Pesticides and the Future

Minimizing Chronic Exposure of Humans and the Environment

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Status of recombinant baculoviruses in insect pest control

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Abstract. Biological pesticides hold the promise of reducing exposure of humans and their environment to chemical pesticides as well as providing a cost effective way to control pests of human health and agriculture. These double-stranded DNA viruses are highly selective for insects and have proven effective in some biological control programs. However, baculoviruses kill their hosts very slowly. By inserting a gene coding for a toxin such as an insect-selective scorpion toxin, or a regulatory enzyme, such as insect-derived juvenile hormone esterase into the genome, the engineered virus causes the infected insect to produce large amounts of the recombinant protein. These proteins kill the insect faster than the wild-type parent virus in the case of toxins, or block feeding in the case of juvenile hormone esterase, leading to more effective biological insecticides. Recent studies have demonstrated dramatic reductions in feeding by insects infected with recombinant viruses. These recombinant viruses show high selectivity for target insects with little or no effect on a variety of beneficial arthropods. These biodegradable materials show promise for incorporation into integrated pest control programs by providing attractive supplements to classical pesticides and transgenic crops. The integration of recombinant viruses may prevent or overcome the mounting problem of insecticide resistance.

1. Introduction

There are many possible approaches to reducing human and environmental exposure to potentially dangerous pesticides. The development of a repertoire of effective pest control agents of improved safety certainly is a viable approach to this problem. In at least the near future, it is likely that we will depend largely upon pesticides for the high level of productivity and profitability currently enjoyed by agriculture. This is not an argument for complacency, because we need pest control agents that present less risk to the public and the ecosystem and which are readily integrated into ecologically-based pest management systems. In insect control one possible approach to improving pesticides involves the use of biological insecticides. This article focuses on developments in one sector of this field – the development of recombinant viral insecticides. As will be discussed later in the chapter, there are many viruses with the potential to provide effective insect control. Among the entomopathogenic viruses, the baculoviruses or nuclear polyhedrosis viruses are the closest to being ready for the agricultural market [6]. Recent advances in genetic engineering have led to recombinant baculoviruses

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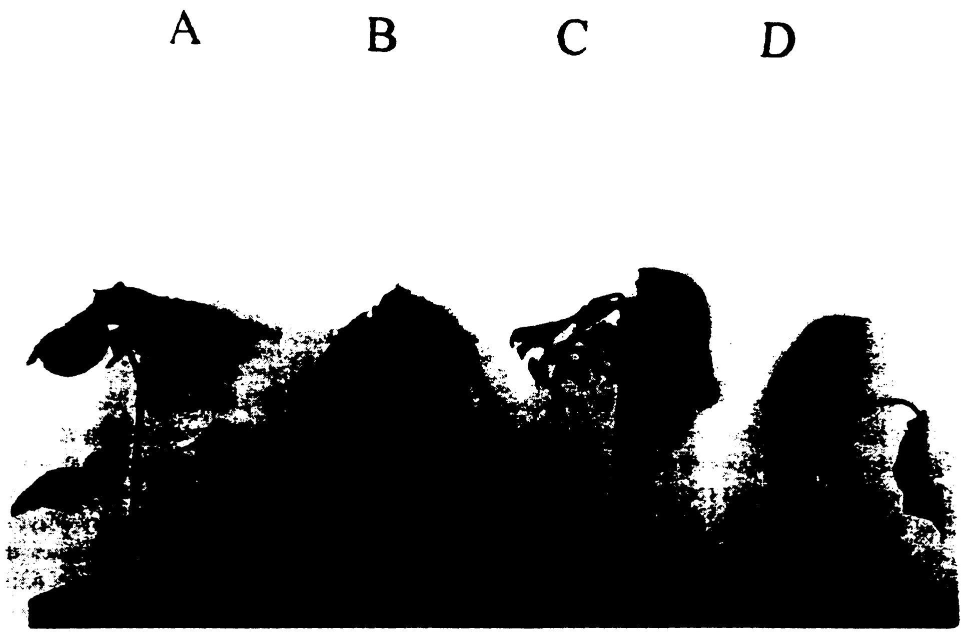

Fig. 1. Reduction in feeding damage to plants caused by a third stadium larva of *Heliothis virescens* following treatment with the virus expressing the toxic peptide AaIT (B), the wildtype baculovirus AcMNPV (C), or water (D). (A) depicts an untreated plant. Computer scans of leaf area indicate that feeding by a larva infected with the recombinant virus is reduced by 80 % compared with an insect infected with the wild-type virus.

that approach the speed of action of classical insecticides. These recombinant viruses result in a dramatic decrease in feeding damage by pest insects (Fig. 1). These viruses are clearly effective, soft on nontarget species, and leave no undesirable residues in the environment. Field tests in several countries are now in their third year. Recombinant viruses appear to compete well with classical insecticides and demonstrate synergistic combinations with some of them, especially the pyrethroids. Recombinant viruses are able to kill some strains of insects that are highly resistant to classical pesticides – in some cases better than nonresistant populations. The economics of production and marketing, success with formulation, competing products, and potential regulatory barriers soon will determine if these materials will have a major impact in agriculture during the next decade [4].

In discussing recombinant baculoviruses, the term biological "insecticide" is used for several reasons. First, unlike classical biological control agents, these viruses are not being designed to establish and recycle for long periods of time. Second, these viruses will be used in an inundative fashion much as classical insecticides are used today. This approach offers several advantages. It will reduce the potential environmental risks posed by a genetically altered organism by limiting recycling. Further, this new approach to insect control can be employed using the same equipment and technologies currently used to apply classical insecticides. This term also implies that the recombinant viruses are at a selective disadvantage, which will lead to a greener and more sustainable agriculture, not an instant panacea. Consequently, repeated application of baculoviral insecticides will require establishing economic thresholds to determine when control of a pest insect by this technology is needed. The recombinant viruses, along with other genetically modified organisms including the crop plants themselves, will present new challenges for toxicologists. Many of the same regulatory, health, and environmental questions that are posed regarding small molecules must now be asked about not only biopolymers, but about biopolymers that have the ability to reproduce. This concern has been discussed in many recent reviews (see [4–6,13,20,26,28,34,36,38]). The purpose of this chapter is to provide a brief introduction to the field emphasizing pest control and safety issues.

2. Description of baculoviruses

Baculoviruses are double-stranded DNA viruses that infect only arthropods. There are no known relatives of these viruses that are pathogenic to other animals or to plants. The majority of the known baculoviruses occur in butterflies and moths, but baculoviral species have also been reported in many insect orders and a few other arthropods. These viruses are important components of many natural control systems. Their potential to cause natural epizootics has been noted many times; they have long been used in organized inundative release programs to control several insect pests. Attempts by Sandoz to market baculoviruses for insect control in the 1970's proved disappointing for many reasons; the biggest obstacle for Sandoz was competition from the introduction of pyrethroid insecticides. This early attempt to market baculoviruses highlighted several problem areas requiring resolution. The key issues included development of reliable, inexpensive production/distribution systems, and improved formulations. However, from the farmer's perspective, there was a clear need for a faster acting biopesticide.

In the natural history of most baculoviruses the insect ingests the viral particle, the protein coat or polyhedron dissolves, and viral particles are released. In susceptible hosts the particles infect a gut cell where they must multiply and spread beyond the gut before the gut cells are shed at the next molt. Once in the body of the insect, the virus spreads throughout the insect using a different phenotype called the budded virus, which lacks a protein coat. Shortly before the insect dies, much of the body mass of the insect is converted into microencapsulated viral particles called polyhedra (plural for polyhedron). The polyhedron protects the virus in the environment. Often the insect climbs to the top of the plant, the remaining internal tissues dissolve, and the insect hangs from the plant as a bag of virus. The bag of virus easily ruptures, raining viral particles onto the foliage below at the slightest disturbance. Although this is a fantastic system for a natural control agent, slow speed of kill severely limits the utility of these viruses as insecticides. Usually insecticides are applied once the number of pests in the field reaches an economic threshold. Certainly one can develop earlier economic thresholds to accommodate the use of wildtype viruses. Initially, however, new insect control agents must usually fit into existing pest management plans; current management plans are primarily based on classical insecticides that kill pests quickly [6,45].

3. Engineering viruses to improve speed of kill

The initial engineering efforts in the baculovirus field addressed primarily the issue of slow speed of kill [45]. Fortunately, baculoviruses have long been used for high level expression of eucaryotic proteins [34,39]. Due to their value in the research community and pharmaceutical industry, transgenic expression systems are well developed [25]. Initial work simply involved adapting existing recombinant technology to a new field. Early studies involved transgenic expression of neurohormones [27]. These efforts were marginally successful. Unfortunately, this field has been dropped by most laboratories. Expression of these powerful chemical mediators offers many conceptual advantages. Early efforts were optimistic, but they were also based on limited knowledge of the physiology and biochemistry of insect neurohormones. We now know that many neurohormones control processes which are not immediately lethal to the insect. Instead these neurohormones are regulated by a variety of factors designed to maintain homeostasis. Also, neurohormones themselves are rapidly degraded. Hopefully work on peptide mediators will be successful in the future if the endeavor is pursued using a more developed, fundamental understanding of neuroendocrinology.

An elegant approach to improving speed of kill involved removing from the baculovirus a gene (EGT) whose function was to prolong the life of the insect to maximize the reproductive capacity of the virus. Deletion of this gene give a virus (EGT) that lacks ecdysone glucose transferase. This modification resulted in a slight improvement in speed of kill compared with wildtype viruses [35]. This result suggests that the virus is well adapted for infection and replication in the field. It also points out that perhaps there are many genes in the virus that could be altered to produce a better insecticide. Thus, viral insecticides may be improved by eliminating from the virus genes that may be important for replication and competition under some natural conditions, but not essential when the virus is used as a classical pesticide.

Another line of research involves the expression of the insect's own enzymes in the virus. One such enzyme is juvenile hormone esterase (JHE). JHE is normally present just before pupation. It removes a terpene hormone from the insect called juvenile hormone. Over-expression of this enzyme slightly increased the speed of kill by the virus [19]. JHE proved to be an excellent reporter enzyme for eucaryotic systems because of its stability in tissue culture and its usefulness in highly sensitive assays [7]. However, in whole insects the recombinant enzyme, like the natural enzyme, is rapidly taken up by pericardial cells, transported to lysosomes, and destroyed [24]. Engineering efforts that interfered with the degradation process of this enzyme improved the speed of action by this virus reducing plant damage by 80 % [9]. This success illustrates that a variety of enzymes and endogenous proteins could be used to disrupt the development of insects in recombinant viruses. Although the mutants with enhanced stability seem to kill insects, at least in part, by a mechanism related to the destruction of juvenile hormone, another mutant is catalytically inactive and kills insects by an unknown mechanism [8]. Recently another member of this family of proteins, chitinase, was reported to speed insect death following virus expression [16].

In concept, the simplest way to enhance the speed of kill of baculoviruses involved the insertion of a gene coding for a rapidly acting toxin into the viral genome. The recombinants arising from this strategy now appear most likely to find commercial use in the near future. For example insertion of insect-selective neurotoxins from mites [41], spiders, and sea anemones [37], or scorpion venom leads to rapid intoxication of the insect [29,31,40].

Many recombinant viruses generated to date have demonstrated potential for effective insect control using genes from the virus, from insects and other animals, and especially genes encoding peptide toxins common in many arthropod venoms. Results of the most recent field trials using the scorpion toxins AaIT and LqhIT2 are promising [4]. Viruses expressing insect-selective neurotoxins are the constructs progressing most rapidly toward commercial development. However, the viruses expressing modified juvenile hormone esterases are more active in reducing insect feeding than the first generation viruses containing scorpion toxin genes.

4. Regulatory issues

Regulatory issues will certainly have a major impact on how recombinant baculoviruses are developed in the near future. The same issues and problems related to classical pesticides which need to be addressed by regulatory agencies apply to recombinant viruses as well. Of course it is essential that regulation be prudent so that human and environmental health are assured. In addition standards of quality control and efficacy of viral preparations also are needed. The high selectivity of baculoviruses for restricted insect groups and the high selectivity of the toxins being expressed by these viruses provide strong evidence that the recombinant viruses will be safe. On the other hand two additional issues need to be examined. Since the recombinant baculoviruses represent a new technology for pest control, it is essential that regulations require sufficient information to make the public feel comfortable with this technology. Also the recombinant baculoviruses represent the vanguard of several new technologies that could be used in agriculture. The precedents set by the registration policies and regulation of recombinant baculoviruses may set the tone for evaluation of other technologies to follow. Some of these technologies may not be as inherently safe as the baculoviruses. Thus one could envision a tiered system in which performance of a technology in initial tests would determine which, if any, more extensive tests are required.

The major advantages of recombinant baculoviruses are their lack of persistence and their high selectivity for target rather than beneficial insects. By their nature, these advantages will limit the market size for these baculovirus products. Thus if the regulatory barriers are too high, industry will simply not invest in these biological approaches to pest control forcing society in both developed and developing countries to continue relying on classical chemical insecticides. None of the recombinant baculoviruses currently conceived for development could bear the registration costs of a classical chemical based on the value of the probable market. Moderate regulatory costs might allow several viruses to be developed by a large company that could be used for major insect markets, such as viruses for controlling the *Heliothis/Helicoverpa* complex. Such a policy will stimulate research on expanding the host range of viruses, which is economically desirable. Unfortunately, this policy may not be optimal for many pest management situations. However, innovative regulatory guidelines involving careful, incisive tests could be performed to insure a high degree of human and environmental safety. This approach would allow the development of a variety of recombinant and wildtype viruses that could be used alone or in combination for insect control. Such an outcome would provide the pest management specialist with a collection of "magic bullets" of high selectivity. Such a procedure would facilitate the involvement of major companies marketing viruses to control pests of the greatest economic importance. This approach may also attract smaller companies marketing more specialized materials that could not support the overhead of a major company. It is critical as well that regulatory guidelines be based on the product to be used (such as a baculovirus expressing an insect selective toxin) rather than the process used to arrive at the product, such as genetic engineering [34]. Guidelines should yield good science addressing real questions rather than simply serving as an exercise in data collection. The agricultural biotechnology field seems to have suffered while the regulatory community struggles with this new technology [32,33]. However, thus far in the United States the regulators appear to have worked effectively with the developers of baculoviral technology to design careful strategies for evaluating the safety of these materials in a cost effective fashion [4,15].

5. Environmental safety

The recombinant viruses currently under development appear to offer no threat to the ecosystem, but regulatory agencies and a variety of investigators are toiling with what experiments can and or should be run to test their safety [38]. When they are evaluated it is critical that their environmental impacts be compared with the environmental impacts expected from classical technologies such as chemical pest control and alternative biological pest control strategies. The major advantage offered by baculoviruses over many classical pesticides is that these viruses are highly selective. This selectivity will avoid pest resurgence and other problems that arise from using products that kill beneficial insects [4,42]. Thus, the economic benefits of baculoviruses from a single application will extend beyond a comparison of their efficacy with that of a classical pesticide. The advantages offered by baculoviruses may continue throughout the growing season by avoiding pest resurgence, beyond the current growing season by limiting resistance, and even beyond the immediate farm by providing refuge for natural enemies and minimizing environmental contamination.

Several studies confirm that baculoviruses will exert minimal effects on nontarget species beyond potentially reducing their food supply [4,44]. The high selectivity of baculoviruses, even among lepidopterous species, indicates that these viruses could, for instance, be used effectively in sensitive forest ecosystems without the nontarget effects that can occur when using classical pesticides or the toxins of *Bacillus thuringiensis*. Extensive work at the Oxford NERC laboratory indicates that the recombinant virus expressing the AaIT gene is relatively safe toward nontarget Lepidoptera [36]. However, it is possible that viruses expressing potent insecticides will increase mortality in some partially permissive species [42]. The practical significance of this is questionable. Engineering viruses to significantly increase host range is currently in the early laboratory stage.

A major concern with any genetically modified organism is that it will become established and replace the existing biota. Fortunately, the same trait that improves the insecticidal properties of the recombinant virus also makes it non-competitive with the wildtype virus. Because the recombinant virus kills quickly there is not sufficient time to produce numerous viral progeny. The faster the virus kills, the less ecologically competitive the virus becomes with the wildtype virus [14]. Only in the rare situation of very high pest density could the recombinant virus temporarily outcompete the wildtype virus. Such a scenario rarely occurs in an agricultural setting. In addition the rapid kill caused by recombinant viruses bypasses the behavioral change that causes insects to climb plants, liquefy and thus spread the virus (Fig. 2A, B). Often infected insects are knocked off the plants because of this change in behavior caused by the toxin [12,23]. This hard cadaver fails to liquefy, minimizing the spread of the virus. Cadavers on the ground may even be subject to greater predation from ground dwellers. Other genetic changes can be used to cripple the recombinant virus in multiple ways [4,6], but such changes are unlikely to be needed to insure environmental safety. The deletion of multiple genes needed for the action of the virus as a pathogen, but not as a viral insecticide, might make the resultant recombinant more active on selected pests.

Modern genetics have taught us that genes are capable of moving. However, for these genes to become established in a population there must be a driving force. In the

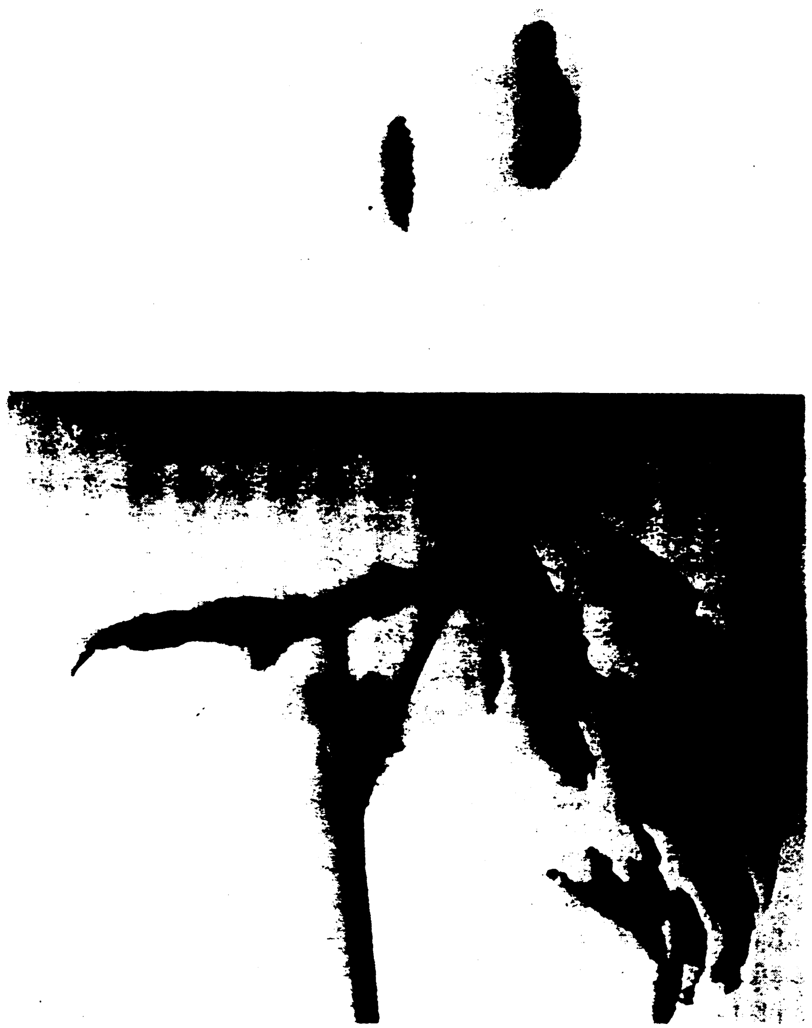

Fig. 2A and B. Bodies of larvae killed by the wildtype virus AcMNPV and recombinant AcMNPV coding for expression of the toxic peptide AaIT. (A) Most larvae infected with the wildtype virus climb near the top of the plant, hang by their prolegs and liquefy. This process leads to spread of the viral infection. (B) Most larvae infected with the recombinant virus are knocked off of the plant due to sublethal symptoms induced by the peptide toxin (larva on the left). They are unable to crawl back on to the host plant. Following death most form hard cadavers on the ground reducing the spread of the virus infection. An insect that died from wild-type viral infection appears on the right of the photo for comparison.

simplest laboratory procedures using plasmid transfection (the basis of genetic engineering), high concentrations of plasmid DNA are forced into a cell specially bred to be receptive. Even under these circumstances, a strong positive selection such as antibiotic resistance conferred on the transfected plasmid is needed to keep the new gene in the bacterial population. Although gene transfers among and between baculoviruses and their hosts have been very difficult to demonstrate in the laboratory, as biologists we know that such transfers will occur given sufficient time and numbers. However to be of biological significance and to establish in the population, the gene in question must confer some survival advantage to its new host. The loss of the AaIT gene from the recombinant virus results in the virus simply returning to the wildtype state. The gaining of the AaIT gene by another virus or by the host will undoubtedly offer no benefit. If the AaIT were to find itself in a new situation where it is able to be expressed, it will act to the detriment of its new host; thus, the gene and its new environment will vanish. Therefore the fact that recombinant viruses are biological insecticides and not biological control agents enhances dramatically their safety to the environment. They will not suffer from problems of long term spread and competition with wild viruses [18]. Their instability in the environment makes it unlikely that one will observe biological effects distant from the site of application. When compared with alternate insect control strategies, the recombinant baculoviruses offer a tremendous advantage for reducing unwanted effects of pesticides while retaining the high level of pest control which allows us to feed the world's population and preserve both land and water resources for natural habitats.

6. Human safety

With classical drugs and pesticides one can think of safety in terms of a therapeutic index or toxicity ratio between the target species and humans. A safety factor of 100-fold seems readily accepted, but some pesticides still in use are almost equally toxic to humans and pest species with safety arising from application technologies. With the recombinant viruses there have been no ill effects detected; thus it is hard to develop such a ratio. There are peptides which if injected are highly toxic to vertebrates such as those from some spiders, cone snails, and the vertebrate-selective scorpion toxins. Even these highly toxic peptides from potentially lethal organisms are harmless by oral or dermal routes. Thus, having the active agent be a peptide gives tremendous selectivity to recombinant viruses. Of course the toxins used in recombinant viruses are not vertebrate toxins. Rather they are highly selective for insects showing no effects when injected directly into a mouse's brain or placed on nerve preparations. They even show no effects on the isolated sodium channel where their potency on the insect channel is many orders of magnitude greater than on the vertebrate channel. These massive levels of safety probably are not needed because it is unlikely that a human will come into contact with the peptide toxin even if widespread use of the virus occurs. The toxin will be produced in pest insects. Only very low levels of the toxin are needed to kill the insect. Furthermore, immediately upon death of the insect, toxins begin to decompose and are rapidly degraded. These toxins will not contaminate aquifers nor will they bioaccumulate.

With each bite of salad we eat thousands of wildtype baculoviruses. Baculoviruses possess many layers of safety. The first layer is that these viruses have a short half-life. Most are readily removed from food by washing or illumination. No bioaccumulation is seen with lipophilic compounds such as polychlorinated biphenyls or translocation by the plant to edible parts as has occurred with systemic pesticides such as aldicarb. If viruses are consumed they probably will remain occluded and pass through the gut. If the infectious particles are released there is no evidence that they can enter a gut cell and multiply. If the viruses are injected in cells there is no indication of replication [4].

An approach under study in the field of gene therapy is the economical production of very large amounts of DNA. Baculoviruses are used extensively as one of the most efficient systems for producing nucleic acids. In hepatic cells treated with massive amounts of DNA, it is sometimes possible to detect baculovirus genes in the cells, but there is no evidence that the viruses can reproduce in vertebrate cells. There are numerous promoters in baculoviruses. Some are quite selective for insect cells while others are more general in their action.

Thus the baculoviruses offer higher levels of safety for humans and other vertebrates than we can reach with classical insecticides. However, the natural origin of the virus and foreign gene do not in themselves confer safety. The evaluation of safety must be based on a firm understanding of the biology and adequate testing. Even with these highly safe biological insecticides, careful handling to prevent the development of allergies in the manufacture and application of these viruses is essential.

7. Public relations

A key factor in the development of any new technology will be public acceptance. Although scientifically these recombinant viruses seem safe to humans and to their environment, it is critical that the public make the ultimate decision regarding application of this technology through their regulatory agencies. In the US, American Cyanamid and DuPont have been very credible in their approaches by keeping the public, regulatory agencies, and interested groups informed at each step in the process [15]. In contrast, this technology has been met with protests in Britain at Oxford despite the Institute's development of these viruses by adopting a logical and methodical system of field tests [3] and being very careful to follow all government regulations [1]. The large difference in public reaction between these two countries points out the need for scientists to take an active role, not only in communicating their results to the public, but also in listening actively to the public's concerns [15].

8. Integrating viruses into agricultural systems

The efficacy and cost effectiveness of recombinant viruses must approach that of classical insecticides if these products are to be used. However, these viruses may offer advantages over classical compounds that will provide a market advantage. A major advantage directly to the farmer is the selectivity of baculoviruses, which avoids problems with the outbreak of secondary pests and pest resurgence. Alleviating these problems reduces the need for multiple applications. Since baculoviruses have very short half-lives and are of no risk to humans, they may be applied shortly before harvest without the fear of residues remaining on food and forage crops. The safety of these viruses to the environment and workers in the fields does not influence the profitability of their use directly. However, with farmers in many areas under pressure to reduce environmental contamination by pesticides and with worries about farm worker health impacting cropping strategies, these viruses may offer very real advantages because of their safety.

One of the advantages of baculoviruses is that they can be applied as a classical insecticide [22]. This allows the farmer to use existing technologies and equipment. By acting like a classical pesticide, only minor modifications of pest management programs will be needed to accommodate these viruses in pest management recommendations. Like classical insecticides it is likely that the efficacy of baculoviruses will vary dramatically, not only with the pest target and climate, but even with the species of crop plant to be protected [13]. It has been noted that the AaIT virus is synergized by several classical insecticides. In the case of pyrethroids there is a mechanistic explanation for this synergistic increase in speed of kill since both AaIT and pyrethroids bind to sites on the sodium channel. Based on binding characteristics one should be able to predict the groups of peptide toxins and classical pesticides which will be synergistic at the level of the nerve channel [21]. The high level of pyrethroids used, for example, on cotton is driven primarily by the need to control members of the noctuiid complex in the genera *Heliothis* and *Heliocoverpa*. If the field application rates of pyrethroids can be reduced ten-fold by applying the virus as a tank mix, environmental contamination and destruction of natural enemies will be significantly reduced. However the low dose of pyrethroids should be sufficient to both speed the kill of the virus and control other minor pests. Other compounds probably show positive interaction with the AaIT virus at a physiological level. This probability could also lead to valuable tank mixes for insect control. It is very unlikely that recombinant viruses will be the sole pest management tool used. They are of course much more compatible than classical pesticides with biological control strategies, but can be used equally with most classical compounds. Management of resistance to classical insecticides is a major concern in agriculture and will be discussed below.

9. Resistance

One of the reoccurring problems with insecticides is the development of resistance by pest insects. Any material with a unique mode of action will be useful in resistance management, and the recombinant viruses fit this criterion. There may even be negatively correlated cross resistance in that the AaIT virus kills pyrethroid resistant insects faster than susceptible populations [22 30]. Thus these viruses could be used to delay development of resistance to pyrethroids and other synthetic chemical insecticides. In addition the recombinant virus when used in a weekly rotation with the toxin of *Bacillus thuringensis* was as effective as weekly applications of BT, thus providing a resistance management tool for an important biological agent [42–44]. The virus could be a supplement to transgenic cotton expressing BT to control non-susceptible pests such as *H. zea* or for late season control of pests such as *H. armigera* [2].

With sufficient time, resistance has developed to most existing control measures. Recombinant viruses probably will be no exception. On the positive side, this technology represents a unique approach to insect control. Thus it is likely that new genetic mechanisms will need to be developed by the pest insects leading to delays in the development of resistance in pest populations. Also it is unlikely that resistance will develop to the expressed peptide since ultimately the virus kills the insect regardless of the insect's susceptibility to the toxin. In the unlikely event that there is direct resistance to the peptide, there is a repertoire of other peptide toxins known to be safe to vertebrates that could be used if regulatory barriers are not too high. On the negative side, resistance is almost a badge of success for any pest control strategy. If a material is a commercial success, then the selection pressure that it generates leads to resistance. In the case of viruses, we already know that there is resistance in the insect population [10]. If there were not resistance, the viruses long ago would have driven the insect hosts into extinction and vanished with them. It will be interesting to see how pest populations will respond to selection pressure from the commercial use of these viruses. Again regulation could determine the success of the field. Conceptually, however, these viruses have an advantage in that they are dynamic biological organisms and either new viral strains can be used for resistant populations, or we may see micro-evolution of the virus in parallel to that of its insect host.

10. Use in developing countries

The major markets for any pesticides are in developed counties and in developing countries with a strong agricultural market designed for export. However, much of the human suffering caused by crop loss occurs in regions where agriculture is more of a subsistence effort. These areas face many problems. Lack of infrastructure precludes the development of sophisticated pest management programs. Lack of education and equipment may make the use of many pesticides very dangerous, and the use of pesticides without analytical capability in the country may be used as a trade barrier to keep the produce of that country from being exported. Many of the areas most in need of pest control either lack international currency or material for barter which precludes purchase of pesticides. Can recombinant or wildtype viruses be used to remedy some of these problems?

A clear disadvantage of baculoviruses from the growers' perspective is their selectivity. Farmers in these regions need a simple, broad spectrum material for use. However, there are likely to be cases where there is a single primary pest and where the avoidance of secondary outbreaks and pest resurgence can lead to a significant increase in pest control. In developed countries much pesticide use is for cosmetic improvements. Absolute control of all pests is not needed in many cases for substantial increases in food production. Thus a situation where a biologically based product can provide a real increase in productivity without pest resurgence and release of secondary pests could be a major advantage. Biopesticides could be used as a trade barrier just like trace concentrations of classical materials. However, if the world community takes a more realistic stand on residues of biologicals, their use could open markets for developing countries that are now closed due to charges of pesticide contamination. Also surface contamination by viruses is much more easily removed by washing or illumination by ultraviolet light than is contamination by classical chemicals that have penetrated the produce.

Of course a major problem that must be overcome is availability of recombinant viruses. Initially, viral pesticides will be expensive and possibly more expensive than classical compounds. This will preclude their use in developing areas. However, the production and application technologies developed for recombinant viruses may make wildtype viruses more useful in developing countries. Several such viruses have been produced in village industries. Engineering of viruses to this point has been directed toward their optimization for major markets in developed countries. One could say that we have engineered "yuppie" viruses. Hopefully some future effort will be directed toward engineering the "volks" or peoples' viruses optimized for use in more marginal agricultural situations. Thus, advanced technology could be used to generate sophisticated biological agents for production by using technology available in developing countries.

11. Barriers to marketing baculoviruses

The efficacy of recombinant baculoviruses in laboratory and greenhouse studies appears competitive with many classical insecticides. One barrier, however, is that regulation has led to initial field tests which are largely administrative exercises in moving an agent from the laboratory to the field. The resulting field trials have been very valuable and certainly are milestones in the development of recombinant pesticides. However, regulatory concerns made the tests so artificial that the major questions of comparative efficacy of these viruses in real cropping situations and in comparison to the latest competing chemicals and recombinant plants were not run. It is first essential to determine if recombinant viruses are only intellectually interesting, or if they represent a viable alternative to classical pesticides. More recent field trials are moving toward answering these practical questions [4,15]. Based on greenhouse studies as well as initial field results, the efficacy of these viruses looks adequate and there are clear avenues to improve the performance of the viruses dramatically.

As with classical pesticides the economic success of these materials is likely to be determined by the quality of their formulation and production costs. However, the techniques needed to optimize formulation and production are somewhat different with biological materials. There is room for innovation in these important areas. The other barriers such as marketing strategies are likely to be minor if these major issues are solved successfully. The agricultural market is a moving target. As recombinant baculoviruses are being developed one also sees the evolution of other biologicals, improved transgenic plants, novel classical chemicals, and a changing political and economic environment for the farmer.

12. Further improvement of baculoviruses

The first generation of recombinant viruses expressing toxins demonstrated the concept that genetic engineering could overcome the inherent problem of slow kill among the viruses. One could liken this virus to the first synthetic pyrethroids developed in 1947. Undoubtedly incremental improvements along many lines will lead to much better materials. Already improved toxins, earlier promoters, and different viral stains have dramatically increased the efficacy of these viruses. It is likely that using synergistic combinations of toxins as scorpions do will lead to further improvements [21]. An earlier review outlines several of the pathways along which these improvements could move [20]. Recombinant technology is certain to impact some of these developments, but many issues such as molecular control of host range will depend upon the generation of more fundamental information.

13. The next viruses

Recombinant baculoviruses represent the vanguard product in the field as the first viruses likely to make it beyond the specialty market [4]. However, it is likely that production, formulation and other technologies developed for the recombinant baculoviruses can be applied to wildtype viruses. With spin-off technologies from the development of recombinant baculoviruses, wildtype viruses are likely to look very good in many pest management systems. Several of the related granulosis viruses appear promising for pest control and advances are being made in their engineering. However, there are many other insect viruses which have attributes which are attractive [6]. None of these viruses share the immediate technological advantages of the baculoviruses. However with credibility gained by the success of the baculoviruses, further research is likely to lead to products based on other insect-selective viruses such as cytoplasmic polyhedrosis viruses, entomopox viruses, and small RNA viruses, which surpass the baculoviruses in efficacy and cost effectiveness [11]. The technology developed for viruses might also improve the efficacy of BT and other biologicals [17].

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References

[1] T. Adler, Mothra meets its match, *Science News*, **146** (1994), 154–155.

[2] J.N. All and M.F. Treacy, Improved control of *Heliothis virescens* and *Helicoverpa zea* with a recombinant form of *Autographa californica* nuclear polyhedrosis virus and interaction with Bollgard Cotton, in: *Proceedings Beltwide Cotton Conference*, P. Duggar and D.A. Richter, eds., National Cotton Council, Memphis, Tenn., 1997.

[3] D.H.L. Bishop, P.F. Entwistle, I.R. Cameron, I.R. Allen and R.D. Possee, Field trials of genetically-engineered baculovirus and Noctuide, in: *The Release of Genetically-Engineered Microorganisms*, M. Sussman, C.H. Collins, F.A. Skinner and D.E. Stewart-Tull, eds., Academic Press, New York, 1988, pp. 143–179.

[4] B.C. Black, L.A. Brennan, P.M. Dierks and I.E. Gard, Commercialization of baculoviruses, in: *The Baculoviruses*, L.K. Miller, ed., Plenum Press, New York, in press.

[5] B.C. Bonning and B.D. Hammock, Development and potential of genetically engineered viral insecticides, *Biotechnology and Genetic Engineering Reviews*, **10** (1992), 453–487.

[6] B.C. Bonning and B.D. Hammock, Development of recombinant baculovirus for insect control, *Ann. Rev. Entomol.*, **41** (1996), 191–210.

[7] B.C. Bonning and B.D. Hammock, Use of juvenile hormone esterase as a novel reporter enzyme in the baculovirus expression system, *J. Virol. Methods*, **51** (1995), 103–113.

[8] B.C. Bonning, K. Hoover, T.F. Booth, S. Duffey and B.D. Hammock, Development of a recombinant baculovirus expressing a modified juvenile hormone esterase with potential for insect control, *Arch. Insect Biochem. Physiol.* **30** (1995), 177–194.

[9] B.C. Bonning, V.K. Ward, M.M.M. van Meer, T.F. Booth and B.D. Hammock, Disruption of lysosomal targeting is associated with insecticidal potency of juvenile hormone esterase, *Proc. Natl. Acad. Sci USA*, **94** (1997), 6007–6012.

[10] D.T. Briese, Insect resistance to baculoviruses, in: *The Biology of Baculoviruses*, II, R.R. Granados and B.A. Frederici, eds., CRC Press, Boca Raton, Florida, 1986, pp. 237–263.

[11] P.D. Christian, T.N. Hanzlik, D.J. Dall and K.H. Gordon, Insect viruses: New strategies for insect control, in: *Molecular Approaches to Fundamental and Applied Entomology*, J. Oakeshott and M.J. Whitten, eds., Springer-Verlag, New York, 1993, pp. 128–163.

[12] J.S. Cory, M.L. Hirst, T. Williams, R.S. Hails, D. Goulson, B.M. Green, T. Carty, R.D. Possee, P.J. Cayley and D.H.L. Bishop, Field trial of genetically improved baculovirus insecticide, *Nature*, 370 (1994), 138–140.

[13] S. Duffey, K. Hoover, B.C. Bonning and B.D. Hammock, The impact of host plant on the efficacy of baculoviruses, in: *Reviews in Pesticide Toxicology*., Vol. 3, R.M. Roe and R.J. Kuhr, eds., Toxicology Communications, Inc., Raleigh, North Carolina, 1995, pp. 137–275.

[14] J.R. Fuxa, S.A. Alaniz, A.R. Richter, L.M. Reilly and B.D. Hammock, Competition between wild-type and recombinant nucleopolyhedro viruses in a greenhouse microcosm, *Biol. Control*, submitted.

[15] I.E. Gard, Field testing a genetically modified baculovirus, *British Crop Protection Council Symposium Proceedings. Microbial Pesticides: Novelty or Necessity?*, 68 (1997), 101–114.

[16] K. Gopalakrishnan, S. Muthukrishnan and K.J. Kramer, Baculovirus-mediated expression of a *Manduca sexta* chitenase gene: Properties of the recombinant protein, *Insect Biochem. Molec. Biol.* 25 (1995), 255–265.

[17] K.J. Hackett, R.B. Henegar, R.F. Whitcomb, D.E. Lynn, R.F. Schroder, J.L. Vaughn and W.W. Cantelo, Distribution of Colorado potato beetle Spiroplasmas in North America, *Biol. Control*, 2 (1992), 218–225.

[18] B.D. Hammock, Virus release evaluation, *Nature*, 355 (1992), 119.

[19] B.D. Hammock, B.C. Bonning, R.D. Possee, T.N. Hanzlik and S. Maeda, Expression and effects of the juvenile hormone esterase in a baculovirus vector, *Nature*, 344 (1990), 458–461.

[20] B.D. Hammock, B.F. McCutchen, J. Beetham, P. Choudary, E. Fowler, R. Ichinose, V.K. Ward, J. Vickers, B.C. Bonning, L.G. Harshman, D. Grant, T. Uematsu and S. Maeda, Development of recombinant viral insecticides by expression of an insect-specific toxin and insect-specific enzyme in nuclear polyhedrosis viruses, *Arch. Insect Biochem. Physiol.*, 22 (1993), 315–344.

[21] R. Herrmann, H. Moskowitz, E. Zlotkin and B.D. Hammock, Positive cooperativity among insecticidal scorpion neurotoxins, *Toxicon*. 33 (1995), 1099–1102.

[22] K. Hoover, R. Herrmann, H. Moskowitz, B. Bonning, S. Duffey, B. McCutchen and B. Hammock, The potential of recombinant baculoviruses as enhanced biopesticides, *Pesticide Outlook*, 7 (1996), 21–27.

[23] K. Hoover, C.M. Schultz, S.S. Lane, B.C. Bonning, S.S. Duffey, B.F. McCutchen and B.D. Hammock, Reduction in damage to cotton plants by a recombinant baculovirus that knocks moribund larvae of *Heliothis virescens* off the plant, *Biol. Control*, 5 (1995), 419–426.

[24] R. Ichinose, A. Nakamura, T. Yamoto, T.F. Booth, S. Maeda and B.D. Hammock, Uptake of juvenile hormone esterase by pericardial cells of *Manduca sexta*, *Insect Biochem. Molec. Biol.* 22 (1992), 893–904.

[25] L.A. King and R.D. Possee, *The Baculovirus Expression System*, Chapman and Hall, London, 1992.

[26] S. Maeda, Expression of foreign genes in insects using baculovirus vectors, *Ann. Rev. Entomol.* 34 (1989), 351–372.

[27] S. Maeda, Increased insecticidal effect by a recombinant baculovirus carrying a synthetic diuretic hormone gene, Biochem. *Biophys. Res. Commun.* 165 (1989), 1177–1183.

[28] S. Maeda and B.D. Hammock, Recombinant baculoviruses expressing foreign genes for insect pest control, in: *Pest Control with Enhanced Environmental Safety*, Vol. 524, S.O. Duke, J.J. Menn and J.R. Plimmer, eds., American Chemical Society, Washington, D.C. 1993, pp. 281–297.

[29] S. Maeda, S.L. Volrath, T.N. Hanzlik, S.A. Harper, D.W. Maddox, K. Majima, B.D. Hammock and E. Fowler, Insecticidal effects of an insect-specific neurotoxin expressed by a recombinant baculovirus, *Virology*, 184 (1991), 777–780.

[30] B.F. McCutchen, M.D. Betana, K. Hoover, R. Herrmann and B.D. Hammock, Interactions of recombinant and wild-type baculoviruses with classical insecticides and pyrethroid-resistant tobacco budworm (Lepidoptera: Noctuidae), *J. Econ. Entomol*, (1997) 9:(5) 1170–1180.

[31] B.F. McCutchen, P.V. Choudary, R. Crenshaw, D. Maddox, S.G. Kamita, N. Palekar, S. Volrath, E. Fowler, B.D. Hammock and S. Maeda, Development of a recombinant baculovirus expressing an insect-selective neurotoxin: Potential for pest control., *Bio/Technology*, 9 (1991), 848–852.

[32] H.I. Miller, A need to reinvent biotechnology regulation at the EPA, *Science*, 266 (1994), 1815–1818.

[33] H.I. Miller and D. Gunary, Serious flaws in the horizontal approach to biotechnology risk, *Science*, 262 (1993), 1500–1501.

[34] L.K. Miller, Baculoviruses as gene expression vectors, *Ann. Rev. Microbiol.* 42 (1988), 177–199.

[35] D.R. O'Reilly and L.K. Miller, A baculovirus blocks insect molting by producing ecdysteroid UDP-Glucosyl transferase, *Science*, 245 (1989), 1110–1112.

[36] D. Possee, M. Hirst, L.D. Jones, D.H.L. Bishop and P.J. Cayley, Field tests of genetically engineered baculoviruses, *British Crop Protection Council Monograph 55, Opportunities for Molecular Biology in Crop Production*, 55 (1993), 23–36.

[37] G.G. Prikhod'ko, M. Robson, J.W. Warmke, C.J. Cohen, M.M.S.P. Wang, V. Warren, G. Kaczorowski, L.H.T.V. d. Ploeg and L.K. Miller, Properties of three baculovirus-expressing genes that encode insect selective toxins, *Biol. Control*, 7 (1996), 236–244.

[38] A. Richards, M. Matthews and P. Christian, Ecological considerations for the environmental impact evaluation of recombinant baculovirus insecticides, *Ann. Rev. Entomol.* in press.

[39] G.E. Smith, M.D. Summers and M.J. Fraser, Production of human beta interferon in insect cells infected with a baculovirus expression vector, *Mol. Cell. Biol.* 3 (1983), 2156–2165.

[40] L.M.D. Stewart, M. Hirst, M.L. Ferber, A.T. Merryweather, P.J. Cayley and R.D. Possee, Construction of an improved baculovirus insecticide containing an insect-specific toxin gene, *Nature*, 352 (1991), 85–88.

[41] M.D. Tomalski and L.K. Miller, Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene, Nature, 352 (1991), 82–85.

[42] M.F. Treacy, ed, *Efficacy and Non-Target Arthropod Safety of an AaIT Gene-Inserted Baculovirus: Results from Field and Laboratory Studies Conducted During 1995–1996*, International Business Communications, Southborough, MA, in press.

[43] M.F. Treacy and J.N. All, Impact of insect-specific AaHIT gene insertion on inherent bioactivity of baculovirus against tobacco budworm, *Heliothis virescens* and cabbage looper, *Trichoplusia ni*, in: *Proceedings Beltwide Cotton Conferences*, P. Duggan and D.A. Richter, eds, National Cotton Council, Memphis, TN, 1996, pp. 911–917.

[44] M.F. Treacy, J.N. All and C.F. Kukel, Invertebrate selectivity of a recombinant baculovirus: Case study on AaHIT gene-inserted *Autographia californica* nuclear polyhedrosis virus, in: *New Developments in Entomology*, K. Bondari, ed, Research Signpost, London, in press.

[45] J.M. Vlak, Genetic engineering of baculoviruses for insect control, in: *Molecular Approaches to Fundamental and Applied Entomology*, J. Oakeshott and M.J. Whitten, eds, Springer-Verlag, NY, 1993, pp. 90–127.