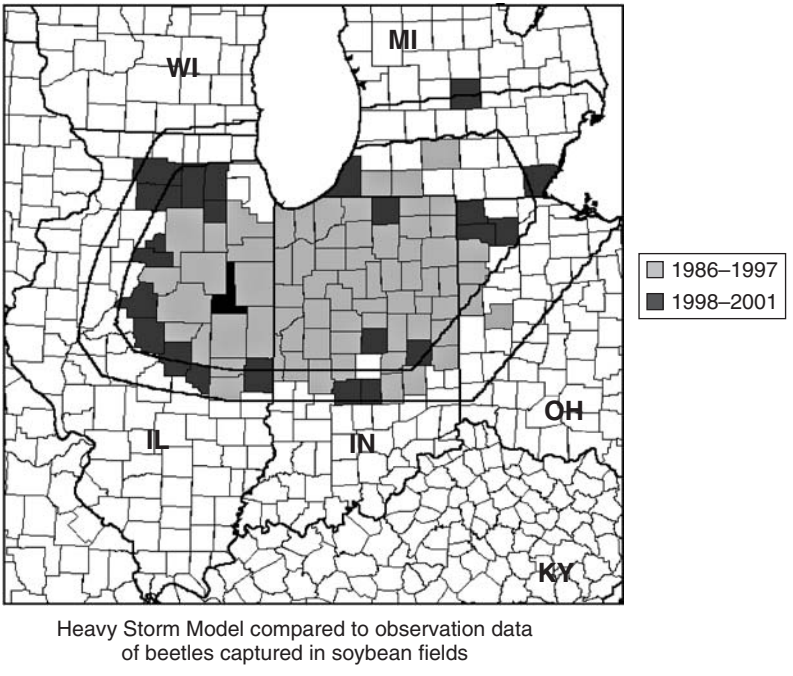

Figure 8.4 Comparison of basic heavy storm model results to observations (20 beetles/100 sweeps or 2.0 beetles/trap/day) with the dark contours representing the 12th (1997) and 16th (2001) years of the simulations.

using meteorological and behavioral information. Figure 8.4 shows one prediction versus the observations through 2001. Since then the resistance has spread into Wisconsin, Iowa, and perhaps Ontario. It is hoped that the genetic basis for the evolution of rotation resistance will be confirmed using satisfactory bioassays to distinguish wild type and rotation-resistant phenotypes (Knolhoff *et al.*, 2006) or by genomic analysis.

Onstad *et al.* (2001b, 2003b) demonstrated that a simple model of population dynamics, behavior, and genetics could account for the evolution of rotation resistance by the WCR from its invasion of eastern Illinois as a wild type *ca.* 1970 to the discovery of its damage in first-year corn in 1987 (Figure 8.5). They also showed that landscape diversity could slow the evolution of resistance to crop rotation (Figure 8.6). In this case, landscape diversity means the proportion of the vegetated area that is not planted to corn or the crop rotated with corn (e.g., soybean). This work caused Onstad *et al.* (2003a) to modify their model of geographic spread to include a variable for landscape diversity. This modification permitted the model to more accurately simulate the observed slowing of the spread of rotation-resistance in regions with greater landscape diversity (Figure 8.7).

Onstad *et al.* (2003b) expanded their simple model to explain how to manage the WCR in a landscape of corn, soybean, and winter wheat where evolution of rotation resistance may occur. They modeled six alternative IRM strategies over a 15-year time horizon (Table 8.1), as well as the typical scenario involving a 2-year rotation of corn and soybean in 85% of the landscape, to investigate their effectiveness from both a biological and an economic perspective.

Each of the alternative IRM strategies has different effects on western corn rootworm survival and behavior relative to the standard 2-year crop rotation (2 year).

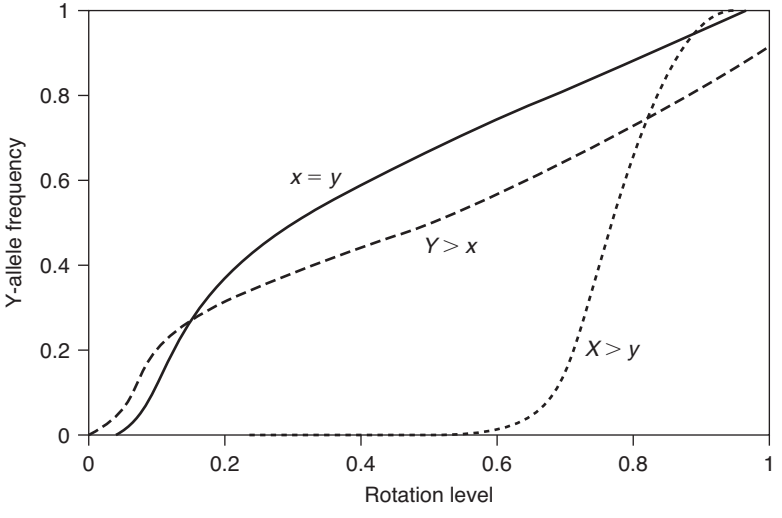

Figure 8.5 Resistance-allele (Y) frequency in year 15 as a function of the level of crop rotation, with 5% extra vegetation, with *X* dominant ($X > y$), *X* and *Y* additive ($x = y$), or *Y* dominant ($Y > x$) (Onstad *et al.*, 2003b, reprinted by permission of Entomological Society of America).

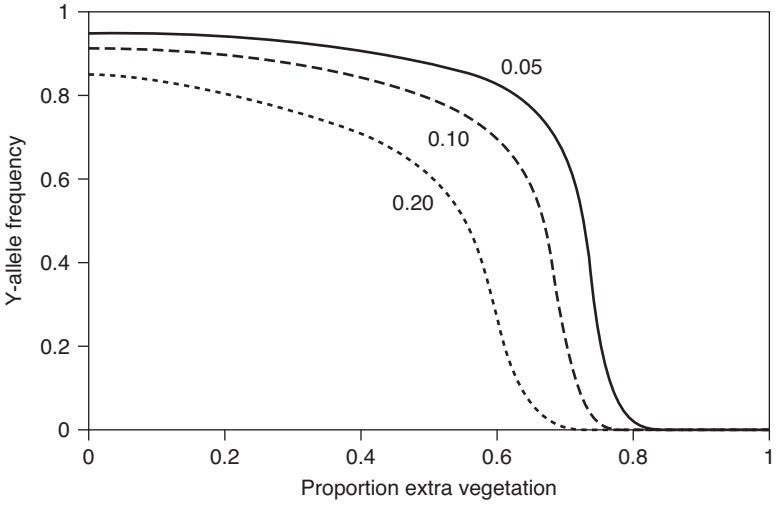

Figure 8.6 Resistance-allele frequency in year 15 produced with additive gene expression as a function of the proportion of extra vegetation when fraction of area in continuous corn is 0.05, 0.10, or 0.20 (Onstad *et al.*, 2003b, reprinted by permission of Entomological Society of America).

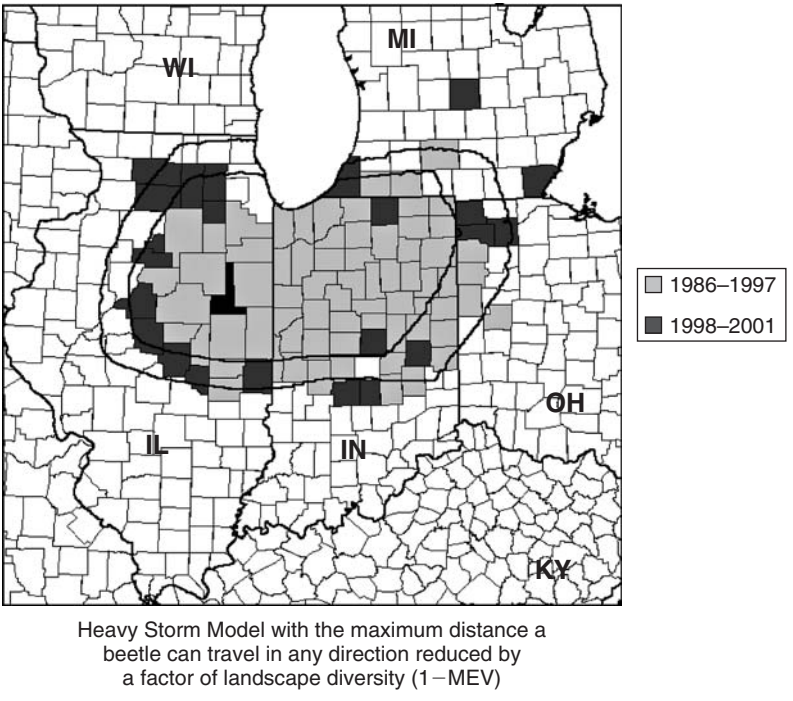

Figure 8.7 Comparison of heavy storm model results to observations (20 beetles/100 sweeps or 2.0 beetles/trap/day) with the dark contours representing the 12th (1997) and 16th (2001) years of the simulations. Rate of spread reduced by landscape diversity (1-MEV) in each county (modified from Onstad *et al.*, 2003a).

Table 8.1 Six alternative IRM strategies simulated by Onstad *et al.* (2003b). All have 2-year rotation of corn (42.5%) and soybean (42.5%) with 10% continuous corn unless indicated otherwise. Labels for Figures 8.8 and 8.9 are given at end of each description.

(A) 3-year rotation with corn (30%) preceded by soybean (30%) or other vegetation (30%), 3 year
(B) Plant transgenic insecticidal corn in rotated fields (90% of neonates die in this rotated corn), Trans
(C) Plant more continuous corn in landscape (increase to 35% of landscape), MCC
(D) Plant repellant soybean (repels 90% of rotation-resistant phenotypes), RSoy
(E) Plant rotated corn that is more attractive (attracts 90% of all phenotypes), Att. Corn
(F) 3-year rotation with corn preceded by a crop that repels 90% of rotation-resistant phenotypes, 3-year UE

Management strategies A–C (Table 8.1) alter the proportions of the landscape in which rootworms will survive, but retain the movement parameters of the typical 2-year crop rotation. The remaining three strategies (D–F, Table 8.1) alter the behavior of the WCR thereby increasing the proportion of eggs laid in locations that will not be rotated to corn the following year. Note that strategies D–F are hypothetical given current technology. In strategy D, the rotation-resistant beetles that are repelled from soybeans lay their eggs randomly throughout the rest of the landscape. This strategy will increase the number of adults that lay eggs in corn while decreasing the number that lay eggs in soybeans. Strategy E causes all beetles to lay more eggs in rotated corn than in other parts of the landscape. With this strategy the number of beetles laying eggs in both continuous corn and soybeans decreases. In strategy F, use of a 3-year rotation with a less attractive crop, such as winter wheat, attempts to prevent rotation resistance by affecting both the survival of larvae and the movement rates of adults. Onstad *et al.* (2003b) investigated a winter wheat crop that repels rotation-resistant beetles, forcing them to lay eggs randomly throughout the rest of the landscape. This increases the proportion of beetles emerging in continuous corn while decreasing those emerging in rotated corn.

Each strategy (Table 8.1) was evaluated according to its effects on resistance allele (Y) frequency, 15-year average larval densities, and the economic costs and benefits of each approach. Generally, resistance to crop rotation evolves in fewer than 15 years and the rate of evolution increases as the level of rotated landscape (selection pressure) increases. When resistance is recessive, all six alternative strategies were effective at preventing evolution of rotation resistance. The two most successful strategies were the use of transgenic rotated corn in a 2-year rotation and a 3-year rotation of corn, soybean, and wheat with unattractive wheat (for oviposition) preceding corn (Figure 8.8). Economically, three alternative strategies were robust solutions to the problem, if technology fees were not too high. Repellant soybeans, attractive rotated corn, and transgenic rotated corn, all in 2-year rotations, were economically valuable approaches (Figure 8.9). Onstad (Chapter 12) discusses the simultaneous management of resistance to crop rotation and transgenic insecticidal corn.

Even the typical 2-year rotation was economical as an IRM strategy when resistance was recessive and it took 14 years for the resistance allele frequency to reach 50% (Figure 8.9). This result occurs because even though the frequency of the resistance allele grows rapidly, population densities do not increase for several years.

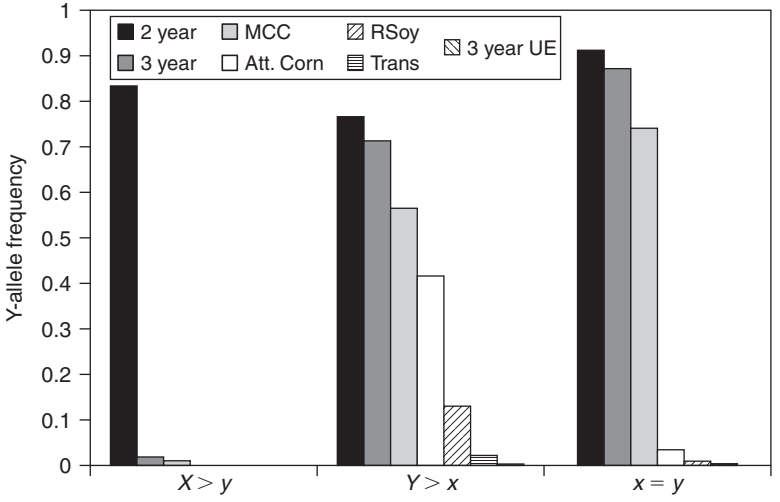

Figure 8.8 Resistance-allele frequency in year 15 for 2-year rotation and six alternative management strategies with three types of gene expression (Onstad *et al.*, 2003b, reprinted by permission of Entomological Society of America).

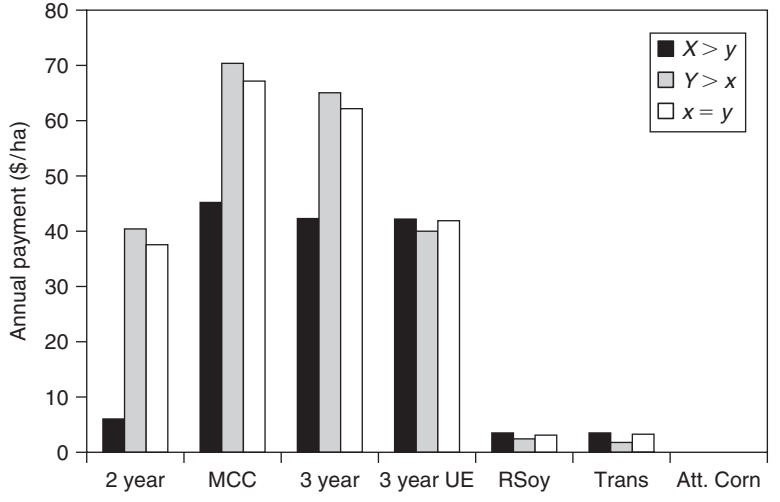

Figure 8.9 Annual payment ($/ha) that would equate the annualized net present value for each management strategy to the strategy (Att. Corn) with the greatest annualized net present value for each type of gene expression (Onstad *et al.*, 2003b, reprinted by permission of Entomological Society of America).

As a result, yield losses on rotated corn in excess of 1% do not occur until years 14 and 15, when losses are 4.2% and 11.7%, respectively. However, since these losses occur far in the future, they are substantially discounted by the net present value criterion, implying that farmers concerned only with economic returns will prefer

to do nothing about the evolution of rotation resistance. This result gives insight into the economic logic that underlies the evolution of rotation resistance among WCR.

It is difficult, if not practically impossible, to halt the evolution of rotation resistance once the resistance allele frequency reaches *ca.* 1%. Thus, the uncertainty in the timing of invasion by the rotation-resistant variant and the initial resistance gene frequency make it more difficult to choose a good strategy. Furthermore, reductions in farmer returns are not the only costs of resistance. If soil insecticide use will greatly increase as a result of rotation resistance, it may be desirable from a social perspective to subsidize farmers in the present, so they have an incentive to change practices now and so delay the development of resistance in the future.

Onstad *et al.* (2003b) determined that under a standard set of assumptions, several alternatives, including transgenic insecticidal corn in rotation, are superior to a typical 2-year rotation with regard to rootworm IPM in areas where rotation resistance is a serious problem. Unless a third crop can be found that is less attractive for rootworm egg laying and economically competitive with corn and soybeans, a 3-year rotation does not appear to be a practical IPM solution in existing problem areas.

Incorporating WCR Biology into IRM

Transgenic and other biotechnological options for corn insect pest management are becoming more common; in 2006, 25% of US cornfields were planted with transgenic insecticidal corn, and 61% of US cornfields were planted with some type of genetically engineered corn (USDA ERS, 2006). The sustainability of these solutions will depend on accurate information about pest biology. In a future in which the transgenic insecticidal crops available to producers may express a range of Bt toxins, studies of rootworm movement, mating and host interactions will be vital to designing refuges that are appropriate to the mechanism of management and the mode of action of a particular transgenic variety.

In addition to understanding rootworm movement and mating dynamics between refuge and transgenic insecticidal corn, the consequences of long-distance transport should be considered. If, and when, resistance arises, containing and managing its spread will benefit from knowledge about patterns of post-mating rootworm dispersal. Local variation in crop phenology, local and regional weather patterns, and the presence of other types of resistance – all may affect rootworm dispersal and the spatial distribution of resistance genes.

Future Resistance

Resistance to crop rotation by the NCR and WCR was unexpected and happened in the absence of any planned strategy to avoid it. The history of worldwide insecticide resistance over the last 60 years teaches us that there are many possible routes to resistance; the same could be said about NCR and WCR beetles – their capacity for resistance should not be underestimated. For growers already dealing with rotation-resistant WCR/NCR, preserving susceptibility to new technology is

of paramount importance, because they have a limited set of available management tools. The continued spread of rotation-resistant WCR into Iowa and the western Corn Belt will place greater pressure on remaining IPM methods and underscore the need to be judicious in the use of management tools.

Seed treatments are becoming a popular vehicle to protect many crops including corn and soybean. The presence of a systemic, neonicotinoid-insecticide seed treatment on corn (targeting secondary pests) and on soybean (in this case, to help manage *Aphis glycines*) could potentially expose two different life stages of rotation-resistant WCR populations to neonicotinoids – once as larvae in corn and again as soybean-feeding WCR adults. This dual exposure could occur over a vast area of the Corn Belt. Exposure of rotation-resistant WCR to insecticide applications targeting *A. glycines* in early-reproductive stage soybean (Myers *et al.*, 2005) has the potential to add another level of resistance risk for WCR in the Midwest.

Though there has never been field-evolved resistance to a Bt protein expressed in a transgenic insecticidal crop (Tabashnik *et al.*, 2003), the threat of rootworm resistance to transgenic corn cultivars is real. Onstad *et al.* (2001a) have modeled various scenarios for WCR resistance to transgenic insecticidal corn. Baseline Bt toxin susceptibility studies indicate significant variation exists among wild WCR populations (Siegfried *et al.*, 2005). Studying Bt protein resistance in laboratory-selected colonies may improve resistance monitoring strategies and early detection (Siegfried *et al.*, 2005).

The potential for novel mechanisms of behavioral resistance cannot be ignored. Recent studies of larval movement capacity and suitability of alternative grassy weed hosts raise the possibility that rootworm larvae may be capable of avoiding transgenic insecticidal corn until they have matured into older larvae that are less susceptible (Oyediran *et al.*, 2005). Feeding on nearby alternate hosts, such as grassy weeds in corn, may allow larvae to grow beyond a stage when they are most susceptible to transgenic insecticidal corn and then move back to the transgenic corn (Hibbard *et al.*, 2005). Clark *et al.* (2006) have recently demonstrated that the feeding behavior of WCR larvae on transgenic versus non-transgenic roots is very different. Some larvae feed on transgenic insecticidal corn roots and exhibit no movement, others sample continuously and are very mobile – these larvae may not feed, suggesting an expression of non-preference. These findings indicate that early instars can differentially respond to transgenic cultivars and raise interesting questions about the potential for behavioral and biochemical mechanisms of resistance to Bt toxin and transgenic insecticidal corn in general.

The phenomenon of delayed adult WCR emergence from transgenic corn varieties is worrying. Absence or displacement in time of adult male or female emergence from some transgenic events may be problematic. If there are declining concentrations of Bt toxin expression in some cultivars, we may already be selecting for WCR populations with variable life-history traits that allow WCR to avoid high toxin concentrations by delaying development until later in the season.

We must be cautious about over-reliance on a single solution or management method. The history of pest management and corn rootworm management in particular suggests that simple solutions are often not simple and once solved, many problems do not stay solved forever!

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Chapter 9

Arthropod Resistance to Crops

David W. Onstad and Lisa M. Knolhoff

Humans have been managing crops for thousands of years, often choosing plants that are more resistant to pests. For millions of years, arthropods have been evolving mechanisms to counteract defenses in plants (Berenbaum, 2001). This chapter describes attempts by agriculturalists and entomologists to manage the resistance by host plants to deal with the real or potential evolution of counteracting resistance by arthropods.

Host plant resistance (HPR) is one of the tactical pillars of integrated pest management (IPM) (Smith, 2005). The standard definition of HPR offered by Painter (1951) is "the relative amount of heritable qualities possessed by the plant which influence the ultimate degree of damage done by the insect in the field." Panda and Khush (1995) further describe it as "any degree of host reaction less than full immunity." HPR under genetic control can be divided into three categories: tolerance, antixenosis, and antibiosis (Panda and Khush, 1995). Plant tolerance has no effect on the insect population; it merely raises the economic threshold for control. Antixenosis is a mechanism by which the plant deters herbivores or reduces their colonization (e.g. leaf trichomes). Antibiosis either kills the herbivore or negatively affects its development after feeding. Note that in some cases, it may be hard to differentiate between antixenosis and antibiosis if the plant exhibits both types of effects (Panda and Khush, 1995). To avoid confusion in the use of the term resistance, throughout this chapter we will use the abbreviation HPR for resistance by plants to insects and reserve the term *resistance* for the case of resistance by insects to crops.

As with most approaches to IPM, the emphasis in HPR work has been on incorporating a mechanism that, by itself or in conjunction with other IPM tactics, can either reduce the pest population, reduce damage to the crop, or both. Long-term durability of a cultivar is only one of many characteristics of the crop that must be considered, weighed, and balanced by plant breeders in a breeding program (Kennedy *et al*., 1987). Gould (1983) provides a valuable discussion of the evolutionary biology of plant–herbivore interactions. Kennedy *et al*. (1987) promote the use of landscape ecology and modeling of IPM and insect resistance management (IRM) during early stages of breeding programs to make breeding decisions more efficient.

From an insect's perspective, there is no difference between crops that have been bred using a traditional approach and those that have been genetically engineered. Evolution of resistance to either technology can occur. The extensive planting of transgenic insecticidal crops in agro-ecosystems does force us to deal with many

issues (Onstad, Chapter 10), but for now we will focus on the similarities of IRM for the two types of HPR crops.

In the following sections we describe a variety of case studies concerning the evolution and management of insect resistance to HPR. Each section is labeled according to the arthropod pest that has evolved resistance to the crop. First, we discuss crops developed using traditional methods. Then we describe three cases involving transgenic insecticidal crops. In the discussion, we address some lessons that can be learned from resistance to crops.

Traditional Crops

Liriomyza trifolii

Hawthorne (1998) used two simple models to predict the evolution of resistance by the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) to a resistant cultivar of chrysanthemum, *Dendranthema grandiflora*. For each insect generation, each model calculates a change in insect performance based on selection intensity, S, and heritability, h^2, (Response = Sh^2). In the first model, heritability was held constant throughout the 10-generation simulation. In the second model, heritability declined due to the effects of linkage disequilibrium. For three types of leafminer populations that differed in their history of host plant use, Hawthorne (1998) compared model predictions of larval survivorship against data from a selection experiment performed on the same leafminer populations.

In general, the predicted evolutionary trajectories of the leafminer populations on the resistant host plant were very similar to the trajectories observed in the selection experiment (Hawthorne, 1998). Two of the populations had a very good match between the predicted and the observed trajectories. The third simulated population evolved too quickly to the resistant crop, due in part to an overestimate of its genetic variance (heritability). Simulations that accounted for reduced heritability, because of factors such as linkage disequilibrium, produced better predictions of all three observed patterns (Hawthorne, 1998). Hawthorne predicted that a resistant cultivar of chrysanthemum would not remain resistant to leafminers in Florida greenhouses. Hawthorne's results indicate that laboratory studies of selection intensity and genetic variance can be used to make rational IRM decisions regarding commercialization of crops with HPR.

Tetranychus urticae

The twospotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), is a generalist herbivore that feeds on many crop and ornamental plants. Hot, dry weather is conducive to spider mite outbreaks. Damage can be seen as chlorosis of the leaves where the mites have been feeding. Its short life cycle and high reproductive potential predispose this mite to evolving resistance to many chemical control methods, so some growers may opt to use HPR plants. The fact that these mites are polyphagous has many implications in devising a resistance management strategy with HPR hosts.

One host of *T. urticae* is cucumber. Gould (1978a) found that adaptation to HPR cultivars of cucumber expressing antibiosis could occur in as little as nine

generations. Antixenosis is not a factor in cucumber HPR, because resistant and susceptible cultivars were equally attractive to mites (Gould, 1979).

Resistance to HPR cucumbers promoted resistance by *T. urticae* to other plant or insecticidal compounds. Once mites were adapted to an HPR cultivar of cucumber, they were predisposed to utilize tobacco and potato as hosts (Gould, 1979), which are both taxonomically and chemically unrelated to cucumbers. In addition, Gould *et al*. (1982) found that mites adapted to HPR cucumber had significantly higher survivorship when exposed to three organophosphate insecticides. The reverse effect, however, was not observed; mites resistant to several insecticides did not have higher survivorship on resistant cucumber varieties than the susceptible mites did.

While adaptation to cucumbers seems to confer advantages with respect to some hosts or chemical controls, there may be either no effect or even a cost associated with adaptation to another host. Fry (1992) found that adaptation to tomato did not significantly increase or decrease ability to survive on tobacco and cucumber. Fry (1990) reported no difference in survival or fecundity on lima bean, a highly preferred host, when comparing bean- and tomato-adapted mites. However, lines of mites that were originally adapted to cucumber and tomato gradually lost the ability to utilize these hosts after acclimation to an attractive host, such as lima bean (Gould, 1979; Fry, 1990; Agrawal, 2000). Reversion of resistance could indicate a possible fitness cost associated with it. Gould (1979) found a small but significant difference in fitness on the original lima bean host after adaptation to cucumber, but Fry (1990) found no difference in survival or fecundity on lima bean after adaptation to tomato.

Antixenosis as an HPR mechanism is likely to affect evolution of resistance. One thing to consider is whether the pest will feed upon resistant cultivars or merely be repelled; resistance is thought to evolve more slowly if the pest simply avoids the resistant cultivar over the susceptible one (Cantelo and Sanford, 1984). If there is a more favorable, alternative host present, this should weaken selection for resistant pests (Cantelo and Sanford, 1984). HPR of tomatoes and broccoli seems to be both behavioral and toxicological, in that mites tended to disperse from these plants and had high mortality on them (Fry, 1989). HPR may take place as an antixenotic mechanism because of morphological features of these hosts: trichomes and wax, respectively (Fry, 1988, 1989). Fry (1989) reported that it took 21 weeks for mites to diverge in survival on broccoli and only 7 weeks for divergence on tomato. Certain morphological features may have a larger effect on the evolution of resistance.

Factors affecting economic decisions by the producer cannot be ignored. Environmental effects, such as amount of water or natural enemies, must be considered in a resistance management strategy because certain regions may experience climatic conditions for which HPR expression is compromised. Gould (1978b) found that mites that were not adapted to HPR cucumbers still destroyed susceptible varieties, but they did no noticeable damage to water-stressed seedlings. In contrast, the resistant mites destroyed HPR seedlings regardless of water stress, but did have lower survivorship on stressed plants (Gould, 1978b).

In determining the adaptation to HPR in the presence of natural enemies, one must ascertain whether those natural enemies will increase or decrease the fitness differential (Gould *et al*., 1991). Generally, adaptation to HPR occurs more slowly with a combination of low HPR and natural enemies than a high level of HPR

alone (Gould *et al*., 1991). Therefore, an IPM-like approach to resistance management could be beneficial.

Finally, one important aspect of IRM is the initial proportion of resistance alleles in a population. Resistance alleles are usually assumed to be rare because they may have some cost associated with them. Gould (1978a, 1979) found that the genetic variation in survivorship on cucumber cultivars was present within a small area, meaning that it is more likely that resistant individuals will encounter each other to mate. With respect to resistance management, Gould (1978a) highlighted the need to test multiple populations of the insect target and to look at population size, mobility, and whether there is mono- or polygenic inheritance of resistance. Fry (1988) found large differences in survivorship on tomato in populations of mites. The genetic variability with respect to resistance seems to be common in many populations.

Mayetiola destructor

The Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), is an important pest of wheat, *Triticum aestivum*. While it also feeds on barley, rye, and some grasses, wheat is the host of most economic interest (Painter, 1951). Damage is caused by larval feeding, resulting in stunted plants with weakened stems. A major cultural control of *M. destructor* is the planting of winter wheat late enough in the fall after most *M. destructor* have been killed by the frost. Besides planting after the "fly-free" date, one of the main tactics for control of *M. destructor* is the use of antibiotic wheat varieties.

Thirty-two genes conferring wheat HPR to *M. destructor* have been identified (Ratcliffe and Hatchett, 1997; Sardesai *et al*., 2005), and all except one are either dominant or partially dominant (Formusoh *et al*., 1996). Approximately a third of the identified HPR genes have been deployed for commercial use (Williams *et al*., 2003). A gene-for-gene relationship exists between wheat antibiosis and *M. destructor* resistance (Hatchett and Gallun, 1970).

The extensive use of HPR in wheat has selected for resistant biotypes in the field (Ratcliffe *et al*., 1994). Resistance to HPR wheat varieties is controlled by single-locus recessive alleles in *M. destructor* (Hatchett and Gallun, 1970; El Bouhssini *et al*., 2001). Biotypes of *M. destructor* are identified by their ability to survive on antibiotic varieties of wheat (Gallun, 1977). The Great Plains (GP) biotype is susceptible to all resistant wheat varieties, and the others (A through O) have resistance to one or some combination of HPR genes in wheat.

Figure 9.1 shows the distribution of biotypes in regions of the United States based on data from Ratcliffe *et al*. (1994, 2000). Insects were collected between 1989–1992 (Figure 9.1a) and 1996–1999 (Figure 9.1b). Biotype L is resistant to all deployed wheat HPR genes, except the recently deployed *H13* (Ratcliffe, 2000), and is often the reference biotype of breeding experiments for new sources of resistance in wheat. Biotype L is the predominant biotype in the eastern half of the United States above 33ºN latitude, including the Midwest (Chen *et al*., 1990; Ratcliffe *et al*., 1994, 2000). Biotype O is the main biotype south of 33ºN latitude in the eastern United States (Ratcliffe *et al*., 2000). Populations from the northwest are more variable, with frequencies of the susceptible GP biotype ranging from

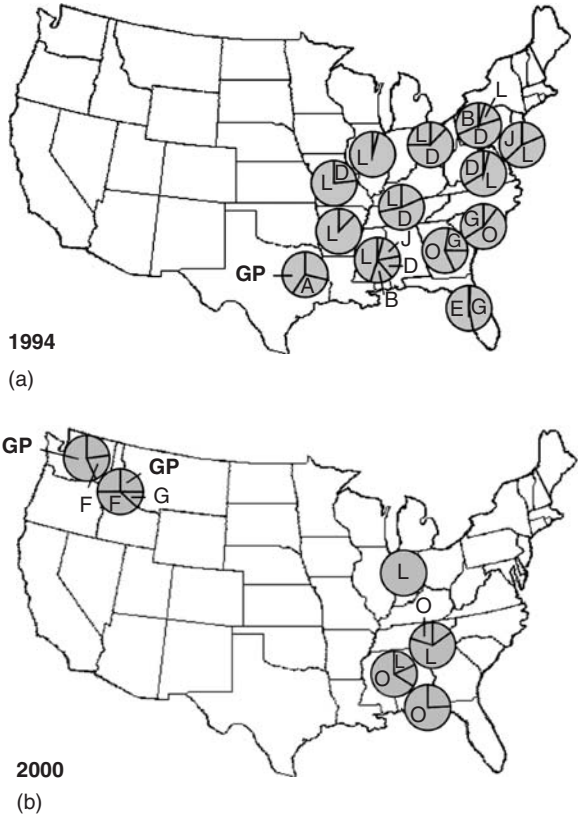

Figure 9.1 Distribution of *M. destructor* biotypes in (a) 1994 and (b) 2000 (data from Ratcliffe *et al*., 1994, 2000). Unmarked portions of pie graphs refer to one or more biotypes individually comprising 10% or less of the total biotype composition for a given area.

25% to 75% and biotypes E, G, F, and O comprising the other sizable portions (Ratcliffe *et al*., 2000).

Because there are numerous antibiosis genes for *M. destructor* in wheat, there are a number of ways that these genes can be deployed. Cox and Hatchett (1986) suggested that sequential release of HPR genes would be simple and effective, but Gould (1986b) recommended the use of wheat cultivars with pyramided HPR genes in addition to a 20% refuge of susceptible plants. Sequential release is the deployment of single HPR gene until it loses efficacy, after which it is replaced by another single HPR gene. Pyramided HPR genes simply refer to multiple genes within a cultivar. Under optimal conditions, the pyramid plus refuge strategy was expected by Gould (1986b) to last at least 400 fly generations. This strategy is similar to that recommended for transgenic insecticidal crops (see section Transgenic Insecticidal Crops). While Foster *et al*. (1991) noted that a pyramid strategy would be quite useful, they discussed potential difficulties in executing it, particularly the time necessary to develop the pyramided cultivars. Because wheat cultivars are generally only grown for up to 10 years, the time required to develop a pyramid may preclude its

use (Foster *et al*., 1991). The authors suggested that if a pyramid is developed, that it should be incorporated into newer cultivars with higher yields. HPR gene pyramiding in wheat has not yet been accomplished (Harris *et al*., 2003).

The utility of a refuge is dependent on the dispersal of adult females seeking oviposition sites. The short adult life span (a few days at most) of *M. destructor* makes dispersal and movement of mated females easier to quantify. Zeiss *et al*. (1993) concluded that any interactions with grasses outside of the tribe Triticeae would be negligible. Indeed, Withers *et al*. (1997) observed the rates of dispersal of mated females in different hosts, and they predicted more than a doubling of dispersal in plots with non-hosts compared to plots with hosts. However, during oviposition, female *M. destructor* do not differentiate by tillage (Del Conte *et al*., 2005) or between resistant and susceptible cultivars (Harris *et al*., 2001). Note that oviposition preference does not necessarily correlate with success of offspring. For example, while triticale (Durum wheat X rye hybrid) experienced a high rate of oviposition, there was low survival of larvae (Harris *et al*., 2001). Additionally, while females do not have a preference for plants with conspecific eggs, they do avoid plants with already infested larvae at a rate proportional to the larval density (Kanno and Harris, 2002). This is probably due to chemical cues from the stressed plants rather than the established larvae themselves (Harris *et al*., 2006). Females also prefer to oviposit on taller, rather than shorter plants (Kanno and Harris, 2002). These aspects of female *M. destructor* behavior allow predictions for a refuge strategy.

The potential of a pest to develop resistance to a control method should not be underestimated. Ratcliffe *et al*. (1994) found some level of resistance to almost all HPR genes in all of the field-collected *M. destructor* populations that they studied in the laboratory. This may mean that there is a relatively high initial frequency of resistance alleles (Gould, 1983). Another factor that contributes to rapid adaptation to antibiotic cultivars of wheat is paternal gene loss in this insect. During spermatogenesis, the paternal chromosomes are eliminated, meaning that males transmit only maternal genes (Gallun and Hatchett, 1969). This reduces the time for *M. destructor* to evolve resistance to antibiotic cultivars (Gould, 1986b). The *M. destructor* genome consists of 2 pairs of autosomes and 2 pairs of sex chromosomes (Stuart and Hatchett, 1988a, b), meaning that linkage of resistance genes may likely occur.

Finally, it is important to consider IRM for *M. destructor* to be part of regionally based IPM. Certainly, economics influence the decisions made by farmers. In an economic analysis of management of *M. destructor* in the southeastern United States, Buntin *et al*. (1992) concluded that, when infestations were light, net returns were greater with the use of susceptible cultivars (with or without insecticide treatment). But when infestations were high, it was desirable to instead plant resistant cultivars. Because it is difficult to predict *M. destructor* outbreaks, Buntin *et al*. (1992) and Buntin (1999) advocate the use of a resistant cultivar with high yield or a susceptible cultivar with preemptive insecticide treatment. In much of the United States, winter wheat planted after the "fly-free" date (determined by latitude) prevents most damage. In the far South, delayed planting is of limited use, as the winter is not sufficiently cold to kill all insects (Buntin *et al*., 1990). Climate affects IRM in another way; certain HPR genes are less effective at high temperatures (Sosa, 1979; Ratanatham and Gallun, 1986; El Bouhssini *et al*., 1999).

Sitodiplosis mosellana

The wheat midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae), is another important gall midge attacking wheat (*T. aestivum*) around the world. Females oviposit eggs on the wheat head directly before anthesis. After hatching, the larvae migrate to and feed on the developing seeds, causing direct economic damage. Recently, an HPR gene called *Sm*1 has been successfully incorporated into commercial cultivars of wheat in Canada. This gene controls an inducible hypersensitive reaction in the seed surface that causes antibiosis against young larvae (Smith *et al*., 2004). Some cultivars also express oviposition deterrence against the wheat midge, but this HPR is less effective and more difficult to incorporate into wheat than *Sm*1 (Lamb *et al*., 2002).

In a field study, Smith *et al*. (2004) evaluated the use of a seed mixture of resistant and susceptible wheat cultivars to maintain susceptibility in the *S. mosellana* population and to conserve a parasitoid that is an effective biological control agent of the *S. mosellana*. They anticipated the evolution of resistance by the midge on HPR wheat because of the high mortality caused by *Sm*1. Smith *et al*. (2004) also believe that a seed mixture creating an interspersed refuge will succeed as an IRM strategy because larvae do not move from the natal plant and adults mate before dispersal. In HPR wheat, few larvae completed development: 2% or less compared with about 80% in susceptible wheat. The densities of mature midge larvae and parasitoids were in proportion to the size of the refuge. A 5% refuge produced about 41 mature parasitoid larvae for each mature *S. mosellana* larva from the resistant wheat. Smith *et al*. (2004) concluded that a seed mixture is a promising strategy for sustaining HPR conferred by *Sm*1 and biocontrol of the *S. mosellana*. Mixtures may be extremely useful in cases such as these, where the targeted pest has low dispersal or mobility.

Schizaphis graminum

The greenbug aphid, *Schizaphis graminum* (Hemiptera: Aphididae) is a pest on wheat (*T. aestivum*) and sorghum (*Sorghum bicolor*), but its crop hosts also include barley, oats, and rye (Puterka and Peters, 1990). At least 70 other non-cultivated grasses have also been reported as hosts for *S. graminum* (Michels, 1986). Alternate hosts such as wild grasses may sustain *S. graminum* populations when preferred hosts such as wheat and sorghum are not present (Sambaraju and Pendleton, 2005). *S. graminum* damages crop plants by feeding on the phloem, resulting in chlorosis of the leaves, and they may transmit plant viruses. Control is usually done through HPR crops, insecticidal applications, and conserving natural enemies. Resistance has been found to both HPR varieties and organophosphate insecticides.

HPR to *S. graminum* exists in wheat and sorghum as antibiosis, antixenosis, and tolerance. Most sources of plant resistance seem to exhibit some combination of HPR mechanisms. For example, the sorghum cultivars tested by Dixon *et al*. (1990) and Schuster and Starks (1973) exhibited all three. Bowling and Wilde (1996) and Teetes *et al*. (1974) found levels of antibiosis and antixenosis in several sorghum cultivars. The HPR wheat cultivars tested by Sambaraju and Pendleton (2005) exhibited tolerance and some antibiosis. Fritts *et al*. (2000) found that it may be difficult to distinguish between antibiosis and antixenosis in some HPR wheat cultivars.

There are eleven documented *S. graminum* biotypes, designated by letters from A to K, although only eight have any relation to HPR varieties (Porter *et al*., 1997). The term "biotype" here will be used to designate strains of insects differing in their capability of infesting certain HPR varieties (Diehl and Bush, 1984). There is considerable variation even within aphid clones for ability to damage certain sorghum cultivars, which may explain why *S. graminum* adapts so quickly to new cultivars (Wilhoit and Mittler, 1991).

There are two schools of thought concerning the evolution of *S. graminum* biotypes. The traditional hypothesis maintains that the use of resistant plant varieties has selected for the biotypes, while a more recent hypothesis claims that biotypes are artifacts of population-level host selection on non-cultivated grasses (Porter *et al*., 1997). Kindler and Hays (1999) suggested that the development of biotype F was due to maintenance of *S. graminum* populations on certain native grasses. Anstead *et al*. (2003) reported a larger host range of non-cultivated grasses with biotype I, which is resistant to a number of HPR varieties of sorghum and wheat. The deployment of HPR wheat varieties has not apparently caused the evolution of new biotypes, because HPR wheat genes were released to growers after their respective biotypes were reported (Porter *et al*., 1997). On the other hand, Bowling *et al*. (1998) noted that as area planted with resistant sorghum cultivars increased in the southern US, so did the proportion of insects in the resistant biotypes. Deployment of HPR cultivars may have caused an increase in resistant alleles already present in a given population of *S. graminum*.

One possible reason for the perceived disconnect between HPR varieties and resistant biotypes is that HPR is scored by plant response to *S. graminum* (Burd and Porter, 2006), rather than a difference in the insect itself. Furthermore, sorghum HPR to *S. graminum* is measured in the length of time it takes for the insect to cause economic damage to the variety (Porter *et al*., 1997). Finally, because cultivars usually have more than one HPR mechanism, it may make determination of the origin of *S. graminum* biotypes more difficult to ascertain.

For these reasons, resistance management of *S. graminum* continues to be a challenge. They advocated sequential deployment of resistance genes because of the time and effort required to pyramid them into a cultivar. Porter *et al*. (2000) found no benefit of pyramiding resistance genes in wheat for control of *S. graminum*, because the HPR genes tested were already susceptible to at least one biotype. Bush *et al*. (1991) found that mixtures of 3:1 resistant:susceptible wheat cultivars reduced *S. graminum* damage. This study, however, did not address the sustainability of this practice, as it lasted for only one season.

Transgenic Insecticidal Crops

Transgenic insecticidal crops have been available to farmers since 1995. The first generation of crops expressed genes from the soil bacterium *Bacillus thuringiensis* (Bt), which has been the source of a variety of manufactured insecticides for much of the 20th century. Bates *et al*. (2005b) and Christou *et al*. (2006) review the history of transgenic insecticidal crops and discuss some of the novel toxins that will likely be incorporated into the next generation of engineered crops. Up to now, gene expression has occurred in the nucleus of most, if not all, cells of the plant.

One approach for the future may be the expression of genes in chloroplasts (Kota *et al*., 1999; De Cosa *et al*., 2001). This approach provides tissue specificity and extremely high expression levels (much higher than for nuclear expression).

Many isolates of Bt produce a crystalline protein that is toxic to insects that ingest it (Frutos *et al*., 1999, Pittendrigh *et al*., Chapter 3). In the midgut, the crystals are solubilized and digested into the delta-endotoxin, which interferes with midgut epithelial cells. This leaves the midgut membrane perforated, and the insect is killed by sepsis. Each identified Bt crystalline protein has a limited range of species that it harms (Pittendrigh *et al*., Chapter 3). Bt toxins are commonly used in chemical applications for insect control, and more recently, genes producing a toxin have been transformed into certain crops. Transformation is the term referring to the insertion of a foreign gene into a new genome. Chemically inducible Bt toxin expression in a plant is feasible (Cao *et al*., 2001, 2006; Bates *et al*., 2005a), but commercially grown transgenic insecticidal cotton and corn crops produce Bt toxin constitutively.

Because of evolution of insect resistance to Bt insecticidal sprays, it has been widely recognized since the introduction of transgenic Bt crops that these products should be used in a judicious manner to prevent resistance from evolving (McGaughey and Whalon, 1992). Several insect species have been selected for resistance to Bt in the laboratory (Tabashnik, 1994). In addition, two lepidopteran pests of crucifers have evolved resistance to Bt sprays: greenhouse populations of *Trichoplusia ni* (Janmaat and Myers, 2003) and field populations of *Plutella xylostella* (Tabashnik *et al*., 1990, 2003). Resistance to Bt in laboratory colonies may not necessarily ensure ability of insects to complete development on Bt crops (Tabashnik *et al*., 2003); nevertheless the potential for resistance to evolve is of great concern. Although there are multiple means of resistance to Bt, the most common mechanism involves some modification of the binding site in the midgut (Ferré and Van Rie, 2002).

In all countries where they are grown, governments regulate transgenic insecticidal crops, though this is rarely the case with traditionally bred HPR crops. Thus, legal restrictions have been placed on the sale, planting, and cultivation of this modern technology. In most countries, corporations producing the transgenic insecticidal crops and selling the seeds are required to implement an IRM strategy for these cultivars. Hurley and Mitchell (Chapter 11) discuss some of the economic aspects of these regulations.

A refuge strategy is the preferred approach to managing resistance on transgenic insecticidal crops if a high dose of the toxin can be consistently expressed by the plants (Gould, 1998). The high dose plus refuge strategy is based on the work of Comins (1977), Taylor and Georghiou (1979) and Tabashnik and Croft (1982). A high dose is defined as one that kills all susceptible homozygotes and most, if not all, heterozygotes. This makes resistance functionally recessive. A refuge of conventional plants is a source of susceptible homozygotes that can mate with any surviving heterozygotes to prevent the production of homozygotes that are resistant to the toxin. The resistance allele must be initially rare to ensure the effectiveness of this approach. Liu and Tabashnik (1997) were the first to experimentally demonstrate the value of a refuge in delaying resistance to a Bt toxin not incorporated into a plant.

The following case studies have similarities concerning IRM, but differ significantly in the agricultural and regulatory history of the three transgenic insecticidal

crops. The first two cases involve registered crops developed by Monsanto: the first failed to succeed commercially because of marketing concern by potato buyers and competition from new insecticides, and the second has become established and accepted as a significant part of agriculture. The third case pertains to a crop that has not yet been commercialized or registered by the US Environmental Protection Agency.

Leptinotarsa decemlineata

The Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), is the most devastating defoliator of potato (*Solanum tuberosum*) around the world (Hare, 1990). Multiple generations may occur per year depending on the latitude and climate. Adults overwinter in the soil in diapause. Both larvae and adults feed on potato leaves. Even though IPM alternatives exist, most potato farmers rely on insecticides (Feldman and Stone, 1997). *L. decemlineata* has a long history of evolving resistance to insecticides (Hare, 1990).

In 1995, transgenic insecticidal potatoes created by Monsanto became the first genetically engineered HPR crop to be registered by the US Environmental Protection Agency for commercial use (Feldman and Stone, 1997). The Bt cultivars were also registered for commercial use in Canada and Russia. The potato expresses a Bt protein toxic to some Coleoptera including *L. decemlineata*. This was the first time that an IRM plan had been submitted to the US Environmental Protection Agency during the registration process for an insecticidal crop. Thus, Monsanto's IRM plan for transgenic insecticidal potato was the first to be developed prior to market introduction (Feldman and Stone, 1997).

The IRM strategy was incorporated within an overall IPM plan (Feldman and Stone, 1997; Hoy, 1999). This permitted potato farmers to reduce insecticide use and take advantage of biological control. Monsanto recognized the importance of adjusting the IRM strategy according to local conditions. The strategy included expression of a high dose in the Bt potatoes, planting of a refuge of susceptible potatoes, and monitoring *L. decemlineata* for survival and resistance. The high dose was important because the Bt potatoes would have to be competitive in the marketplace with the very effective systemic insecticide imidacloprid. Selection for resistance to the Bt toxin (not the transgenic insecticidal plant) had already occurred in a laboratory study (Whalon *et al*., 1993).

The choice of refuge configuration was the most difficult problem during the development of Monsanto's IRM strategy. Monsanto realized that research was needed to investigate the complexity and uncertainty of larval and adult behavior and to learn how farmers would deal with Bt potato under realistic conditions. In addition to a refuge block near a Bt potato field, Monsanto considered using a seed mixture to randomly insert the refuge within the Bt potato field to ensure grower compliance with the IRM strategy.

Field observations, laboratory experiments, and modeling contributed to the decision making regarding seed mixtures. Both older larvae and adults move frequently between potato plants. Hoy and Head (1995) measured a positive genetic correlation between larval avoidance and larval tolerance to the Bt toxin that could lead to more rapid evolution of resistance. In addition, Ferro (1993) evaluated the

effects of seed mixtures with a high-dose Bt potato and susceptible potato as well as a low-dose strategy that could delay maturation of *L. decemlineata*. He concluded that a seed mixture would not be effective in delaying resistance evolution. He advocated the use of a low-dose Bt potato crop that would reduce the number of generations of *L. decemlineata* per year, but warned that non-random mating amongst susceptible and resistant beetles could occur because of the delays in maturation. Therefore, for several reasons, the seed mixture idea was abandoned.

Monsanto's IRM plan required the use of Bt potato in an annual rotation with a non-Bt crop (temporal refuge) and a 20% spatial refuge. Potato growers could not apply a foliar Bt application for *L. decemlineata* control on refuge fields, but they could treat the refuge with other insecticides to prevent damage by *L. decemlineata* according to local IPM recommendations. Monsanto's transgenic insecticidal potatoes were annually planted on less than 20,000 hectares from 1995 to 2001. At its maximum, Bt potato accounted for less than 5% of total potato production in United States. The Bt cultivars never became broadly established in the IPM programs imagined by Monsanto.

Pectinophora gossypiella

The pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), is a very destructive, cosmopolitan pest and the most serious lepidopteran pest of cotton (*Gossypium hirsutum*) in the southwestern United States (Henneberry and Naranjo, 1998). Female moths lay eggs on cotton plants and larvae bore into cotton bolls where they eat cotton seeds. At least four generations per year occur in the United States. Larvae do not move from plant to plant (Carrière *et al*., 2005) and they overwinter in diapause. Although *P. gossypiella* can infest 70 plant species in seven families, it feeds almost exclusively on cotton in Arizona (Henneberry and Naranjo, 1998).

Transgenic insecticidal cotton expressing Bt toxin was first grown on a large scale in Arizona in 1996. Since then, a large team of Arizona scientists has been studying the evolution of resistance by *P. gossypiella* to Bt cotton. A coordinated research and educational effort called the Arizona Bt Cotton Working Group includes the University of Arizona, the Arizona Department of Agriculture, the US Department of Agriculture's Western Cotton Research Laboratory, the Arizona Cotton Research and Protection Council, and the Arizona Cotton Growers Association (Carrière *et al*., 2001a).

Before Bt cotton was commercialized in the United States, the US Environmental Protection Agency granted a registration to Monsanto that included IRM requirements based on a refuge strategy. The percentage of cotton fields required to be refuges depends on whether refuges are planted inside or outside of the Bt cotton fields (Carrière *et al*., 2005). For refuges outside of Bt cotton fields, refuge size is either 5% non-Bt cotton if the refuge is not sprayed with insecticides effective against *P. gossypiella* or 20% non-Bt cotton if the refuge is sprayed with such insecticides. Refuges inside Bt cotton fields must be at least 5% of the field and they can be planted as one row of conventional cotton for every six to ten rows of Bt cotton (Carrière *et al*., 2005). The internal refuge block and Bt cotton block can be treated with any insecticides, except Bt, as long as the refuge and Bt cotton are both treated.

The risk of resistance by *P. gossypiella* to Bt cotton was initially considered high in Arizona for several reasons. Lab selection with Bt toxin in artificial diet quickly produced strains resistant to Bt cotton. From 1998 to 2003 in most of the major cotton areas of Arizona, Bt cotton was planted in *ca.* 60–80% of the fields (Carrière *et al*., 2005). Bt cotton produces a high dose of toxin that kills almost 100% of susceptible *P. gossypiella* larvae that ingest it. In Arizona, *P. gossypiella* has no other host plants and has up to five generations per year. All of these conditions favor the rapid evolution of resistance.

Subsequent observations on the *P. gossypiella* and Bt cotton system challenged the early predictions of fast evolution of resistance. For example, on non-Bt cotton, the Arizona team observed significant fitness costs for homozygous resistant individuals relative to homozygous susceptible ones (Carrière *et al*., 2001b, c, 2004). Also, survival of homozygous resistant individuals is lower on Bt cotton then on conventional cotton (Carrière and Tabashnik, 2001).

The Arizona IRM team monitored resistance to Bt cotton by collecting infested bolls from 10 to 17 cotton fields in Arizona per year (Tabashnik *et al*., 2005b). The progeny of field-collected *P. gossypiella* from each site were tested using bioassays involving an average of 2,541 larvae per year. Neonates were tested on artificial diet containing a dose of toxin that kills all larvae but the homozygous resistant ones (Tabashnik *et al*., 2000, 2002, 2005a). In 1999 and 2000, no larvae survived on diet treated with a diagnostic concentration of Bt toxin (Tabashnik *et al*., 2005b). Figure 9.2 presents the results of this monitoring program. The highest frequency of resistance to the Bt toxin occurred in 1997 with a mean resistance-allele frequency of 0.16. Five of ten cotton fields sampled in 1997 yielded one or more resistant larvae that survived exposure to the diagnostic concentration of

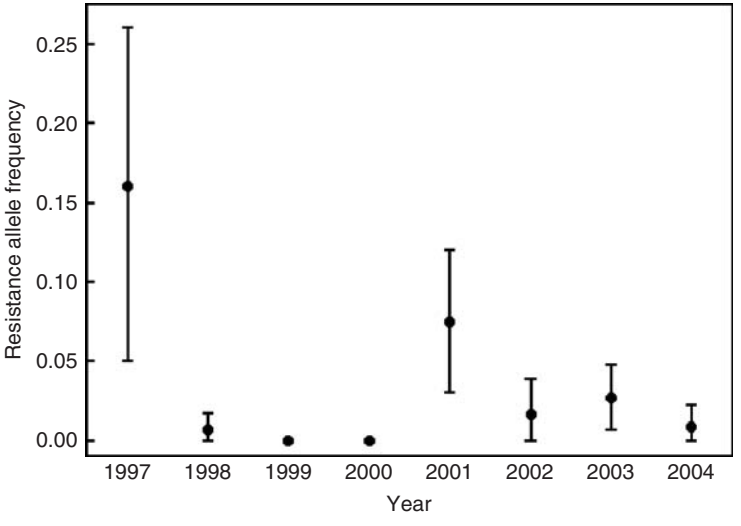

Figure 9.2 The mean allele frequency (with 95% confidence intervals) for resistance to transgenic insecticidal cotton by *P. gossypiella* in Arizona from 1997 to 2004. (from Tabashnik *et al*., 2005b; copyright 2005, National Academy of Sciences, USA.)

toxin (Tabashnik *et al*., 2000). The resistance-allele frequency decreased in 1998 to 0.0070 and then varied over the next 6 years, with no net increase from 1997 to 2004 (Figure 9.2).

Tabashnik *et al*. (2006) also screened the *P. gossypiella* population for resistance to Bt cotton with a DNA-based method. The technique uses polymerase chain reaction primers that specifically amplify three mutant alleles of a cadherin gene linked with resistance to Bt cotton to detect single resistance alleles in heterozygotes. Tabashnik *et al*. (2006) found no resistance alleles in 5,571 insects derived from 59 cotton fields in three states during 2001–2005.

Additional data support the conclusion that *P. gossypiella* resistance to Bt cotton in Arizona did not increase from 1997 to 2004. Field efficacy of Bt cotton has remained very high with greater than 99% of larvae killed (Dennehy *et al*., 2004). No control failures caused by resistance have been reported, and regional declines of *P. gossypiella* have occurred in areas of Arizona with high use of Bt cotton (Carrière *et al*., 2003). In six major cotton-growing counties of Arizona, non-Bt cotton refuges on the average accounted for 14–78% of the fields planted with cotton per county from 2000 to 2003 (Figure 9.3). Furthermore, 88% of the fields, in 5 out of 6 years, appeared to be in compliance with refuge requirements (Carrière *et al*., 2005). Therefore, the Arizona IRM team concluded that the high dose/refuge strategy has helped to delay *P. gossypiella* resistance to Bt cotton (Tabashnik *et al*., 2005b).

Plutella xylostella

The diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is a major pest of crucifers (Brassicaceae) in many parts of the world (Head and Savinelli, Chapter 5). Damage is caused by larval feeding on the leaves and around buds. The insect passes through many generations per year. *P. xylostella* has evolved resistance to numerous synthetic insecticides (Talekar and Shelton, 1993), so it has become the focus of many IRM studies. Because *P. xylostella* was the first insect to evolve resistance to Bt sprays in the field (Shelton *et al*., 1993; Tabashnik, 1994) and because it can be studied under greenhouse conditions, a team at Cornell University used the insect as a model system to examine the assumptions underlying IRM strategies in transgenic insecticidal crops.

Before they could perform the IRM experiments, the Cornell University team had to develop both transgenic insecticidal broccoli (*Brassica oleracea*) and laboratory strains of *P. xylostella* resistant to the transgenic Bt cultivars. The team developed transgenic Bt cultivars with high expression of one or two Bt toxins (Metz *et al*., 1995; Cao *et al*., 1999a, b). Resistant *P. xylostella* were collected from areas where control with Bt products was failing. Colonies derived from these insects were subsequently selected on transgenic Bt broccoli plants (Zhao *et al*., 2002).

The resistant strains of *P. xylostella* can be used to study the dynamics of resistance to Bt transgenic crops. Resistance to Bt toxin is an autosomal recessive trait in the moth (Zhao *et al*., 2000; Tang *et al*., 1997). Zhao *et al*. (2002) concluded that initial frequencies of resistance alleles are underestimated when using transgenic broccoli with high Bt expression as a screen; they recommended a diet assay that would yield fewer false negatives. Zhao *et al*. (2000) found that after selection for

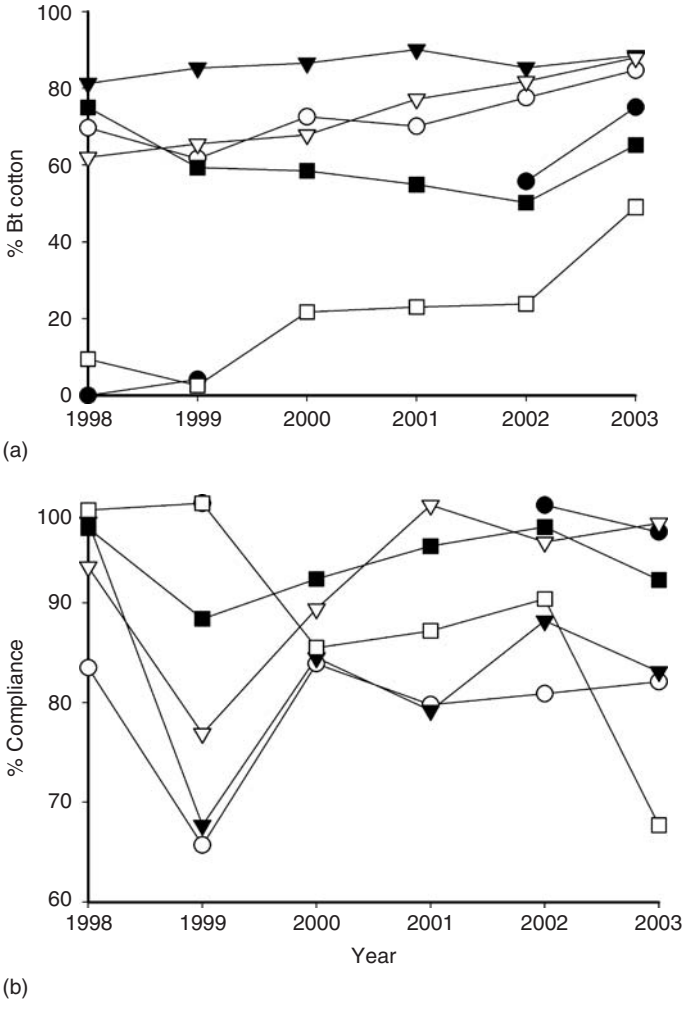

Figure 9.3 Percentage of Bt cotton grown in six counties (a) and percentage compliance (b) during 1998–2003. (from Carriere *et al*., 2006 Copyright Society of Chemical Industry. Reproduced with permission.)

resistance to one Bt toxin is stopped, there is a drop in resistance. However, baseline susceptibility to Bt was not restored after ten generations without selection.

The Cornell University team was the first to experimentally evaluate the refuge strategy IRM for transgenic insecticidal crops under realistic conditions. The team varied the size and arrangement of the refuge in the field and in the greenhouse. Field tests were possible because *P. xylostella* does not overwinter in New York; released resistant insects would die in the fall. Each field plot contained 300 broccoli plants, and the initial resistance-allele frequency was either 0.12 or 0.8 (Shelton *et al*., 2000). In the greenhouse, Tang *et al*. (2001) used cloth cages containing 30 potted broccoli plants. In the greenhouse experiments, the initial

resistance-allele frequencies were either 0.007–0.0125 (Tang *et al*., 2001) or 0.10–0.34 (Zhao *et al*., 2003, 2005).

All of the experiments demonstrated the effectiveness of separate refuges to delay evolution of insect resistance to Bt broccoli (Shelton *et al*., 2000; Tang *et al*., 2001; Zhao *et al*., 2003, 2005). There is no ovipositional preference between Bt and non-Bt broccoli cultivars (Tang *et al*., 1999). *P. xylostella* larvae tend to disperse more readily from Bt plants than from non-Bt broccoli plants (Tang *et al*., 2001), increasing the chance that larvae will receive a sub-lethal dose of the toxin. This means that refuge plants should be separated from (rather than randomly mixed with) Bt broccoli plants to prevent or delay resistance (Shelton *et al*., 2000; Tang *et al*., 2001).

Zhao *et al*. (2003, 2005) evaluated the deployment of refuges plus the pyramiding of Bt genes within plants (Cao *et al*., 2002). They concluded that pyramids of insecticidal genes in Bt broccoli is the most effective strategy for delaying resistance (Zhao *et al*., 2003), but that this benefit is lost in the presence of transgenic insecticidal crops containing only one of the genes in the pyramid (Zhao *et al*., 2005).

Discussion

This chapter describes a wide variety of cases in which an arthropod species has overcome HPR designed by humans to control crop pests. Resistance by arthropods to HPR can occur to both traditionally bred crops and transgenic insecticidal crops (Gould, 1998). Much of the focus of HPR is on antibiosis, and more recently, production of extremely high doses of toxins in transgenic insecticidal crops. IRM will be needed whether the HPR is caused by antibiosis or by antixenosis.

Mechanisms of antibiosis and the resulting toxicological resistance are much better understood. Major resistance genes have been identified, most notably in insects that are targets of transgenic insecticidal crops. These genes and the resistant insect colonies based upon them can be used to study the dynamics of resistance and the efficacy of various management strategies. Examples of this are *M. destructor* on wheat and *P. xylostella* on broccoli.

The behavior of the arthropod influences how quickly it will evolve resistance to either antibiosis or antixenosis. An HPR variety that deters an herbivore is expected to exert less selection pressure for resistance than one in which the herbivore has no preference between it and the susceptible variety. This does not mean, however, that an herbivore will not evolve behavioral resistance. Indeed, *T. urticae* still evolved resistance to morphological features of tomatoes and broccoli previously shown to deter mites. *S. graminum* has evolved resistance to many sorghum and wheat cultivars, most of which have some level of antixenotic effect. Exactly what causes these behavioral changes is yet to be discovered.

The history of Gould's modeling work exemplifies the predictions needed to make scientific, preventative, and some would say, "proactive" IRM strategies for HPR. In his first major modeling study, Gould (1984) evaluated the effects of crops expressing one trait for toxicity and one for repellency (Castillo-Chavez *et al*., 1988). He was the first entomologist to study management of behavioral resistance. He found that behavior modification could be valuable in IRM, but that coordination of strategy implementation would be important because of the off-farm externalities caused by repelling mobile pests to neighboring fields. In later work,

he focused on crops with two toxin traits. Gould (1986a) used the model to evaluate sequential deployment of single-gene crops, single-gene cultivar mixtures, and a pyramided two-gene cultivar. He concluded that no single IRM strategy was the best or most durable for all modeled scenarios. Model results indicated that the pyramided crop would be more durable if it is planted along with a plot of the cultivar lacking HPR. Gould (1986b) applied this model to the evaluation of IRM for *M. destructor* on HPR wheat (discussed in section on traditional crops along with the alternative model by Cox and Hatchett, 1986). He extended this analysis of pyramided crops containing two toxins by investigating the role of fitness costs in resistance evolution (Gould *et al*., 2006). He concluded that, when an IRM plan includes a 10–20% refuge of cultivars lacking any HPR and the rest of the cultivars are only pyramids of two toxin genes, the risk of resistance is often low when resistant insects experience fitness costs on the susceptible cultivars. However, the IRM plan often fails when the landscape includes a single-gene cultivar (Gould *et al*., 2006). Again, the need for coordination of strategy implementation is critical for the success of IRM; some agency or stakeholder association must restrict the planting of single-gene cultivars to ensure the durability of pyramided crops. In all of his modeling studies, Gould has emphasized the point that specific IRM plans can only be derived from pest and cropping-system specific models. Additional views concerning IRM models can be found in Chapters 10–12.

Gould (1988) was one of the first scientists to emphasize the need for IRM plans for transgenic insecticidal crops. By the middle of the 1980s, scientists had successfully transformed plants to express Bt toxin, but it was uncertain that anyone in industry, government or academia had thought of rational approaches to the wise use of such plants in commercial agriculture. Gould was concerned about the intense selection pressure likely to occur when very toxic plants express the toxin in every crop tissue throughout the entire growing season. He urged genetic engineers to develop crops that express the toxin only in some tissues and only for a short time. He even challenged these laboratory scientists to have plant expression of the toxin induced only after the pest causes substantial damage (Gould, 1988). Nevertheless, 18 years later, no commercialized transgenic insecticidal cotton or corn plant has been purposefully engineered to limit exposure of a pest to a toxin to short periods and in only a few plant tissues.

As with any IRM program, stakeholders should expect and plan for complexity and complications in HPR. Traditional HPR is typically not coordinated in any area wide IPM programs, although effective cultivars may be recognized by many individual farmers and extensively planted across a large region. Management of resistance to traditionally bred or genetically engineered crops will face the complexity of agricultural and natural landscapes. Regions will likely consist of crop cultivars having one, two, or more traits with lethal or sub-lethal effects on the targeted arthropod. IRM strategies cannot be based on assumptions of single traits with simple effects. Stakeholders should expect expression of insecticidal traits to vary over the growing season because toxins may increase, and particularly, decrease during certain plant processes and stages. Just as likely, expression of HPR traits can vary in different plant tissues, producing a mosaic of toxin levels within each plant.

When we manage arthropod resistance to HPR, we should address the following questions. How can we develop or acquire a kind of plant that improves IRM? What

kind of toxins should it have? How much of each toxin should the plant produce? In what tissues? Should the toxins be pyramided within a given plant or should HPR cultivars be planted in rotation or grown sequentially over multiple seasons? To lower the probability of evolution of resistance, how should the plant and its toxin change over a growing season? Of course, solutions to the IRM problem must still allow adequate yield and crop quality in the field where other stresses may be occurring.

Carrière *et al.* (2001a) describe the efforts by the Arizona Bt Cotton Working Group to develop a rational and scientific IRM strategy for *P. gossypiella*. From the beginning, the Working Group knew that they were in the middle of a large-scale field experiment testing the high dose plus refuge strategy and testing their ability to implement and comply with the US Environmental Protection Agency's regulation of Bt cotton. Given the high stakes involved, the Working Group was under pressure to produce educational and scientific results that would not only successfully implement the required IRM strategy in the cotton fields of Arizona but also improve the US Environmental Protection Agency's regulation of Bt cotton. The report by Carrière *et al.* (2001a) records some of their recommendations to the Agency based on their laboratory and field studies. They also draw some conclusions about coordination of activities. For example, they state that IRM should be a primary objective for grower-funded, commodity-based organizations. The Arizona Bt Cotton Working Group was one way to engage such stakeholder groups.

Under what conditions would government-imposed IRM requirements for traditionally bred HPR crops be beneficial to society? For instance, would wheat growers benefit from a required IRM strategy for *M. destructor* on HPR wheat? Certainly the mandated coordination of efforts and compliance with the strategy would impose unknown, but substantial, cost on growers and society (Hurley and Mitchell, Chapter 11), but would the benefits in terms of decreased loss of harvested yield from old, but still resistant, cultivars and lower cost of reduced breeding programs exceed these costs over the long term? Part of the question becomes, over what time horizon do we evaluate the economics (Mitchell and Onstad, Chapter 2)?

Two important conclusions can be drawn from field and modeling studies concerning resistance to HPR crops. First, refuges of susceptible cultivars are effective in delaying the evolution of resistance. This was demonstrated with the field and greenhouse studies of *P. xylostella*, with the field studies of *P. gossypiella*, and with Gould's modeling of *M. destructor*. Second, proper monitoring of resistance and of resistant biotypes or strains requires a significant amount of work. This is exemplified by the work on *P. gossypiella* and *S. graminum*. Chapters 10 and 13 explore these issues more fully.

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Chapter 10

The Role of Environment in Insect Resistance Management

David W. Onstad

Traditionally, research concerning insect resistance management (IRM) has emphasized the targeted pest species and the toxin or toxic plant that it may evolve resistance to. Two other components of every system that are often given little if any consideration are humans and the environment (Figure 10.1). The roles of stakeholders, policy makers, and pest-management decision makers are discussed by Onstad (Chapter 1), Mitchell and Onstad (Chapter 2), Head and Savinelli (Chapter 5), and Hurley and Mitchell (Chapter 11). The structure of the environment, the dynamic abiotic and biotic conditions, the community of species that interact with the targeted pest, and the landscape-influenced behavior of these species are all the subject of this chapter. Thus, the environment affects the pest and the crop or toxin over time and represents the distribution of these components over space.

With the potential for arthropods to evolve resistance to many environmental components (pathogens, crops, parasitoids; Chapter 1) and even to the human-designed landscape (Chapter 8), the focus on the environment as a critical factor in IRM has increased. Certainly, researchers have always known that weather could affect pesticide residues and that natural enemies could be harmed by pesticides, but few scientists studied how dynamic, heterogeneous environments influenced important arthropod behaviors. I present the following case studies and ideas to promote a greater appreciation for the environment and to advocate

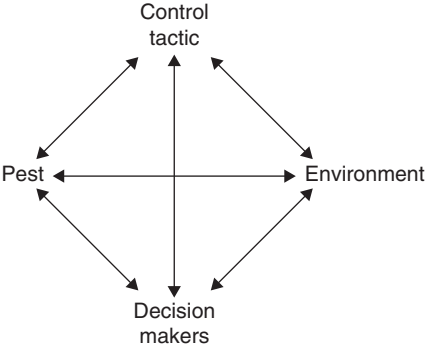

Figure 10.1 Four major components of a pest-management system and their interactions.

the inclusion of rigorous investigations of the role of the environment in all future IRM research.

Landscape Structure and Design

Landscapes are typically heterogeneous and dynamic. Natural variability and variation exist, but humans can also design the landscape. Design may include the location and characteristics of water supplies that may be sources of vectors of human disease. Or a crop landscape may include a structured refuge designed in the form of blocks, within-field strips, or mosaics of plants. Crop rotation on a schedule, that is not modified based on observations, can also be considered part of the landscape design. Too often we either accept the landscape design as a given that cannot be changed or we take it for granted. Croft and Dunley (1993) review several case studies of how landscape structure may influence IRM. Most of these studies focused on the influence of natural areas or untreated habitat on immigration of susceptible arthropods into treated areas. They conclude that the proximity and heterogeneity of habitat for pests and natural enemies influence dispersal and gene flow, which significantly affect IRM (Onstad and Guse, Chapter 4).

Farmers affect landscape structure by deciding whether or not to fertilize, cultivate, or irrigate crop fields. Onstad and Guse investigated the problems of IRM in irrigated fields of corn (*Zea mays*) infested by *Diatraea grandiosella* and *Ostrinia nubilalis* (Guse *et al*., 2002; Onstad *et al*., 2002). The two species of stalk-boring Lepidoptera have different adult behaviors, particularly mating, oviposition, and male moth dispersal. Furthermore, these behaviors differ in irrigated and non-irrigated cornfields. Irrigation occurs primarily in western and southern corn-growing regions of the United States. Guse *et al*. (2002) discovered that these adult behaviors strongly influenced the evolution of resistance to transgenic insecticidal corn. Therefore, Guse *et al*. (2002) concluded that the interactions of landscape and insect behavior must be understood to properly develop IRM plans. Guse *et al*. (2002) also concluded that a strip refuge (i.e., rows within a field) did not change the time for resistance to evolve in the *O. nubilalis*; however, row-strip refuges cannot be recommended for the *D. grandiosella* because the row strips permit evolution to occur more quickly.

Immigration to treated areas from either refuges (two-way gene flow) or a much larger source population (gene flow only into treated area) was considered in an abstract way by early IRM modelers (Comins, 1977; Taylor and Georghiou, 1979; Tabashnik and Croft, 1982). This early modeling work affirmed the value of a refuge strategy for IRM. A refuge is a pest habitat that is a source of susceptible individuals that can mate with any survivors in the treated habitat. The number of susceptibles emerging from the refuge is expected to be larger than the number of resistant individuals emerging in the treated area.

Several more-sophisticated, abstract analyses have been performed since the early modeling efforts. In one of the first simulations of population dynamics and genetics over explicitly-defined space, Caprio and Tabashnik (1992) provided an excellent analysis of dispersal and evolution in a finite population spread over many patches. Peck and Ellner (1997) used both a single-field model and a spatially-explicit multiple-field model to study the influence of gene flow and population

growth rate on resistance evolution. They focused on the consequences of using pest-density thresholds to initiate pesticide treatments in each field. Lenormand and Raymond (1998) evaluated older population genetics analyses relative to scenarios with treated and untreated refuges. They promoted an IRM perspective based on a threshold division of the environment that limits resistance evolution. Mohammed-Awel et al. (2007) performed equilibrium analyses on the scenario in which refuges are used for IRM but a transgenic insecticidal crop is planted only on a small fraction of the landscape. In this case, immigration of susceptible individuals from non-refuge populations of the pest may occur. They generally argued that refuges and immigration from the large source population may not be compatible. However, when they modeled a typical scenario with an injection of resistant individuals (typical initial resistance allele frequency above zero), a refuge is valuable even with immigration.

A variety of studies have been performed on specific systems. All of the results demonstrate that refuges delay the evolution of resistance. These studies give us a full appreciation of the complexities of dispersal within heterogeneous environments. For example, using two models of *Helicoverpa zea* on cotton (*Gossypium hirsutum*), Caprio (2001) studied the relationship between the resolution of habitat patchiness and pest dispersal, mating, and oviposition. One model was a stochastic spatially-explicit simulation and the second was a simpler deterministic model. He concluded that refuge deployment must account for demographic characteristics of the targeted pest.

Peck *et al*. (1999) created a stochastic, spatially-explicit model, to explore the regional evolution of resistance by *Heliothis virescens* to transgenic insecticidal cotton. They discovered that the spatial scale and the temporal pattern of refuges of non-toxic plants significantly affect the evolution of resistance. The time required for resistance to evolve was significantly longer in regions where the same fields were used as a refuge year after year, compared with regions where the refuge fields are changed randomly from year to year. Spring and summer dispersal of adults amongst wild host plants and cotton could significantly affect evolution of resistance to transgenic cotton depending on distances flown.

Storer *et al*. (2003a, b) used a stochastic, spatially-explicit simulation model to examine the role of spatial processes in the evolution of resistance in *H. zea* on transgenic insecticidal corn and cotton. They modified and extended the model of *H. virescens* created by Peck *et al*. (1999). They found that selection for resistance is more intense in transgenic cotton fields than in transgenic corn fields. The results also indicated that local gene frequencies are highly dependent on local deployment levels of transgenic insecticidal crops despite the high mobility of the adult insects. Storer *et al*. (2003b) found that the proportion of the landscape planted to corn significantly affected the evolution of resistance. On a local scale, *H. zea* populations in clusters of fields containing high levels of transgenic crops experience more rapid evolution than populations in neighboring clusters of fields with smaller patches of transgenic crops. Storer *et al*. (2003a) concluded that farm-level refuge requirements are important for managing the risk of resistance.

Storer (2003) created a stochastic, spatially-explicit model of *Diabrotica virgifera virgifera* on transgenic insecticidal corn. Storer compared the rate at which the resistance allele evolved under different refuge deployment scenarios. For a given

refuge size, the model indicated that placing the non-resistant refuge in a block within a rootworm-resistant field would likely delay evolution longer than planting the refuge in separate fields in varying locations. Planting the refuge in the same location each year delays resistance even further.

Sisterson *et al*. (2004) used a stochastic, spatially-explicit model of *Pectinophora gossypiella* on transgenic insecticidal cotton to investigate the influence of population size on resistance evolution. They focused on interactions of carrying capacity for the pest, region size, dispersal, and percentage of fields planted with transgenic cotton. The time to resistance decreased as region size increased, because larger regions were more likely to have at least one field in which resistance evolved rapidly and served as a source from which resistance spread throughout the region. They found resistance evolution was affected by interactions between carrying capacity, dispersal, and the percentage of fields planted with transgenic cotton. Sisterson *et al*. (2005) used the same model to study the influence of relative abundance of refuge and the temporal and spatial distributions of refuge in the region on resistance evolution. They found that these three factors significantly affected evolution of resistance to transgenic insecticidal cotton. Resistance was delayed the longest when refuges had fixed locations over years and were distributed throughout the region to prevent isolation of transgenic cotton.

Carrière *et al*. (2004a) used a spatially-explicit analysis with geographic information systems and demographic data to study the zone of influence of refuges for *P. gossypiella* near transgenic insecticidal cotton. They defined a zone of influence as the area over which a refuge can increase a pest's density. For *P. gossypiella*, the typical refuge had the greatest impact on pest density within 0.75 km from a transgenic cotton field, but the zones of influence varied with conditions such as the relative abundance of transgenic cotton in the area.

Spatial Mosaics

Spatial mosaics of toxic plants are generally not recommended for use in an IRM strategy. This is based on the idea that mixing two kinds of toxic crops or applying different pesticides on two or more different areas of the same local region may cause the pest to evolve resistance to both toxins simultaneously. However, mosaics exist throughout natural and managed environments. For example, mosaics of toxic plants and non-toxic (refuge) plants are encouraged for IRM. In addition, mosaics that take advantage of negative cross-resistance are also being promoted for IRM strategies (Pittendrigh *et al*., 2004; Chapter 6). In this case, one toxin harms susceptible pests and the other toxin only harms those that evolve resistance to the first toxin. Thus, designing a landscape to include a spatial mosaic is a rational approach to IRM.

What has not been considered in most previous research is the unplanned mosaic resulting from variable gene expression within a single field of a transgenic insecticidal crop. Soil moisture, soil nutrients, herbivory, and topography all vary over space and influence the growth of plants and the production of toxin in these plants. Hence, expression of the gene for toxin production may vary between plants in a single cultivar over a crop field creating a spatial mosaic of various doses of toxin. Furthermore, many transgenic insecticidal cultivars that express the same toxin gene may have variable toxin production in the field (Vaughn *et al*.,

2005; Gray *et al*., 2007). As neighboring farmers plant multiple cultivars, even on their own farms, spatial mosaics are created. The consequences of this variability for IRM have not been explored.

Spatial mosaics also exist in traditional host plant resistance and in transgenic insecticidal crops that have variable defenses and toxin levels in different plant tissues. In this case the mosaic is not plant-to-plant but within every plant. Many plants evolve different defenses for reproductive and vegetative organs. Often defenses are induced by initial injury. Furthermore, plant breeders may breed for protection of only one plant organ that is vulnerable to arthropod damage. Thus, toxin levels in traditionally bred plants are expected to be mosaics of various toxins mixed with non-toxic tissues. In transgenic crops, toxins are often constitutively expressed throughout the plant because of the use of particular promoters and transformations in their development. Even in the transgenic insecticidal plants, however, within-plant mosaics may occur. Horner *et al*. (2003) observed modified feeding behavior by *H. zea* on transgenic insecticidal corn ears. The larvae seemed to be responding to the variable toxicity of the kernels, 25% of which are expected to be non-toxic based on sexual reproduction involving the single locus with a dominant toxin allele. Horner *et al*. (2003) concluded that behavioral resistance could evolve under these conditions. Onstad and Gould (1998a) modeled the scenario in which none of the corn silks and kernels express the toxin gene and found that the mosaic did not significantly affect evolution of resistance by *O. nubilalis*. Thus, within-plant mosaics must be identified before crop commercialization so IRM strategies can account for them.

Chilcutt and Tabashnik (2004) describe another kind of spatial mosaic associated with transgenic insecticidal crops. These mosaics are formed when pollen from transgenic crops drifts into adjacent refuge fields and fertilizes the same plant species. Refuge plants then have non-toxic vegetative tissue and seeds with variable toxin levels. Chilcutt and Tabashnik (2004) measured toxin levels in corn kernels fertilized in refuge borders next to transgenic cornfields. They found low to moderate toxin levels in seeds within 31 m of the transgenic cornfield. If pests eat the seeds, as *O. nubilalis* and *H. zea* do, then the lower and variable toxin doses in contaminated refuges could produce higher survival rates for heterozygotes relative to susceptible homozygotes. Consequences for IRM would need to be explored.

Additional types of mosaics exist when alternate host plants, particularly weeds, occur in the crop field or in adjacent areas. Transgenic insecticidal crops may be planted in an unplanned mixture with weeds. There is concern, for example, that IRM for *D. virgifera virgifera* on transgenic insecticidal corn can be affected by weeds in cornfields. *D. virgifera virgifera* larvae can survive on almost all grass species for 10 days and develop to at least the final larval stadium on most grass species (Clark and Hibbard, 2004; Oyediran *et al*., 2004; Wilson and Hibbard, 2004). IRM strategies for transgenic insecticidal crops should consider the influence of weed control.

Seed Mixtures: Designed Mosaics with Non-Toxic Plants

A seed mixture or seed blend is an intentionally produced, randomly mixed set of two or more kinds of crop seed. One component is toxic seed, such as transgenic insecticidal seed, and the other component is non-toxic seed of the same crop.

Thus, for transgenic insecticidal crops, the refuge plants randomly grow within the transgenic crop field. Mallet and Porter (1992) and Tabashnik (1994) were two of the first to evaluate the value of seed mixtures for IRM. Both projects used simple abstract models to study a variety of scenarios and assumptions. Tabashnik (1994) extended the more limited analysis of Mallet and Porter (1992) and concluded that, although refuge planted as a seed mixture could delay evolution of resistance relative to the case without any refuge, the relative advantage of separate refuge versus mixed refuge depends on assumptions about pest movement, mating, and inheritance of resistance. If adult dispersal is significant and random mating occurs across large blocks of fields, then separate block refuges may be superior or at least less risky than seed mixtures.

Several models and empirical studies of specific systems have contributed to the debate regarding the value of seed mixtures in IRM (Wilhoit, 1991; Caprio, 1994; Peck *et al*., 1999). In an analysis of a model, Onstad and Gould (1998a) concluded that separate block refuges were less risky than seed mixtures because of the uncertainty concerning larval movement and mortality for *O. nubilalis* in corn. Davis and Onstad (2000) tested the Onstad and Gould model using field data for transgenic insecticidal corn and found that separate block refuges would delay evolution of resistance better than seed mixtures. Arpaia *et al*. (1998) modified the model of Mallet and Porter (1992) to predict the evolution of resistance by *Leptinotarsa decemlineata* in seed mixtures of transgenic insecticidal eggplant (*Solanum* sp.). They concluded that with partial dominance of resistance and 10% non-toxic plants in the fields, only the typical, high level of adult dispersal or a significant fitness cost for resistant beetles could ensure the significant delay in the evolution of resistance. Shelton *et al*. (2000) and Tang *et al*. (2001) observed *Plutella xylostella* on transgenic insecticidal broccoli (*Brassica oleracea*) in greenhouse and field studies and concluded that separate block refuges are superior to mixtures of plants for IRM. Onstad (2006) created a deterministic IRM model of *D. virgifera virgifera* that emphasized processes during the larval stage to evaluate seed mixtures of non-toxic and transgenic insecticidal corn for IRM. The model included many of the same calculations and processes modeled by Onstad and Gould (1998a) and Davis and Onstad (2000) for *O. nubilalis*. In addition, this model simulated larval survival on insecticidal plants in two ways: (1) in a traditional density-independent fashion, and (2) with a survival function that increases with population density (Onstad, 2006). The positively density-dependent survival is based on the idea that wounding allows subsequent feeders to survive on internal non-toxic tissues. This may be a reasonable representation of many situations in which herbivorous arthropods feed on toxic surface tissues of roots, fruit, seeds, etc., before feeding on the rest of the plant. Simulation results indicated that, with either type of survival function, seed mixtures are a reasonable alternative to separate block refuges for *D. virgifera virgifera* IRM (Onstad, 2006).

Seed mixtures have advantages and disadvantages relative to IRM. Seed mixtures may produce extra selection imposed on feeding stages that can move from plant to plant but not from field to field (i.e., larval and nymphal stages). The selection occurs through differential survival of heterozygotes and susceptible homozygotes leaving the toxic plants and finding a non-toxic plant in the field. The heterozygotes may tend to survive more than the homozygotes. On the other

hand, mixtures may ensure random mating even when mating by adults from different fields of toxic and non-toxic plants is not random. Seed mixtures also tend to ensure compliance with mandated minimum amounts of refuge and provide refuge plants similar in quality to transgenic insecticidal plants. The non-toxic plants are similar in quality because they must be planted at the same time and at the same site as the toxic plants. Biological models, such as the one created by Tabashnik (1994), usually do not address the issues of refuge quality and compliance by farmers with legal or recommended standards.

Evolution of Resistance to Crop Rotation

Onstad *et al*. (2001) used a model to explore several hypotheses concerning the evolution of behavioral resistance to crop rotation by *D. virgifera virgifera* (Chapter 8). Their primary interest was to determine how the landscape design interacts with alternative genetic systems to influence evolution of the pest. In this case, landscape design is determined by cropping practices and the annual switching from corn (*Zea mays*) to soybean (*Glycine max*) and back again. They modeled both a 2-allele as well as a 3-allele genetic system: an X allele for no movement out of corn, a Y allele for the tendency to move to all types of vegetation, and a Z allele for the tendency to move only to the crop most commonly rotated with corn, soybean. The landscape consists of four patches: continuously planted corn, rotated corn, soybean rotated with corn, and extra vegetation. Landscape diversity is represented by the extra vegetation (not rotated with corn) in the landscape. The rotation level, R, equals the sum of the proportions of soybean and rotated corn and tends to be greater than 0.9 in the region where the rotation-resistant phenotype was first detected (Onstad *et al*., 2001).

Because adult feeding in crops other than corn is associated with egg laying outside of corn, the terms monophagy (X-phenotype) and polyphagy (Y-phenotype) may help focus attention on the locations where adult insects are present. Wild-type, monophagous, individuals move from the natal cornfield and distribute themselves (and their eggs) uniformly across the two patches of corn. Polyphagous individuals move into all patches according to their proportional representation in the region. After emerging in a cornfield, the soybean specialists (Z-phenotype) move only to the soybean patch. If allele expression is additive, the heterozygotes are either polyphagous (XY and YZ) or oligophagous (XZ) with movement only to corn and soybean.

For the X-Y system, the Y-allele frequency only increases at very high levels of rotation. When X or Y is dominant, $R = 0.77$ is the threshold that determines whether Y disappears or eventually becomes fixed at 1. For the additive scenario, where the XY-phenotype is expressed, the Y allele disappears below $R = 0.726$ and it becomes fixed above $R = 0.844$. Between these values of R, stable polymorphisms exist with the most prevalent genotype switching from XX to XY to YY as R increases. The greater the value of R, the faster the Y-allele frequency increases.

In the X-Z system, only the additive case permits the Z-allele frequency to increase in the population. When Z and X are additive, Z disappears for $R < 0.79$. For $R > 0.79$, stable polymorphisms occur, and the Z-allele frequency increases at a faster rate as R increases. The maximum Z-allele frequency is 53% when there

is no continuous corn and rotation level, R, is at its highest value. At R = 0.90, the Z-allele frequency stabilizes at 42% after 25 years and the intermediate oligophagous phenotype (XZ) is the most prevalent. With R<0.93, the frequency of Z does not increase when Z is recessive and disappears when Z is dominant.

Given that Z increases only when X and Z are additive, Onstad *et al*. (2001) evaluated only three scenarios for the 3-allele system: constant additive X and Z and allele Y with variable expression. When Y is recessive to both X and Z, the results are the same as those described above for the additive X-Z system. When all are additive, Z disappears from the system, and the later simulated years mimic the results of the 2-allele, additive X-Y system described above, indicating that Y is superior to Z. When Y is dominant to the other alleles and R $\geqslant$ 0.80, the polyphagous phenotypes are most prevalent. As R increases, the final simulated frequencies of X and Z both decline to just over 1%.

As the level of rotation increases and the evolution of behavioral resistance to crop rotation occurs, the winners in these simulations are often polyphagous adults. These results match field observations that *D. virgifera virgifera* adults move into a variety of crops (not just soybean) in the areas where resistance to corn-soybean rotation has been reported (Spencer and Levine, Chapter 8). This may mean that the new strains behave most like YY or XY genotypes. The oligophagous XZ-phenotype may be prevalent in reality, but field observations and the model results for the 3-allele system suggest the superiority of the polyphagous insects to the oligophagous or soybean-specialist phenotypes.

Onstad *et al*. (2001) discovered that the diversity of the landscape does not influence the X-Z system, but it does have a significant effect on the evolution of polyphagy in the X-Y system. The Y-allele frequency and the polyphagous phenotypes decrease as the proportions of either extra vegetation or continuous corn increase. Onstad *et al*. (2003) tested this conclusion and its consequences for IRM by using observations from a large region of the north central part of the United States. Observations of the geographic spread of the variant resistant to crop rotation supported the hypothesis that landscape diversity slows the spread of the variant. Movement by the variant to and egg laying in vegetation that is neither corn nor a crop rotated with corn creates a significant fitness cost for the variant in regions with greater landscape diversity.

Temporal Dynamics of the Environment and Management

Just as toxin gene expression in transgenic insecticidal crops varies over space, it is likely to vary over a single growing season and fluctuate from year to year. As the environment changes over time and as the crop changes over time, so will the selection pressure on the targeted pest. The timing of management activities also can determine the outcome of pest management and alter the evolution of resistance. Trap crops may be more or less attractive to pests depending upon the planting date and maturation of the trap crop relative to other crops in the landscape (see the next section concerning IRM strategies and trap crops). Another example is IRM for transgenic insecticidal sweet corn. After harvest, the crop canopy is destroyed to kill any resistant insects (and susceptibles) that may have survived on the leaves, stems and ears.

Carrière *et al*. (2001) investigated the manipulation of cotton planting date and other cultural control methods to control *P. gossypiella* and to delay evolution of resistance to transgenic insecticidal cotton. They used statewide pheromone trapping, climatic data, and deterministic simulation models in their analysis. Most adults emerge from diapause too early to reproduce successfully on cotton in Arizona. Therefore, Carrière *et al*. (2001) developed a method for predicting the fraction of suicidal emergence resulting from adoption of a given cotton planting date. Model results indicated that manipulation of planting date and implementation of other cultural control methods reduce the rate of application of insecticides and delay the evolution of resistance to transgenic cotton by *P. gossypiella*.

Onstad and Gould (1998b) studied how corn-plant senescence and resulting re-allocation of nitrogen from vegetative tissues to kernels could affect toxin concentration (titer) in corn tissues consumed by *O. nubilalis*. They found that, under many conditions, resistance evolved faster because of titer decline, but the original toxicity and dominance of resistance before titer decline can influence the results. In some cases, resistance evolved more slowly than the case without titer decline. The influence of titer decline is affected by the proportion of larvae that enter diapause in the first generation and the timing of this phenomenon.

Alternative Refuges and Trap-Crop Strategies

Two special kinds of cropping systems must be considered in IRM strategies for transgenic insecticidal crops. Refuges provide susceptible pests to the landscape. To be most effective for IRM, refuges consisting of the same crop as the transgenic crop should be phenologically similar to the transgenic crop. Unfortunately, under commercial production practices, this cannot be assured because refuges are often planted much later than the more valuable transgenic crop. Furthermore, refuges may differ from transgenic crops in other ways because of the quality of the soil in which they are planted. The second category of cropping system considered in this section is trap cropping. Trap crops that attract and harm pests are essentially the opposite of refuges. In order to be attractive, trap crops may need to be phenologically much different than the standard crop.

Several groups have demonstrated that for some pests, adequate refuges for transgenic insecticidal crops can be found in other crops. The best evidence for this is for pests of cotton. In the United States, Gould *et al*. (2002) concluded that local corn can serve as a refuge for *H. zea* on transgenic cotton during mid-summer. In China, Wu *et al*. (2002) discovered that early and late-planted corn provided adequate refuge for the third and fourth generations of *Helicoverpa armigera*, but not for the second generation. They also concluded that non-cotton crops provided a natural refuge for the second to fourth generation *H. armigera*, but function of the refuge depends on the proportion of transgenic insecticidal cotton. In India, two groups investigated the role of non-cotton crops as sources of *H. armigera* (Ravi *et al*., 2005; Subramanian and Mohankumar, 2006). Ravi *et al*. (2005) found that several other crops typically grown in a crop mosaic with cotton could be considered refuges for *H. armigera*.

Pittendrigh *et al*. (2004) proposed the use of negative cross-resistance to change the standard refuge strategy for IRM, which is a passive approach, to an active

refuge strategy. They used a mathematical model to demonstrate that when the crop cultivar in the refuge expresses a toxin that only harms resistant insects, the refuge can actively inhibit the evolution of resistance (Pittendrigh *et al*. Chapter 6).

Carrière *et al*. (2005) evaluated the role of fitness of *P. gossypiella* on refuge plants to determine whether there could be an effect of fitness cost for resistance on evolution of the insect in transgenic insecticidal cotton. They postulated that, because fitness costs may vary among host plants, choosing refuge cultivars that increase the dominance or magnitude of costs could help to delay resistance. It is known that some cotton cultivars have higher concentrations of toxic phytochemicals than other cultivars. For example, Carrière *et al*. (2004b) found that gossypol increased the magnitude and dominance of some fitness costs for resistant *P. gossypiella*. Carrière *et al*. (2005) discovered that costs of resistance primarily affected survival and were recessive on two cultivars, but the magnitude of the survival cost did not differ between cultivars. They used a model to show that differences in fitness costs between hypothetical cultivars could affect resistance evolution, thus supporting the conclusions of Pittendrigh *et al*. (2004).

Trap crops may be useful in IRM in several ways. One approach is to use a toxic crop as the dead-end trap crop. Alstad and Andow (1995) proposed using early-planted transgenic insecticidal corn to attract *O. nubilalis* away from conventional cornfields. Greater than average oviposition in the transgenic trap crop would significantly decrease the local pest population (Pilcher and Rice, 2003), but enough *O. nubilalis* would persist in conventional cornfields to provide the susceptible population to mate with any resistant moths arising from the trap crop. A second approach is to use an alternative crop as a dead-end trap crop to improve integrated pest management (IPM) (Midega *et al*., 2006; Shelton and Badenes-Perez, 2006).

A third possibility is to use a trap crop, which may not be lethal to the pest, as an attractant to form aggregation sites for mixing and mating of all pest phenotypes. For example, Hellmich *et al*. (1998) investigated methods to attract *O. nubilalis* adults to small-grain crops to determine whether these crops could be managed to influence *O. nubilalis* aggregation behavior near transgenic insecticidal corn. They concluded that timing canopy closure of a small-grain crop to coincide with peak *O. nubilalis* flight could maximize *O. nubilalis* aggregation. However, because of the lack of success stories and the potential for the evolution of behavioral resistance to the trap crops, Frutos *et al*. (1999) questioned the value of trap crops for IRM strategies.

To improve local IRM strategies for transgenic insecticidal cotton in Australia, Sequeira and Playford (2001) examined the suitability of several field crops (*Vigna angularis*, *Sorghum bicolor*, *Zea mays*, *Cajanus cajan* and *Helianthus annuus*) as refuges for transgenic insecticidal cotton. They based their assessment on the relative production of *Helicoverpa* pupae in each crop. Field assessments showed that *Cajanus cajan* has the greatest potential as a refuge for transgenic cotton. They concluded that postharvest cultivation in cotton fields is largely ineffective for resistance management under Queensland conditions. Sequeira and Playford (2001) proposed an IRM strategy that includes refuge-crop options and the use of late-season trap crops of *Cajanus cajan* as an alternative to postharvest cotton cultivation.

Natural Enemies

Because pests rarely evolve resistance to their natural enemies (see Chapter 14 for exceptions), the focus in this section is on the influence of natural enemies on the evolution of pest resistance to toxins in pesticides or host plants. With regard to host plant resistance, Gould *et al*. (1991) took the lead in this subject when they published their conceptual and mathematical models. These models arose during a period in ecological and entomological research when the study of tritrophic interactions was very popular. The most commonly studied tritrophic system consisted of a plant, an herbivore, and a natural enemy. Gould *et al*. (1991) realized at the start of their work that hypotheses derived from the deterministic models would be significantly influenced by a variety of interacting ecological, behavioral, and genetic processes acting over a single or multiple generations. Their simplest conclusion was that natural enemies that increase differential fitness between susceptible and resistant phenotypes on host plants will accelerate evolution of resistance; those that decrease the differential will delay resistance.

To evaluate the hypotheses postulated by Gould *et al*. (1991), Gould and his colleagues performed a series of experiments using transgenic insecticidal tobacco (*Nicotiana tobacum*) and potato (*Solanum tuberosum*) (Table 10.1). Johnson and Gould (1992) conducted field experiments to examine interactions of *H. virescens*, its natural enemies, and transgenic insecticidal tobacco plants considered partially resistant to *H. virescens*. They then calibrated a model to study the influence of natural enemies on evolution of resistance to transgenic tobacco. Simulation results indicated that biological control could accelerate evolution to resistant plants. Johnson *et al*. (1997a, b) carried out controlled studies of a parasitoid species and a pathogenic fungus that attack *H. virescens* on tobacco. They concluded that the parasitoid would likely delay the evolution of resistance to transgenic tobacco, while the pathogen would likely promote the evolution of resistance. Arpaia *et al*. (1997) investigated predation of *L. decemlineata* on transgenic insecticidal potato plants in greenhouse and field studies. They included predation rates in a mathematical model to simulate impact of natural enemies on the evolution of resistance by *L. decemlineata* to transgenic potato. Simulations also included refuges of conventional potato plants. Results showed that predation could decrease the rate of evolution. Mallampalli *et al*. (2005) performed field studies to calibrate a simulation model of *L. decemlineata* on transgenic insecticidal potatoes to determine the influence of predation on IRM. They discovered that different prey species for a generalist predator that also eats *L. decemlineata* have different effects on evolution of resistance to transgenic potato: one alternate prey species delays resistance while the other accelerates the evolution of resistance.

Gassmann *et al*. (2006) demonstrated the effectiveness of entomopathogenic nematodes for reducing the relative fitness of resistant *P. gossypiella* on cotton. The nematodes attack the larvae and reduced the fitness of resistant moths more than susceptible moths. Fitness was the same without nematodes. Gassmann *et al*. (2006) concluded that nematodes could delay resistance by *P. gossypiella* to transgenic insecticidal cotton.

In a good demonstration of modeling based on alternative representations of nature, Wilhoit (1991) used two hypothetical models to demonstrate how seed

Table 10.1 Effects of biological control on specific IRM systems for crops or toxins

Pest	Crop/toxin	Natural enemies	Effect on evolution to crop/toxin*	Reference
Heliothis virescens	Tobacco	Predators, parasitoids	Accelerate	Johnson and Gould (1992)
H. virescens eggs	Tobacco/soybean	Predators, parasitoids	No effect	Gould *et al*. (1991)
H. virescens larvae	Tobacco/soybean	Predators, parasitoids	Accelerate	Gould *et al*. (1991)
H. virescens pupae, adults	Tobacco/soybean	Predators, parasitoids	Delay/acc.	Gould *et al*. (1991)
H. virescens	Tobacco	Parasitoid	Delay	Johnson *et al*. (1997a)
H. virescens	Tobacco	Pathogen	Accelerate	Johnson *et al*. (1997a, b)
Epilachna varivestis	Bean	Predators	No effect/acc.	Gould *et al*. (1991)
Aphids	Abstract crop	Predator	Delay	Wilhoit (1991)
Leptinotarsa decemlineata	Potato	Predator	Delay	Arpaia *et al*. (1997)
L. decemlineata	Potato	Predator	Delay/acc.	Mallampalli *et al*. (2005)
Plutella xylostella	*Bacillus thuringiensis*	Parasitoid	Delay	Chilcutt and Tabashnik (1999)
P. xylostella	*B. thuringiensis*	Virus	Delay	Raymond *et al*. (2007)
Pectinophora gossypiella	Cotton	Nematode	Delay	Gassmann *et al*. (2006)

*The natural enemies can delay, accelerate, or have no effect on the evolution of resistance by the pest to the toxin or toxic crop.
Note: acc.: accelerate.

mixtures of toxic and non-toxic plants in combination with biological control could delay or prevent the evolution of resistance by aphids. Wilhoit (1991) created a simple deterministic model and a complex stochastic model. The deterministic non-linear model simulated competition between two asexual phenotypes in a field of resistant and susceptible plants. It included mating, sexual reproduction and inheritance in only one generation at the end of the season. The stochastic simulation model included many non-linear equations for plant growth, aphid behavior and predation by a natural enemy. The model simulated plant-to-plant movement by both predators and aphids. In both models, the rate of immigration into the field from overwintering sites could differ between the two aphid phenotypes.

Wilhoit (1991) determined that the seed mixture reduced the probability of the resistant (superior) phenotype dominating the aphid population. He also discovered that the resistant aphid could be excluded by the susceptible phenotype because of delayed arrival time by the former into the field. Wilhoit stated that this late-arrival disadvantage is likely to happen when aphids reach the field by random immigration and when resistant aphids are initially less numerous. The effect also depends on mortality due to predation increasing as density increases. In both models, the end-of-season sexual activity and genetics had little effect on the results compared to the competition during the season between asexual aphids.

Heimpel *et al*. (2005) extended the abstract work of Gould *et al*. (1991) and modeled the influence of egg mortality on the high-dose/refuge strategy for IRM. The high dose in the transgenic insecticidal crop is expected to kill all susceptible homozygotes and heterozygotes. A refuge of non-toxic plants provides a source of susceptible individuals to mate with any resistant homozygotes arising from the transgenic field (Chapter 9). Heimpel *et al*. (2005) modeled various levels and forms of pest egg mortality: density independence, positive density dependence, and inverse density dependence. Resistance is modeled as a single locus with a fully recessive allele that confers complete resistance with no fitness cost. Heimpel *et al*. (2005) found that both the magnitude and form of egg mortality can influence the rate of resistance evolution. The importance of egg mortality depends on other ecological processes in the pest population.

Chilcutt and Tabashnik (1999) simulated a model of the interactions of a microbial insecticide containing *Bacillus thuringiensis* and a parasitoid in the control of *P. xylostella*. They also modeled the population genetics of *P. xylostella* and its evolution of resistance to *B. thuringiensis*. Chilcutt and Tabashnik (1999) concluded that the use of parasitoids could slow the evolution of resistance to the microbial insecticide by decreasing the number of generations in which insecticide treatment is required. Raymond *et al*. (2007) studied the same pest and microbial insecticide but evaluated the influence of a nucleopolyhedrovirus instead of a parasitoid as the parasite. They found that the virus increased the fitness costs for *P. xylostella*. Raymond *et al*. (2007) then used a model to investigate how the virus can be used to delay the evolution of resistance to *B. thuringiensis*. One option is to apply the virus only to refuges not sprayed with *B. thuringiensis*. They did not model simultaneous evolution of resistance to both pathogens.

Conclusions

Much of the research summarized above indicates that insect behavior in relation to the environment is critical for understanding and implementing IRM strategies. Clearly, landscapes affect pest and natural enemy behavior. Behaviors of nymphs and larvae as well as adults must be studied in the heterogeneous environments of real systems. Even though most examples presented in this chapter describe crop ecosystems, the same conclusions can be drawn for livestock and public-health systems (Chapter 7). Behavior must be measured at small scales, such as animal-to-animal or plant-to-plant, and at large scales encompassing many fields, lakes, or human communities.

The term source-sink dynamics has been used to describe the behavioral and demographic dynamics of pest populations in transgenic insecticidal crops (sinks) and refuges (sources) (Caprio, 2001; Carrière *et al*., 2004a). It is true that the refuge tends to produce more susceptible arthropods than the transgenic crop field, but the sink designation should not prevent us from realizing that even the transgenic crop field may be a source for some types of pests under some conditions. Certainly, the transgenic crop can be a source for resistant individuals. Furthermore, natural enemies in the refuge may reduce its effectiveness as a source.

Given that the environment also determines how we implement IPM, there is a connection between IRM and IPM that is more than just coordination of goals and activities (Chapter 1). IPM and IRM will affect each other indirectly through changes in the environment. The two main approaches to IPM are design and control. Design selects the components of the environment and the spatial and temporal patterns that will be used throughout the time horizon. Control selects the amount of inputs to the environment during each period of decision making. Monitoring often affects control decisions. Both design and control influence IRM and make strategies dynamic and complex as does the natural variability of the environment over time and space.

Biological control is useful for IPM and may significantly affect IRM as Table 10.1 indicates. Interactions of natural enemies with other control tactics imposing selection pressure on pests can complicate IRM strategies. As stakeholders attempt to improve IPM by taking advantage of both biological control and host plant resistance, these interactions will become even more important in the future. The extensive and interesting history of the genetic modification and use of toxin-resistant natural enemies could not be incorporated into this chapter. Good reviews can be found in publications by Hoy (1990, 2003).

Refuge quality and quantity are fundamental issues for IRM. This has become more apparent with the commercialization of transgenic insecticidal crops. As Hurley and Mitchell (Chapter 11) discuss, compliance with refuge requirements is not a simple activity. Even when the amount of refuge is satisfactory, the quality of the refuge as a source of susceptible insects may be inadequate. Refuges planted separately from the transgenic crop depend on the farmer to follow recommendations and requirements. However, when toxic and non-toxic seeds are both included in the same bag before delivery to the farmer, seed mixtures tend to ensure compliance with mandated minimum amounts of refuge and provide

refuge plants similar in quality to transgenic insecticidal plants. Thus, seed mixtures can be a qualitatively different approach to IRM compared to separate block refuges.

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Chapter 11

Insect Resistance Management: Adoption and Compliance

Terrance M. Hurley and Paul D. Mitchell

It is easy to think of insect resistance management as a purely evolutionary process driven solely by insect biology and behavior because effective pest management exploits biological or behavioral adaptations commonly observed in the targeted species. But, it is the act of pest management that drives the evolution of insect resistance and pest management is fundamentally a human activity in agricultural production. Therefore, thinking of insect resistance as purely driven by insect biology and behavior ignores other important aspects of the problem such as human behavior.

The purpose of this chapter is to discuss aspects of human behavior that affect the evolution of insect resistance to management and how a better understanding of this behavior can be used to improve insect resistance management (IRM). While IRM can be thought of in terms of individual farmers, Clark and Carlson (1990) find that individual farmers treat insect resistance as a common property problem, which means they do not have the incentive to manage it appropriately from a societal perspective (see Mitchell and Onstad, Chapter 2). Therefore, this chapter focuses on the problem from a public policy perspective. From this perspective, government regulators like the US Environmental Protection Agency (EPA) or stakeholder groups like the Arizona Cotton Growers Association are interested in formulating and implementing IRM policies in order to promote pest management practices that provide a greater benefit to society or association members. Since pest management decisions are ultimately made by farmers, the regulator or stakeholder group can only influence IRM indirectly. This creates what is referred to as a principal-agent problem (Laffont and Martimort, 2001). The principal (e.g. the EPA or Arizona Cotton Growers Association) would like the agent (e.g. farmers) to use prescribed management strategies that may not be wholly in the interest of the agent. Therefore, the agent's response to the principal's prescription plays an important role in principal's ability to achieve his/her objectives. This principal-agent problem can be further complicated by the fact that farmer decisions are influenced by the decisions of seed, chemical, and other farm input suppliers through which regulators may choose to act.

EPA regulation of transgenic insecticidal crops like transgenic insecticidal corn and cotton offers a useful illustration of the nature of the problem. Transgenic

insecticidal crops are genetically engineered to express insecticidal proteins. The EPA has determined that these insecticidal proteins are safer for human health and the environment, so it would like to promote sustainable use through IRM (US EPA, 1998; Berwald *et al*., 2006). EPA IRM requirements obligate farmers to plant conventional crop varieties (i.e. refuge) along with transgenic insecticidal varieties and also dictate where refuge varieties should be planted in relation to transgenic insecticidal varieties (i.e. refuge configuration). The EPA implements these requirements by dictating the types of contractual arrangements transgenic insecticidal seed providers must enter into with farmers. Farmers who plant the prescribed refuge forgo the production benefits of transgenic insecticidal crops on refuge land, which can be costly. They must also devote costly management and planting time to meeting EPA configuration requirements. These additional production costs give farmers an incentive to ignore EPA IRM requirements. If some farmers do not comply with EPA requirements, there may not be enough refuge planted in a suitable configuration to meet IRM objectives for all or part of the pest population.

This example illustrates how one aspect of human behavior (i.e. regulatory compliance behavior) can influence the success of IRM. Another aspect of human behavior that this chapter will explore is technology adoption behavior by farmers. The evolution of resistance to pest management depends crucially on the extent to which an insect population is exposed to a particular management strategy. The extent to which an insect population is exposed to management depends on how many farmers choose to adopt the strategy and how intensively they use it.

The remainder of the chapter outlines a basic conceptual model for framing the effects of farmer adoption and compliance behavior on IRM. We then discuss factors that have been identified as influencing adoption and compliance behavior. Simulations of the basic model are used to illustrate how adoption and compliance behavior constrain IRM policies, while alternative approaches used to characterize adoption and compliance behavior are discussed and opportunities for future research are proposed. The conclusions reiterate key implications.

Conceptual Framework

A basic model of the evolution of resistance is useful for framing the discussion of how farmer behavior affects IRM. Consider a simplified agricultural production region with a single pest. A proportion of the pest population in the region is managed. Let $1 \geqslant \phi_t \geqslant 0$ be the proportion of managed pests in period t where the period reflects discrete pest generations. Following standard Hardy–Weinberg assumptions, suppose a randomly mating pest population with non-overlapping generations and resistance conferred by a single, non-sex linked gene (Hartl, 1988). There are two types of alleles: resistant and susceptible. The proportion of resistant alleles in period t is $1 \geqslant r_t \geqslant 0$. The Hardy–Weinberg model implies the proportion of each genotype can be represented by $[r_t^2,\ 2r_t(1-r_t),\ (1-r_t)^2]$ with elements corresponding to the proportion of resistant homozygous, heterozygous, and susceptible homozygous pests.

The Hardy–Weinberg model assumes no selection pressure–survival rates are the same for all genotypes. Pest management selects for resistant pests by eliminating susceptible pests. Let $[1,\ 1-hs,\ 1-s]$ be the genotypic survival rates for

managed pests relative to unmanaged pests with elements corresponding to resistant homozygous, heterozygous, and susceptible homozygous pests, where $1 \geqslant h \geqslant 0$ is a dominance coefficient for resistance to management and $1 \geqslant s \geqslant 0$ is a selection coefficient. For $h=0$, the survival rates of resistant homozygous and heterozygous pests are the same, which occurs when resistance is a completely dominant trait. For $h=1$, the survival rates of susceptible homozygous and heterozygous pests are the same, which occurs when resistance is a completely recessive trait. Values between 0 and 1 represent varying degrees of incomplete dominance with values closer to zero representing a higher degree of dominance. Given the proportion of the pest population managed in period t, the genotypic survival rates are $[1, 1-\phi_t hs, 1-\phi_t s]$.

Since each surviving pest contributes two alleles, resistant homozygous pests contribute two resistant alleles, and heterozygous pests contribute one resistant allele, the proportion of resistant alleles in the subsequent period is

$$r_{t+1} = \frac{r_t^2 + (1-\phi_t hs) r_t (1-r_t)}{r_t^2 + 2(1-\phi_t hs) r_t (1-r_t) + (1-\phi_t s)(1-r_t)^2} \tag{11.1}$$

Equation (11.1) with parameters h, and s, and the initial condition r_0 describes a dynamic biological system where the evolution of resistance, r_t, is controlled by the proportion of managed pests, ϕ_t for all t. While this chapter focuses on managing resistance by controlling the proportion of the pest population exposed to management (i.e. the notion of refuge), it is also possible to think of managing resistance by influencing selection (s) and dominance (h). For example, a regulator may restrict pesticide application rates to influence s or decline to register pesticides where resistance exhibits a relatively high degree of dominance to influence h.

From a naive perspective, insect resistance in this system can be effectively managed by choosing ϕ_t for all t of interest to meet desired objectives. From a more practical perspective, the choice of ϕ_t is constrained by human behavior, which is influenced by economic, environmental, sociological, and psychological factors, as well as insect biology and behavior.

Human Behavior

Turning again to the EPA example of the regulation of transgenic insecticidal crops, many of the models developed to help guide IRM policy have assumed $\phi_t = \phi$ for all t where $1-\phi$ represents the refuge requirement set by the EPA. These models often focus on describing how fast resistance evolves as ϕ is varied given alternative assumptions regarding important biological parameters (e.g. h and s). While such assumptions are convenient in terms of studying how biological factors affect insect resistance, they do not withstand empirical scrutiny. In the context of transgenic insecticidal crops, assuming $\phi_t=\phi$ for all t means that the pest management strategy is fully adopted by all farmers and that all farmers fully comply with EPA IRM requirements.

There is substantial evidence to refute the assumption that farmers will fully adopted transgenic insecticidal crops for pest management. For example, Figure 11.1

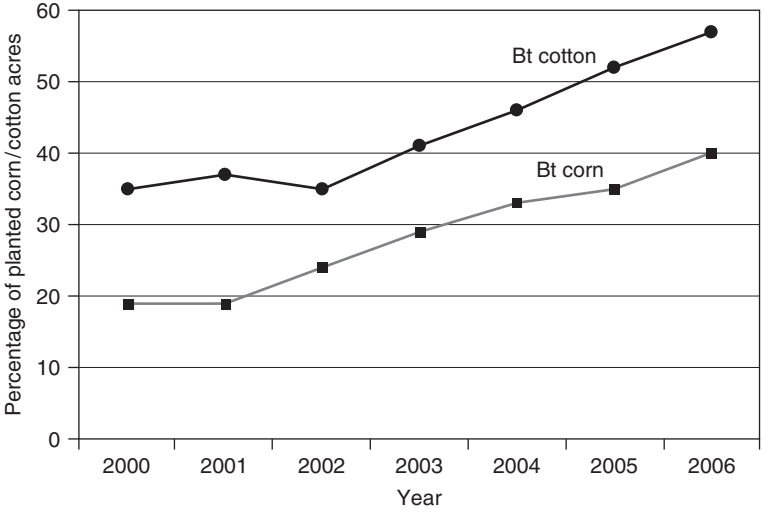

Figure 11.1 Bt corn and cotton adoption trends in the US from 2001 to 2006.

shows the proportion of United States corn and cotton fields planted to transgenic insecticidal corn and cotton from 2000 to 2006 (USDA-NASS, 2001–2006). What is more important to recognize from this figure is that even after a decade of availability, farmers still have not fully adopted these crops, though adoption has trended upward. Minnesota and Wisconsin farm survey data collected in 2003 further reveals that farmers who planted transgenic insecticidal corn only did so on about half their corn fields on average (Merrill *et al*., 2005). Therefore, full adoption fails to characterize both individual and aggregate farmer behavior.

There is also substantial evidence to refute the assumption that farmers who adopt transgenic insecticidal crops will fully comply with EPA IRM regulations. Since 2001, estimates of farmer compliance with EPA IRM requirements have ranged from 72% to 96% (Jaffe 2003a, b; ABSTC, 2005; Merrill *et al*., 2005).

The adoption pattern witnessed with the release of transgenic insecticidal crops is not idiosyncratic. Neither is the compliance pattern witnessed with the EPA IRM requirements. There is a substantial literature that documents and explains systematic trends in the adoption of new production technologies including new pest management strategies. Also, there is a substantial literature that documents and explains why farmers who choose to adopt a particular production practice may not fully utilize it. Finally, there is a substantial literature that documents and explains regulatory compliance. It is instructive to briefly review this literature to better understand the likely implications of its observations and explanations for IRM.

Adoption Behavior

There are two important facets of adoption behavior, referred to as extensive and intensive adoption (Feder *et al*., 1985). Extensive adoption refers to a farmer's decision to initially adopt a particular production practice. Intensive adoption

refers to the degree to which a farmer utilizes a particular production practice once it has been adopted. To initially adopt a new production practice, a farmer must take time to learn about the new practice and how to implement it on his farm. He may also have to invest in new equipment. This time and investment are costly and must be borne regardless of the degree to which a farmer utilizes the new practice. Once a farmer has borne these costs, there are additional costs that depend on the degree to which the farmer utilizes the practice. For example, the cost of managing pests using a chemical pesticide increases as the amount of treated area increases. Costs that do not vary with the intensity of utilization are referred as fixed costs, while costs that vary with the intensity of utilization are referred to as variable costs. Only variable costs are important in terms of intensive adoption. For extensive adoption, fixed and variable costs are important.

Adoption of new production practices exhibits a predictable sigmoidal time trend (e.g. Griliches, 1957). The rate of adoption is initially slow, but increasing, eventually reaches a peak, and begins to decline to ultimately become zero or even negative when new and superior practices are introduced. A variety of economic and sociological theories explains this trend and identifies influential factors.

Sociological explanations of this trend describe adoption as a sequential process (Rogers, 1983). First, farmers become aware of a new technology. Awareness leads them to seek information. The decision to adopt the new technology then follows from an evaluation of this information. Five important characteristics are identified as playing a crucial role in the adoption decision (i) the perception that the new technology is better due to either economic or social factors, (ii) the compatibility of the technology with tradition and past experience, (iii) the complexity of the technology, (iv) the feasibility of experimenting with the technology, and (v) the visibility of the results of the technology. Ultimately, sigmoidal adoption patterns follow from individual differences among farmers in terms of these characteristics, with farmers often cast into categories such as "innovators," "early adopters," "early majority," "late majority," and "laggards" depending on how rapidly they adopt new production practices.

Economic explanations of the trend are generally consistent with sociological explanations, though greater emphasis is placed on factors such as profitability and risk. All else equal, economists argue that farmers will choose the most profitable production practices. Of particular importance in terms of profitability for extensive adoption decisions are the fixed costs of adoption (e.g. Just *et al*., 1980; Feder and O'Marra, 1981). However, when considering a new practice, there is considerable uncertainty regarding profitability due to a lack of experience, which introduces the notion of risk (e.g. Heibert, 1974; Linder *et al*., 1979). Economists think of risk in terms of risk preferences and perceptions. Risk preferences characterize the degree to which an individual does not like variability or does not like to gamble. Individuals who do not like variability are referred to as risk averse. Economists typically assume an individual's degree of risk aversion is stable overtime. However, different individuals exhibit differing degrees of risk aversion, which can drive differences in risky behavior such as the adoption of a new pest management strategy. Risk perceptions characterize the likelihood of unknown outcomes; e.g., the probability that a cotton farmer will experience a severe pest infestation in the coming year. Different individuals may have different risk perceptions because they have

different personal experiences. Furthermore, risk perceptions can evolve overtime as individuals have new experiences. Therefore, individual difference in risky behavior can also be explained by different risk perceptions. Sigmoidal adoption trends follow from inherent differences in profitability, risk preferences, and risk perceptions.

Profitability and risk can also explain intensive adoption patterns (e.g. Feder, 1979; Horowitz and Lichtenberg, 1993, 1994; Hurley *et al*., 2004), though there are important differences in perspective. Decisions for intensive adoption ignore fixed costs, which is not the case for extensive adoption. Intensive adoption decisions offer a mechanism for managing risk through diversification, while extensive decisions are seen as characterized by additional risk due to a lack of experience with and uncertainty about the profitability of the new practice.

Several additional specific factors are commonly identified as important determinants of farmer adoption behavior: credit constraints, farm structure or size, human capital, labor supply, and physical environment (Feder *et al*., 1985). Credit constraints limit a farmer's ability to invest in the skills and equipment needed to adopt new production practices especially when there are high fixed costs (e.g. El-Osta, H., and Morehart, M. 1999). In terms of farm size or structure, when the fixed costs of adoption are large, profitability will tend to be higher for larger farms because they can spread these fixed costs over higher levels of production (Just *et al*., 1980; Feder and O'Marra, 1981). Human capital refers to the skills and experience a farmer has acquired, often measured by a farmer's years of formal education and farming. Higher levels of human capital are often associated with increased profitability. Additionally, farmers with more human capital tend to be better at collecting and critically evaluating new information, which means they are able to identify and adopt profitable new production practices more rapidly (e.g. Schultz, 1964, 1981). Labor supply refers to a farmer's own labor, as well as unpaid family labor and hired labor. For labor intensive production practices like integrated pest management (IPM), a lack of adequate labor can deter adoption (McNamara *et al*., 1991). Alternatively, labor saving production practices like planting herbicide tolerant crops are more likely to be adopted when labor resources are scarce. Heterogeneity in the physical environment influences profitability and ultimately the adoption of new production practices (e.g. Green *et al*., 1996; Thrikawala *et al*., 1999). For example, farmers with inherently more productive soils often find pest management is more profitable because the increased yield potential also results in increased loss potential from pest damage.

Recent research on factors affecting the adoption of new pest management practices focuses on IPM and transgenic herbicide tolerant and insecticidal crops. In terms of IPM, Fernandez-Cornejo *et al*. (1994) found that for vegetable growers, adopters tend to be less risk averse and use more managerial time. Farm size is positively related to adoption, as is the availability of unpaid farm labor. In terms of transgenic herbicide tolerant and insecticidal crops, Carpenter and Gianessi (1999) found that the adoption of herbicide tolerant soybean depends more on simplicity, flexibility, and the fit with existing production practices than on profitability. Alexander *et al*. (2002) found that more risk averse farmers were less likely to adopt transgenic insecticidal corn, while risk aversion was not related to the likelihood of adoption of herbicide tolerant soybean. Hubbell *et al*. (2001) found that adoption of transgenic insecticidal cotton was negatively

influenced by the price of cotton seed and a farmer's share of income derived from cotton production, while more education, total cotton area, and experience with insect resistance positively influenced adoption. Fernandez-Cornejo and McBride (2002) found that farmers with larger farms, production and marketing contracts, more education, and more severe pest problems were more likely to adopt transgenic insecticidal corn active against stalk-boring lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*). Payne *et al*. (2003) found that, for transgenic insecticidal corn active against pests such as the western and northern corn rootworm (*Diabrotica virgifera virgifera* and *Diabrotica barberi*), the likelihood of adoption increased with a farmer's age and farm size up to about 49 years of age and 1,175 hectares, but then decreased. Farmers who specialized in corn production, had experienced more severe rootworm (*Diabrotica* sp.) problems, and had used insecticides to control these pest species were also more likely to adopt. Farmers who performed more off-farm work or lived in regions where more corn is exported to foreign countries were less likely to adopt.

Compliance Behavior

Extensive and intensive decisions are as important for compliance behavior as they are for adoption behavior. Extensive compliance refers to the choice of whether or not to comply with a regulation, while intensive compliance refers to the degree of compliance with a regulation. For example, the EPA dictates refuge size and configuration requirements for transgenic insecticidal crops. Merrill *et al*. (2005) found that while some Minnesota and Wisconsin corn farmers chose to plant sufficient refuge in 2003, they did not always meet configuration requirements. Alternatively, some farmers did not plant enough refuge, but met configuration requirements with what they did plant. As with adoption, intensive compliance decisions depend on variable costs, while extensive decisions depend on variable and fixed costs. Economists, sociologists, and psychologists have all sought explanations for observed patterns in regulatory compliance.

Dominant psychological perspectives on compliance behavior include cognitive and social learning theories. Cognitive theory emphasizes the internal characteristics of an individual that influence compliance behavior, such as individual morality and moral development (e.g. Kohlberg, 1969, 1984; Levine and Tapp, 1977; Tapp and Kohlberg, 1977). Individuals with a higher degree of morality or moral development are more likely to comply with regulations. Social learning theory places less emphasis on individuals and more emphasis on the social interactions that shape behavior through forces like peer suasion (e.g. Aronfreed, 1968, 1969; Bandura, 1969; Mischel and Mischel, 1976; Akers *et al*., 1979; Akers, 1985). Individuals are more likely to comply with a regulation when they perceive that others are also complying with the regulation.

Dominant sociological perspectives include normative and instrumental theories (Tyler, 1990). Normative theory explains compliance behavior in terms of social justice and morality such that an individual's perceptions of the appropriateness and fairness of a regulation and the legitimacy of the regulator are key factors. Individuals are more likely to comply with a regulation that they perceive is appropriate and fair. They are also more likely to comply if they accept the regulator's authority. Instrumental

theory stresses individual self-interest in terms of the rewards an individual receives from ignoring a regulation and the likelihood and severity of sanctions for non-compliant behavior. Individuals are more likely to comply when the cost of compliance is relatively low or the likelihood and severity of sanctions is high.

Like instrumental sociologists, economists have tended to emphasize individual self-interest to explain compliance behavior (Becker, 1968). Specifically, economists have focused on the cost of complying with a regulation and the likelihood and severity of sanctions for non-compliant behavior. Risk is also an important facet of compliance behavior because whether or not an individual faces sanctions for non-compliant behavior is usually uncertain.

Empirically, two clear results emerge from the literature. First, compliance behavior is influenced by economic factors like compliance costs, and the likelihood and severity of sanctions for non-compliant behavior. Second, these economic factors are not sufficient to fully explain compliance behavior. Generally, regulatory compliance is higher than what economic and instrumental sociological theories predict. Therefore, recent efforts to improve theories of compliance behavior integrate models developed by economists, sociologists, and psychologists. For example, Sutinen and Kuperan (1999) propose a theory of compliance behavior that includes compliance costs; the likelihood and severity of sanctions for non-compliant behavior; individual morality; moral suasion; and legitimacy in terms of the fairness and efficiency of the regulatory process, and of the fairness and effectiveness of regulatory outcomes.

Recent studies of compliance with EPA IRM regulations for transgenic insecticidal crops include Jaffe (2003a, b); Stratus Agri-Marketing (2003); ABSTC (2005); Merrill *et al*. (2005); Carriere *et al*. (2005). Data for these studies come from a variety of sources including farmer surveys (mail, personal, and telephone) and field surveys. All assess whether farmers plant enough refuge. Several also consider farmer compliance with EPA configuration requirements. The results of these studies indicate that larger farms tend to have higher compliance rates. Refuge size requirements are frequently not binding (i.e. many farmers plant more refuge than required), which can be explained by typical patterns in intensive adoption behavior. Compliance rates have increased overtime with increased IRM awareness. Compliance rates for transgenic insecticidal corn in the Corn Belt of the United States are higher than for transgenic insecticidal corn in the Cotton Belt where the EPA refuge size requirement is higher. Compliance with refuge size requirements is positively correlated with refuge configuration requirements such that farmers who do not meet size requirements are more likely to not meet configuration requirements.

Implications of Human Behavior

The common adoption and compliance patterns identified in the literature ultimately constrain the ability of regulators to implement an IRM policy that increases the societal benefits of pest management. However, it is not immediately clear whether these constraints will mitigate or exacerbate the evolution of insect resistance. To understand why, several rudimentary modifications to the conceptual model described by equation (11.1) are explored. After the implications of

these rudimentary modifications are explored, alternative methods that have been used to provide a richer characterization of adoption and compliance behavior are reviewed and opportunities for future research are proposed.

Two distinct types of IRM policies are explored within the framework of equation (11.1). The first type is referred to as a refuge policy, while the second is referred to as an IPM policy. The refuge policy is analogous to the EPA's strategy for managing insect resistance to transgenic insecticidal crops where farmers are required to leave a proportion of the pest population unmanaged by planting refuge. The IPM policy is analogous to IPM strategies in which control tactics are used only when pest abundance exceeds the economic threshold. The primary distinction between these alternative policies in the context of the equation (11.1) is the temporal pattern of the proportion of managed pests. For the refuge policy, the regulator tries to assure that only a proportion of the pest population is managed every generation. For the IPM policy, the regulator tries to ensure that the pest population is only managed in generations when pests are relatively abundant and likely to cause significant crop damage.

Refuge Policy

Consider the basic model in equation (11.1) where

$$\phi_t = \phi \tag{11.2}$$

for all t such that $1.0 \geqslant 1-\phi \geqslant 0.0$ is the mandated percentage of refuge. This version of the model is referred to as Full Adoption & Compliance because it assumes that farmers fully and immediately adopted a new pest management strategy and fully comply with the mandated refuge requirement.

Alternatively, suppose

$$\phi_t = a(t)\phi \tag{11.2a}$$

where $a(t)$ represents the proportion of extensive and intensive farmer adoption of a new pest management strategy in period t. Assume

$$a(t) = \frac{1}{1 + e^{\alpha_a + \beta_a t}} \tag{11.3}$$

where α_a and β_a are parameters that describe how rapidly farmers adopt and utilize this new strategy overtime. Note that the parameters are subscripted with a to indicate that they are related to adoption. For the transgenic insecticidal corn adoption trend observed in Figure 11.1, ordinary least squares estimates assuming two generations of pest per year are $\alpha_a = 1.37$ and $\beta_a = -0.078$. Figure 11.2 shows the predicted adoption rates for 50 periods given these estimates. These predicted adoption rates follow the typical pattern. The adoption rate initially increases overtime at an increasing rate (up to about 40%). The adoption rate then continues to increase (above 40%), but at a decreasing rate. This version of the model is referred to as Partial Adoption & Full Compliance because it assumes that not all farmers immediately adopt the new pest management strategy, but those that do fully comply with the mandated refuge requirement.

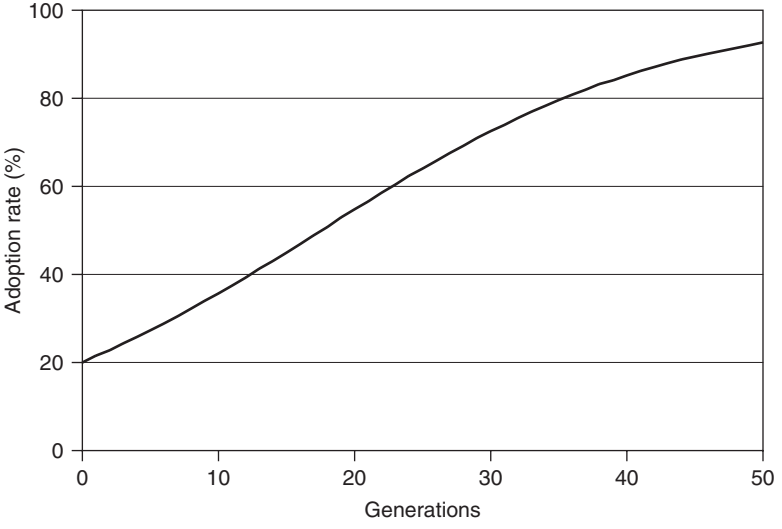

Figure 11.2 Estimated adoption function.

Now, let $c(\phi)$ be the proportion of pests managed by farmers that comply with the mandated refuge requirement such that $c(\phi)\phi$ reflects the proportion of the pest population exposed to management by compliant farmers and $1 - c(\phi)$ reflects the proportion of the population exposed to management by non-compliant farmers. Adding these two proportions yields the total proportion of the population exposed to management:

$$\phi_t = c(\phi)\phi + 1 - c(\phi) \tag{11.2b}$$

For this specification, non-compliant farmers are assumed to manage all their pests (i.e. they plant no refuge), while compliant farmers abide by the mandated refuge requirement.

The proportion of compliant farmers is assumed to be decreasing in ϕ because compliance costs are increasing in ϕ as compliant farmers must forgo the benefits of pest management on more hectares. This assumption is also consistent with results reported by ABSTC (2005) for transgenic insecticidal corn and cotton. Assume

$$c(\phi) = \frac{1}{1 + e^{\alpha_c + \beta_c \phi}} \tag{11.4}$$

where α_c and β_c are parameters that describe how sensitive farmer compliance is to the mandated refuge requirement. Note that the parameters are subscripted with c to indicate that they are related to compliance. In 2001, compliance rates for transgenic insecticidal corn refuge size requirements in the Corn and Cotton Belts of the United States were 87% and 77% (Hurley, 2005). Refuge requirements for these regions were 20% and 50%. Assuming these differences in compliance rates can be explained solely by the difference in refuge requirements implies $\alpha_c = -2.36$ and $\beta_c = 2.31$. Figure 11.3 shows the predicted compliance

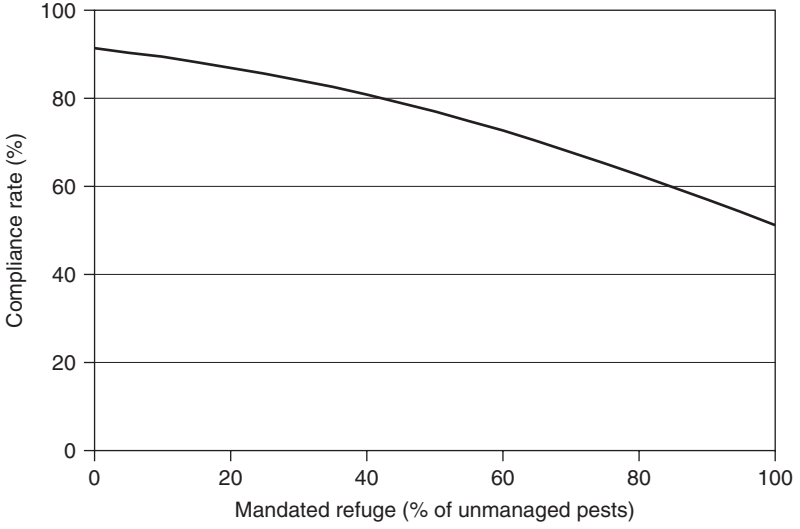

Figure 11.3 Estimated compliance function.

rates as the refuge requirement increases from 0.0 to 1.0 given these estimates. In this figure, the compliance rate falls from about 90% to 50% as the refuge requirement increases from 0% to 100%. This version of the model is referred to as Full Adoption & Partial Compliance because it assumes that all farmers immediately adopt the new pest management strategy, but only a portion of farmers fully comply with the mandated refuge requirement.

Finally, suppose

$$\phi_t = a(t)[c(\phi)\phi + 1 - c(\phi)] \tag{11.2c}$$

where $a(t)$ and $c(\phi)$ are as previously defined. This version of the model is referred to as Partial Adoption & Compliance because it assumes that only a portion of farmers adopt the new pest management strategy overtime and only a portion of farmers adopt and fully comply with the mandated refuge requirement.

Figure 11.4 shows the minimum feasible refuge requirement needed to maintain the proportion of resistant alleles below 0.5 for 50 periods for each version of the model as the selection (s) and dominance (h) coefficients vary between 0 and 1 and initial resistance is $r_0 = 0.001$. Note that variations in initial resistance do not qualitatively change the reported results. In some cases, it is not possible to maintain the proportion of resistant alleles below 0.5 for 50 periods even when the refuge requirement is 100 percent due to non-compliance. Therefore, Figure 11.5 shows the proportion of resistant alleles after 50 periods given the refuge requirement in Figure 11.4. Comparing the results of these four versions of the model provides insight into the potential implications of ignoring farmer behavior when trying to identify a refuge policy that maintains the proportion of resistant alleles below 0.5 for 50 periods.

Figures 11.4 and 11.5 reveal a consistent pattern in the refuge requirement and resistance for all four versions of the model. When selection or degree of dominance is low (e.g. s is close to zero or h is close to 1, resistance is recessive),

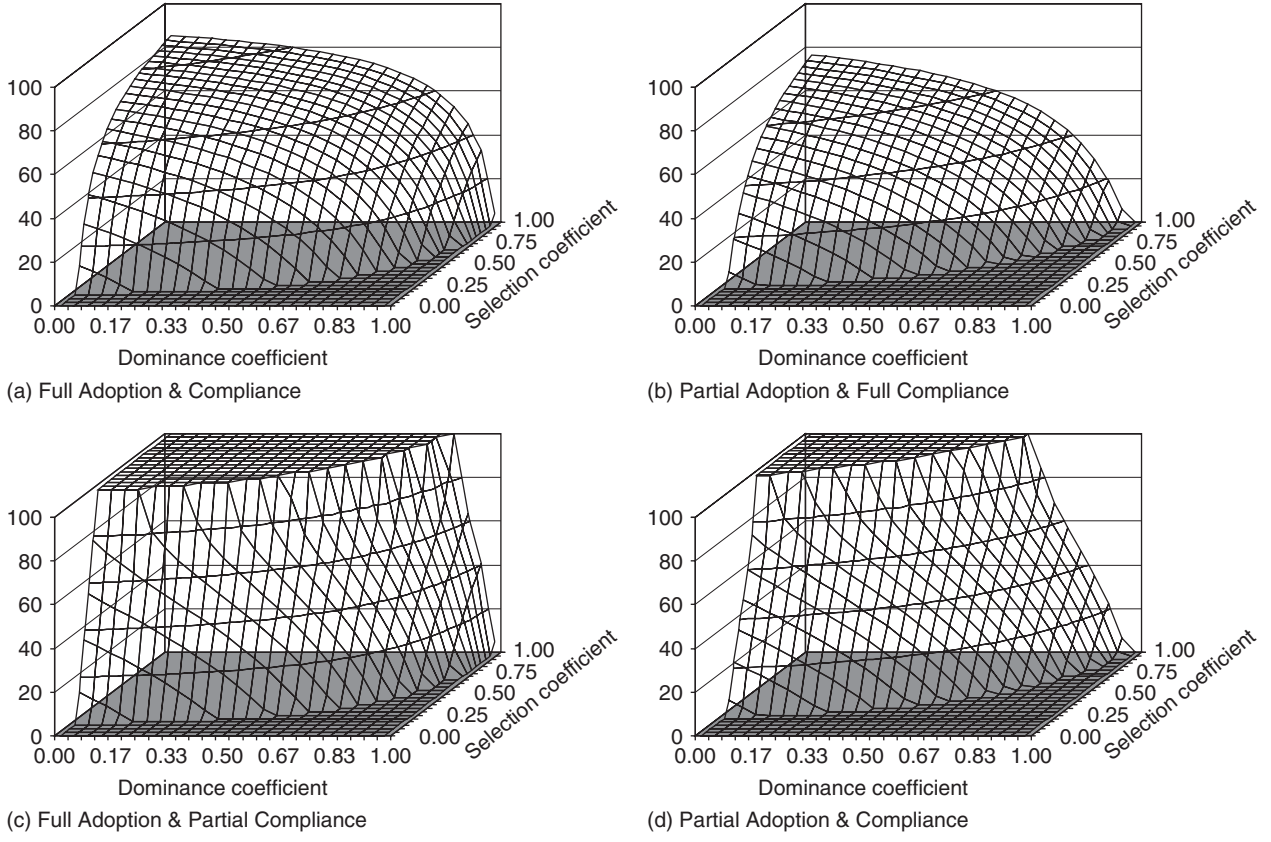

Figure 11.4 Minimum feasible refuge required to maintain the proportion of resistant alleles below 0.5 for 50 periods.

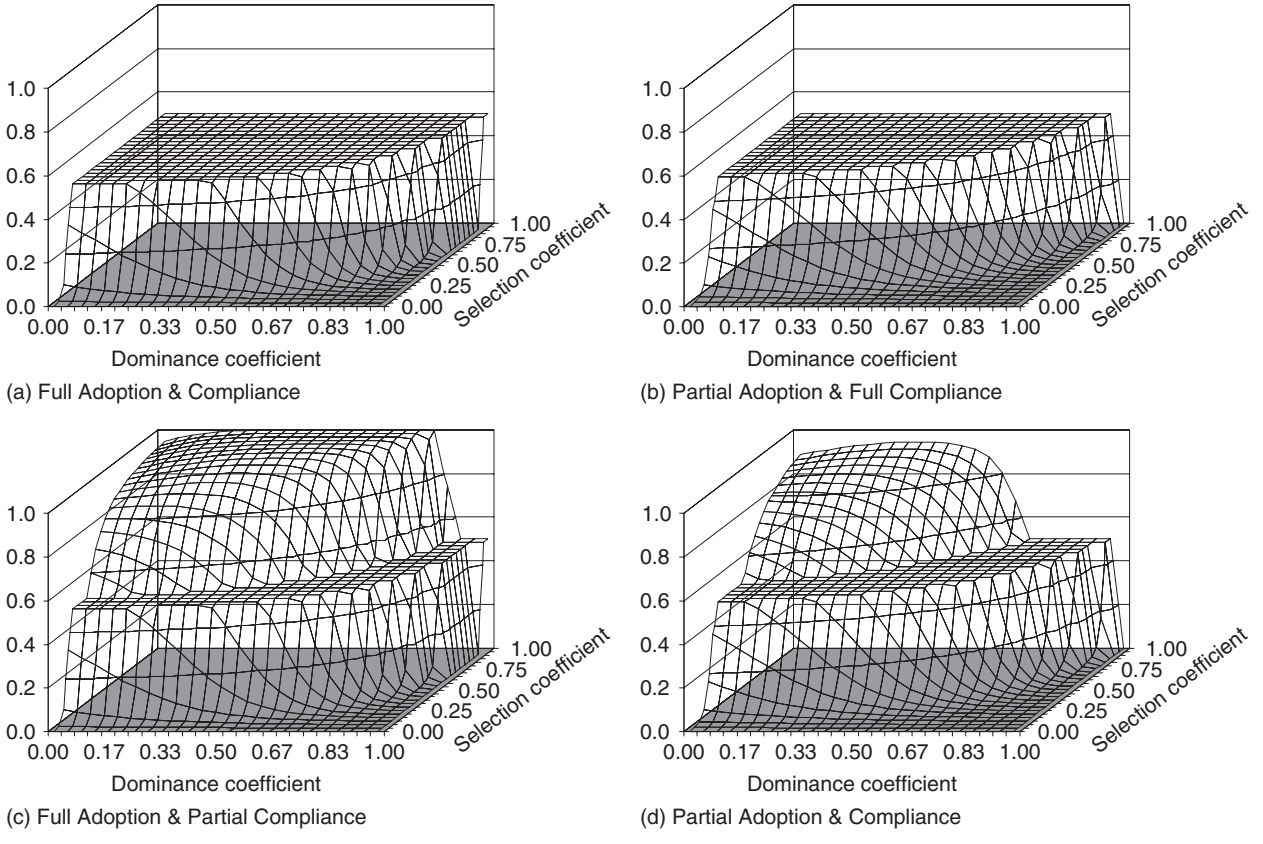

Figure 11.5 Proportion of resistant alleles after 50 periods with the minimum feasible refuge requirement.

resistance is of little concern and there is no need for refuge. Alternatively, as selection and the degree of dominance increase (s approaches 1 and h approaches 0, resistance is dominant), increasing the refuge requirement helps slow the evolution of insect resistance. From a practical standpoint, it is important to recognize that a pest management strategy with a low selection coefficient will not be attractive to farmers because it will not be efficacious.

Comparing alternative versions of the model provides insight into how ignoring the implications of farmer adoption and compliance behavior can bias policy prescriptions. Comparing Figures 11.4 (a) and 11.5 (a) Full Adoption & Compliance to Figures 11.4 (b) and 11.5 (b) Partial Adoption & Full Compliance shows that not accounting for adoption typically results in overestimates of the refuge requirement and resistance. Alternatively, comparing Figures 11.4 (a) and 11.5 (a) Full Adoption & Compliance to Figures 11.4 (c) and 11.5 (c) Full Adoption & Partial Compliance shows that not accounting for non-compliance typically leads to underestimates of the refuge requirement and resistance. Finally, comparing Figures 11.4 (a) and 11.5 (a) Full Adoption & Compliance to Figures 11.4 (d) and 11.5 (d) Partial Adoption & Compliance shows that not accounting for adoption and non-compliance can result in over or underestimates of the refuge requirement and resistance depending on the degree of selection and dominance. It typically leads to overestimates for relatively low selection or dominance, while it typically leads to underestimates for relatively high selection and dominance. For example, when $s = 0.95$ and $h = 0.8$, the minimum feasible percentage of refuge required to maintain resistance below 0.5 for 50 periods is 60% assuming Full Adoption & Compliance, but only 49% assuming Partial Adoption & Compliance. Alternatively, when $s = 1.0$ and $h = 0.75$, the minimum feasible percentage of refuge required to maintain resistance below 0.5 for 50 periods is 66% assuming Full Adoption & Compliance, but 68% assuming Partial Adoption & Compliance.

The results of this analysis are intuitive. Since farmers tend to gradually rather than fully adopt new production practices overtime, assuming full adoption overestimates pest exposure to management and the evolution of resistance. Since not all farmers will comply with mandated refuge requirements, assuming full compliance underestimates pest exposure to management and the evolution of resistance. Given these two opposing effects, the net bias relative to ignoring these effects is generally indeterminate and must be resolved empirically.

IPM Policy

Now consider the basic model outlined above where

$$\phi_t = \begin{cases} 1, & \text{for } n_t > \phi \\ 0, & \text{otherwise} \end{cases} \tag{11.2d}$$

for all t, where $n_t \geq 0$ reflects pest abundance (e.g. pests per plant or the proportion of plants infested) in period t and $\phi \geq 0.0$ is now a management threshold mandated by the regulator. That is, farmers are allowed to manage the pest only if pest abundance exceeds the mandated threshold. For this version of Full Adoption & Compliance, the regulator manages insect resistance by restricting pest management

to periods when pest abundance is relatively high by setting the threshold ϕ. For the Partial Adoption & Full Compliance version of the IPM policy model, assume

$$\phi_t = \begin{cases} a(t), & \text{for } n_t > \phi \\ 0, & \text{otherwise} \end{cases} \tag{11.2e}$$

where $a(t)$ is the proportion of extensive and intensive farmer adoption of a new pest management strategy in period t. For the Full Adoption & Partial Compliance version, assume

$$\phi_t = \begin{cases} 1, & \text{for } n_t > \phi \\ 1 - c(\phi), & \text{otherwise} \end{cases} \tag{11.2f}$$

where $c(\phi)$ is the proportion of pests managed by farmers that comply with the mandated threshold requirement. Finally, for the Partial Adoption & Compliance version, assume

$$\phi_t = \begin{cases} a(t), & \text{for } n_t > \phi \\ a(t)(1 - c(\phi)), & \text{otherwise} \end{cases} \tag{11.2g}$$

where $a(t)$ and $c(\phi)$ are as previously defined. For the partial adoption versions of the model, the adoption rate determines the proportion of pests managed by farmers when pest abundance exceeds the threshold requirement. The remaining proportion of the pest population is unmanaged because some farmers choose not to adopt the new management strategy. For the partial compliance versions of the model, the compliance rate determines the proportion of pests managed by farmers even when pest abundance does not exceed the threshold requirement. There are also a proportion of unmanaged pests in some generations attributable to the portion of farmers who comply with the threshold requirement.

To operationalize the IPM policy versions of the model, the evolution of pest abundance must be described in addition to the population genetics. Consider the common growth function

$$n_{t+1} = r\left(1 - \frac{n_t}{K}\right)n_t + n_t \tag{11.5}$$

where r is the population's intrinsic rate of growth and K is the population's carrying capacity. Equation (11.5) describes the population dynamics assuming no pest management. To account for the effect of pest management on this dynamic, n_t must be adjusted to reflect the proportion of pests remaining in the population due to management,

$$\omega_t = r_t^2 + 2r_t(1 - r_t)(1 - \phi_t hs) + (1 - r_t)^2(1 - \phi_t s) \tag{11.6}$$

such that equation (11.5) can be written as

$$n_{t+1} = r\left(1 - \frac{\omega_t n_t}{K}\right)\omega_t n_t + \omega_t n_t \tag{11.7}$$

Figure 11.6 shows the minimum feasible threshold requirement ($K \geqslant \phi \geqslant 0.0$) needed to maintain the proportion of resistant alleles below 0.5 for 50 periods for each version of the model as the selection (s) and dominance (h) coefficients vary between 0 and 1, initial resistance is $r_0 = 0.001$, intrinsic rate of growth is $r = 1$, carrying capacity is $K = 1$, and initial pest pressure equals the carrying capacity (qualitatively similar results are obtained for variations in the parameters). While the general pattern of results and comparisons of different versions of the models is similar to Figure 11.4, there is a notable difference. With the IPM policy, comparing Figure 11.6 (a) Full Adoption & Compliance to Figure 11.6 (c) Full Adoption & Partial Compliance reveals that not accounting for compliance behavior can result in over or underestimating the minimum threshold requirement. Assuming full compliance tends to lead to overestimates when selection is relatively high, dominance is relatively low, and the selection and dominance coefficients are nearly equal. The implication of this result is that, in some cases, non-compliance can actually slow the evolution of resistance such that regulatory policy does not need to be as restrictive, which is contrary to the results obtained for the effect of non-compliance on a refuge policy.

Figure 11.7 helps explain why non-compliance can slow the evolution of resistance with an IPM-based IRM policy. The top of the figure shows the evolution of pest abundance overtime for the Full Adoption & Compliance and Full Adoption & Partial Compliance versions of the model when the threshold requirement is $\phi = 0.072$, selection coefficient is $s = 0.875$, dominance coefficient is $h = 0.958$, initial resistance is $r_0 = 0.001$, intrinsic rate of growth is $r = 1$, carrying capacity is $K = 1$ and initial pest pressure equals the carrying capacity. The bottom of the figure shows the evolution of the proportion of resistant alleles.

First note that, in Figure 11.7, the proportion of resistant alleles after 50 periods is lower for the Full Adoption & Partial Compliance version of the model. Therefore, ignoring compliance behavior could result in mandating an IPM threshold that is more restrictive than necessary to maintain resistance below 0.5 for 50 periods. This result emerges because when pest abundance is relatively low, no pest management occurs under Full Adoption & Compliance, but a modicum of management occurs under Full Adoption & Partial Compliance due to non-compliant farmers. This modicum of management has the effect of modestly increasing resistance. But, it also has the effect of slowing population growth, which reduces pest abundance and the likelihood of all farmers employing pest management in future periods. By delaying pest management by all farmers in the future (e.g. in generation 9), the evolution of resistance is slowed. In Figure 11.7, the effect of increased resistance due to non-compliance is outweighed by the effect of decreased resistance due to delayed pest management by all farmers over 50 periods.

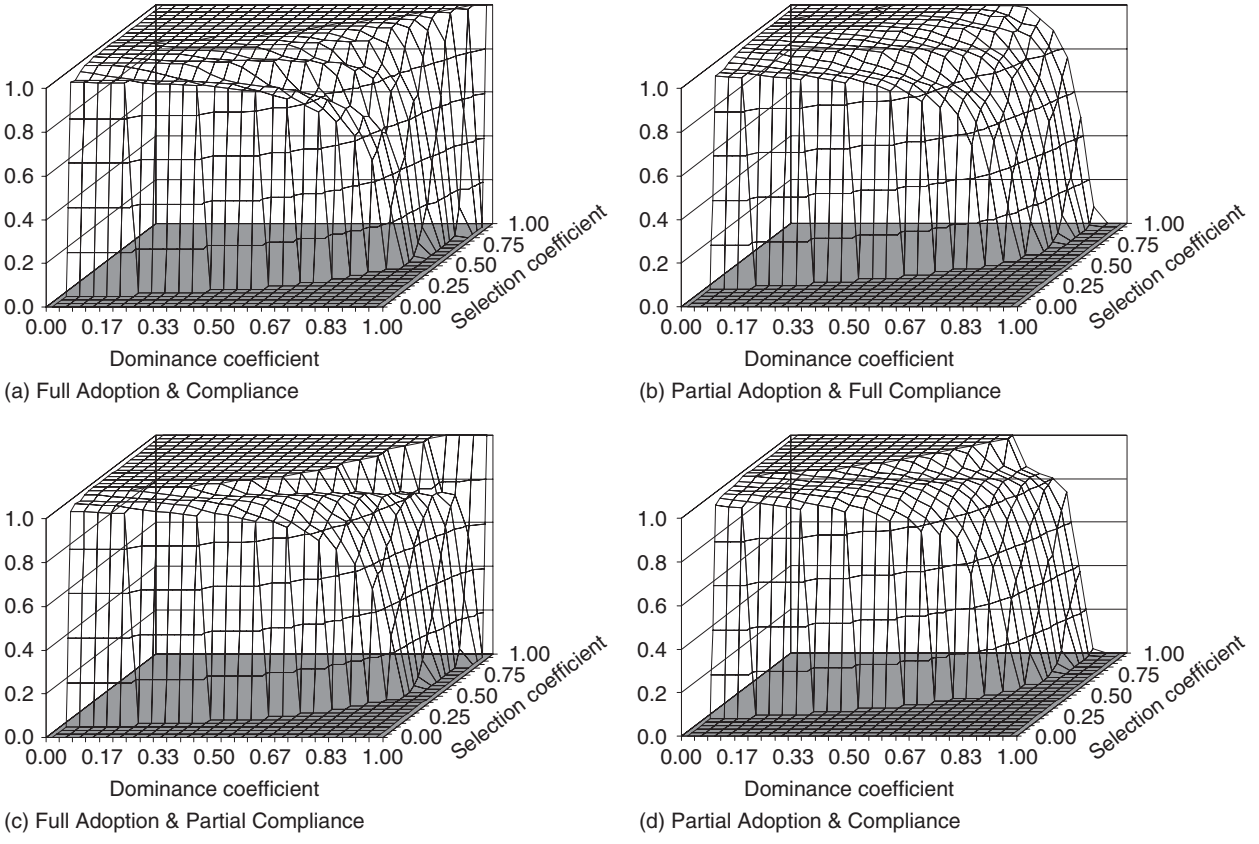

Figure 11.6 Minimum feasible treatment threshold required to maintain the proportion of resistant alleles below 0.5 for 50 periods.

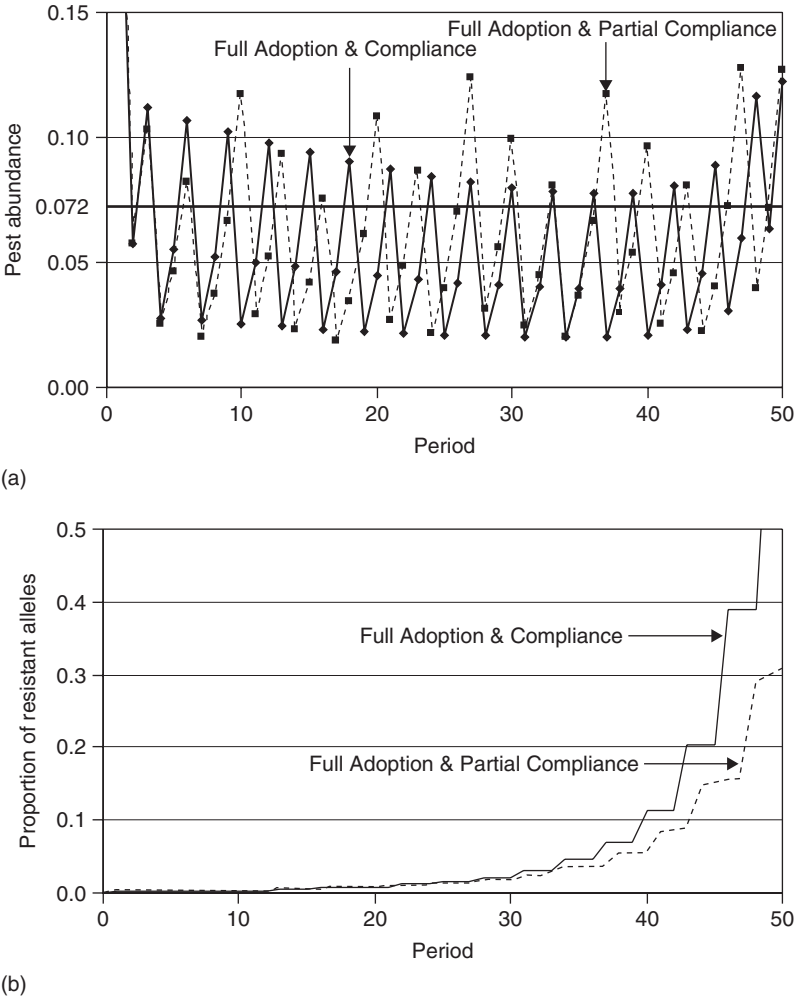

Figure 11.7 Evolution of pest abundance and proportion of resistant alleles over 50 periods with a treatment threshold of 0.072.

Characterizations of Adoption and Compliance: A Review

Exploring the effectiveness of refuge and IPM-based IRM policies in the context of this basic model highlights the importance of having a better understanding of human behavior. For the rudimentary adoption and compliance models, farmer adoption and compliance behavior can either mitigate or exacerbate the evolution of resistance depending on important biological factors. Therefore, it is not possible to make general recommendations regarding how regulatory policy should be adjusted to account for this behavior. Instead, reasonable policy recommendations require a better understanding of the specific factors affecting farmer adoption and compliance decisions for a given pest, management strategy, and regulatory policy.

There are two general approaches that have been employed to provide a richer description of adoption and compliance behavior. One approach attempts to describe farmer adoption or compliance behavior by constructing models based on fundamental economic principles (Peck and Ellner, 1997; Hurley *et al*., 2001; Hurley *et al*., 2002; Mitchell *et al*., 2002; Livingston *et al*., 2004; Hurley, 2005; Mitchell and Hurley, 2006). These models can then be incorporated into biological models of the evolution of resistance and operationalized using the best available data. The other approach uses farmer survey data to estimate, rather than construct, farmer adoption functions that are conditional on important economic, sociological, and regulatory factors (Hubbell *et al*., 2001; Hurley *et al*., 2006). These estimated functions can then be incorporated into biological models of the evolution of resistance.

Construction Approaches

The simplest models used to construct farmer adoption behavior have used concepts like economic thresholds (e.g. Peck and Ellner, 1997; Hurley *et al*., 2001, 2002; Crowder *et al*., 2006). In these models, farmers are assumed to use pest management only when pest abundance exceeds some threshold. The threshold in these models is a fixed parameter and does not take into account losses in pest management efficacy due to the evolution of resistance.

More complex constructions of farmer adoption behavior dynamically adjust adoption decisions overtime based on pest abundance and a loss in pest management efficacy due to the evolution of resistance (e.g. Livingston *et al*., 2004). These models define the returns to pest management as the difference in crop revenues minus the cost of management:

$$\pi_t(x_t) = P_y Y(n^S(n_t, r_t, x_t)) - C(x_t) \tag{11.8}$$

where $\pi_t(\cdot)$ is the net return to pest management; $P_y > 0$ is the price received per unit of crop yield; $Y(\cdot) > 0$ is the crop yield; $n^S(\cdot,\cdot,\cdot) \geqslant 0$ is pest abundance after management; $n_t \geqslant 0$ is pest abundance prior to management; $x_t \geqslant 0$ is the intensity of pest management usually related to the proportion of the pest population managed (e.g. percentage of crop land not planted to refuge) or selection coefficient (e.g. rate of insecticide application); and $C(\cdot) \geqslant 0$ is the cost of pest management. Crop yield is modeled as a non-increasing function of pest abundance after management. Pest abundance after management is modeled as a non-decreasing function of pest abundance prior to management and resistance, and a non-increasing function of the intensity of pest management. Management costs are modeled as an increasing function of the intensity of pest management. The models then assume farmers choose a management intensity, x_t, that maximizes the net return, $\pi_t(x_t)$, where the management intensity may be constrained by regulatory policy. For example, if x_t is the proportion of crop land managed for pests and $1-\phi$ is the regulator's refuge requirement, the farmer is constrained to maximizing $\pi_t(x_t)$ by choosing $\phi \geqslant x_t \geqslant 0$.

This more complex strategy improves on the use of static thresholds by making a farmer's adoption decision dependent on the evolution of resistance as well as the pest population dynamics. In terms of the factors that have been identified as influencing adoption behavior, it does not reflect important economic and sociological factors that influence extensive adoption decisions. It also does not

account for the inherent variability of pest abundance and the risk associated with this variability.

Hurley (2005) addresses the issue of variability in pest abundance and the risk it entails by making n_t a random variable that depends on pests surviving management in the previous period. To account for differences in risk attitudes across individual farmers, it assumes

$$a(t) = 1 \Bigg/ \left[1 + \exp\left(\lambda_a + \gamma_a \left(\frac{E\pi_t(x_t) - E\pi_t(0)}{E\pi_t(0)}\right)\right)\right] \tag{11.9}$$

where λ_a and γ_a are parameters that can be used to calibrate the model to observed adoption patterns and $E\pi_t(\cdot)$ reflects the expected net return. With this specification, adoption is possible even when the expected net return to adoption is negative, which could result from risk aversion. The analysis also incorporates compliance behavior by assuming

$$c(\phi) = 1 \Bigg/ \left[1 + \exp\left(\lambda_c + \gamma_c(1 - \phi)\left(\frac{E\pi_t(x_t) - E\pi_t(0)}{E\pi_t(x_t)}\right)\right)\right] \tag{11.10}$$

where λ_c and γ_c are parameters that can be used to calibrate the model to observed compliance patterns and $1-\phi$ is the refuge requirement. Note that with this specification

$$(1 - \phi)\left(\frac{E\pi_t(x_t) - E\pi_t(0)}{E\pi_t(x_t)}\right) \tag{11.11}$$

provides a more descriptive characterization of compliance costs than the rudimentary model used earlier in this section because it more explicitly accounts for the difference in the expected returns from managing pests $E\pi_t(x_t)$, and not managing pests in refuge, $E\pi_t(0)$. The larger this difference, the more costly a hectare of refuge is to a farmer and the less likely the farmer is to comply with the refuge requirement. The model is operationalized by setting $E\pi_t(\cdot)$ to the weighted average of $\pi_t(x_t)$ over previous periods, which implies farmers use past experiences to form expectations about the net return to pest management in the current period.

Mitchell *et al*. (2002) provides an even richer description of farmer adoption and compliance behavior that includes regulatory policy instruments for encouraging compliance: compulsory refuge insurance, voluntary refuge insurance with subsidized premiums, and enforcement with monitoring and sanctions for non-compliant behavior. It also accounts for risk using more traditional economic approaches. For the case of enforcement with monitoring and sanctions for non-compliant behavior, a farmer's net return can be written as

$$\begin{aligned}\pi_t(x_t) = {} & qU(P_y Y(n^S(n_t, r_t, x_t)) - C(x_t) - F(x_t, \phi)) \\ & + (1-q)U(P_y Y(n^S(n_t, r_t, x_t)) - C(x_t))\end{aligned} \tag{11.12}$$

where q is the probability that a farmer is monitored for compliance; $U(\cdot)$ is a concave transformation function referred to by economists as the utility function; and $F(\cdot,\cdot)$ is the monetary value of sanctions imposed on farmers who are monitored for non-compliance. The concavity of $U(\cdot)$ implies farmers do not like variability or are risk averse. Mitchell *et al*. (2002) assumes

$$F(x_t, \phi) = \begin{cases} f, & \text{for } x_t > \phi \\ 0, & \text{otherwise} \end{cases} \tag{11.13}$$

where f is a fine, x_t is the proportion of transgenic crop, and $1-\phi$ is the refuge requirement. This specification implies that farmers must pay a fine if they are monitored and found to have exceeded the refuge requirement. Mitchell and Hurley (2006) extend this model further to simultaneously model both farmer adoption and compliance behavior. The model includes farmer demand for transgenic insecticidal corn seed and the pricing decision of a monopolistic seed company that also uses a fine program to ensure that farmers buying transgenic insecticidal corn plant the required refuge. While these models were used to evaluate the feasibility of implementing policies to ensure regulatory compliance, they were not integrated with a biological model of the evolution of resistance to evaluate the effect of policy levers, like the frequency and severity of sanctions for non-compliant behavior, on the efficacy of IRM.

With net returns defined as in equation (11.12), a farmer's pest management choice, x_t, to maximize net returns, $\pi_t(x_t)$, defines his level of adoption and compliance. This choice depends crucially on the price received for the crop, P_y, the cost of pest management, $C(\cdot)$, and risk attitudes, $U(\cdot)$. It also depends on the regulator's policy choices including the restrictiveness of the regulatory policy, ϕ, the likelihood of sanctions, q, and severity of sanctions, f. Finally, the farmer's adoption and compliance behavior will depend on important biological factors including crop response to pests, $Y(\cdot)$, the frequency and severity of pests, n_t, and the efficacy of pest management, $n^S(\cdot,\cdot,\cdot)$, which in turn depends on the evolution of resistance.

Estimation Approaches

Hubbell *et al*. (2001) and Hurley *et al*. (2006) use farmer survey data to estimate adoption functions. The survey data used by Hubbell *et al*. (2001) asks farmers if they adopted transgenic insecticidal cotton at the current price and if so, how much they planted. If farmers had not adopted transgenic insecticidal cotton at the current price, the survey asked hypothetically if they would have adopted it at a lower price, and if so, how much they would have planted. Econometric methods are used with the survey data to estimate both extensive and intensive adoption functions that depend on the price of transgenic insecticidal cotton seed as well as other important socio-economic factors. Hubbell *et al*. (2001) used the estimated adoption function to evaluate how government subsidies for transgenic insecticidal cotton could be used to increase adoption while reducing the use of other insecticides. The results were not used to evaluate the effect of adoption behavior on IRM policy.

There are a number of important differences between Hurley *et al*. (2006) and Hubbell *et al*. (2001). Hurley *et al*. (2006) focuses on farmer adoption of transgenic insecticidal corn active against western and northern corn rootworm (*D. virgifera virgifera* and *D. barberi*) and stalk-boring lepidopteran species such as European corn borer (*Ostrinia nubilalis*). Also, the survey data it uses were collected before, not after, the commercial release of the product. Therefore, the survey questions were purely hypothetical, which provided an opportunity to focus on the effect of regulatory policy on adoption. Specifically, the survey described a new transgenic insecticidal corn variety that provided rootworm control or rootworm and European corn borer control. It indicated whether the transgenic insecticidal corn crop would be approved for foreign export. It described the refuge requirements the farmer would be obligated to follow if they planted the new variety. The refuge requirements varied across surveys in terms of size, configuration, and whether supplemental refuge insecticide treatments were permitted in years of heavy infestation. Finally, it told farmers what the additional seed costs would be, which also varied across surveys. Farmers were then asked if they would adopt the new variety if it were available. Econometric methods were used to estimate an adoption function that depended on the price of transgenic insecticidal corn, the spectrum of control, export market approval, and regulatory policy.

Hurley *et al*. (2006) found that adoption increases if the corn hybrid controlled European corn borer as well as rootworm species and was approved for export, while it decreased as the size of refuge increased, if supplemental refuge insecticide treatments were not permitted. The adoption function was used to estimate compliance costs for alternative regulatory policies. The results were not used to evaluate the effect of adoption behavior on IRM policy.

Each of these approaches to characterizing farmer adoption and compliance behavior has had its advantages and disadvantages. Applications of the construction approach have done a good job of integrating important economic aspects of the problem like profitability with important biological aspects of the problem like pest population dynamics and genetics, which has resulted in rich and dynamic descriptions of farmer adoption and compliance behavior. They have not done as well integrating less tangible sociological and psychological factors. Applications of the estimation approach have done a good job integrating important economic and sociological factors, but have not done as well in terms of integrating biological factors. Furthermore, estimation applications have yet to take advantage of the benefits of longitudinal surveys, which would offer a richer description of temporal variability in farmer adoption and compliance behavior.

Opportunities for Future Research

The development and commercial release of the first transgenic insecticidal crops served to reinvigorate IRM research, which has resulted in important advances in understanding how complex interactions between pest biology and behavior, and farmer adoption and compliance behavior can affect IRM. Still, there is a wealth of opportunities to further advance this understanding.

Constructive approaches to modeling adoption and compliance behavior could be further developed to more accurately describe extensive patterns of adoption. Two

possible ways to accomplish this objective are to add fixed cost and farmer experience to the problem. In terms of fixed cost, equation (11.8) can be modified to

$$\pi_t(x_t) = P_y Y(n^S(n_t, r_t, x_t)) - C(x_t) - FC(x_t, \ldots, x_1) \tag{11.14}$$

where $FC(x_t, \ldots, x_1)$ is the fixed cost of adoption. For equation (11.14), $FC(x_t, \ldots, x_1) > 0$ if the pest management strategy is used in period t ($x_t > 0$), but has not been used in prior periods ($x_t' = 0$ for $t' = 1, \ldots, t-1$); otherwise, $FC(x_t, \ldots, x_1) = 0$. This specification of fixed costs implies that they are only incurred in the first period a farmer chooses to adopt the technology. In terms of farmer experience, equation (11.8) can be modified to

$$\pi_t(x_t) = \int_{\varepsilon} U(P_y Y(n^S(n_t, r_t, x_t)) - C(x_t) + \varepsilon_t)\varphi_t(\varepsilon, x_t)\mathrm{d}\varepsilon \tag{11.15}$$

where ε is a random variable with probability density function $\varphi_t(\varepsilon, x_t)$. To capture the effect of increasing experience overtime, assume the mean of ε is zero and the variance $\sigma_t^2(x_t)$ is decreasing overtime and increasing in x_t. Such assumptions would imply that the risk associated with employing a new pest management strategy by choosing $x_t > 0$ would decrease overtime, which would tend to increase adoption assuming $U(\cdot)$ is concave because farmers are risk averse.

Estimation approaches could be improved by employing longitudinal survey data that includes more extensive information on farmer perceptions of the frequency and severity of pests. With longitudinal data, it becomes possible to directly measure the effect of farmer experience on adoption behavior. With more extensive information on farmer perceptions of the frequency and severity of pests, it becomes possible to estimate adoption equations that can be integrated with pest population dynamics and genetics. Estimation approaches based on well-constructed survey data might also be employed to obtain a better understanding of the sensitivity of farmer compliance to alternative IRM policies.

The majority of work on the effect of human behavior on IRM has focused on farmer adoption and compliance without regard for other important actors like chemical and seed companies. Alix and Zilberman (2003) and Noonan (2003) note that new pesticides are often patented by chemical companies, which gives them exclusive control over distribution for a specified time period. This exclusive control provides an incentive to charge higher prices in order to achieve higher returns from pesticide sales. These higher prices slow adoption by increasing the cost of pest management to farmers, which slows the evolution of resistance. Incorporating this observation into an IRM model, Alix-Garcia and Zilberman (2005) argue that regulatory policy may in fact be unnecessary. For transgenic insecticidal crops, several companies can have control over the distribution of a particular toxin, which leads to a situation referred to by economists as oligopoly. An oligopolist also has an incentive to charge a higher price to increase returns, but is more constrained in terms of how high of a price it can charge because its competitors may choose to charge a lower price. Therefore, a better understanding of the benefits of IRM policy can be obtained from a better understanding of how companies that supply pest management products and services interact with each other and farmers.

Conclusions

Pest management is a fundamental human activity in agricultural production. This activity is inherently constrained by the evolution of insect resistance. IRM has the potential to increase societal benefits achieved through pest management. Farmers are unlikely to effectively manage insect resistance to the benefit of society on their own due to the commons nature of the problem (see Mitchell and Onstad, Chapter 2). Therefore, public policy may play an important role in helping manage insect resistance to the benefit of society. An understanding of insect biology and behavior is not enough to design effective IRM policies because predictable farmer behavior also plays an important role in the evolution of resistance. Furthermore, this predictable farmer behavior depends in complex ways on important economic, sociological, and psychological factors, and pest behavior and biology. These complex interactions limit the possibility of using sweeping generalities to help guide IRM policy, which ensures the continual importance of both practical and innovative IRM research.

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Chapter 12

Modeling for Prediction and Management

David W. Onstad

Modeling plays a critical role in understanding and managing resistance. In the study of complex ecological systems, modeling can be used to identify important gaps in knowledge, predict consequences of management, assess risks, and perform virtual experiments that are impossible to perform in reality because of cost, logistics, or ethics. To make predictions about evolution, insect population dynamics, and pest management, scientists need to use the best possible information and logic available and integrate them using mathematics. Once the model is made, scientists can study the system dynamics over time and space. The wide variety of models used in insect resistance management (IRM) is similar to the range of models used in population ecology and integrated pest management (IPM).

Modeling is no longer simply an academic exercise. Stakeholders concerned about public health and environmental protection are interested in IRM modeling. For instance, governments and developers of transgenic insecticidal crops have focused on modeling since transgenic crops began to be regulated. The United States Environmental Protection Agency (US-EPA) has even taken steps to formalize procedures and frameworks for model development and evaluation, so that the model-based predictions made by registrants can be adequately interpreted and evaluated. Clarifying models and their results for all stakeholders is another issue of concern to the US-EPA. The hope is to make the evaluations of IRM plans more transparent, with the process based on generally accepted standards for models and data. Since 2001, workshops about model design and validation have been sponsored by the US-EPA's Office of Pesticide Program and National Risk Management Research Laboratory (Glaser and Matten, 2003). The purpose of this chapter is to describe the process of modeling so that models can be clearly understood and evaluated.

Model Development and Evaluation

What is Modeling?

Modeling is the process of creating a conceptual, diagrammatic, algorithmic, or mathematical representation of reality. We all use conceptual models when we try to mentally predict what will happen in situations that we experience every day. We try to understand causes and imagine the effects. Computer programs and mathematical models describe population processes that cause changes to occur in

the quality and quantity of populations. We calculate or work through the model to answer questions, test hypotheses, or make decisions. Much of model analysis involves understanding your ecological system and your goals well enough to follow leads provided by early calculations.

The model is given credibility when it is supported by theory and/or data. Greater credibility is achieved by testing the model against independent data and finding an adequate match between model results and observations. To create a model that is credible, one must use logic and the best data available during its calibration. The best models are made by the most logical, critical, and careful scientists with access to the best data. Logic helps eliminate mistakes.

The analysis of some models is like performing a traditional scientific experiment (Royama, 1971). The typical laboratory or field experiment involves conditions that are held constant, other conditions that are allowed to vary over time or space, and treatments that are evaluated. Replication and multiple trials are performed to account for the variation that cannot be controlled. With a model, particularly a deterministic one, the scientist has complete control over all conditions. Stochastic models require replication because of the variability of results. Thus, a model is an experimental design and the calculation of the model is the experimental trial. Hypotheses are derived from the modeler's interpretation and generalization of the results. The entire process of modeling is summarized in Table 12.1.

Table 12.1 The process of modeling

I. Select subject and purpose of model
   a. Determine time horizon
   b. Determine maximum spatial boundaries

II. Review existing models and literature about experiments
   a. Find quantitative information; note the scales of the data, especially time
   b. Identify relevant theories
   c. Take advantage of techniques used in existing models

III. Create mathematical functions from logic and data
   a. Convert data to appropriate units
   b. Interpolate – where does change occur?
   c. Extrapolate – what are logical limits?
   d. Compromises may be required because of interactions between functions and processes occurring at different time scales

IV. Verification
   a. Check logic of entire model
   b. Check conversion of math into computer code

V. Validation
   a. Relate to goal of model
   b. Test model against independent data

VI. Analysis and experimentation
   a. Sensitivity analyses
   b. Assess risk
   c. Evaluate economics

The Role of the Goal

There are an infinite number of representations of any given ecological system. All will be simplifications. Our goal or the one given to us by stakeholders or supervisors determines which subset of the possible models should be considered. Of course, deadlines and budgets also determine which models are attempted. Do we have enough time and money to make a credible model given the goal? The goal not only directs us during the creation of the model, it also helps others understand and evaluate the model and its results. Therefore, it is important to clearly express the goal and purpose for making the model(s). *Every model must be based on a goal and judged according to that goal.*

Given the goal, the preferable approach would be to create at least two different models of the system. If they both provide the same results with regard to your most important questions, then you have more confidence in your results. The answer and solution may be robust to changes in your model influenced by budget, deadlines, and personal bias.

Kinds of Models

Models used for IRM are usually labeled according to several major characteristics that are easily identified. Some models can be solved without a computer while others require a computer. Each model is based on a particular style of mathematics. Characteristics that currently seem important are discussed below.

One conceptual division of models is based on whether a model incorporates variability in processes or conditions. This variability is formally represented by probability distributions in stochastic models that account for variability within a population or variability in the environment. Deterministic models do not contain any probability distributions with random variables. They are often justified by focusing on mean values for large populations. Stochastic models contain one or more functions that are based on a random variable with a random number generator providing a value each time the function is calculated. The underlying probability distributions are either based on data or assumed to be a particular type.

Sometimes choices are made regarding the use of linear and non-linear functions in models. Linear functions consist of multipliers of a variable such as insect density that do not change as density changes. For example, if oviposition is linear with respect to female density, then 1 female will lay 10 eggs and 100 females will lay 1000 eggs. A non-linear function causes density, for instance, to produce different effects: 1 female lays 10 eggs but 100 females only lay 500 eggs. Well-known non-linear functions are (a) dose response for toxin mortality in each genotype, (b) density-dependent, competition-based survival, (c) mating, (d) density-dependent dispersal by larvae or adults, and (e) oviposition. Modelers typically explain why they include or ignore density-dependent and other non-linear functions.

To aggregate or dis-aggregate, that is the question, at least in many situations and models. Aggregation combines variables into one or a few variables or reduces space from many units into one or a few patches. With aggregation we omit age or stage structure. Dis-aggregation enables the modeling of each life stage, each sex,

or each genotype. The chosen level of simplification should be based on the purpose of the model and the availability of data to support the dis-aggregation.

Choices must be made concerning the representation of time and space. Modelers can choose to make time discrete or continuous. Typical time steps for calculation in IRM models are a day or a pest generation. Several choices can be made concerning the representation of space. Often space is discretized into explicit patches, but it can also be continuous or essentially one location without dimensions. Modelers must choose to make space either homogeneous or heterogeneous. For heterogeneous landscapes, models usually consist of many units; each unit representing a plant, a plot, a field, or some other rational area.

Simplicity, Generality, Realism, and Precision

The goal helps us focus on the real ecological system and sharpens the focus on the major components of interest. However, you must always remember that *Every model of the same system has the same total number of implicit and explicit assumptions.*

One model of a pest and its environment may explicitly include only one natural enemy and only temperature as a climatic influence. Another model of the same pest may include two natural enemies and no weather. A third may not include natural enemies, but it may focus on the variety of host plants, some with partial resistance. Of course, real systems include most if not all of these factors. And each model rests on implicit assumptions about the influence of factors not explicitly described with mathematical functions.

The recognition of implicit and explicit assumptions are particularly important for evaluating the representation of space in IRM models. Every model that does not explicitly represent space and its heterogeneity, implicitly considers space to either be uniform or random depending on the explicit functions for the modeled components.

In general, if another model can add a function (explicit assumption) for a new process or component, then another model without that function can be considered to have one more implicit assumption regarding the same ecological system. In other words, when we state that a modeler or model ignores some aspect of the system, then we are also implying that an implicit assumption has been made about that factor.

Why is this viewpoint important? Some modelers that make simple models do not mention the many implicit assumptions that they had to make about the real system. This is a serious concern when they claim that the simple model is generally applicable to many ecological systems. These same modelers claim that complex models have too many explicit functions. Yet the viewpoint expressed above sees both simple and complex models having the same number of assumptions. Thus, both kinds are complex. One kind is just more easily solved mathematically rather than numerically on a computer.

Onstad (1988) addressed the issues of simplicity, generality, realism, and precision. He concluded that generality is not a property of a model that can be identified nor proclaimed at the time of a model's creation. A model earns a designation of

being general after it has been tested against many systems. A model's accuracy and precision can be evaluated when it is tested against independent data. Complex and simple models can produce the same degree of accuracy and precision. Modeling and experimentation in basic science are similar, because each is a simplification of reality. Both can be faulted at times for being too simple or unrealistic.

Since all models are simplifications of reality, can one model be more real than another? Certainly! A model that permits all individuals to reproduce without accounting for the differences of males and females and immatures and adults cannot be considered as real as a model that has more realistic reproduction. For example, exponential growth of a population may seem to work fine at some gross scales of time, but the same function cannot be used when only immatures are alive over a given period. *Models should be made as realistic and as simple as possible to achieve the goal.*

The realism of a model can usually be determined at its creation. Its generality and precision must be determined as it is tested. The value of a model and whether it should be more or less simple are determined by how well it helped the modeler achieve the goal and satisfy the stakeholders.

Validation

As mentioned above, validation helps modelers and stakeholders determine the accuracy, precision, and credibility of a model relative to its purpose. If a model is tested against data from a variety of systems, then its generality can also be determined. Individual processes, such as larval survival or inter-field dispersal of males, can be tested, or the predictions and observations of major variables representing the dynamics of an entire system over time and space (e.g., allele frequency, pest density) can be compared. The comparison of predictions to observations may simply involve evaluations of qualitative patterns that can be presented in plots of model results and field data. Or the validation may involve statistical analysis of the quantitative data and model output. Tabashnik (1990) provides a good review of efforts to validate or test IRM models.

Two issues are important in validation of IRM models. First, it is often difficult to collect the allele-frequency data over enough pest generations (years) to test predictions in the field. For species that can be reared under laboratory conditions, there is greater potential for extensive model validation. For other species, partial validation through testing of population dynamics and individual processes is usually the most that can be expected. Second, models can only be tested against independent data collected in the field, greenhouse, or laboratory. Data are independent when they have not contributed to the calibration or construction of the model. Early validation studies for specific systems were performed by Taylor *et al.* (1983), Tabashnik and Croft (1985), Tabashnik (1986), Denholm *et al.* (1987), and Mason *et al.* (1989).

More recently, several modelers have demonstrated proper and informative validation techniques. Storer (2003) created a spatially-explicit stochastic IRM model primarily to study the evolution of resistance by *Diabrotica virgifera virgifera* to transgenic insecticidal corn. He was able to test several ecological processes against

field data of population dynamics. Furthermore, he tested the entire model against historical field data concerning the pest's evolution of resistance to specific insecticides. Carrière and Roff (1995) tested a simple model simulating the simultaneous evolution of diapause propensity and insecticide resistance in *Choristoneura rosaceana*. Differences between the optimal solution to the model and the field data allowed them to explain the population genetics of the system. The IRM model created by Zhao *et al*. (2005) failed to match the population dynamics of *Plutella xylostella* in greenhouse studies, but the predicted changes in allele frequencies were generally similar to those observed over 26 generations. Boivin *et al*. (2005) created a phenological model of *Cydia pomonella* that represented populations of a susceptible and two insecticide-resistant homozygous genotypes. Model simulations for each genotype were compared with pheromone trap catches recorded in field populations over an 8-year period.

Sensitivity Analysis

A sensitivity analysis permits the modeler and stakeholders to determine how sensitive the model results are to small changes in model parameters. The less sensitive the model results are to these changes, the more confidence we have in the decisions that are made based on the model. Model results and recommendations are considered robust when they are not sensitive to changes in the model.

Usually modelers focus on the parameters that they are uncertain about. When model results are sensitive to small changes in a parameter, such as fecundity or survival, and the values are not known with great certainty, the sensitivity analysis can be used to guide future data collection and experimentation to reduce the uncertainty.

The sensitivity analysis will likely be influenced by the goal of the modeler. Thus, the goal and the procedures for every analysis should be clearly presented in reports. Sensitivity analyses can also investigate synergistic effects due to changes to multiple parameters and the substitution of mathematical functions representing complete ecological or genetic processes.

Risk Assessment for IRM

Risk assessment is important in IRM because stakeholders need to understand the consequences of implementing alternative IRM strategies in an uncertain future (Andow and Zwahlen, 2006). Modeling can contribute to these assessments. What is the risk that a population will evolve resistance to a management tactic? Associated with this risk are the economic risks that farmers face when a pest evolves resistance (Chapters 2 and 11) and the risk that substituted tactics will harm the environment. The three basic steps to a risk assessment: problem formulation, analysis, and risk characterization are essentially the same as those used in an IRM modeling project (Jensen and Bourgeron, 2001). They both start with stakeholders selecting a goal and accepting system boundaries for the assessment. The time horizon and the spatial scale must be clear (Chapters 2 and 4). Ecological risk assessment identifies the management tactic (the stressor) as the threat to the pest population (the receptor). In other risk-assessment jargon, the endpoint consists of the entity, which is the

targeted population; and the characteristic of the population that is measured is the allele frequency or some other measure of resistance.

The IRM model calculates the exposure of the population to the tactic and the level of selection pressure (the effect on the stressor). With deterministic models, the emphasis is on the expected or mean threat, exposure, and selection pressure; whereas, with stochastic models, the modeler can use the probabilities of a range of threats, exposures and selection events to calculate the probabilities of change to the population. Of course, determining the probabilities of the threats, exposures, and selection events can be difficult.

IRM Models

The historical development of modeling for IRM coincides with the development of scientific IRM strategies (Taylor, 1983; Tabashnik, 1990). If models did not provide the foundation of IRM strategies, their results at least supported them from the beginning. Sometimes abstract models have been used to study the evolution of resistance and the consequences of management practices (Taylor, 1983; Tabashnik, 1990). Several abstract models are discussed by Onstad and Guse (Chapter 4), Pittendrigh *et al*. (Chapter 6), and Onstad (Chapter 10).

Various authors have advocated the creation of species and system-specific models to develop the most credible results for applied IRM (Roush and McKenzie, 1987; Kennedy *et al*., 1987; McKenzie and Batterham, 1994; McKenzie, 1996). They generally support the points made about realistic models discussed above. Species-specific models are discussed by Onstad and Knolhoff (Chapter 9) and Onstad (Chapter 10). The following two topics demonstrate how models can be used to explore issues not normally considered in traditional population-genetics models.

Effects of Pest Phenology

Models can be used to investigate how pest maturation influences the efficacy of IRM tactics. Follett *et al*. (1993) developed a model to predict the rate of evolution in *Leptinotarsa decemlineata* on potato. DeSouza *et al*. (1995) created a model for *Helicoverpa armigera* on cotton. Both studies showed that the timing of insecticide applications relative to the period in which the pest is in diapause has a significant effect on the evolution of resistance to insecticides. For some pests in some cropping systems, diapause can provide a temporal refuge. DeSouza *et al*. (1995) discovered that the effect of diapause was different in Australia and India; diapause conserved resistance in Australia but conserved susceptibility in India.

Two modeling studies investigated the consequences of developmental delays caused by pests feeding on transgenic insecticidal crops. Ferro (1993) modeled the effects of transgenic potato on *L. decemlineata*. He concluded a low toxin dose in the potato crop could reduce the number of generations of *L. decemlineata* per year, but warned that non-random mating amongst susceptible and resistant beetles could occur because of the delays in maturation. Peck *et al*. (1999) found that transgenic cotton can either increase or decrease the rate of resistance evolution for *Heliothis virescens*.

Carrière *et al*. (2001) used a model to demonstrate how cultural-control tactics and cotton-planting date could be changed to delay evolution of resistance by *Pectinophora gossypiella* to transgenic insecticidal cotton. Carrière *et al*. (2001) showed that adult emergence in the spring should be the focus of both IPM and IRM.

Mitchell and Onstad (2005) developed a model for *Diabrotica barberi* to examine the effect of prolonged egg diapause on the evolution of resistance to transgenic insecticidal corn. They attempted to mimic conditions found in the prolonged-diapause problem area near the Minnesota, South Dakota, and Iowa borders (Spencer and Levine, Chapter 8). Results indicated that toxin dose and farmer management practices, such as insecticide use on refuge corn and the pattern of crop rotation, generally have a larger impact on the evolution of resistance than many parameters concerning population dynamics and genetics. In the region where prolonged diapause already exists, increasing prolonged diapause (increasing hatch rates after two and/or three winters while holding total hatch constant), tends to increase resistance to transgenic corn.

Some models even explore the simultaneous evolution of pest maturation and resistance to a toxin. Carrière and Roff (1995) used two models to study the simultaneous evolution of diapause propensity and insecticide resistance in *Choristoneura rosaceana*. One model was based on ecological optimality theory and the other was a quantitative-genetic model simulated with two threshold traits. The models predicted that more of the population would enter diapause early in the summer because of insecticide applications. Refuges occupied during diapause may permit larvae to escape insecticide. The evolution of the timing of events in a pest's life cycle will likely be an important research subject in the future.

Complex Biological Models with Simple Economic Analyses

Models that include economic factors, management processes, and even human behavior are described by Mitchell and Onstad (Chapter 2) and Hurley and Mitchell (Chapter 11). The following models combine complex biological models with relatively simple economic functions to calculate the benefits of certain strategies for IRM.

Gutierrez *et al*. (1979) were perhaps the first to combine complex biological processes and economics into a model for IRM. They modeled the population dynamics of the pest, the dynamics of crop growth, the population genetics of insecticide resistance, and economics of harvested crop yield and insecticide use. Gutierrez *et al*. (1979) evaluated a scenario in which the insecticide is applied according to a predetermined schedule and a second scenario in which insecticide applications are adjusted each generation based on observations of pest density and resistance allele frequency. They concluded that sampling (second scenario) delayed the evolution of resistance but not significantly.

Since the mid-1990s, Onstad has led a group of modelers using deterministic simulation models to evaluate IRM for pests of corn, *Zea mays*. These data-based models tend to have intermediate complexity with age structure and behavior explicitly incorporated. Some models have daily time steps and more details that permit hypotheses to be addressed concerning intra-generational issues. Alternative models for the same species have life stages and processes aggregated to permit

the use of a time step equal to the period of the insect's generation. The purpose of these models was to study the evolution of the pest, to evaluate the alternative strategies and tactics for IRM, and to demonstrate how some IRM strategies economically compare to other IPM practices. In addition to the models described below, other modeling efforts by this group are described in Chapters 8 and 10.

Onstad's original focus was on IRM for *Ostrinia nubilalis* in transgenic insecticidal cornfields (Onstad and Gould, 1998a). In these deterministic models with a daily time step, mating is random in the landscape and eggs are oviposited uniformly across the cornfields. Onstad and Gould (1998a) evaluated block, row-strip, and corn-ear refuges, seed mixtures, and sequential planting of transgenic insecticidal and non-transgenic corn as IRM tactics. Onstad and Gould (1998a, b) created a model that simulated toxin concentration decline in plants during senescence. The daily time step permitted the simulation of various synchronies and asynchronies between toxin dose and larval maturation. Seasonal dynamics of toxin concentrations significantly increased the risk of evolution of resistance. Onstad and Guse (1999) extended the model to include density-dependent larval mortality and the economics of crop damage. Onstad and Guse economically evaluated the block and row-strip refuges and concluded that a 20% refuge along with a constant high dose of toxin was a robust strategy for *O. nubilalis* IRM. Both studies emphasized the need for research on seasonal decline in toxin concentration in transgenic insecticidal crops before commercialization (Onstad and Gould, 1998b; Onstad and Guse, 1999).

Onstad *et al*. (2002) created a different model for *O. nubilalis* IRM that had a time step of one generation. They used the model to study the effects of insecticide use in refuge corn on evolution of resistance to both transgenic insecticidal corn as well as to the insecticide. Figure 12.1 clearly demonstrates the importance of limiting

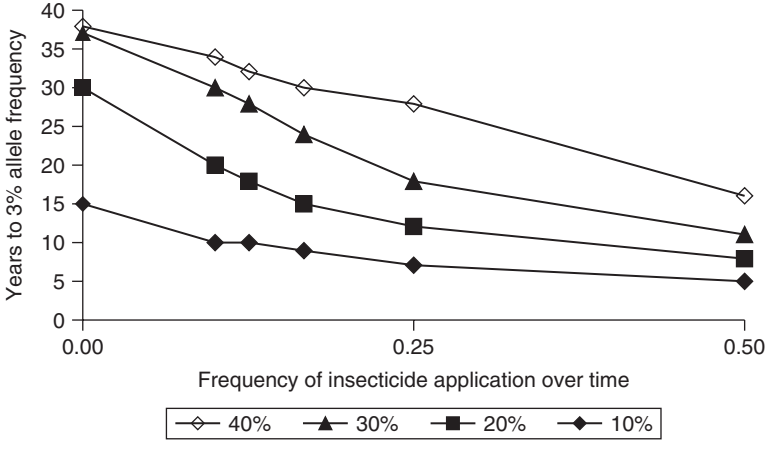

Figure 12.1 Number of years required for *O. nubilalis* to evolve resistance to transgenic insecticidal corn (3% allele frequency) as a function of the proportion of generations treated in the refuge for four refuge sizes. Initial allele frequency is 0.001, with a practical high-dose crop, partially recessive expression of the transgenic resistance allele, and 90% insecticide efficacy with no evolution of insecticide resistance. Reprinted from Onstad *et al*. (2002) by permission of Entomological Society of America.

insecticide use in the refuge. Evolution to transgenic insecticidal corn occurs faster as either the refuge size decreases or as the frequency of insecticide use in the refuge increases. For example, with a 20% refuge, the number of years to resistance decreases from 30 to 15 years when the insecticide use changes form none (0.0) to every fourth generation (0.25), which is equivalent to one application every other year. A consistently sprayed refuge, consisting of less than 40% of the cornfields, was an inadequate IRM strategy for *O. nubilalis* even when a low efficacy insecticide (70% mortality) was used.

Onstad *et al*. (2001a) investigated IRM for *D. virgifera virgifera* in cropping systems with transgenic insecticidal corn. They used a deterministic model that included two non-linear functions: density-dependent larval mortality due to competition, predation or parasitism and a density-dependent function for calculating the proportion of female beetles that mate each day in each field. Results indicated that toxin concentrations at intermediate levels in the transgenic plants would be worse for IRM than either higher or lower doses. Sensitivity analyses demonstrated that the function for density-dependent survival was important, but the non-linear mating function had little effect on the results.

Onstad *et al*. (2001b, 2003) modeled the evolution of behavioral resistance in *D. virgifera virgifera*. One of the most interesting results of the modeling by Onstad *et al*. (2001b) was the discovery that landscape diversity, represented by the proportional area of vegetation in the environment that is not part of the annual corn–soybean rotation, reduces the rate of evolution of rotation resistance. Essentially, in this very simple deterministic model, the oviposition on lands not rotated with cornfields is a fitness cost for resistant beetles. Onstad *et al*. (2001b) also used the 3-allele, single-locus model to determine that a soybean specialist would be less fit than a polyphagous phenotype that oviposits on lands covered by a wide variety of vegetation (Chapter 10).

Onstad *et al*. (2003) used a model to analyze the economics of IRM for rotation-resistant *D. virgifera virgifera*. They explored the economic consequences for six alternative IRM scenarios and compared them to the standard 2-year rotation of corn and soybean. They concluded that IRM utilizing transgenic insecticidal corn in the 2-year rotation is a robust, efficient strategy.

Crowder and Onstad investigated the simultaneous evolution of resistance by *D. virgifera virgifera* to crop rotation and transgenic insecticidal corn (Crowder and Onstad, 2005, Crowder *et al*., 2005a,b,2006). At the same time, they evaluated how management of the cropping system (design and control of what is planted, where, and when) influences IRM for both problems. Thus, the deterministic models, which were extensions of the models created by Onstad *et al*. (2001a, 2003), considered a two gene system: one for behavioral resistance and one for toxicological resistance. These models included mating by females before dispersal to oviposit, mating by males before and after dispersal from the field of emergence, differential, density-independent, field-to-field dispersal based on gender and phenotype, and density-dependent larval survival. Crowder and Onstad determined that a robust strategy for delaying resistance to transgenic corn in areas where rotation-resistance is a problem is to plant transgenic corn in rotated cornfields. This also helps delay resistance to crop rotation.

Crowder *et al*. (2005a, 2006) investigated the economics of IRM strategies for *D. virgifera virgifera* in areas with crop rotation and transgenic insecticidal corn. In areas with and without populations adapted to a 2-year rotation of corn and soybean (rotation-resistant), the standard management strategy is the planting of 80% of a cornfield (rotated and continuous) to a transgenic corn hybrid each year. In each area, Crowder *et al*. (2005a) also studied dynamic management strategies where the adoption of transgenic corn increased over time in a region. They also analyzed management strategies for a single field that is the first to adopt transgenic corn within a larger unmanaged region. In all areas, increasing the expression of the toxin in the plant increased economic returns for farmers. In areas without rotation-resistance, planting 80% transgenic insecticidal corn in the continuous cornfield each year generated the greatest returns with an intermediate or greater toxin dose. In areas with alleles for rotation-resistance at low initial levels, a 2-year rotation of non-transgenic corn and soybean may be the most economical strategy if resistance to crop rotation is recessive. If resistance to crop rotation is additive or dominant, planting transgenic insecticidal corn in the rotated cornfield was the most effective strategy. In areas where rotation-resistance is already a severe problem, planting transgenic insecticidal corn in the rotated cornfield each year was always the most economical strategy. In some cases the strategies that increased the proportion of transgenic insecticidal corn in the region over time increased returns compared with the standard strategy. With these strategies, the evolution of resistance to crop rotation occurred more rapidly but resistance to transgenic insecticidal corn was delayed compared with the standard management strategy. In areas not managed by a regional norm, increasing the proportion of transgenic corn and increasing toxin dose in the managed field generally increased returns.

Crowder *et al*. (2006) explored the use of sampling and economic thresholds to improve IPM and IRM when transgenic insecticidal corn is used for *D. virgifera virgifera* management. In the model, transgenic insecticidal corn was planted only when adult densities in the previous year were above a threshold calculated from single-season economics for IPM. The use of economic thresholds slightly slowed the evolution of resistance to transgenic insecticidal corn. In areas with or without rotation-resistant phenotypes, the use of sampling and economic thresholds generated similar returns compared with strategies of planting transgenic corn every year. Because many transgenic insecticidal crops are extremely effective, farmers may be inclined to plant transgenic crops every year rather than implementing costly and time-consuming sampling protocols.

Conclusions

Some models will emphasize evolution of resistance, while others will focus on economic outcomes. For management of insect resistance, we need both types of models. In a single chapter, it is impossible to adequately describe or even represent all the important models that have been created to study and improve IRM. Certainly any student of IRM must become familiar with the modeling styles and accomplishments of the scientists who have been working on models for a significant part of their careers. If we consider a publication history spanning more than

10 years to be an indication of a scholar's serious commitment to IRM modeling, then we can say that Caprio, Carrière, Curtis, Gould, Roush, and Tabashnik, are among the scientists that deserve our attention. I hope that their work has been adequately represented throughout the book.

Modeling can be a valuable tool in predicting the consequences of IRM strategies deployed under a given set of environmental, economic, and social conditions. This chapter was written to help scientists and stakeholders to understand how models are created, tested and analyzed. Everyone should realize that models and associated analytical procedures must be considered in the same manner as reports about experiments. The reader must be able to duplicate the model and its calculation, judge its credibility, and interpret its analysis.

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Chapter 13

Monitoring Resistance

Bruce H. Stanley

Susceptibility and Tolerance

The goal of an Insect Resistance Management (IRM) program is to keep the proportion of susceptible organisms in a population as large as possible. Ideally, all individuals in the population are susceptible to a toxin, crop trait or cultural practice to which they are exposed, although sometimes a population may contain some tolerant individuals before it is ever exposed. In this chapter, the emphasis of discussion is on assessing susceptibility of agricultural pests to toxins, the area of the author's expertise, and little focus will be made to other organisms or systems. However, concepts presented here can be generalized to most other systems.

The tolerance of an individual arthropod to a substance, such as a pesticide, is governed by the phenotypic expression of its genotype (Andow and Ives, 2002). The set of phenotypes within a population of arthropods is continually changing through time (Martinson *et al*., 1991; Kranthi *et al*., 2002) and space as a result of the response to the abiotic and biotic (age, gender, density, mating, and reproduction (Sparks *et al*., 1990; Bouvier, *et al*., 2002) environment the individual experiences. This has led some researchers to incubate their test subjects in field conditions (Schouest and Miller, 1991).

The Concept of a Distribution of Tolerances

When dealing with a population of arthropods, each individual is likely to vary in some fashion that effects intoxication or expression of a response. Accordingly, this will result in a statistical distribution of response probabilities that is a function of the stimulus, e.g., toxicant level. Because a monitoring program will most likely not measure the entire population, the responses in the part of the population sampled will follow a statistical sampling distribution. Even if the response to a non-destructive stimulus is measured on a single individual through time, the response will most likely follow a distribution because the response will be affected by the attributes of the individual that are sensitive to the ever-changing environmental conditions in which the individual finds itself. This is one reason why it is so important in a resistance-monitoring program to conduct the evaluation under a standard environmental regime.

The typical pattern of susceptibility of organisms to a harmful or burdensome stimulus is that first all are observed to be susceptible, and then eventually through evolution by natural selection, they all are observed to be tolerant. This results in a shift of the tolerance distribution to higher rates as shown in Figure 13.1. Note that the variance in the population tends to be small before and after selection.

Tolerance is usually governed by a small set of genes, and in many cases by one primary gene. This results in a very characteristic shape of response when plotted against dose if a single mode-of-action dominates, and a smoother response when many factors are involved in defining susceptibility to an insecticide. Head and Savinelli (Chapter 5) describe the importance of classifying toxins according to mode of action. For a monogenic trait, one might expect a chair-shaped tolerance distribution representing a mixture of the susceptible and tolerant individuals (i.e., two distributions of susceptibility). The height of the "seat" of the "chair" is the proportion of susceptible individuals. Because a population is usually assayed over a finite set of doses, a graph of the percent response against treatment rate or stimulus intensity will look like a shallow sigmoid curve, i.e., a typical dose–response curve with a shallow slope. When many genes are involved in determining tolerance there will not be a clear separation among populations, and one would expect a wider range of susceptibilities. This too will result in a shallow, sigmoidal tolerance distribution.

Care must be used when interpreting a plot of percent response versus treatment rate or stimulus intensity. Chilcutt and Tabashnik (1995) conducted an extensive review of the literature and did not observe a relationship between potency

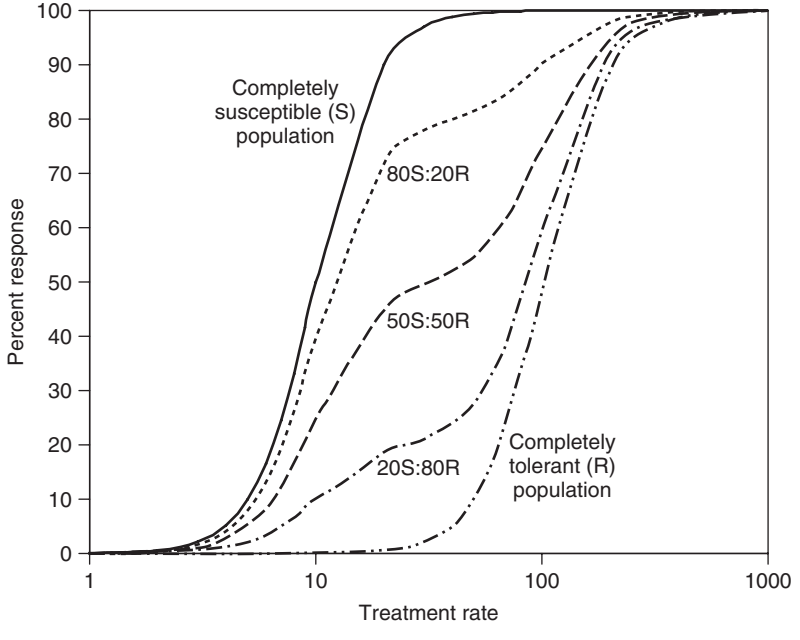

Figure 13.1 Hypothetical dose–response curve (i.e., tolerance distribution) for mixtures of susceptible and tolerant individuals.

(e.g., the LD_{50}) and the slope of dose–response curves. So, although theoretically plausible, the empirical evidence does not support a relationship between potency and the dose–response curve slope. Regardless, the researcher needs to maximize his or her understanding of population susceptibility from the data he or she generates, and needs to check it for consistency with reasonable models.

Typically, the distribution of tolerances tends to be approximately lognormally or loglogistically distributed. The lognormal distribution tends to be very similar to the loglogistic distribution. Both are approximately normal on the logarithmic scale (any base), but the loglogistic distribution tends to have slightly thicker "tails". Details of both the lognormal and loglogistic distribution can be found in Finney (1971) and McCullagh and Nelder (1989). Because the loglogistic distribution tends to be mathematically simpler, we will assume the loglogistic distribution in the following discussion.

Timing of Monitoring and Treatment

Care must be taken when a treatment, such as a pesticide, is applied before a population has reproduced. The survivors are from the more tolerant individuals of the pest population, and their tolerance distribution will appear to have shifted to the right or "more resistant". The individuals making up this population may still be "genetically susceptible", and upon reproducing may generate a normal susceptible population. If the genes involved now confer some tolerance on the off-spring then one might observe a slow progression towards a stable tolerant population. This illustrates the importance of resistance monitoring. It allows a researcher to understand how the susceptibility of a population may be shifting. This apparent shift in the tolerance of a population previously treated at rate R units is illustrated in Figure 13.2. Assuming a loglogistic ("logit") tolerance distribution, the density of individual tolerances, $f_{D|R}$, and the tolerance distribution for the population, $F_{D|R}$, of a treatment at dosage D units would be

$$f_{D|R} = \begin{cases} 0 & \text{if} \quad D \leq R \\ \dfrac{b \cdot F_D \cdot (1 - F_D)}{D^{b+1} \cdot F_R} & \text{if} \quad D > R \end{cases}$$

and

$$F_{D|R} = \begin{cases} 0 & \text{if} \quad D \leq R \\ \dfrac{F_D}{F_R} & \text{if} \quad D > R \end{cases}$$

respectively, where $F_X = \dfrac{X^b}{X^b + \left(\text{Dose}_{50}\right)^b}$

is the tolerance distribution at rate X ($X = D$ or R), D is any dose, R is the dose of the treatment, $LD_{50} = Dose_{50}$, is the median lethal dose, and $LD_{90} = r \cdot Dose_{50}$

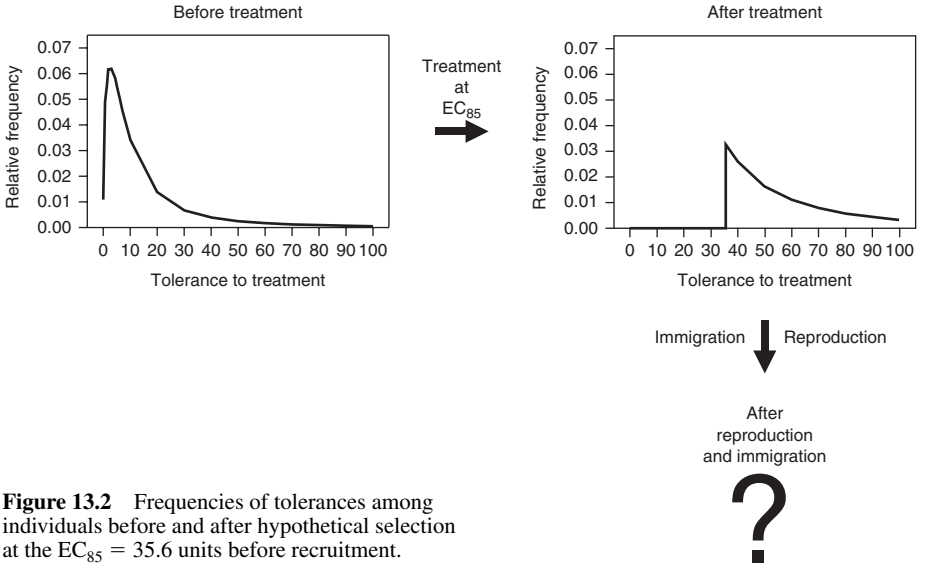

Figure 13.2 Frequencies of tolerances among individuals before and after hypothetical selection at the EC_{85} = 35.6 units before recruitment.

units is the dose eliciting a 90% effect, r is the ratio of the LD_{90} to LD_{50}, and b is the slope of the logit versus log dose line. Parameter b is calculated as

$$b = \frac{\log(r)}{\log\left(\frac{P}{1-P}\right)} = \frac{\log(5)}{\log(9)} = 0.73 \quad \text{for an } LD_{50} \text{ of 10 and } LD_{90} \text{ of 50 units}$$

This is shown in Figure 13.2 for an EC_{50} of 10 units and EC_{90} of 50 units and a previous treatment at an EC_{85} of 35.6 units. (Note that LD and EC are interchangeable.) The theoretical shape of the tolerances of the residual population is very similar to that of the unexposed population (Figure 13.2). It is unlikely that a researcher would be able to discriminate the two tolerance distribution shapes using conventional methods. Accordingly, one could erroneously assume that a significant shift in tolerance has occurred. This is one reason that researchers only get concerned when the LD_{50} shifts by a factor of some larger number, such as 10. However, in a monitoring program any shift should be investigated using follow-up testing.

Visualizing Tolerance

Monitoring programs will generate a large amount of information. This information should be summarized in a way that allows easy communication of the results and allows changes in tolerance to be readily recognized. One method that lends itself well to visualizing changes in the distribution of tolerances is the probability plot. Probability plots are easy to generate and communicate graphically.

The following is a recipe for creating an empirical probability plot of the distribution of population tolerances in a resistance-monitoring program:

1. Rank the dose–response quantiles (e.g., LD_{50}s) from low to high

2. Calculate the sample-size adjusted percentile for each quantile (e.g., $\% = 100(r + 0.5)/(n + 1)$)
3. Plot the adjusted percentiles against the ranked quantiles.

This method is illustrated in a later section in the case study in which probability plotting was used to assess the geographic variability of *Helicoverpa armigera* susceptibility to cypermethrin in six West African countries.

Quantifying Tolerance

A population of organisms can be very heterogeneous in its response to a stimulus and the population will vary in response relative to its environment. Also, the individuals in a population are often exposed to a mixture of stimuli that can, to varying degrees, elicit similar responses. However, the treatment or trait of interest usually elicits a significant, distinct response. Accordingly, researchers need to balance the resources spent measuring a response with the likelihood that it is stable. The goal is to get a representative measurement at a reasonable cost. Researchers must ask a series of questions to ensure that the tolerances are measured logically. Is the susceptibility stable? If so, is the population already tolerant? Does the frequency of tolerance observed in the laboratory reflect the frequency in the field? Researchers should maximize the effectiveness of their monitoring program relative to all of these questions.

An effective monitoring assay should do the following:

- Remove the natural response or mortality
- Quantify the level of tolerance
- Allow ranking of sites in order of tolerance
- Allow tracking of changes in tolerance through time
- Test hypotheses and explore sources of variability in tolerance.

If these requirements are met in a monitoring assay, the researcher will be able to readily assess changes in susceptibility and respond quickly with changes in a management strategy.

Single, Discriminating-Dose Approach

The discriminating or diagnostic-dose assay has been by far the most widely used method for monitoring susceptibility in the field. It is easy, relatively resource efficient, and supplies a clear "answer". A local population is either susceptible or tolerant. The goal of the discriminating-dose assay is to identify that the status of the population's susceptibility has changed, ideally, in time for remedial action to be taken.

The three important considerations for designing a single, discriminating-dose monitoring program are

1. Establishing the "diagnostic dose" to separate individuals with susceptible phenotypes from the resistant phenotypes.

2. Determining the sample size to be collected at each location.
3. Determining the appropriate response to a survivor of the discriminating dose.

Extensive work has been done on the diagnostic-dose monitoring approach. Very notable examples are the *Heliothis* susceptibility monitoring programs (Staetz, 1985; Staetz *et al*., 1988; Plapp *et al*., 1990b). Knight *et al*. (1990b) and Marcon *et al*. (2000) discuss the development of diagnostic doses for mites and *Ostrinia*, respectively. Venette *et al*. (2002) assess the discriminating-dose approach thoroughly. They point out that the discriminating-dose approach is most likely to detect resistance determined by a dominant allele. Venette *et al*. (2002) conclude that an average of 1,400 individuals would need to be randomly sampled to detect a 1% rate of the phenotypic expression of resistance.

Dose–Response Approach

The most precise method to assess the susceptibility of a population to a compound or trait is the classical dose–response bioassay (Busvine, 1971; Finney, 1971). This is a regression of responses, usually using the probit or logit transformation, against the logarithm of the dosage. It is an oversimplification to assume that all populations follow either a logit (i.e., loglogistic) or probit (i.e., lognormal) tolerance distribution. As was previously illustrated, the population surviving after exposure to a trait or chemical will be a truncated tolerance distribution that may or may not recover to the pretreatment distribution. And the tolerance distribution of a population is ever-changing in response to the environment it finds itself in. Accordingly, the researcher must be flexible in assuming and fitting a tolerance distribution to his data.

There is much literature, both in the biological and statistical disciplines, describing the virtues and limitations of the probit and logit (Finney, 1971; McCullagh and Nelder, 1989). In essence, the two methods ensure that there are only responses for non-zero dosages and that the shape of the tolerance curve is sigmoid with a long positive tail. Both the logit curves and probit curves have a clear theoretical genesis, but one is hard pressed to consider one to be logically superior to the other. This author tends to favor the logit, i.e., loglogistic tolerance distribution, simply because it lends itself to easier mathematical manipulation.

There are a number of ways a tolerance model can be fit to a set of data. These include linear regression on transformed data, minimum chi-square regression (Berksen, 1955; Smith *et al*., 1984), maximum likelihood (Finney, 1971; McCullagh and Nelder, 1989), and maximum quasi-likelihood (Wedderburn, 1974; McCullagh and Nelder, 1989) of which maximum likelihood is a special case. All of the methods have their strengths. And one should select the appropriate method for one's needs. If in doubt, it is highly recommended that the researcher work with a biostatistician. Because of the time and resources that it takes to conduct a bioassay, one should try to extract as much information with the least bias as possible from the data.

Background or natural response should be accounted for in any bioassay. If it can be assumed that the factors affecting background response are independent of those affecting tolerance to the compound or trait, then the background response is

easily removed from effect of the chemical or trait using Abbott's formula (Abbott, 1925) or the equivalent Schneider-Orelli formula (Schneider-Orelli, 1947).

The design of multiple-dose bioassays is well described (Finney, 1971; Smith and Robertson, 1984; Robertson, *et al*., 1984; Smith, *et al*., 1984). The author has found the DOSESCREEN (Smith and Robertson, 1984) approach particularly useful.

A useful modification is to design to the relative length of confidence interval of the EC_x value rather than the absolute length.

The Two-Dose Approach

A simplification of the multiple-dose monitoring approach that has proved very informative and cost-effective in susceptibility monitoring has been the "Two-Dose" approach. In reality, it requires three treatments, i.e., an untreated control group to assess background mortality, a lower discriminating dose that allows a shift in susceptibility to be detected, and a higher discriminating dose that allows the researcher to determine what proportion of the population is tolerant to the treatment. This method is simple and requires only a modest number of test subjects. The two-dose approach is only slightly more expensive than a single discriminating dose monitoring program, because only one additional treatment is added. Most of the expense of a bioassay is in finding the sample site and collecting the samples.

The point estimate for the LD_{50} is estimated as a perfect fit through the two responses adjusted for background mortality using Abbott's formula (Abbott, 1925). The formula for the LD_{50} estimate adjusted for background response is given as

$$LD_{50} = 10^{\left[\log_{10}[L] + \frac{\log_{10}\left[\frac{S_L}{S_C - S_L}\right]}{\log_{10}\left[\frac{S_L \cdot (S_C - S_H)}{S_H \cdot (S_C - S_L)}\right]} \cdot (\log_{10}[H] - \log_{10}[L])\right]}$$

wherein the exponent C is zero dose (control), L is lower discriminating dose (e.g., 5 mg), H is higher discriminating dose (e.g., 30 mg). The reduced bias estimate of the percentage of larvae surviving exposure to dose D, $S_D = 100 \times (a_D + 0.5)/(n_D + 1)$, where a_D is the number of larvae alive after exposure to dose D, and n_D is the number of larvae exposed to dose D ($D = C$, L, or H).

Since the responses at the two dosages are binomial proportions, confidence intervals can be created around the responses. If the confidence intervals around the two dosages are connected, approximate confidence bands around the tolerance curve, and thus any quantile such as the LD_{50} (or EC_{50}), can be approximated. The approach is to find the dose where the lower and upper confidence interval of a binomial proportion with mean equal to the fitted curve crosses the percentile of interest, e.g. the EC_{50} is estimated from 50% survival as shown in Figure 13.3.

The parameter estimates from a two-dose bioassay are used just like those generated by a multiple-dose bioassay. They generally will not be as precise as those estimated from a multiple-dose assay, and will often be in Category I (Table 13.1). However, the two-dose bioassay is very cost efficient, and lends itself well

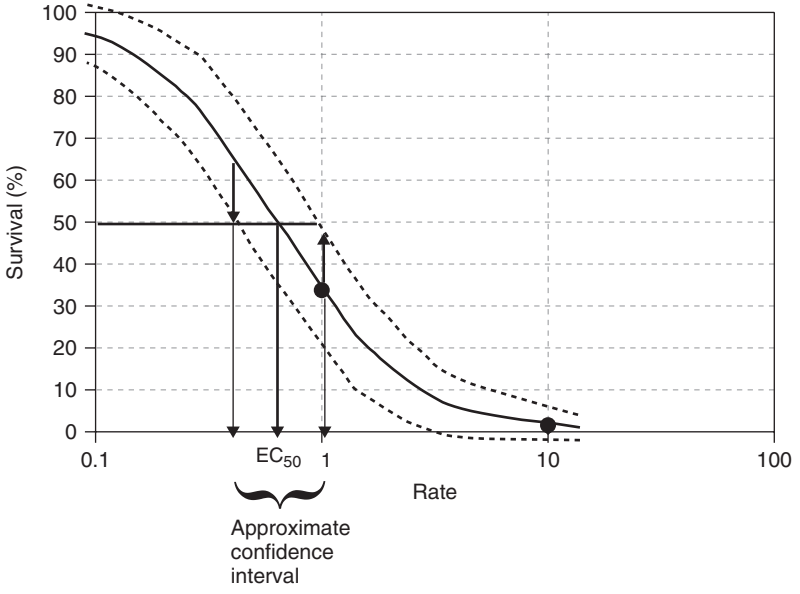

Figure 13.3 Two-dose bioassay showing data points and arrows pointing to the EC_{50} and its confidence interval.

Table 13.1 Recipe for conducting a two-dose bioassay

1. Work with number alive as the endpoint to reduce misclassification error.
2. Define four categories of local results:
   - i *Highly Susceptible*: Survival at the low dose is zero.
   - ii *Intermediate*: Survival at the low dose is less than the control.
   - iii *Highly Tolerant*: Survival at the high dose is equal to the control.
   - iv *Inconsistent*: Survival at the high dose is less than the low dose or greater than the control.
3. Estimate the LD_{50} for the tests in the Intermediate category using logit analysis techniques that adjust for background mortality using Abbott's formula (Abbott, 1925).
4. Use probability plot techniques and non-parametric, rank-based analysis methods to understand the changes in the distribution of susceptibility over space and time or among management approaches.

to a monitoring program. It is recommended that additional follow-up testing using multiple-dose bioassays be conducted for populations falling into Category 2.ii (Table 13.1) to understand the real tolerance distribution of a population.

Other Methods for Quantifying Tolerance

As the biochemical mechanisms for resistance are discovered, scientists can use this knowledge to create techniques for identifying biochemical markers in the tissues of resistant arthropods (ffrench-Constant and Roush, 1990). The marker may be an allele for a resistance gene, or it may be a chemical created by the resistance gene. Because of the greater certainty in these procedures, lower sample sizes

will likely be required to obtain the same precision as for methods described above (Venette *et al*., 2002). However, unless a resistance mechanism is known *a priori* for a new insecticide, multiple biochemical assays may be needed to discover tolerance in the field (ffrench-Constant and Roush, 1990).

Venette *et al*. (2002) summarize the feral screen and F_2 screen for measuring tolerance in wild populations of pests. Andow and Ives (2002) describe the statistics and costs for these methods for *Ostrinia nubilalis* on transgenic insecticidal corn. They state that the F_2 screen is the most labor-intensive monitoring method. Zhao *et al*. (2002) used an F_2 screen for *Plutella xylostella* on transgenic insecticidal broccoli (*Brassica oleracea*). They concluded that using transgenic plants expressing a high level of toxin may underestimate the frequency of resistance alleles with high false negatives or fail to detect true resistance alleles. Zhao *et al*. (2002) urged scientists to carefully validate the screening method for each insect–crop system before use of an F_2 screen.

An important new technique is the in-field screen (Tabashnik *et al*., 2000; Venette *et al*., 2000; Andow and Ives, 2002; Venette *et al*., 2002). This approach uses the toxic crop field as the diagnostic or discriminatory dose to distinguish resistant from susceptible individuals. In essence, the survivors in the toxic field indicate the level of tolerance relative to the survivors in a field of non-toxic plants. Because actual crop fields are used to screen insects, the costs are lower than those for other methods (Andow and Ives, 2002).

Monitoring as Part of Resistance Management

To be effective, resistance monitoring must be calibrated and broadly coordinated with clear and efficient communication. All of the information needs to flow into a decision-making body that will take action based upon the analysis and distribution of measurements. Before the process starts, the decision-making body needs to understand the costs and ensure that the stakeholders can afford the monitoring program. If not, the decision makers need to re-evaluate the goals to determine if their needs can be met with a subset of the program or a different approach. At the end of each cycle, a prudent manager should evaluate whether the measurements can be obtained more efficiently, and adjust the program before the next cycle. In all adjustments the manager should modify the scheme to preserve as much value as possible from any earlier work. In essence, this is the "adaptive management" with "adaptive management interventions" approach set forth by Andow and Ives (2002). Sometimes, the decision makers may decide that no resistance management is cost effective, and will let the progression of resistance unfold. They may choose to continue monitoring to track the status of the system.

Accordingly, the manager leading a resistance-monitoring program should:

1. Insure that a method (bioassay) is available to detect tolerance.
2. Make an assessment that the monitoring program is logistically and cost feasible.
3. Insure that the detection method can be used correctly by everyone involved.
4. Insure that everyone communicates accurately and promptly.
5. Insure all of the information is synthesized to yield an accurate picture from which decisions can be made.

6. Have an action plan in place to respond in some way to the results. (Usually, monitoring is not a goal unto itself.)
7. Review, to assess whether the next iteration might be done more efficiently.

Energy and resources focused upon the logistics of the monitoring program will insure a successful program. Timing is a key issue. Insuring that information flows accurately and promptly will greatly enhance the feasibility and usefulness of any resistance-monitoring program.

Logistics, Costs, and Practical Issues

Monitoring and its associated logistics have a cost, which can be substantial. As discussed by Andow and Ives (2002), the manager needs to assess the potential cost of a monitoring program against the benefit of information generated by it to determine if it is worthwhile. This suggests that the manager should map-out the entire program and his actions for each of the possible outcomes. This could be an iterative process where the manager tailors his monitoring program to fit comfortably within a budget. Usually, the manager can optimize the program within the cost constraints to achieve a very cost-effective program. The manager should also explore novel ways to conduct the monitoring, e.g., new bioassays or contracting opportunities, to maximize the chances of a successful program. If the manager decides that monitoring is not worth the cost, then other strategies may be needed to determine when to implement change or how to accomplish stakeholders' goals.

There are two considerations associated with any set of measurements. The first is the central tendency and intrinsic variability among the items being measured. This is the true magnitude and heterogeneity among the items being measured. The second is any variability and bias added to the actual values by the measurement technique. This is the error associated with the measurement system. The goal of a monitoring program is to minimize the error and bias, so that you get an accurate understanding of the magnitude and variability of the system. The manager must balance the resources spent on reducing the bias and error and the cost. This will yield an optimum monitoring program relative to the goals of the project.

A philosophy that lends itself well to resistance monitoring is the adequate precision approach. Under this approach, the manager assesses early in the program how precisely effectiveness of a treatment or trait to a population must be measured. The program is designed to measure to this level of precision. However, because there are often unforeseen needs that arise over the course of a program that require a bit higher precision, the manager should design the program to be slightly more precise than needed to insure that the measurement will be sufficient for all needs. Of course, the program needs to be below the budgeted costs, so that it is feasible over the time horizon defined by stakeholders' goals.

A key resource in any monitoring program is time. The manager should design a program that allows him to assess the state of the system, decide upon a course of action, and implement program adjustments in time to influence the population genetics to preserve the effectiveness of the treatment or trait. This can be achieved by clearly communicating program goals and response options, and automating reporting electronically to promote near real-time population status assessments.

Again, a cost–benefit analysis is appropriate so that the manager is confident that the effectiveness of the communication effort is worth the cost.

All of the preceding points regarding standardization, logistics and precision are achievable through planning. It is highly advised that the manager budget some time and resources for planning before initiating the monitoring program. One of the simplest methods to promote feasibility and responsiveness is to standardize all aspects of the program and communication. Also, attention to the logistics of the program will pay great dividends towards feasibility. The key is to have a program that is cost effective and meets all of the goals of the program. Although good planning can be the key to a successful resistance-monitoring program, the manager should not expend unreasonable time or resource in planning. Planning should not be an end unto itself, and it should be in balance with all of the other activities in the monitoring program.

The importance of training and communication in the success of a resistance-monitoring program cannot be over emphasized. Because the natural variation in response is usually already high, there is great payback in reducing procedural deviations among workers and errors due to poor communication. All of these problems mask an already variable and, hopefully, small change in response. If the change in susceptibility is large, then monitoring adds only minimal value. The manager is already confronting the need for a major change in the management of his system and may need to adapt his monitoring approach to a new set of goals and responses.

Effective and timely communication is important, particularly in a large geographical area, because decisions need to be implemented quickly to manage susceptibility. Anything that promotes communication approaching real time has value. There are many logistic considerations involved in changing a management program, so quick, effective communication allows time for critical changes to be made quickly and correctly. This maximizes the chances that an efficacy management program will be successful.

It is important to allocate time and resources in a monitoring program to benchmark the status of the program. The manager needs to verify that the monitoring program is not drifting and that all parties involved are communicating well and ready to respond to any changes in susceptibility. Inevitably, the manager will want to fine-tune the program while it is in progress. This can best be done by developing and testing possible changes in a research environment outside of an existing monitoring program. Proposed changes can then be incorporated simultaneously in all locations. This insures that the monitoring results are broadly comparable and have an expected and well-delineated shift in measured response. This avoids a drift in response over a wide geographic range that could easily be misinterpreted as a change in population susceptibility.

Major Limitations: Sampling and Surveying

Determining how to quantify tolerance in a population requires significant scientific work, but the primary costs of monitoring are the sampling and handling of all the insects or their tissues. Thousands of sample units must be checked for a chemical, a gene, or tested in a bioassay. This section focuses on the feasibility of collecting the tens of thousands of samples needed for precise estimation of tolerance in a population over time and space.

The goal of the project determines the region over which resistance will be monitored, the time horizon during which the population will be sampled (Chapter 2), and the resolution of sampling in space and time. The larger the region and the longer the time horizon, the greater effort devoted to and the cost of the monitoring program. The resolution indicates how small the areas and time intervals will be for sampling. For example, will samples be collected monthly or yearly? Will samples be taken from every field, farm, or county? Obviously, as the resolution becomes finer with smaller units of time and space sampled, the greater the cost.

A good general book on insect or animal surveys should be consulted for a description of the various types of sampling approaches that may be valuable in IRM. The collections may be obtained from simple random sampling or from stratified sampling. Geographic stratification can be based on farm, county, watershed, community, or any other rational or political division of the world.

The likelihood that a monitoring program will detect the first tolerant individual is very low. This has been discussed in detail by Roush and Miller (1986), Andow and Ives (2002) and Venette *et al*. (2002). The formula (Roush and Miller, 1986) for the randomly selected sample size necessary to detect 1 tolerant individual in a population of 1,000,000 individuals with probability 0.95 is

$$n = \frac{\log\left[1 - P(x \geq 1)\right]}{\log\left[1 - f\right]} = \frac{\log\left[1 - 0.95\right]}{\log\left[1 - 0.000001\right]} = 2{,}995{,}731 \quad \text{individuals}$$

where x is the number of resistant individuals in the sample, and f is the frequency of the resistant phenotype. It is very unlikely that any monitoring program will have the capability to make this feasible. Andow and Ives (2002) extended this analysis using more complicated models. They determined that one must detect recessive resistant alleles at a frequency less than 5×10^{-3} to provide enough time to implement an adaptive management program. If the resistance allele is dominant, then it must be detected at a frequency less than 1×10^{-7}.

As can be surmised from the above analyses of gene frequencies, it will be difficult to detect resistance in the field unless a monitoring program collects large numbers of individuals using pheromone-baited traps (Riedl *et al*., 1985; Haynes *et al*., 1987; Shearer *et al*., 1994) or attractant baits (Siegfried *et al*., 2004). Even with this possibility, it is very unlikely that the manager is going to be able to sample over the entire range of the population. Accordingly, with organisms with large reproductive power, such as arthropods, it is likely that the tolerant gene will become quickly fixed in the population, at least locally. Eradication of the tolerant gene will then become challenging and costly.

Turning Measurement into Action

Unless the goal of one's effort is solely to assess the geographic and temporal susceptibility of a population to a stimulus, the decision makers should have a plan of action developed that is tied to the observed susceptibility. This may range from slight changes in use patterns to completely removing the product or trait from a region. The decision makers should continually refine contingencies as they learn more about the system. In practice, the most challenging aspect of a resistance

management program is responding in a time frame that allows changes to purposefully and deliberately affect the monitored system. Having a plan in place allows one to react quickly and in a coordinated manner to the observed results from a monitoring program. The uninterrupted continuation of the monitoring program should also allow an assessment of whether remediation actions have improved the susceptibility of a population to the product or trait.

Examples of Monitoring Projects

There have been many insect resistance-monitoring programs in the past. They each had their specific goals and were constrained by resources, time and commitment. Their reports and methodology provide useful guides to designing effective monitoring programs. Some have examined the variation in susceptibility over large spatial scales (Knight and Hull, 1990; Knight *et al*., 1990a; Carriere *et al*., 2001). Many different taxanomic groups have been studied, particularly heliothines (Riley, 1990; Rogers *et al*., 1990; Kanga *et al*., 1995; Bailey *et al*., 2001; Wu *et al*., 2006) and other Lepidoptera (Leeper, 1984; Zhao *et al*., 2006). Croft *et al*. (1989), Grafton-Cardwell and Vehrs (1995), Grafton-Cardwell *et al*. (2004), Hockland *et al*. (1992), Nauen and Elbert (2003) and Sanderson and Rousch (1992) examined homopterans. Dennehy *et al*. (1990) and Reissig *et al*. (1987) examined mites. Edwards and Hoy (1995) monitored parasitoids. Peterson (2005) gives an extension service perspective on monitoring resistance.

Once pyrethroid resistance was suspected in the region, a group of entomologist in the southeastern United States investigated the problem with a monitoring project (Plapp *et al*., 1990a, b). They used pheromone traps to collect large numbers of adule male *Heliothis virescens*. Over 3 years and five states, they collected over 55,000 moths: no more than 13,000 in one state in 1 year. Plapp *et al*. (1990b) believed that their samples were large enough to base valid conclusions concerning the occurrence of resistance. This is certainly the case given that resistance allowed at least 10–20% of the male moths to survive higher doses of pyrethroid. Even though the standard deviations for their estimates of survival were large, with these high levels of survival it was impossible to not discover resistance in the population. Clearly, Plapp *et al*. (1990a, b) were not trying to find the earliest indications of resistance (Roush and Miller, 1986).

Later, in studies described by Plapp *et al*. (1990a), field experiments demonstrated that estimates of resistance based on larval sampling yielded more accurate results than those based on the sampling of adults. However, higher accuracy at one site based on more difficult larval sampling must be balanced with the greater precision provided by the huge numbers of easily captured male moths.

Steven N. Irving, PhD, led a very efficient and innovative resistance-monitoring study in Africa in 1998 as part of an initiative by Insecticide Resistance Action Committee International (IRAC). The concern was that field control failures of the pyrethroids against larval *Helicoverpa armigera* in Western Africa were due to resistance. The goal of the monitoring study was to survey cypermethrin efficacy against larval *H. armigera* in a number of countries in Western Africa to determine whether the control failures were due to the build-up of tolerance in *H. armigera* to the pyrethroids. There was a relatively small budget and the monitoring had to be implemented

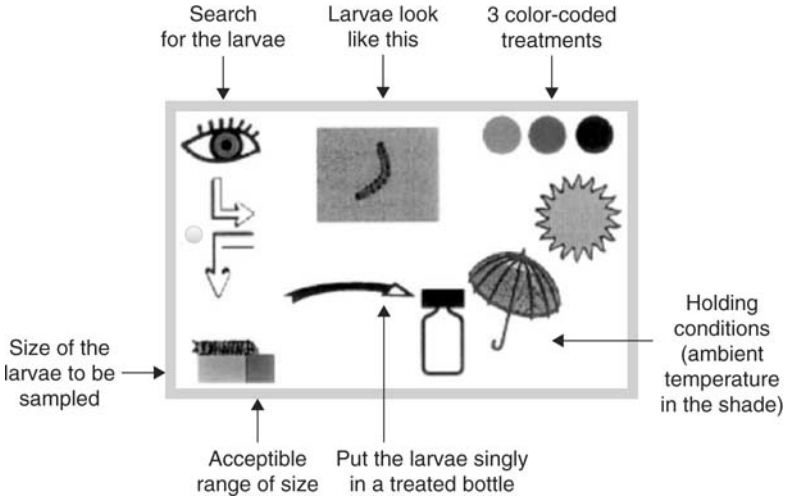

Figure 13.4 Instruction card used by Dr. Steven N. Irving (IRAC) to monitor cypermethrin susceptibility in West Africa.

in rural farming regions where a number of languages are spoken. Prior to initiating the study, Irving organized a small team of bioassay and statistics experts to design the monitoring plan. They decided to use the two-dose (plus control) method to monitor susceptibility. To minimize variability, Irving prepared all of the test vials from a common source of cypermethrin in France and shipped the prepared, color-coded vials to his investigators in Africa. He also sent instructions for use of the vials in the form of simple, plastic-coated cards with a pictorial representation of bioassay procedures (see Figure 13.4). He then trained the local investigators to use the vials in accordance with the instructions, and had the raw data sheets sent back to France for analysis. A graphical summary of the results is presented in Figure 13.5. Irving demonstrated that the pyrethroid cypermethrin was still efficacious. Efficacy was greatest in Senegal and Tchad and weakest in Burkino Faso and Benin. Irving did not observe a relationship between the estimated slopes and EC_{50}s. This highly coordinated effort allowed data from very rural, multicultural regions to be generated uniformly, summarized expertly and reported in the same field season within budget.

Conclusion

Monitoring involves two major steps. First, the arthropod population must be sampled or an indirect estimate of resistance must be measured (e.g., yield loss, control failure). Second, the sample must then be tested or interpreted based on criteria designed to make a judgement about resistance (e.g., bioassay). Some newer approaches, such as the in-field and F_2 screens, try to combine the two steps. In addition to providing an overview of the kinds of assays and statistics that can be used in monitoring programs, this chapter has highlighted three issues that are critical for the

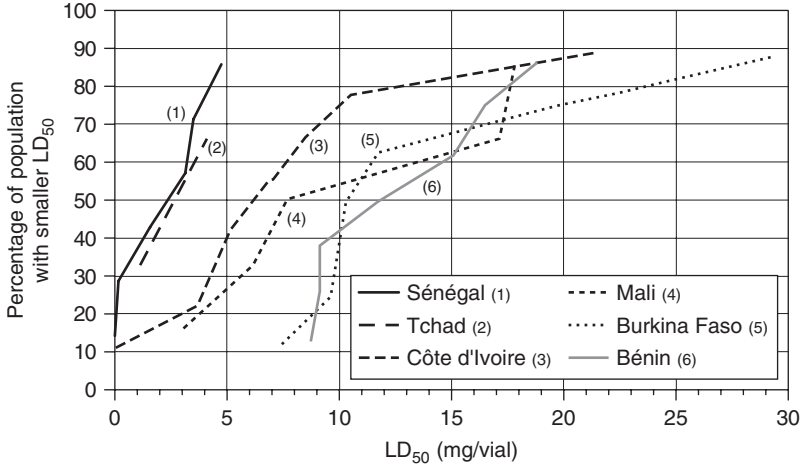

Figure 13.5 Probability plots representing the ranges of susceptibility of different field populations from six West African countries in a monitoring study sponsored by IRAC International and managed by Dr. Steven N. Irving.

economic evaluation of all plans: goals, precision, and cost. These issues pertain to the monitoring of all forms of resistance described in this book.

Monitoring can be an important aspect of a resistance management program. It reveals the current state of the system and allows the magnitude and direction of changes to be tracked. Monitoring may be valuable for assessing population status, understanding potential risks, determining whether a resistance management program is stabilizing the efficacy of a compound, trait or program, and projecting future trends.

Coordination amongst workers and amongst stakeholders is critical for successful resistance monitoring (Brent, 1986). Whether only one agency or corporation chooses to monitor on its own or whether multiple agencies, corporations, public health organizations, farmers, or ranchers all contribute to the monitoring effort, coordination is and communication must be maintained. Thus, coordination for monitoring is no different than the overall coordination required for the implementation of an effective IRM plan.

Since most monitoring programs will involve some type of biological assay, details of a number of techniques were given, to help managers maximize the value of the information generated. In practice, this author has found the two-dose approach very useful. It requires only slightly more resources than a single-dose assay, and gives much of the information given by multiple-dose assays. It has been very useful in practice.

Resistance monitoring has been widely and successfully used in a number of pest management systems. Monitoring should be a tool in a larger decision-making strategy. The strategy should be understood by all participants, and attention should be given to effective communication so that the whole process can nimbly respond to changes in the status of the system under study.

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Chapter 14

The Future of Insect Resistance Management

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The first thirteen chapters of this book have provided a variety of directions for exciting future work in insect resistance management (IRM). By the future, I mean the next few years, not the next few decades. Techniques, hypotheses, strategies, and problems are all changing too much to predict where we will be in a decade. As society places greater responsibility on integrated pest management (IPM) to protect crops, livestock, humans, and the environment, IRM will also change to account for society's goals.

This final chapter presents several case studies that provide additional lessons for IRM scholars. Then I describe recommendations for IRM practitioners that can guide IRM over the next several years.

Case Studies

One of the most serious pests of potato (*Solanum tuberosum*) around the world is *Leptinotarsa decemlineata*. Roush and Tingey (1992) described the biological and management factors that contribute to evolution of resistance by *L. decemlineata* and proposed an IRM strategy for insecticides. Three important biological characteristics of this species include its 40-fold population growth during each generation, the tendency for the populations to inhabit only one or a few treated crops, and the existence of a single mechanism capable of causing broad cross-resistance. The use of soil insecticides and the treatment of all active stages of the pest are the management practices that contribute to the evolution of the pest. Based on this knowledge of the pest and the agroecosystem, Roush and Tingey (1992) recommended that insect resistance can be managed by the replacement of soil insecticides with foliar applications and the rotation of insecticides after each generation. In addition, monitoring of pest densities and applying insecticides only when needed along field edges reduces insecticide use (Roush and Tingey, 1992). They also recommended the use of annual rotation of potatoes with non-host plants to reduce the pest population in alternating years. Crop rotation also supports the reduction in insecticide use, thereby lowering the selection pressure. Thus, the IRM plan reduces population growth, the proportion of each population treated during a generation, and the frequency of insecticide use.

Chemical insecticides, pheromones, and viral insecticides are used to control *Cydia pomonella*, a major orchard pest found in most temperate regions of the world. Eberle and Jehle (2006) describe the first documented case of field resistance by an insect to a commercially-applied baculovirus. The *C. pomonella granulovirus* (CpGV) is a species-specific and extremely virulent pathogen. Several commercial CpGV products are used for microbial control on 100,000 ha of apple orchards (*Malus domestica*) in Europe (Eberle and Jehle, 2006). However, in 2005, reduced susceptibility to CpGV was documented in several populations inhabiting organic apple orchards where control failed despite intensive CpGV application. Eberle and Jehle (2006) performed experiments to understand the inheritance of resistance, which is stably inherited even under non-selective conditions in the laboratory. A resistant strain of *Cydia pomonella* showed 100 times lower susceptibility in bioassays. Eberle and Jehle (2006) concluded that inheritance of resistance to the virus is due primarily to a trait that is incompletely dominant. Additional work is needed to determine whether resistant populations represent previously existing local variations in susceptibility or they evolved during repeated CpGV applications.

In a series of studies, Boivin *et al*. (2003, 2004, 2005) investigated the evolution of changes in maturation in *Cydia pomonella* in France. Boivin *et al*. (2003) investigated pleiotropic effects of resistance on maturation. Resistant homozygotes had significantly shorter developmental times relative to susceptible homozygotes. The resistant moths had much higher propensity for diapause than susceptible phenotypes. Boivin *et al*. (2004) investigated whether genetic variation associated with selection for insecticide resistance may be a source of divergence in the photoperiodic timing of diapause through pleiotropic interactions. Boivin *et al*. (2004) observed higher critical photoperiods for diapause induction in resistant homozygotes than in other genotypes. Boivin *et al*. (2005) then compared model simulations for each genotype with pheromone-trap catches recorded in insecticide-treated orchards over an 8-year period. They found a significant delay in adult emergence relative to the prediction of the model for susceptible homozygotes. The delay was positively correlated with increasing frequencies of insecticide resistance in the sampled field population. The predicted emergence for resistant homozygotes matched those recorded in the field. Boivin *et al*. (2005) suggested that phenological modeling can be used as a forecasting tool for IRM.

Tetranychus urticae is the most common pest of orchards and a frequent target of pesticide applications. This mite has a long history of evolving resistance to acaricides. Flexner *et al*. (1989) determined that fitness costs and immigration of susceptibles could cause reversion of acaricide resistance when selection pressure is relaxed. Flexner *et al*. (1995) also concluded that the immigration of susceptible mites into pear orchards (*Pyrus* sp.) could be important for IRM. They studied the dynamics of resistance in *Tetranychus urticae* in pear orchards for 7 years. They compared five treatments involving two acaricides: (1 and 2) consecutive use of one acaricide (two applications per year), (3) alternation of both within a single year, (4) rotation of both on a yearly basis, and (5) a combination at half rates of both acaricides. Flexner *et al*. (1995) concluded that field durability of the acaricides was not extended by rotations or half-rate combinations compared with consecutive uses, but benefits from these programs may occur because of slow registration of

new acaricides. Alternate, consecutive uses may give greater than 33% longer control compared with control from other programs. However, this advantage depends on which acaricide is used first, because one acaricide conferred cross-resistance to the other. Flexner *et al*. (1995) concluded that better IPM, including the use of economic thresholds and biological control, could reduce the number of applications and delay resistance.

Few scientists have performed field experiments in which the population dynamics and behavior of a pest are modified. These studies are even more difficult when immigration of susceptible insects is manipulated. Imai (1987) performed field experiments to determine how immigration of susceptibles could reduce insecticide resistance in a population of *Musca domestica.* Imai (1987) released 163,000 susceptible flies to mate with resistant flies at a waste disposal site in late 1977. Five months after the releases, susceptibility increased in the field population. For a second experiment, between 31,000 and 46,000 susceptible pupae were used in each of five releases in late 1980. Genetic markers were used to permit morphological identification of susceptible flies. The field population became more susceptible within 6 months after the second series of susceptible-fly releases.

The number of independent origins of insecticide-resistance alleles is often debated. To deal with this issue, we must understand both arthropod movement and genetics. ffrench-Constant *et al*. (1996) offered evidence for multiple independent origins of resistance in two *Drosophila* species, two beetle species, and the *Bemisia tabaci* complex. The repeated replacement of the same amino acid in the resistance to dieldrin (*Rdl*) gene, conferring resistance to cyclodiene insecticides, is a model system which can be used to study the origins of resistance alleles. They used this technique, plus an examination of flanking sequence data, to produce evidence for multiple origins of the same amino acid replacement. These results should be compared to studies of amplified esterases and insensitive acetylcholinesterase in *Culex* mosquitoes (Chapter 7). ffrench-Constant *et al*. (1996) also discussed the importance of life history in determining the likely origin and spread of different resistance alleles.

Guidelines for Managing Insect Resistance

The following section highlights rules that emphasize management. Ecology, molecular biology, and population genetics are important foundations for IRM, but success in the real world depends on using knowledge to accomplish important goals. These rules can be used to keep our attention on the management side of IRM. They are not listed in order of importance or in the sequence that they might be followed. They all are important and should be considered simultaneously.

A. Consider IRM within an IPM Framework

As explained in Chapter 1, IRM will be more effective and more valuable when it is incorporated into IPM. The best IRM will take advantage of the best IPM, including all design and control opportunities. Both IPM and IRM must deal with problems related to pest phenology and pest behavior, and sometimes these problems can be turned into opportunities to improve management. IRM may complicate IPM

because of the use of refuges for susceptibles and tactics involving negative cross-resistance, but these complications will be less than those caused by the evolution of resistance. As with IPM, IRM cannot simply rely upon and reuse old tactics. New IRM strategies must be proposed, evaluated, implemented, and evaluated again to improve management of insect resistance.

However, the relationship between IPM and IRM may not always be easy, particularly if stakeholders and scientists continue to view the goals of IPM and IRM as separate issues. For example, Onstad *et al*. (2003) warned about the conflicts that will continue to exist, if IRM is kept separate from and perhaps even elevated above IPM. They stated,

> Management recommendations and decisions by growers must be made even when we do not know how evolution will develop and when new phenotypes will invade our landscapes. IPM must address management concerns without being overwhelmed by these uncertainties and being overridden by IRM. In its best sense, IPM has accounted for both the ecological and economic factors of a problem. Too often in the past, evolutionary changes in pest populations have caused scientists to emphasize IRM over IPM, as if one gene and one risky tactic was more important than basic IPM. Perhaps this narrow focus on IRM makes sense when only one tactic is available. But some believe that IPM cannot be true IPM if it permits evolution of resistance. From this perspective the extensive use of crop rotation (for insect control), though very effective for many years, was not truly IPM. However, resistance by insects to highly effective IPM strategies is inevitable, but long-term success for IPM is not. Thus, we must constantly strive for better approaches that combine landscape design, host plant resistance, biological control, and other feasible tactics for pest management.

B. Choose Explicit Goals and a Time Horizon

Decision makers must have clear goals with explicit descriptions of the time horizon and the area over which IPM and IRM will be implemented and evaluated. A time horizon is a time period during which we observe, measure, and manage an ecological or agricultural system. A time horizon permits the specification of a clear point in time (at end of horizon) at which a final decision will be made. Thus, at the end of a given time horizon, the effectiveness of management is evaluated. For example, when you invest money in stocks or bonds, your goal is to earn a significant return on your investment over a time horizon. During the period, stock values will rise and fall, but the primary issue is whether you will have significantly more money at the end of the time horizon when you want to spend the money.

A time horizon also defines the set of observations and calculations that can be compared with those from another scenario for the same period. Time horizons and spatial scales are rarely specified, even in a vague way, except in model simulations. Modelers encourage stakeholders to be more rigorous in the specification of their goals, time horizons, and spatial scales (Chapters 2 and 4).

Goals and management plans can take many forms. Is the goal to delay evolution of a gene over 20 generations in one county? Is the goal to control the pest over

40 generations in the cotton belt of the United States? Is the goal to minimize cost, including damage and control costs, on one farm until retirement of the farmer? Each stakeholder may have a goal: some similar and some different. These will influence the goals of the real agencies, corporations, and individuals that make the actual IPM and IRM decisions. Thus, political and economic factors influence which goals are to be emphasized.

C. Account for the Issues Related to Coordination of Stakeholders

Coordination is critical for successful IRM (Chapter 1). At a minimum, coordination permits management of resistance across a large region and over a long period of time. The coordination may lead to actual cooperation amongst stakeholders and the sharing of resources and costs. Otherwise coordination can be implemented, possibly less effectively, by a centralized agency with legal authority to require certain IRM practices. Mosquito control agencies are an obvious example of the latter scenario. In the interest of public health, one or two agencies coordinate regional control of mosquito populations and manage the resistance to control tactics.

In agriculture, the Arizona case with transgenic insecticidal cotton (*Gossypium hirsutum*) and *Pectinophora gossypiella* clearly demonstrates the value of cooperation amongst public agencies and groups of growers. Data collection and education were facilitated by this cooperation (Chapter 9). Caprio (1994) demonstrated how cooperation by farmers influences evolution of resistance in one of his models. His results suggest that some stakeholders will not always benefit directly from cooperation. Nevertheless, coordination may be able to take advantage of many resources and behaviors not typically included in models. For instance, the coordination of IRM and IPM across major cropping systems for major pests in Arizona, including *Bemisia*, is a lesson that should be learned by all working on future IRM problems (Chapter 5).

D. Adjust the IRM Plan for Local Social and Environmental Conditions

The example of *Bemisia* IPM and IRM in Arizona also demonstrates how management can be adjusted and optimized for local, social, and environmental conditions (Chapter 5). The scientists devising strategies accounted for the needs of local farmers growing multiple crops. The work of Onstad *et al*. (2002) showed that IRM for transgenic insecticidal crops must be different under irrigated and non-irrigated conditions. In the United States, transgenic insecticidal corn is regulated differently in regions with transgenic insecticidal cotton than it is in regions without cotton. In Texas where cotton is grown, refuges must be 50% of the cornfields, whereas in northern states the refuge can be 20% of cornfields. Chapter 10 describes many other situations in which the environment must be considered in the management of insect resistance. The bottom line is that one to three strategic options for IRM do not cover all the pests in all environments managed according to all possible goals of stakeholders. IRM strategies must be flexible and dynamic.

E. Evaluate the Economics of Each IRM Strategy

Management requires goals and a time horizon, and both of these must reflect the interests and values of the stakeholders and decision makers. Human values may

not always be quantifiable and capable of being expressed in traditional economic terms, but economics can certainly be a starting point for discussion of these values, trade-offs, and differences amongst stakeholders. It is often difficult not only to place an economic value on natural resources and ecosystem services, but also to justify an extremely narrow focus on only one resource, such as pest susceptibility, without considering the consequences. At a minimum, stakeholders should discuss how they value goods and services in the present versus those in the future and which aspects of the pest management system they value the most (Chapter 2). Some stakeholders will value ecosystem services more than they value crop yield, while others will value crop yield more.

Economics provides methods for allocating resources and time to solve multiple problems with efficiency and equity. This allocation of resources occurs at various scales, which may or may not be apparent to the stakeholder. For example, scarce resources can be allocated for (1) choices related to IRM for a single pest, (2) choices for IPM concerning a single pest on a single crop, (3) protection of multiple crops from multiple pests, and (4) crop protection and other important environmental problems within the overall economic system. Without the use of economic analyses, allocations will likely be less equitable and efficient. Will our grandchildren believe that we allocated too much or too little for IRM compared to managing global climate change?

Understanding economics and human behavior will also help scientists better predict cooperation and compliance with IRM strategies and regulations (Chapter 11). Farmers will likely consider the economics of IRM adoption and compliance in the context of their overall business and farming operation.

F. Predict the Risks and Implement Preventative IRM

Scientific IRM must provide support for strategies that delay the evolution of resistance or at least manage it within the goals of the stakeholders (Chapter 1). Scientific IRM uses general hypotheses and models to initiate plans and then obtains and analyzes data specific to the pest species and the management system to improve the strategies.

Roush (1997, 1999), Curtis *et al*. (1993), and Siegfried *et al*. (1998) advocate preventative IRM instead of curative or reactive strategies. Roush (1997) states, "The substitution of new toxicants to replace those that have failed is not resistance management, as it does nothing to preserve susceptibility." Curtis *et al*. (1993) conclude that reactive IRM and the switching of chemicals "can hardly be called a strategy for resistance management."

Prediction is the foundation for prevention. Although Gould (1998) and Carrière (2003) have demonstrated how the history of species in natural environments can be used to explain and predict the consequences of IRM, they would agree that modeling is one of the best tools for predicting future consequences and scenarios (Chapter 12). For example, Caprio (1998) used a stochastic model to evaluate five IRM strategies: sequential introduction of two toxins, rotations and mosaics of toxins, and half- and full-rate mixtures of the toxins. When an economic threshold for applying an insecticide based on pest density was adopted along with

refuges, Caprio (1998) demonstrated that full-rate mixtures have the potential to effectively delay resistance evolution. Simulations comparing monogenic and polygenic inheritance of resistance indicated that resistance took twice as long to evolve in the polygenic simulations. Caprio *et al*. (2006) used a similar model to investigate the history and assess the future risks of insecticide resistance by *Diabrotica virgifera virgifera*.

Carrière and Tabashnik used a combination of modeling and empirical work to explain the success of IRM for a *Pectinophora gossypiella*. In 1997, extensive plantings of transgenic insecticidal cotton began to exert selection pressure on populations in Arizona. Carrière and Tabashnik (2001) used models to understand how the high-dose/refuge IRM strategy could be used to delay and even reverse resistance. (The high dose of toxin is expected to kill almost all heterozygotes, making the resistance gene functionally recessive.) Monitoring for resistance indicated that mean frequency of resistance by *P. gossypiella* did not increase over an 8-year period (Tabashnik *et al*., 2005). Tabashnik *et al*. (2005) concluded that this delay in resistance can be explained by four factors. Arizona farmers complied with IRM regulations by planting non-toxic cotton refuges, which were sources of susceptible moths. With regard to population genetics of the pest, resistance is recessive, but fitness costs are significant. Tabashnik *et al*. (2005) also considered incomplete resistance, or the limited survival of resistant homozygotes on transgenic cotton, to be another factor causing the delay in resistance evolution.

G. Understand, and Possibly, Alter Pest Behavior

Case studies throughout the book have demonstrated the importance of pest behavior in IRM (particularly Chapters 1 and 8). Waldstein *et al*. (2001) measured the rate at which larvae of *Choristoneura rosaceana* abandoned feeding sites on apple branches. Larvae frequently changed feeding sites, switching from older leaves to actively growing foliage with sublethal insecticide residues. Waldstein *et al*. (2001) postulated that this behavior may increase larval survival and could slow evolution of resistance to insecticide by providing a refuge for susceptible insects. At a minimum, behavior must be understood to improve IRM or prevent disasters (Gould, 1991). In some cases, we can take advantage of pest behavior to cause the targeted pest to react to management in a certain way. However, we should also be aware that pest behavior can evolve. One of the oldest examples of behavioral resistance was avoidance of malathion baits in *Musca domestica* (Schmidt and LaBrecque, 1959). Gould (1984) was the first to model the evolution of behavioral resistance.

Onstad and Buschman (2006) used a model of *Ostrinia nubilalis* to evaluate the effectiveness of oviposition deterrence in transgenic insecticidal cornfields (*Zea mays*) for IRM. The population genetics of two genes was simulated: one for resistance to transgenic insecticidal corn and one for insensitivity to deterrence. They simulated two types of hypothetical deterrence: one has moths reducing their oviposition because of lost opportunities to lay eggs and the other has the deterred moths moving to the refuge to lay the eggs. Oviposition deterrence was clearly effective in extending the time to resistance to transgenic insecticidal corn. The

time to 50% frequency for the allele for resistance to transgenic corn was similar for the two types of simulated deterrence, but the pest densities were 100-fold higher when the deterred moths oviposited in the refuge.

H. Monitor Resistance Only When the Benefits Are Worth the Costs

To some extent, monitoring programs for IRM are pessimistic. If a scientifically based IRM strategy is implemented and complied with, we should have faith that resistance will not evolve (Hurley and Mitchell, Chapter 11; Roush, 1989). Unfortunately, the ability of arthropods to evolve and the chaotic and stochastic dynamics of natural systems should not be underestimated. Thus, scientists and stakeholders will always consider designing and implementing monitoring plans.

Stanley (Chapter 13) states that monitoring programs can be very expensive if rare resistant arthropods are sampled for. We can more cheaply monitor for high levels of resistance in a population. Thus, it is feasible to determine whether a control tactic has already failed or will fail relatively quickly. On the other hand, we will monitor what we value. Hence, the more valuable the damage caused by the pest, the more likely we can justify the cost of a monitoring program. For this reason, we should expect greater monitoring for resistance in vectors of human and livestock diseases. The World Health Organization (WHO) and the Insecticide Resistance Action Committee (Nauen, 2007) encourage the monitoring of vectors of human-disease using bioassays designed or approved by the WHO, but the feasibility and costs of surveys are not highlighted in most planning documents.

Nyrop *et al*. (1986) suggested that the cost of sampling and the uncertainty of the information provided by the sample be incorporated into cost-benefit analyses for pest-control decision making. Without such a procedure, sampling too often is perceived as free by many scientists and stakeholders.

Sampling can be more easily justified if it is a normal part of IPM. Stakeholders may already sample for insects or the damage caused by the pests. Additional steps could be added to the process, such as increasing the number of units sampled or sending specimens to a central agency for processing. Sampling that is valuable for IPM may be adjusted to contribute to IRM. One approach would be to use the thousands of farmers or ranchers as the monitors for the consequences of resistance. This would permit the feasible monitoring of a much larger region to determine when resistance becomes a problem. In this scenario, much greater coordination and training would be required than that needed for IPM monitoring.

I. Prepare for Evolution of Detoxification, Behavioral Modification, and Mechanisms of Maturation

Arthropods can evolve resistance in many ways. Every control tactic used against the pests can be overcome by modification of more than one mechanism. Chapter 3 presents the wide variety of molecular, biochemical, and anatomical mechanisms of detoxification. Chapters 1, 7, 8, and 9 provide examples of new behaviors that have evolved in arthropods. Arthropods can also be selected for changes in maturation that either shorten or prolong one or more stages in their life cycles (Chapters 8 and 12). *Cydia pomonella* evolving in many apple orchards demonstrates the

significance of this phenomenon. Changes in maturation, particularly diapause, permit the pest to avoid harm from a control tactic (Chapter 8, Carrière *et al*., 1995).

Of course, two or more mechanisms can evolve simultaneously. Thus, scientists must be prepared to deal with a variety of mechanisms. In fact, every process in a population dynamics model can be a mechanism of resistance evolution and be represented in a population genetics model with genetic variation that can be selected for or against. The more details the demographic and behavioral model contains, the more complicated the genetic model can be. The key is to determine which processes are under the greatest selection pressure and how much genetic variation exists for that mechanism. Perhaps some day, we will have a large database with information about genetic variation that will help us make predictions.

Databases with information about resistance mechanisms may also allow us to more easily take advantage of negative cross-resistance (Chapter 6). Genomic information concerning intoxication and detoxification will obviously be valuable knowledge (Chapter 3).

J. Do Not Delay Implementing IRM

Chapters 1, 8, and 9 clearly demonstrate that arthropods can evolve resistance to host-plant resistance, cultural control (crop rotation), and biological control as well as pesticides. Once we accept the high probability of resistance evolving to effective pest management, we must consider how quickly we should implement IRM. Certainly, economics and local social and cultural conditions influence implementation (Guidelines D and E). Nevertheless, the work of several authors support the argument that IRM should be implemented relatively soon after a control tactic becomes part of an IPM program. We should not wait until we understand the pest and its ecosystem perfectly.

Croft (1990) stated that many scientists believe that, although genetics, biochemistry and physiology determine whether resistance evolves, these are usually not the primary determinants influencing the rate of resistance evolution. Instead, it is the ecological genetics of resistance (including gene flow and population dynamics of selection) that largely determines the progression of resistance evolution.

McCafferty (1999) reviewed the status of resistance by Lepidopteran species in the genera *Heliothis* and *Helicoverpa*. Pest species in these genera are polyphagous, distributed worldwide, and have evolved resistance to a variety of insecticides. McCafferty (1999) concluded that, despite our rapidly increasing knowledge of the biochemical and molecular nature of resistance, the most effective IRM for the heliothine Lepidoptera remains a strict control of insecticide use. He cited the cotton program in Israel, which included a dramatic reduction in the number of insecticide applications and the use of several IPM tactics, as the most successful IRM program for these pests.

Roush (1989) clearly stated that stakeholders should not wait until genetic or molecular mechanisms of resistance or fitness costs are understood before implementing IRM. He argued that most IRM strategies should be effective against most, if not all, mechanisms and that the strategy can and should evolve as more knowledge is learned about the system. He even believed that species- and system-specific modeling would not be necessary to choose and implement the initial

IRM strategy for an insecticide. His main concerns about implementation are social issues, such as stakeholder compliance with a rational strategy (Roush, 1994).

Conclusion

The guidelines expressed above are clearly not meant to prescribe how a farmer or any other individual stakeholder should implement IRM. These are guidelines for scientists, regulators, and leaders of stakeholder groups to assist with decision making. Plans, policies and strategies are not easy to develop when we must understand dynamic, heterogeneous situations and prepare for an uncertain future.

We must learn to take advantage of biology, economics, and predictions to manage insect resistance effectively. Much hard work remains to be done, and we need all the help we can get. We know that arthropods can become resistant to any control tactic, including cultural control, such as crop rotation, and microbial control, exemplified by the cases of *Cydia pomonella* and its virus and *Hypolimnas bolina* and *Wolbachia* (Charlat *et al*., 2007). Thus, the study of IRM should no longer be considered an activity only for those that choose to work on or with pesticides. IRM should be given much more attention in courses and training programs at universities throughout the world.

I encourage students, scholars, and practitioners to focus on the positive, perhaps even optimistic, aspects of insect resistance management. We are far from delaying many future cases of resistance, but we are at least learning to implement and even improve IRM. People coordinating, regulating, and implementing IRM on all the continents deserve our praise for their efforts.

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