DEVELOPMENT OF INSECT RESISTANCE TO BIOPESTICIDES

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ABSTRACT – Resistance to a number of different microorganisms has been documented in wild insect populations and deliberately induced in laboratory populations and domesticated insects. In recent years, rapid progress in introduction of toxin genes from insecticidal bacilli into agricultural crops has raised concern that resistance to insecticidal bacteria may reduce the usefulness of these valuable microbial control agents. Mechanisms which have been implicated in resistance of insects to pathogens include changes in behavior, cuticle, and midgut cell turnover, reduced binding affinity of midgut proteins, and maturation immunity. Development of resistance to pathogens, particularly to those which do not act by means of a toxin, is expected to be of a different nature than resistance to chemical insecticides, and may proceed more slowly.


DESENVOLVIMENTO DA RESISTÊNCIA DOS INSETOS AOS BIOPESTICIDAS

RESUMO: A resistência a uma série de diferentes microrganismos tem sido comprovada em grupos de insetos selvagens e foi deliberadamente induzida em populações de laboratório e insetos domesticados. Nos últimos anos, o rápido progresso na introdução de genes de toxinas de bacilos inseticidas em culturas agrícolas aumentou a preocupação de que a resistência a bactérias inseticidas pode reduzir a utilidade destes potentes agentes de controle microbiano. Os mecanismos que têm provocado a resistência de insetos aos patógenos incluem mudanças no comportamento, na cutícula, mudança no ciclo das células do intestino médio, redução da afinidade de proteínas no intestino médio e imunidade à maturação. O desenvolvimento da resistência aos patógenos, em especial aos que não atuam por meio de uma toxina, deverá ser de natureza diferente da resistência aos inseticidas químicos e poderá progredir mais lentamente.

Termos de indexação: Bioinsecticidas, Seleção para resistência, Mecanismos de resistência, Resistência em vetores, Resistência em populações naturais.

INTRODUCTION: THEORETICAL BASIS FOR RESISTENCE

Intraspecific variation in susceptibility to pathogens among animal and plant populations is a universal phenomenon. Many of these differences are genetically based, and therefore strong continuous selection pressure would be expected to lead to development of a population which requires a higher concentration of pathogens to kill a representative proportion of the host population. Why, then, does any animal or plant population remain susceptible to pathogens? First, the interaction between a pathogen and its host is a very old, complex, and dynamic relationship. Two genetic pools interact in this relationship, that of the host and that of the pathogen. In most cases, the generation time of the pathogen is much shorter than that of the host, the genetic compliment of the pathogen is much simpler (e.g. haploid) permitting immediate expression of a mutation, and the pathogen’s genotypic and phenotypic makeup may be quite plastic. As an example, two granulosis...
viruses of *Pieris* spp. exhibit 97.7% DNA homology, but are very different in virulence to *P. brassicae*, although not in virulence to *P. rapae* (Crock 1981). Second, a high level of mortality due to pathogens may not be common in most natural situations. Epizootics are certainly seen in insect populations, but these events are virtually always followed by a period of reduced host population during which the proportion of hosts infected is small. These periods of reduced pathogen mortality relax the pressure on the genetic pool of the host permitting the replenishment of susceptibility in a significant proportion of the population (Evans 1986; Briese 1986). Third, the assumption that resistance to a pathogen is of significant benefit to the host may be false. Successful pathogens are well adapted to the normal physiology and behavior of the host, taking advantage of cuticular lipids, midgut proteins, gut pH, feeding behavior, or other aspects of the host to gain entrance. In order to become resistant to attack by the pathogen, the host insect is obliged to make a change in a physiological or behavioral parameter which presumably is in place because it confers fitness on the host. In the absence of the pathogen, or during periods when the pathogen exerts a low level of pressure on the host population, resistance may in fact be detrimental. Resistance to chemical insecticides has been shown to result in reduced fitness (Roush & Plapp 1982). Finally, in highly mobile insects, migration will lead to constant infusion of susceptible genotypes into the selected population (Anderson 1986).

There are several differences between the nature and use of chemical insecticides and microbial insecticides which are important to the consideration of the possibility of resistance to pathogens. The nature of chemical insecticides is novel; for the most part these molecules have never been encountered by the insect target prior to their development by man. Chemical agents often kill by interaction with a single host system. And finally, chemical insecticides are relatively inexpensive and stable to storage, permitting their use over large areas in multiple applications. Microbials, in contrast, are organisms with which the host insect has had contact for perhaps millions of years, which may interact with many host systems before causing death, and which are both expensive and unstable to storage and to environmental exposure. Therefore these agents, to date, have not been used widely or frequently. The insecticidal protein toxins of the Bacilli, e.g. *Bacillus thuringiensis*, represent possible exceptions to these principles, in that they are essentially chemical insecticides, produced by a microbial agent. They are, however, very complex molecules of great variety with complex modes of action, apparently with a long history of interactions with insects, and unstable to environmental exposure. These toxins therefore share some characteristics with both chemical insecticides and microbial pathogens. The remainder of pathogenic microorganisms, however, are very different in their interactions with their hosts from chemical insecticides, and the development of resistance to these organisms would be expected to be slower and of a different nature than resistance toward chemicals (Boman 1981).

A change in the response of an insect population following selection pressure may be reflected in a reduced proportion of insects which succumb to a given discriminating dose of the pathogen, and/or an increase in the LC$_{50}$ or LD$_{50}$. Insect populations, particularly wild populations, consist of a mixture of individuals with varying susceptibility, and elimination of the most susceptible individuals may lead to changed response without the presence of true "resistance". The elimination of the susceptible proportion of the population leads to a significantly steeper dosage-mortality regression slope, suggesting reduction in genetic variability. In field populations, immigration of susceptible
individuals may be expected to lead to a return of genetic variability within a few generations (Briese 1981, 1986). Most studies of resistance have involved laboratory populations, although in some cases these were recently derived from field populations. Genetic variability in these populations is much reduced from that present in the field, and there is no opportunity for immigration of susceptibles. Therefore the stability of resistance induced in laboratory populations, or the failure to induce resistance under deliberate selection, may not actually reflect the field situation but rather the narrow genetic base of the test population. Laboratory populations are often more resistant than field populations, due to interbreeding and unintentional selection with the pathogen during long periods of laboratory culture. Real shifts in overall resistance to baculoviruses have been demonstrated, however, in some cases with a known genetic base. No pattern of genetic basis for resistance has appeared; complex or simple genetic bases have been described, and either dominant or recessive alleles may be involved (Briese 1981, 1986).

DELIBERATE SELECTION FOR RESISTANCE

Deliberate selection of “domesticated” insects for resistance to microbial pathogens is a long-standing practice. In the 1860’s Pasteur selected a strain of the silkworm, Bombyx mori, for resistance to Nosema bombycis, and among the 700 or so races of the domestic silkworm in Japan and China, are several which have been selected over many generations for resistance to viral, fungal, bacterial and protozoan diseases. In one case, selection of the silkworm with cytoplasmic polyhedrosis virus produced a rapid increase in resistance to 10-20 fold within eight generations, probably due to reduction in genetic variability within the selected population (Watanabe 1967 reviewed by Briese 1981). Up to 50-fold resistance was induced in the silkworm to two B. thuringiensis products, Bacotopeine and Thuricide (Aizawa et al. 1962, Aizawa 1971). Strains of the honey bee, Apis mellifera, have been bred for resistance to American Foulbrood disease, caused by the bacterium Bacillus larvae (Rothenbuhler & Thompson 1956, Hoage & Rothenbuhler 1966). Resistance to “hairless-black syndrome” of bees, a viral disease, has been enhanced by deliberate selection (Kulincevic & Rothenbuhler 1975), and differences among bee races in susceptibility to fungi and protozoa have also been reported (Briese 1981). Domesticated insects are unique in that their breeding is totally controlled, in the case of silkworms, or at least readily monitored, in the case of the honey bee. Maintenance of resistance in these populations is therefore different than in populations of wild insects.

RESISTANCE IN VECTORS

Because of their importance to man, the interaction of vectored mammalian pathogens (which also infect the insect vector) with the biting insects has been studied extensively. Resistance to malarial parasites and to vectored filarial nematodes in mosquitoes has been well documented, and in nearly all cases appears due to a dominant gene (Briese 1981, Anderson 1986). Deliberate or unintentional selection of laboratory mosquito populations for resistance to pathogens has produced mixed results. The practice of returning uninfected mosquito pupae to the breeding colony following challenge with mosquito-parasitic nematodes led to resistance to the nematodes in two cases. Anopheles quadrivaculatus larvae from a selected colony were found to require a much higher nematode: mosquito larva ratio to produce a high level of infection by Dixinemis petersoni than did unselected larvae (Woodard & Fukuda 1977). Culex quinquefasciatus similarly developed resistance to Romanomermis culcivorax following 300
generations of selection (Petersen 1978). In the first case, behavioral changes in selected larvae were observed, which led to reduced efficiency of parasite penetration. Selection of mosquitoes with Bacillus thuringiensis var. israelensis has not led to greatly enhanced or permanent resistance. Laboratory populations of C. quinquefasciatus, recently established from field populations, were treated with B. thuringiensis var. israelensis at varying levels of selection over 32 generations, producing only a 5-7-fold increase in resistance. Almost complete recovery of susceptibility was found after 3 generations without selection pressure (Vasquez-Garcia 1983). A two-fold increase in resistance was observed in only one of three Aedes aegypti populations following 14 generations of selection with B. thuringiensis var. israelensis (Goldman et al. 1986). A similar 1.9-fold increase in LC_{50} was produced by selecting A. aegypti over 25 generations in another study (Gharib & Szalay-Marzso 1986). Following seven years of extensive use of B. thuringiensis var. israelensis in the West African Ochocerciasis Control Programme, susceptibility of the black fly, Simulium damnosum, has remained unchanged (Kurtak et al. 1989).

Selection of a laboratory population of C. quinquefasciatus with Bacillus sphaericus toxin over two years at 80-90% mortality led to 3-fold resistance to the purified toxin but only 1.4- to 1.8-fold resistance to the spores of this organism. The mosquito colony selected with B. sphaericus toxin exhibited a steeper dosage/mortality slope and decline in vigor of the colony, suggesting loss in genetic heterogeneity.

RESISTANCE TO VIRUSES IN WILD POPULATIONS

Among wild populations of insects, fewer cases of resistance to pathogens have been documented, perhaps only because of lack of study. Resistance to infection by a granulosis virus was observed in a population of European larch budmoth, Eucosoma griseana, following an epizootic of this virus (Martignoni 1957), and similar changes in susceptibility to nuclear polyhedrosis virus occurred in populations of the California oakworm, Phygoaedia californica, following an epizootic (Martignoni and Schmit 1961). Although the latter study was published almost 30 years ago, it still represents one of the most carefully analysed studies of resistance in a field population. The authors presented the hypothesis that resistance, followed by gradual return to susceptibility, may be responsible for the cyclic occurrence of viral epizootics in insect populations. Field populations of pea aphids, Acrythosiphon pisum, were found to contain a biotype naturally resistant to the fungal pathogen, Erynia neoaphidis (Milner 1982). Significant differences in infectivity of fungal isolates were also found.

RESISTANCE TO BACILLUS THURINGIENSIS

Because of the commercial importance and widespread use of lepidopteran-toxic B. thuringiensis products, and recent progress in introduction of B. thuringiensis toxin genes into crop plants, a number of studies have examined the possibility of induction of resistance in target insects to these toxins. Highly variable levels of control of the stored grain pest, Ploidia interpunctella, were found in wild populations from grain bins treated with B. thuringiensis and in laboratory populations (Kinsinger & Mcgaughey 1979). Failure to control this pest in grain bins with B. thuringiensis was later shown to be due to resistance, in some cases apparently developed over a single year's treatment (Mcgaughey 1985, 1990). Using a laboratory colony developed from a field population which was not adequately controlled by B. thuringiensis, Mcgaughey (1985) selected with B. thuringiensis to produce an increase of 30-fold in resistance in two generations and 100-fold
after 15 generations. Enhanced resistance developed similarly in four other selected colonies. Slopes of the dose-mortality relations did not shift significantly, suggesting development of true resistance, rather than simple elimination of susceptibles from the population. In further studies, a resistance level of up to 250-fold was developed in one colony. Selection of the almond moth, Cadra cautella, however, led to only 7-fold increase in resistance over 21 generations (McGaughey & Beeman 1988). The resistance trait appeared to be recessive or partially recessive. Maintenance of resistance after release of selection pressure was variable, with decline in resistance in some but not all colonies (McGaughey 1990). Resistance in P. interpunctella is apparently restricted to the toxins present in var. Kurstaki strain HD1 (Dipel), against which these colonies were selected. Larvae selected for resistance to Dipel were also resistant to cloned HD1 and HD73 toxins expressed in E. coli, but these larvae were not resistant to toxins of several other types, in particular those produced by var. aitawai (McGaughey & Johnson 1987, Han et al. 1988). Resistance in this insect therefore appears to be specific for the types of B. thuringiensis toxins against which it was selected. Interestingly, a highly elevated level of resistance to granulosis virus was also demonstrated in P. interpunctella (Hunter & Vigneswaren 1983; pers. commun. cited in Briese 1986). As a stored grain pest, P. interpunctella encounters microbial pathogens in an almost constant environment, without the sunlight load or seasonality experienced by field crop pests. These environmental factors, and perhaps intrinsic genetic heterogeneity of this insect, may make it particularly susceptible to the development of resistance.

A tobacco budworm, Heliothis virescens, colony was selected with a 130 kDa B. thuringiensis var. kurstaki HD1 toxin expressed in Pseudomonas fluorescens. Gradual increase in resistance up to 24-fold was observed by the seventh generation. Probit line slopes were identical between selected and nonselected lines, again suggesting the development of true resistance. The selected strain was only ca. 4-fold resistant to purified HD1 toxin and to Dipel containing this toxin. Thus, P. fluorescens and the endotoxin acted synergistically, or the larvae were most resistant to the toxin in the form in which it was used in selection, i.e. in the P. fluorescens cell. The resistance trait was stable over two generations of relaxed selection (Stone et al. 1989).

Tabashnik et al. (1990) have recently reported the first well documented case of resistance following use of commercial B. thuringiensis products against a field crop pest. Populations of the diamondback moth, Plutella xylostella, from farms in Hawaii which were repeatedly treated with Dipel, were found to be up to 41-fold more resistant than laboratory populations or field populations which had minimal exposure to B. thuringiensis. The recommended field application rate was effective against 60-100% of unselected larvae, but killed only 34-35% of the insects from treated fields. Approximately two-fold increase in resistance was found over three years in another field treated with Javeline, containing B. thuringiensis strain NRD-12.

MECHANISMS LEADING TO RESISTANCE

Resistance can be caused by a change at any point in the complex interactions between a pathogen and its host, preceding the lethal insult to the host (Table 1). Behavioral changes, for example, apparently led to resistance of mosquito larvae to parasitic nematodes (Woodard & Fukuda 1977). Larvae from the selected colony defended themselves very actively against penetration by the nematodes. Bees from colonies resistant to American foulbrood disease clean the combs and remove diseased larvae more efficiently (Rothenbuhler 1964). Changes in the cuticle

TABLE 1. Changes which may lead to resistance of an insect to a pathogen

1. Behavior which avoids contact with the pathogen.
2. Cuticular changes which render the insect less vulnerable to penetration.
3. Enhanced humoral or cellular immune response.
4. For ingested pathogens, changes in the midgut including antimicrobial properties of gut juice, pH, enzymes, peritrophic membrane, or midgut cell turnover time, enzymatic detoxification.
5. Maturation immunity, expressed as enhanced development rate and more rapid cellular turnover.
6. Altered target, e.g. membrane glycoprotein receptors.
7. Intracellular alterations, including internalization, transport, lysosomal acidification, and target of action mutants.
8. Enhanced intracellular defenses, including detoxification and intracellular immune responses. Exocytosis.

1 Modified from Boman (1981).

of the host may lead to enhanced protection from pathogens such as fungi (Koidsumi 1957, Koidsumi & Wada 1955). Even if a pathogen is successful in penetrating the cuticle, the cellular and humoral immune systems of the host may be enhanced to lead to more efficient encapsulation or other response to invasion (Boman 1981).

In some cases, maturation immunity appears to be involved in resistance. In many insect species, increasingly larger pathogen dosages are required to produce mortality with each larval instar, and the rate of this dosage increase may or may not parallel the increase in size of the larvae. Honey bee larvae from colonies susceptible to American Foulbrood disease can be infected by this bacterium during the first 48 hr of age, whereas larvae from resistant colonies become resistant by 36 hr (Bamrick & Rothenbuhler 1961). This resistance appeared to be related to the rate of development of the peritrophic membrane (Bamrick 1964; Davidson 1973). Maturation immunity to baculoviruses has been well documented, and was correlated with increase in larval weight in three lepidopteran species. A sharp increase in resistance of the larvae was observed at mid-fifth instar in four species, probably related to the fact that the full viral cycle requires more time than remaining before pupation. Full maturation resistance was expressed at ca. 30% of maximum larval weight (Evans 1981, 1986; Briese 1986). Maturation resistance to viruses may involve "dilution" effects, e.g. reduced probability of attachment of viral particles to the midgut cells in the midgut of older larvae, due to increase in the volume: surface area ratio. Additional defense mechanisms may also be operative (Briese 1986).

For pathogens which gain access to the host through the gut, the peritrophic membrane, gut juice, and rapid midgut epithelial cell turnover are all potential barriers to infection. The midgut barrier is probably the primary mechanism of resistance to baculoviruses. Discharge of infected cells into the lumen, followed by regeneration of uninfected cells, is an important component of this resistance, and this turnover is related to maturation immunity as well. The rapid turnover of these cells at metamorphosis may be the cause for greatly reduced susceptibility of insects during this period (Briese 1986).

Bacillus thuringiensis delta-endotoxins and B. sphaericus mosquito larvicial toxins require activation by midgut proteases at high pH to achieve full toxicity, and changes in the proteolytic activity or midgut pH would be expected to change susceptibility of the larvae. Proteases from B. sphaericus toxin-resistant Aedes aegypti larvae were found to be similar to those of susceptible C. quinquefasciatus larvae in their ability to

activate this toxin (Aly et al. 1986; Davidson et al. 1987b; Broadwell & Baumann 1987). Johnson et al. (1990) found that midgut proteases of susceptible and resistant strains of P. interpunctella were similar in their ability to activate B. thuringiensis protoxin, suggesting that resistance of these larvae was not expressed at the level of midgut proteolytic activity.

Certain viruses and bacterial toxins have recently been shown to bind to glycoproteins on the membrane of midgut cells (Uchima et al. 1988, Knowles & Ellar 1986; Haider & Ellar 1987, Davidson et al. 1987a, Davidson 1988). A subtle change in these receptor glycoproteins may lead to failure of the viral particle or toxin particle to bind to the midgut cell, circumventing the pathogenesis process. Resistance in P. interpunctella to B. thuringiensis delta-endotoxin was found to be correlated with a 50-fold reduction in affinity of the membrane receptor for the toxin type (Cry IA(b)), used to select for resistance, however the population of receptors for this toxin on the cell membrane was unchanged. The resistant insects were susceptible to a second type of toxin (Cry IA(c)), not used for selection, and an increase in concentration of binding sites for this toxin was found in resistant insects (McGaughey & Johnson 1987; Han et al. 1988; Rie et al. 1990). Two distinct molecular changes have apparently occurred in these insects; a reduction in affinity (but not concentration) of one binding site, with a concurrent increase in concentration of a second, distinct binding site (Rie et al. 1990). These results suggest that the B. thuringiensis toxin receptor has a necessary function in the physiology of the insect, and that alteration of this receptor may be detrimental to the insect such that compensation occurs by increase in concentration of another receptor. This may be an example of the situation in which resistance to a pathogen may not necessarily be beneficial to the insect.

Once a toxin or virus gains access to a susceptible cell, there may still be a number of points at which a change in host response may lead to survival of the cell, and ultimately the insect host. If the toxin acts at the level of the cell membrane, as has been postulated for some B. thuringiensis toxins (Knowles & Ellar 1987), the formation of transmembrane pores, followed by loss of ionic integrity of the cell, may be the only step after receptor binding at which resistance may be expressed. To date, evidence has not been found for resistance to B. thuringiensis at this level. For toxins or viruses which exert their activity process. Clones of cultured Spodoptera frugiperda cells were selected which showed a high level of resistance to NPV, even though they adsorbed the virus equally as well as susceptible lines (Crawford & Sheehan 1983). In the case of toxins, steps at which intoxication may fail include toxin internalization and transport, lysosomal acidification, and the final lethal mode of action. Resistance expressed at each of these steps has been demonstrated in mammalian cell lines selected for resistance to toxins (reviewed by Saelinger 1990), but to date these steps have not been demonstrated in within the cell, however, there are many steps at which physiological changes may lead to abortion of the infection or intoxication insect cells. Cultured lepidopteran cells were selected for resistance to B. thuringiensis delta-endotoxin, but the cause for this resistance is unknown (Johnson 1984). Cultured C. quinquefasciatus mosquito cells selected for resistance to the B. sphaericus toxin bound and internalized the toxin in a manner similar to susceptible cells (Schroeder et al. 1989). Resistance in this line is therefore apparently not due to a change in a receptor, but rather to a failure in a necessary step in the intoxication which occurs within the cell. Recent observations have led us to suggest that these toxin-resistant cells may enzymatically degrade fluorescent-labelled B. sphaericus toxin, or may remove it from the cell by exocytosis. Removal of toxin by

exocytosis is reminiscent of the mechanism by which multiple-drug resistance is expressed in many tumor cell lines (Endicott & Ling 1989).

**CONCLUSIONS**

The rapid progress in recent years in introduction of *B. thuringiensis* delta-endotoxin genes into crop plants (Vaeck et al. 1987), as well as the manipulation of many other pathogens to increase their usefulness and persistence in the field, have focused attention on microbial insecticides. The most attractive features of microbial insecticides are narrow host range, environmental safety, and the apparent lack of development of resistance to these organisms. As can be seen from this review, this latter feature may be an artifact of the current patterns of use of these agents. The economic stimulus to introduce plants bearing the *B. thuringiensis* toxin genes into widespread use is great. Introduction of a new plant variety costs, on average, under $1 million, whereas the introduction of a new insecticide costs in excess of $25 million (Meusen & Warren 1989). In recognition of the potential for development of resistance to *B. thuringiensis* toxins, the B. t. Management Working Group was established, made up of representatives of companies engaged in production of *B. thuringiensis* products or in genetic engineering of plants containing these toxins. This group funds research on resistance to *B. thuringiensis* toxins and shares published and unpublished research on the topic. The aim of this group is identification of ways to avoid or minimize resistance to these valuable insecticidal agents.

In summary, there are several points at which resistance to a pathogen may be expressed (Table 1). Change in the host physiology or behavior at any of these points may lead to resistance, but may also lead to loss in overall fitness of the insect in the absence of selection pressure from the pathogen. Speaking from experience with chemical insecticides used against mosquitoes, Brown (1980) stated, “We can expect resistance to come sooner or later to any larvicide we use”. Although resistance to microbials may be different in character and perhaps slower in appearance than resistance to chemical insecticides, evidence to date suggests that such resistance is certainly a possibility. Careful management of these valuable microbial agents, as well as their own characteristic properties, may be very important in maintaining their usefulness in the long term.

**REFERENCES**


STHONE, T.B.; SIMS, S.R.; MARRONE, P.G. Selection of tobacco budworm for resistance to a genetically engineered *Pseudomonas fluorescens* containing the delta-endotoxin of *Bacillus thuringiensis* subsp. *kurstaki*. *Journal of Invertebrate Pathology*, v.53, p.228-234, 1989.


