BENEFITS AND RISKS OF RECOMBINANT BACULOVIRUSES FOR CONTROL OF HELIOTHINES

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Abstract

The tobacco budworm (*Heliothis virescens*) and the pink bollworm, (*Helicoverpa zea*) are key pests of Texas cotton. Genetically altered baculoviruses present an opportunity for implementation of inundative biological control in a cost effective manner. Field trials conducted in San Angelo, Texas demonstrated the efficacy of several recombinant baculoviruses to control Heliothines infesting cotton with few adverse side effects. Compared to plots sprayed with conventional insecticides, plots treated with baculovirus exhibited no secondary outbreaks of cotton aphid, an abundance of natural enemies, and budworm/bollworm damage levels equivalent to conventional control tactics. Laboratory and field bioassays of natural enemies exposed to various recombinant baculoviruses could not detect any adverse effects on predator life history characteristics.

Introduction

Lepidopterous insects are common and often serious pests of cotton grown in the United States. Increasing awareness of environmental problems and widespread pest resistance pose a severe threat to conventional synthetic insecticide-based management programs aimed at controlling these pests (National Research Council 1986, Roush & Tabashnik 1990). If cotton production is to remain a prominent component of United States agriculture, alternate methods for the control of leptidopterous pests must be found.

Biological control has received most of the attention in developing environmentally benign methods of Lepidoptera control. Probably the most frequently tried method of achieving control with natural enemies in annual crops has been by augmentative releases of artificially reared parasitoids or predators (King *et al*. 1986). While the technical feasibility of suppressing lepidopteran pest populations in several crops by this method has been demonstrated, the results have not always been consistent and the economic feasibility dubious at best, when compared with insecticides (King *et al.* 1986). Another alternative to conventional synthetic insecticides is the microbial insecticide *Bacillus thuringiensis* or the expression of its toxins within genetically altered cotton. While the usefulness of *B. thuringiensis* has increased due to the development of new strains (Gasser & Fraley 1989, Lindow *et al.* 1989), laboratory selection experiments have produced resistance to *B. thuringiensis* in some insects (McGaughey 1985, McGaughey & Beeman 1988). Furthermore, development of resistance to *B. thuringiensis* has been documented in field populations of diamondback moth, *Plutella xylostella* (L.) (Tabashnik *et al.* 1990). Therefore, alternative strategies are desperately needed both in case of failure of *B. thuringiensis* and to use in resistance management programs to maintain it.

An alternative tactic for control of lepidopterous pests is the use of nuclear polyhedrosis viruses (NPVs) (Entwistle & Evans, 1985 for review). Applications of these viruses have greatly increased levels of Lepidoptera mortality in a variety of situations, but the major limitation of NPVs as a control procedure for agricultural pests is the extensive time required for death of infected insects (Hammock *et al.* 1993). The resulting crop destruction has seriously limited the use of NPVs in augmentative biological control or as biological insecticides. To address this problem, NPVs have been genetically modified to express insecticidal proteins resulting in a significant increase in their speed of action (McCutchen *et al.* 1991, Maeda *et al.* 1991, McCutchen & Hammock 1994). These genetically altered NPVs may provide agricultural scientists with a potent tool in combating outbreaks of arthropod pests attacking crops.

As with other forms of biological control (Howarth 1991, Greathead 1995, Hopper 1995, Andow *et al.* 1995), there currently exist several unresolved risks associated with large-scale agricultural applications of NPVs that include their effects to non-target organisms and their ability to displace naturally occurring NPVs. Because of these potential risks, there is widespread agreement for the need of detailed testing of recombinant NPVs prior to their release in pest management programs (Williamson 1991, Levidow 1992, Wood & Granados 1991). The goals of our current research were to (1) document the benefits in terms of pest efficacy of recombinant baculoviruses and (2) quantify the direct effects of recombinant baculoviruses on several non-target natural enemies.

Methods

These studies were conducted in commercial DPL 5557 cotton fields planted on 29 May, 1997 and located near Wall, Texas. Thirty-two plots of 12 rows by 50 feet were established, each separated by two rows or five row feed on a side as an untreated buffer.

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Six formulations of baculovirus, supplied by DuPont Agricultural Chemicals (Stine-Haskell Research Center; Newark, DE), were evaluated in each of two replicated studies. These were HzLqhIT2-ie1 (*Heliothis zea* virus with a gene coding for *Leiurus quinquestriatus hebraeus* scorpion toxin with an early, ie1, promotor), AcLqhIT2-ie1 (*Autographa californica* virus with a gene coding for *L. quinquestriatus hebraeus* scorpion toxin and an ie1 promotor), a combination of the HzLqhIT2-ie1 and AcLqhIT2-ie1, Hz wild-type, Ac wild-type, and a combination of the Hz wild-type and Ac wild-type. In addition, a set of plots were treated with a chemical standard (Tracer or Asana) and a second set of plots were left untreated. These 7 treatments were applied to 4 plots randomly distributed throughout the field.

The viral and chemical standard applications were coordinated with a significant egg lay of the target *H. virescens/H. zea* complex. Two applications occurred three days apart with each spray cycle. The first spray occurred on 28 July, 1997 with the second one falling on 31 July, 1997. The second significant egg lay came in early September. For this cycle the first spray came on 1 September, 1997 and the second on 4 September 1997. Natural enemy and *H. virescens/H. zea* sampling occurred both 24- and 48-hours after each spray application. Hence, for each spray cycle, there were 4 separate days of sampling.

Sampling was done by bending a maximum of 30 individual cotton plants into a white, 5-gallon bucket and shaking vigorously 4-5 times against the walls of the bucket. An aspirator was used to capture the natural enemies from the bucket with an emphasis placed upon the more common of the predators. The contents of the aspirator were then emptied into an empty plastic jar. After identify each natural enemy to genus or family (spiders) DNA was extracted from each specimen. Whether viral DNA associated with the baculovirus applications was found in the non-target natural enemy tissues was determined using the polymerase chain reaction (PCR).

Results

Field efficacy of each of the treatments were assessed by damage (numbers of damaged squares per 25 sampled) and yield (kg of lint per ha). Damage estimates ranged from below 1.0 (chemical and the HzLqh treatments) to 2.6 (the AcLqh and the Ac-wild type plus Hz-wild type treatments) The HzLqh recombinant viruses (alone and in combination with the AcLqh virus) performed better then their wild-type counterparts. Yields were the highest in the chemical control treatments (425 kg), followed by the AcLzh plus HzLqh (365 kg) and the Hz-wild type (320 kg) treatments. Each of the remaining treatments yielded approximately 320 kg of lint per hectare. However, yields from conventional insecticide plots were statistically indistinguishable from yields in plots treated with HzLqh recombinant virus. Thus, the HzLqh recombinant virus provided a level of plant protection statistically indistinguishable from that afforded by conventional insecticides.

Natural enemy densities in the virus treated plots were significantly greater than the natural enemy densities in plots treated with conventional insecticides. In terms of assessing the risk of recombinant technology on nontarget natural enemies, the densities of natural enemies in plots treated with recombinant baculoviruses were statistically indistinguishable from those treated with wild-type viruses. Thus, the recombinant baculoviruses do not appear to adversely affect natural enemy population densities different from the effects exerted by wild-type baculoviruses.

Risks associated with indirect effects of recombinant baculoviruses may include (1) the abilities of natural enemies to harbor recombinant DNA after consuming an infected host, and (2) subsequent but inadvertent movement of the recombinant DNA. Laboratory, maximum challenge tests against *Solenopsis invicta, Hippodamia convergens*, and *Geocoris punctipes* indicated that between 0.0 and 12.8% of workers or adults that consume virus infected H. virescens larvae harbor the recombinant DNA 48 hours after consumption of the infected prey. For each of the viruses tested, however, recombinant DNA were never recovered in F1 progeny or in *S. invicta* queens. Thus, it appears as though vertical transmissions of recombinant viruses are unlikely. Results from the field trial support these findings. Molecular analyses of *Orius* spp., *H. convergens*, and Thomisid spiders collected after the field trials never recovered any recombinant DNA.

Summary

Laboratory and field results reported here suggest that recombinant technology may be capable of producing inundative biological control agents that are effective for use in the field. Further, our current data suggest that recombinant technology does not pose an immediate threat to nontarget organisms. While previous studies conducted by McCutchen *et al.* (1996) suggested that recombinant baculoviruses may adversely affect parasitoid life histories, our studies with a wide range of predators find few detectable differences between effects from several recombinant and wild-type baculoviruses.

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