2001

Wildtype and recombinant baculoviruses for management of insect pests

Anthony John Boughton
Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Agricultural Science Commons, Agriculture Commons, Agronomy and Crop Sciences Commons, Entomology Commons, and the Microbiology Commons

Recommended Citation
https://lib.dr.iastate.edu/rtd/1099

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®
Wildtype and recombinant baculoviruses for management of insect pests

by

Anthony John Boughton

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Entomology
Major Professor: Bryony C. Bonning

Iowa State University
Ames, Iowa
2001
Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of
Anthony John Boughton
has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College
TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

CHAPTER 1. GENERAL INTRODUCTION
- Introduction 1
- Research Problem 2
- Literature Review 3
- Dissertation Organization 24
- References 25

CHAPTER 2. CHARACTERIZATION OF A NUCLEOPOLYHEDROVIRUS FROM THE BLACK CUTWORM, AGROTIS IPSILON (LEPIDOPTERA: NOCTUIDAE)
- Abstract 37
- Introduction 38
- Materials and Methods 39
- Results 43
- Discussion 44
- Acknowledgments 46
- References 46

CHAPTER 3. POTENTIAL OF AGROTIS IPSILON NUCLEOPOLYHEDROVIRUS FOR SUPPRESSION OF THE BLACK CUTWORM (LEPIDOPTERA: NOCTUIDAE) AND EFFECT OF AN OPTICAL BRIGHTENER ON VIRUS EFFICACY
- Abstract 53
- Introduction 54
- Materials and Methods 55
- Results 61
- Discussion 63
- Acknowledgments 67
- References Cited 68

CHAPTER 4. EFFECTS OF A NEW CLASS OF PROTEASE-EXPRESSING RECOMBINANT BACULOVIRUS ON LIFE HISTORY TRAITS OF THE PREDATOR COLEOMEGILLA MACULATA (COLEOPTERA: COCCINELLIDAE)
- Abstract 81
- Introduction 82
LIST OF FIGURES

CHAPTER 2.

Figure 1. Comparison of restriction enzyme digest profiles of AgipMNPV DNA (Ag, and AgJ), with those of AcMNPV C6 DNA (Ac) and RoMNPV R1 DNA (Ro). Purified DNA was digested with Hind III or Eco RI and electrophoresed on 0.8% agarose gel for 14 h at 75 V. 1-kb DNA ladder (1Kb) and λDNA Hind III (λHIII) size standards are shown.

Figure 2. Electron micrographs of AgipMNPV in tissue samples from Agrotis ipsilon. (a) Nucleus of infected hemocyte showing polyhedral occlusion bodies (POB) and virogenic stroma (VS). Nuclear membrane (NM). Bar = 2 μm. (b) POB in nucleus of fat body cell, showing virions (V) in longitudinal and transverse section. Each virion contains multiple nucleocapsids (NC). Bar = 200 nm. (c) Nucleus of fat body cell showing fibrillar bodies (FB). Nucleocapsids (NC) can be seen associating with de novo synthesized membrane (M) prior to packaging into mature virions. Bar = 500 nm.

CHAPTER 3.

Figure 1. Percent mortality resulting from various doses of AgipMNPV in water (Agip) or AgipMNPV in 0.5% M2R (Agip M2R), as predicted by probit analysis of LD50 data. Agip data plotted with solid symbols, Agip M2R data plotted with open symbols.

Figure 2. Back-transformed mean feeding damage ratings averaged across four replicates, for the eight treatments used in the greenhouse studies. Damage rating of 5 corresponds to cut seedlings while 1 corresponds to undamaged seedlings (see Methods). Bars show back-transformed standard errors. Columns with the same letter do not differ at p = 0.05 significance level by Tukey's means separation test. Columns with different letters are different at p = 0.05 significance level.

Figure 3. Trays of corn seedlings from the first replicate of the greenhouse studies, showing typical feeding damage observed after seven days for trays receiving different treatments. Treatments were as follows: (A) water only, (B) 0.5% M2R, (C) bait only, (D) bait + 0.5% M2R, (E) AgipMNPV in water, (F) AgipMNPV in 0.5% M2R, (G) bait + AgipMNPV, (H) bait + AgipMNPV in 0.5% M2R.

Figure 4. Back-transformed mean feeding damage ratings averaged across blocks for the five treatments used in the first and second field trials. Damage rating of 5 corresponds to cut seedlings while 1 corresponds to undamaged seedlings (see Methods). Bars show back-transformed standard errors.
CHAPTER 4.

Figure 1. Impact of AcMLF9. ScathL on *C. maculata* survival. Survival over time of *C. maculata* larvae fed on four different feeding regimes. Data for *C. maculata* larvae fed on ECB eggs and aphids are presented until the point of adult emergence only, when feeding was discontinued. Data from first replicate of survival studies. 40 *C. maculata* larvae per treatment.

Figure 2. Impact of AcMLF9. ScathL on *C. maculata* survival. Survival over time of *C. maculata* larvae fed on four different feeding regimes. Data for *C. maculata* larvae fed on ECB eggs and aphids are presented until the point of adult emergence only, when feeding was discontinued. Data from second replicate of survival studies. 20 *C. maculata* larvae per treatment.
LIST OF TABLES

CHAPTER 2.
Table 1. Preliminary Lethal Concentration Data For AgipMNPV Against First Instar Lepidoptera 50

CHAPTER 3.
Table 1. Lethal dose data for AgipMNPV in 3rd-instar A. ipsilon 73
Table 2. Pooled lethal dose data for AgipMNPV in 3rd-instar A. ipsilon 74
Table 3. Survival time data for AgipMNPV in 3rd-instar A. ipsilon 75
Table 4. Factorial analysis of variance showing the factors explaining significant amounts of variation in feeding damage under greenhouse conditions 76

CHAPTER 4.
Table 1. Time to death and survival proportions for C. maculata larvae fed on different feeding regimes 95
ABSTRACT

Wildtype and recombinant baculoviruses have potential for managing many serious agricultural pests. The black cutworm, *Agrotis ipsilon* (Hufnagel), is a serious pest of many crops worldwide. We have characterized a new baculovirus, the *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV), that was isolated from *A. ipsilon*. Restriction enzyme analysis showed AgipMNPV to be distinct from previously described nucleopolyhedroviruses, while electron micrographs of AgipMNPV polyhedra showed that virions contained multiple nucleocapsids. AgipMNPV was highly active against *A. ipsilon*. Four of seven other noctuid species tested, were also susceptible to infection by AgipMNPV. Studies were performed to assess the potential of AgipMNPV and a viral enhancing agent, M2R, for suppression of *A. ipsilon*. AgipMNPV was highly active against third-instar *A. ipsilon*. The optical brightener M2R significantly reduced LD$_{50}$ estimates by 160-fold, but had no direct effect on survival time estimates. In greenhouse and field trials, AgipMNPV significantly reduced feeding damage to corn seedlings caused by third-instar *A. ipsilon*, but there were no improvements in virus performance attributable to the inclusion of M2R in AgipMNPV formulations. In an appropriately designed pest management program, AgipMNPV could be used to suppress populations of *A. ipsilon*.

AcMLF9.ScathL is a new recombinant baculovirus that expresses a basement membrane degrading protease. Laboratory studies were conducted to assess potential negative impacts on the predator *Coleomegilla maculata* (Degeer), arising from consumption of *Heliothis virescens* F. larvae infected with AcMLF9.ScathL. Control groups of *C. maculata* were fed mock-infected *H. virescens*, *H. virescens* infected with wildtype AcMNPV, or European corn borer, *Ostrinia nubilalis* (Hübner), eggs and green peach aphids, *Myzus persicae* (Sulzer). There was no significant difference in *C. maculata* survival between the three *H. virescens* feeding regimes. Mean survival time of *C. maculata* larvae fed on mock-infected *H. virescens* was significantly longer than *C. maculata* fed on virus-infected...
*H. virescens*, possibly due to lower nutritional quality of virus-infected prey. There were no significant differences in survival times between *C. maculata* fed *H. virescens* infected with AcMLF9.ScathL or AcMNPV. These data suggest no greater threat to nontarget organisms from the use of AcMLF9.ScathL as a microbial insecticide than would occur with AcMNPV.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Billions of dollars worth of crops are lost to insect pests annually and crop losses to insects constitute one of the main factors limiting worldwide agricultural production. The insecticide market is dominated by sales of synthetic chemical insecticides. Of an annual global insecticide market of $7,635 million, synthetic chemical insecticides account for 95% of sales, the remaining $122 million of the market consisting of biologically-based insecticides such as insect pathogenic bacteria, fungi, or viruses or biologically-derived compounds (Powell & Rhodes 1994).

Recently there has been increased interest in use of biologically-based insecticides (Bonning & Hammock 1992). There have been several factors driving this shift, not least of which have been the time and cost involved in developing new chemical insecticides. Nowadays it can take up to 10 years to screen the 10,000 or more novel compounds that typically have to be evaluated in order to identify one or two compounds with suitable activity, persistence and specificity parameters for commercialization (Hunter-Fujita et al. 1998d). This process is expensive and may cost upward of $200 million. Given that exclusive patent rights only last 20 years, of which 10 years are occupied with research and development and five years are concerned with recouping costs, this means there may only be a five year period during which a new chemical insecticide is profitable. Problems with insect resistance to insecticides and in particular the increasing frequency of insects exhibiting cross-resistance to different classes of chemical insecticides have also become more common in recent years (Georghiou & Taylor 1986). Finally, heightened public concern about health risks associated with insecticide residues on food and in drinking water, together with increased worries about the detrimental effects of chemical insecticides on wildlife, have further driven interest in less toxic, more environmentally benign, biologically-based insecticides (Federici 1993).
It was estimated that these driving forces would lead to a 30% increase in the market for biologically-based insecticides by the end of 2000, to about $510 million, and that insect viruses would account for $5-6 million of the market (Georgis 1997).

Thirteen families of viruses contain members that are capable of infecting insect hosts (Hunter-Fujita et al. 1998a). Amongst these families, the Baculoviridae show the most promise for use in insect pest management applications. Baculoviruses are highly pathogenic, infect many serious agricultural pests, and are more stable in the environment than families of non-occluded entomopathogenic viruses (Hunter-Fujita et al. 1998a). In addition, baculoviruses are arthropod-specific and have no activity against vertebrates or plants, which is advantageous from an environmental risk perspective (Laird et al. 1990).

**Research Problem**

Baculoviruses are ubiquitous in natural systems inhabited by suitable arthropod hosts. Although many of these baculoviruses will have hosts that are of no economic importance, it is likely that some of these baculoviruses will be active against insect pests. Only through detailed laboratory studies can the pathogenicity, host range and potential utility of new viruses for use in pest management be determined. Even for viruses which are identified in the laboratory as having potential for use in insect pest management, their true utility can only be accurately gauged through greenhouse and field trials. Often a virus that exhibits promise in the laboratory fails to live up to expectations in the field owing to problems of low activity, persistence or speed of kill. To some extent these inherent limitations of baculoviruses can be overcome, either through the use of viral enhancing agents to improve baculovirus activity (Hamm 1999), or through genetic engineering of baculoviruses to improve insecticidal efficacy (Possee et al. 1997). However, although genetic engineering can be used to improve the utility of baculoviruses, this approach raises important questions regarding the risks posed to nontarget
organisms in the environment by recombinant baculoviruses relative to unaltered, wildtype viruses (Richards et al. 1998).

Four issues relating to the use of wildtype and recombinant baculoviruses in insect pest management are addressed in this dissertation. Firstly, identification, characterization and host range studies for a new baculovirus isolated from an important agricultural pest, the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Secondly, greenhouse and field trials to evaluate the potential of this virus for use in managing populations of the black cutworm. Thirdly, the feasibility of using a viral enhancing agent to improve the performance of this virus. Fourthly, laboratory studies to examine the risks posed to an insect predator, *Coleomegilla maculata* (Degeer) (Coleoptera: Coccinellidae), by the use of a newly developed protease-expressing recombinant baculovirus.

**Literature Review**

**Baculovirus biology**

The Baculoviridae is a large family of occluded, double stranded DNA viruses. The DNA genome is packaged into structures called nucleocapsids. One or more nucleocapsids surrounded by an associated membrane, constitutes a structure known as a virion. Depending upon the baculovirus in question, one or more virions are then encased within a protective crystalline protein matrix, forming an occlusion body. This protein occlusion body serves to protect the virus from attritional factors in the environment such as freezing/thawing, high temperatures, desiccation etc., and in so doing makes baculoviruses more stable in the environment than non-occluded families of insect viruses (Hunter-Fujita et al. 1998b). Environmental persistence is crucial for successful baculovirus transmission.

Two genera are recognized within the family Baculoviridae, the nucleopolyhedroviruses (NPVs) and the granuloviruses (GVs) (Volkman et al. 1995). These
two genera are differentiated on the basis of the arrangements of nucleocapsids and virions within occlusion bodies, the protein composition of the occlusion body, and the location within an infected cell where the occlusion body forms. GVs have a single nucleocapsid per virion, and one virion per occlusion body. The occlusion bodies are called granules, are composed largely of the protein granulin, and form in the cytoplasm of host cells during the infection cycle. Two different types of NPVs are recognized. Single-capsid nucleopolyhedroviruses (SNPVs) have a single nucleocapsid per virion, but many virions per occlusion, while multi-capsid nucleopolyhedroviruses (MNPVs) have many nucleocapsids per virion and many virions per occlusion. The occlusion bodies of NPVs are known as polyhedra, are composed largely of the protein polyhedrin, and form in the nuclei of host cells during the infection cycle.

Baculoviruses are named after the insect species from which they were first isolated, followed by the appropriate baculovirus subgroup. Thus the multicapsid nucleopolyhedrovirus of the alfalfa looper, Autographa californica (Speyer), is designated Autographa californica MNPV (AcMNPV).

Baculoviruses are arthropod-specific, only causing infections in insects and certain groups of crustaceans. There are no baculoviruses that infect vertebrates or plants. Within the class Insecta, baculoviruses primarily infect insects in the order Lepidoptera, although insects in the orders Hymenoptera and Diptera are also infected. Baculoviruses have been isolated from hosts in many different insect families, but most baculoviruses will typically only infect one or a few closely related hosts in the same family. Baculoviruses cause acute infections in their hosts, leading to death in 4-14 days. Baculoviruses exist in two morphologically distinct forms during different stages of the life cycle (Granados 1980). Virus particles derived from occlusion bodies are referred to as occlusion derived virus (ODV), and they are responsible for initiating infection of cells in the host midgut. The subsequent spread of the virus within the host and infection of other body tissues, is accomplished by a second viral phenotype known as budded virus.
The life cycle begins when a susceptible host ingests foliage contaminated with viral occlusion bodies. The occlusion bodies pass down the alimentary canal of the insect and under the alkaline conditions of the midgut the occlusion bodies dissolve, releasing virions into the lumen of the gut. The peritrophic membrane is a permeable membrane composed of chitin and protein that lines the midgut of many insects (Lehane 1997). The membrane has a protective role and serves to shield the delicate columnar cells of the midgut epithelium from abrasion by food particles. The membrane also serves to prevent pathogenic microorganisms such as bacteria from contacting the midgut epithelium (Lehane 1997). Baculovirus virions are able to pass through the peritrophic membrane, but the presence of the membrane serves to exclude a large proportion of the virions within the lumen of the midgut from contacting the cells of the midgut epithelium (Wang & Granados 2000). As such the peritrophic membrane acts as a partial barrier to baculovirus infection. Some virions do pass through the peritrophic membrane though, and when they contact the cells of the midgut epithelium, the virion envelope fuses with the plasma membrane of the midgut cell, allowing the nucleocapsid to enter the cytoplasm of the host cell (Kawanishi et al. 1972; Granados 1978). The nucleocapsid then moves to the nucleus, and undergoes one round of replication, before the progeny nucleocapsids pass out through the nuclear membrane into the cytoplasm. These nucleocapsids acquire an envelope of plasma membrane as they bud out of the cell (Keddie et al. 1989), giving rise to budded virus.

Subsequent movement of budded virus within the insect is limited by the basement membrane. The basement membrane is a fibrous extracellular matrix that surrounds the tissues of all animals, providing structural support, a filtration function and a surface for cell attachment (Rohrback & Rohrback 1993). Although the basement membrane is porous, the pores are too small to allow free passage of budded virus particles. Indeed accumulations of budded virus particles in the space between the midgut epithelium and the basement membrane have been documented (Hess & Falcon 1987). Thus, basement membrane constitutes a barrier to baculovirus infection, and there was much debate over how budded virus was able to
disseminate baculovirus infections within insect hosts. Recent studies have shown that baculovirus infections spread via the tracheal system of insects (Engelhard et al. 1994; Washburn et al. 1995). The tracheal system is the primary system concerned with gaseous exchange in insects, and consists of an extensive network of hollow tubes called tracheae, that are invaginations of the insect cuticle, and ramify throughout the insect body. Tracheae open to the air at small pores in the insect cuticle called spiracles, and internally the tracheae divide and subdivide forming ever finer tubes, eventually ending with a cell called a tracheoblast. Tracheoblasts give rise to processes called tracheoles which are the finest branches of the tracheal system. Tracheoles penetrate the basement membrane and associate intimately with the cells of the body tissues. Oxygen diffuses through the tracheae and is ultimately carried to the tissues via the tracheoles. Using recombinant baculoviruses expressing a \textit{lacZ} reporter gene, it has been demonstrated that budded virus enters tracheoles associated with cells of the midgut epithelium and subsequently spreads throughout the tracheal system via the epidermal cells that underlie the cuticular lining of the tracheae (Engelhard et al. 1994). Budded virus then disseminates infection to the other body tissues via the tracheoles that supply these tissues with oxygen. In this way, budded virus is able to gain access to body tissues without having to pass through basement membrane.

Baculovirus genes are expressed in at least three temporally distinct phases (early/late/very late) during the infection cycle. Virions are produced during the late phase of the infection cycle, and subsequent expression of very late phase genes, which code for the proteins granulin and polyhedrin, leads to the occlusion of virions within granules or polyhedra respectively. Following the production of occluded virus, infected host cells lyse. Ultimately the host dies, and viral chitinases and proteases weaken the cuticle of the dead host, eventually causing the cadaver to lyse, releasing occluded virus back into the environment.
Baculoviruses in pest management

For a long time it has been known that baculoviruses can be used to manage insect pests (Bishop et al. 1989; Fuxa 1990; Fuxa 1991a; Hunter-Fujita et al. 1998c). One of the first examples of the use of baculoviruses in this role was in 1892 when an NPV was introduced into pine forests in Germany to control populations of *Lymantria monacha* (L.) (Moscardi 1999). Since then, baculoviruses have been used in many parts of the world to control a large number of different pest species, the majority of which have been lepidopterans (Entwistle 1998; Moscardi 1999). There are four main strategies for the use of baculoviruses in pest management, these strategies being introduction-establishment, inoculative augmentation, inundative augmentation and conservation (Fuxa 1990).

Introduction-establishment is a strategy that involves the introduction of a baculovirus into an area where it previously did not exist, resulting in more or less permanent suppression of the pest. Probably the best example of a baculovirus working in this way, was that of the NPV of the European spruce sawfly *Gilpinia hercyniae* Hartig (Cunningham & Entwistle 1981). This pest was accidentally introduced into Canada from Europe and resulted in severe losses in forest systems. However, in the 1930's an NPV of *G. hercyniae* was accidentally introduced into Canada in a consignment of parasitoids that had been imported from Scandinavia for the control of this pest. Following the introduction of the NPV, problems with *G. hercyniae* rapidly declined, and for the last 50 years no management tactics have been necessary for this pest.

Inoculative augmentation is a strategy in which baculoviruses are introduced into the environment on a periodic basis. Typically one or a few small-scale applications of virus are made against pest populations at the beginning of the growing season. This virus replicates in hosts and persists in the environment throughout the growing season, bringing about regulation of subsequent generations of the pest. Inoculative augmentation is the strategy most commonly used for managing insect pests with baculoviruses and there are many examples of
the successful use of baculoviruses in this manner (Fuxa 1990). The best example of inoculative augmentation and also the most widespread use of a baculovirus in pest management, is the use of an NPV against the velvet bean caterpillar, *Anticarsia gemmatalis* (Hübner) on soybean in Brazil (Moscardi 1999). *A. gemmatalis* is the primary pest of soybean in Brazil, and management of this insect pest is necessary every growing season to prevent economic losses. Historically, several applications of chemical insecticides were made each year across huge areas of soybean in order to control *A. gemmatalis*. However in 1980 a pilot program overseen by the Brazilian Organization for Agricultural Research was started to evaluate the potential of *A. gemmatalis* MNPV (AgMNPV) for managing populations of *A. gemmatalis*. Field trials carried out during the first two years of the program, conclusively demonstrated that applications of AgMNPV reduced larval populations by over 80%, giving soybean yields comparable to those in insecticide treated control plots. Subsequent soybean yield data from the last 20 years has confirmed the value of AgMNPV as a bioinsecticide. In 1991, five companies started commercialization of AgMNPV. Virus was initially produced in several ways including in the laboratory using larvae reared on artificial diet, in screened field cages and by collection of dead larvae from farmers' soybean fields. It subsequently became clear though that laboratory and field cage production were too expensive, and production was switched entirely to field collection. Using this approach, one person can collect sufficient AgMNPV-killed cadavers in a day to treat about 100 hectares, which translates to a final cost to the farmer for the finished virus formulation of $1.20-1.50 per hectare, which is cheaper than the cost of chemical insecticides. During the 1997/98 growing season, AgMNPV was applied to approximately 1.2 million hectares of soybean in Brazil. Another example of the successful use of inoculative augmentation, is the utilization of *Lymantria dispar* MNPV (LdMNPV) for suppression of the gypsy moth, *Lymantria dispar* (L.) in forest systems in the United States (Cunningham 1998). In 1978 an LdMNPV-based viral formulation called Gypchek was registered by the US Forest Service, and by 1996 Gypchek had been applied to 19,200
hectares in USA. Improvements have been made to the formulation, by means of feeding stimulants, sticking agents, and ultraviolet (UV) light protectants, but the main factor limiting the use of Gypchek continues to be the lack of a commercial source of the virus.

Inundative augmentation involves massive applications of baculoviruses targeted against significant pest populations that are predicted to exceed the economic injury level. There is no expectation of virus establishment, persistence, or regulation of subsequent generations of the pest. The aim is simply to bring about high mortality within the targeted population. Use of pathogens in this way mirrors the goals behind the use of chemical insecticides, and led to the appearance of the term microbial insecticide. However when baculoviruses are used as microbial insecticides, great emphasis is placed on one of their weakest pest management attributes, namely their slow speed of kill. Thus when baculoviruses are used in inundative augmentation programs, it is difficult for them to compete with fast-acting chemical insecticides (Fuxa 1990). For this reason there are fewer examples of the successful use of baculoviruses in inundative augmentation programs. The best example of a baculovirus that has been used in this fashion is *Helicoverpa zea* SNPV (HzSNPV) (Cunningham 1998; Moscardi 1999). HzSNPV is highly active against *Helicoverpa zea* (Boddie) and also four other closely related species, *Heliothis virescens* (F.), *Helicoverpa armigera* (Hübner), *Helicoverpa punctigera* (Wallengren), and *Helicoverpa assulta* (Guenée). Together these *Heliothis/Helicoverpa* species form one of the most economically significant agricultural pest complexes in the world. Globally these species attack over 60 important crops including grasses such as maize and sorghum, various legumes and other important crops such as tomatoes, tobacco and cotton. In 1975, HzSNPV under the trade name Elcar®, became the first baculovirus insecticide to be registered in the USA. Between 1975 and 1980, approximately one million hectares of crops were treated with Elcar®. After this time though, sales decreased dramatically, primarily due to the appearance on the market of a new group of chemical insecticides, the synthetic pyrethroids. Elcar® is no longer available commercially, but in 1996 a new liquid formulation
of HzSNPV under the trade name Gemstar® was introduced for use against *H. virescens* and *H. zea* on cotton in USA.

Conservation is the last of the four strategies for baculovirus use. Conservation involves making changes to cultural practices within agricultural systems so as to maximize virus persistence and optimize the conditions in the environment that are required for virus transmission and the outbreak of epizootics. However, conservation has been utilized to only a limited degree, probably as a consequence of the fact that little research has been devoted towards understanding how to utilize this strategy. One example of the use of conservation was changes that were made to the grazing schedule in pastures in Louisiana, to increase the prevalence of NPV's and enhance virus regulation of damaging populations of *Spodoptera frugiperda* (J.E. Smith) (Fuxa & Geaghan 1983).

**Factors limiting the use of baculoviruses in pest management**

Although there are many examples of the successful use of baculoviruses for the management of insect pests, there are undeniably factors that limit their use in this role. Four main factors, namely low stability, slow speed of kill, problems with large-scale production, and high host specificity, limit baculovirus use in pest management (Fuxa 1990). Problems of low stability and slow speed of kill arise directly from the inherent biological characteristics of baculoviruses. Meanwhile problems that have emerged with virus production and high host specificity are largely a function of economic and market place pressures.

Although baculoviruses may persist for years in the environment in soil, they have low stability when exposed to sunlight. UV-A (320-400 nm) and UV-B (290-320 nm) radiation in the ultra violet region of the electromagnetic spectrum induces molecular changes in the DNA of baculovirus genomes (Hunter-Fujita et al. 1998e). These changes block normal DNA synthesis, leading to high rates of mutation which render baculoviruses inactive. Baculovirus
activity can be completely lost in 24 hours (Cantwell 1967), but more commonly the half-life is 2-5 days (Morris 1971; Jaques 1985).

Slow speed of kill is the second serious problem that reduces the utility of baculoviruses for use in pest management. Owing to the nature of the baculovirus infection cycle and the fact that it takes time to establish systemic infections, wildtype baculoviruses take from 4-14 days to kill their hosts. During this time infected insects continue to feed and cause crop damage. Certain baculoviruses contain genes for enzymes such as ecddysteroid glucosyl transferase (EGT) which, when expressed in the host insect, alter hormonal control of development. EGT prevents moulting and in so doing allows the host to feed for a longer period of time, which provides additional nutritional reserves for virus replication (O'Reilly & Miller 1991). It is clearly undesirable that insects continue to feed and cause damage for several days after application of a viral insecticide.

At present baculovirus production is still accomplished primarily in vivo, using infected insect hosts reared on artificial diets (Moscardi 1999). In vivo production is labor-intensive, time-consuming and requires insect rearing facilities (Hawtin & Possee 1993). In developing countries where labor costs are low, in vivo baculovirus production is financially feasible on a small to moderate scale (Hawtin & Possee 1993; Hunter-Fujita et al. 1998f). However in developed countries, automation of in vivo baculovirus production is obligatory if higher wage costs are to be offset (Hunter-Fujita et al. 1998f). Otherwise the baculovirus product is too expensive to be cost-competitive with chemical insecticides (Moscardi 1999). Industry is interested in large-scale production of baculoviruses by means of cell culture in huge bioreactors, but the absence of low cost cell culture medium and suitable cell lines make this strategy unfeasible at the present time (Federici 1993; Hawtin & Possee 1993; Possee et al. 1997; Moscardi 1999). Absence of a cost effective, large scale supply of LdMNPV is the main factor limiting greater use of Gypchek against gypsy moths in forest systems in North America (Cunningham 1998).
In theory high specificity of insecticides is desirable in integrated pest management (IPM) programs because of reduced impacts on beneficial natural enemies such as insect predators and parasitoids, and non-target organisms such as pollinators (Fuxa 1990). Because natural enemy populations are not directly impacted by applications of baculoviruses, insect predators and parasitoids can continue to regulate populations of secondary pests within the agricultural system. All too often, applications of broad-spectrum chemical insecticides decimate not only populations of the targeted pest, but also natural enemy populations. In the absence of regulation by natural enemies, populations of secondary pests explode, giving rise to secondary pest outbreaks (Pedigo 1996). Broad-spectrum insecticides can thus elevate pests which are normally of little importance to pests of major economic significance. Despite this positive aspect of the high specificity of baculoviruses, there is a down side. Baculoviruses are active primarily against lepidopterans and most viruses infect one or a few closely related host species in the same family. If a crop is attacked by several different pest species, it is unlikely that applications of one baculovirus will control all of these pests, even if these species are all lepidopterans. Control might be achieved by applying several baculoviruses, but this strategy cannot be used if the pests belong to other insect orders, such as Coleoptera, Hemiptera and Homoptera, which contain many of the common agricultural pests. In contrast, it is often possible to select a single chemical insecticide that will be effective against all the insect pests attacking a crop. Thus when contemplating the use of baculoviruses, farmers are faced with the prospect of having to buy several baculovirus pest control products, making more spray applications, and having to buy traditional insecticides to control non-lepidopteran pests. These considerations, coupled with the higher initial costs of baculoviruses relative to chemical insecticides have generated a certain amount of resistance to the adoption of baculoviruses by farmers. Meanwhile, from an industry perspective, the high specificity of individual baculoviruses, translates to limited market share. Many large agrochemical companies require potential products to have certain minimum earnings projections prior to commercialization. If a
product cannot generate this minimum level of sales, commercialization will not proceed even if the product has good biological activity and is financially competitive with rival products (Fuxa 1990).

**Strategies to overcome inherent baculovirus limitations**

The two main biological factors limiting the use of baculoviruses are their low persistence and slow speed of kill, and both of these problems have been partially overcome. Compounds have been evaluated for inclusion in baculovirus formulations to increase persistence, while alteration of baculoviruses using genetic engineering has enabled the production of recombinant baculoviruses with increased rates of kill.

**Optical brighteners**

A wide variety of UV protectants including particulates, dyes, reflectors and absorbers have been evaluated for inclusion in baculovirus formulations as potential UV protectants to improve field persistence (Ignoffo et al. 1997; Tamez-Guerra et al. 2000). Among these different compounds a group of stilbene disulfonic acid derivatives known as optical or fluorescent brighteners have shown particular promise. Optical brighteners absorb radiation in the non-visible UV part of the electromagnetic spectrum and re-emit this radiation as visible light in the blue part of the spectrum. These compounds have long been used in the textiles and detergents industries where their fluorescence activity makes clothes appear whiter and brighter.

Shapiro (1992) initially investigated optical brighteners as possible UV protectants for inclusion in formulations LdMNPV. These studies revealed that as predicted, optical brighteners provided baculoviruses with good protection from UV degradation, with 8 of 23 compounds providing complete protection of virus from UV degradation (Shapiro 1992). Subsequently it was noticed that in addition to providing UV protection, optical brighteners
also enhanced virus-induced mortality in gypsy moth larvae (Shapiro & Robertson 1992). This enhancement in virus activity was independent of improvements attributable to reduced UV degradation, because these benefits were seen even when virus formulations were not exposed to UV radiation. When second instar *L. dispar* were allowed to feed on artificial diet laced with LdMNPV and an optical brightener, lethal concentrations were reduced 529-1,670 fold relative to the virus alone, and the time taken for half of the larvae to die from virus infection (*LT* 50) was lowered from 11.2 days for the virus alone to 6.0 days for the virus and brightener (Shapiro & Robertson 1992). In addition, optical brighteners were also found to greatly enhance the activity of LdMNPV against later instar gypsy moth larvae (Webb et al. 1994a). Subsequent laboratory studies with gypsy moth larvae documented similar reductions in lethal concentrations of LdMNPV attributable to optical brighteners (Farrar et al. 1995; Shapiro & Argauer 1995; Argauer & Shapiro 1997).

The enhancing effect of optical brighteners has also been documented in other virus-host systems. Optical brighteners reduced lethal concentrations of *Spodoptera frugiperda* MNPV against *S. frugiperda* (Hamm & Shapiro 1992), *Pseudoplusia includens* SNPV against *Pseudoplusia includens* (Walker) (Zou & Young 1996), and *Spodoptera exigua* MNPV against *Spodoptera exigua* (Hübner) (Shapiro 2000). Optical brighteners significantly reduced lethal concentrations of *Anagatha falcifera* MNPV against *Trichoplusia ni* (Hübner), *H. virescens*, *H. zea* and *S. exigua* (Vail et al. 1996), and lethal doses of *Choristoneura fumiferana* MNPV in *Choristoneura occidentalis* Freeman (Li & Otvos 1999). The activities of AcMNPV, *Heliothis armigera* SNPV, AfMNPV, and *Galleria melonella* NPV against *S. frugiperda* (Shapiro & Hamm 1999) and *S. exigua* (Shapiro 2000) were also significantly improved by the inclusion of optical brighteners in virus formulations. Many of these studies also found that inclusion of optical brighteners in baculovirus formulations, reduced *LT* 50 values relative to the same dose of virus alone (Shapiro...
Field studies have generally shown beneficial effects arising from the inclusion of brighteners in baculovirus formulations. Field trials in oak woodland using spray applications of LdMNPV and an optical brightener, caused significantly greater mortality in third and fourth instar gypsy moth larvae than was observed with the same rate of virus alone (Webb et al. 1994a), or allowed similar levels of larval mortality to be achieved using 10-fold less virus (Webb et al. 1994b; Thorpe et al. 1998). Inclusion of optical brightener in LdMNPV formulations also reduced LT$_{50}$'s from 15.8 days to 10.5 days. In addition, application of solutions of brightener alone significantly increased virus-induced larval mortality in control plots, indicating that the brightener had the potential to enhance mortality caused by native NPV. In field tests on soybean, inclusion of 1% optical brightener in spray applications of PiSNPV, significantly increased mortality in $P.\ inchicola$ larvae, by 1.7-2.3 fold against manual infestations of second, third and fourth instars, and by 1.5-2.0 fold against naturally occurring populations (Zou & Young 1996). Field trials in cotton, consisting of spray treatments of AfMNPV and 1% optical brightener, in which leaves were subsequently harvested at various times post application and fed to $H.\ zea$ larvae in the laboratory, showed higher mortality with leaves sprayed with virus-brightener than with leaves sprayed with virus alone (Vail et al. 1999).

Despite the large amount of work that has been done during the last decade on optical brighteners as enhancers of baculovirus infections, relatively little was known about the mechanism of enhancement. Shapiro (1992) hypothesized that optical brighteners might somehow interfere with the synthesis of chitin microfibrils and in so doing, disrupt the integrity of the peritrophic membrane and facilitate virus-infection. One study in which a recombinant baculovirus expressing a reporter gene was used to elucidate the effects of optical brighteners on viral pathogenesis in $T.\ ni$ and $H.\ virescens$, suggested that viral enhancement
by optical brighteners resulted from inhibition of the shedding of infected columnar epithelial cells from the midgut of infected insects (Washburn et al. 1998). Only recently has it been shown that viral enhancement by optical brighteners is a direct result of disintegration of the peritrophic membrane (Wang & Granados 2000). Optical brighteners competitively bind to the chitin-binding proteins that normally maintain the integrity of the peritrophic membrane.

**Recombinant baculoviruses**

Approximately 20 recombinant baculoviruses have been successfully engineered for improved insecticidal efficacy (Harrison & Bonning 2000). In all cases the goal has been to reduce the period of time following application of viral insecticides during which virus-infected pests continue to feed. In most cases this has been accomplished by increasing baculovirus speed of kill. In theory though, any protein which brings about a reduction in feeding by the pest, whether by paralysis, change in behavior or other means, has potential for incorporation into recombinant baculovirus insecticides. Genes coding for peptide hormones, enzymes and insect-specific toxins have been used to develop recombinant baculoviruses with increased insecticidal activity.

Hormones play vital roles within insects by regulating important life processes and controlling growth and development. Genes coding for peptide hormones have been introduced into recombinant baculoviruses, as a means of increasing insecticidal efficacy. The first recombinant baculovirus with increased insecticidal activity, incorporated an insect diuretic hormone to disrupt osmoregulation (Maeda 1989). The diuretic hormone of the tobacco hornworm, *Manduca sexta* (Haworth), was introduced into *Bombyx mori* NPV and silkworm larvae, *Bombyx mori* (L.) infected with this recombinant BmNPV, died 20% faster and exhibited a 30% reduction in hemolymph volume relative to larvae infected with wildtype BmNPV (Maeda 1989). Attempts have also been made to incorporate insect eclosion, or moulting hormone, into a recombinant baculovirus, in an effort to disrupt normal development
and bring about death. A gene for the eclosion hormone of the *M. sexta* was introduced into AcMNPV, however although the recombinant baculovirus was seen to cause premature moult of infected larvae of *M. sexta* and *S. frugiperda*, increased larval mortality relative to wildtype AcMNPV was not observed (Eldridge et al. 1991).

Enzymes also play vital roles in insects, and several attempts have been made to introduce genes into baculoviruses that code for enzymes involved in the hormonal control of insect development. Juvenile hormone esterase (JHE) is one such enzyme. JHE controls the hydrolysis and inactivation of insect juvenile hormone (JH). High hemolymph levels of JH play a crucial role in maintaining the larval condition, while a low hemolymph titer of JH leads to molts to the pupal stage. Since most feeding damage to crops tends to be done by immature insects, reduction in JH levels by over expression of JHE in a baculovirus, leading to premature pupal molts, was an obvious strategy to investigate with recombinant baculoviruses. Initial attempts focused on incorporating a JHE coding sequence from *H. virescens* into AcMNPV (Hammock et al. 1990). *T. ni* larvae infected with this virus exhibited reduced growth and feeding, but these effects were small and were only observed in first instars. Subsequent investigations in *M. sexta*, showed that JHE is rapidly removed from the hemocoel of Lepidoptera (Ichinose et al. 1992). Recombinant baculoviruses which contained genes for mutated, but catalytically active forms of JHE (Bonning et al. 1997; Bonning et al. 1999) or catalytically inactive JHE (Bonning et al. 1995), exhibited significant improvements in insecticidal activity over wildtype AcMNPV and AcMNPV expressing unaltered JHE. The reasons for the increased insecticidal activity of these JHE-mutant viruses remain to be established.

In some cases recombinant baculoviruses have been produced by the deletion of genes from the virus genome, rather than by the incorporation of foreign genes. An example of this strategy is the production of recombinant baculoviruses by the deletion of the viral *egt* gene (O'Reilly & Miller 1991; O'Reilly et al. 1992). The *egt* gene encodes an enzyme called
ecdysteroid glucosyl transferase (EGT) which catalyzes the glycosylation of ecdysteroid molting hormones, and in so doing renders them catalytically inactive, preventing them from triggering molting and pupation of the infected host (O'Reilly & Miller 1989). The egt gene thus prolongs the larval stage of baculovirus infected larvae, enabling them to feed for a longer period of time, thereby promoting virus production. Deletion of the egt gene from viral genomes reduces the length of time infected larvae feed. Fourth instar *S. frugiperda* infected with an egt deletion mutant, molted earlier and exhibited lower feeding activity relative to larvae infected with wildtype AcMNPV (O'Reilly et al. 1992).

The first recombinant baculovirus expressing a neurotoxin incorporated a coding sequence for an insect-specific toxin (AaIT) of the Algerian scorpion, *Androctonus australis* Hector, into AcMNPV (Maeda et al. 1991; McCutchen et al. 1991; Stewart et al. 1991). AaIT causes changes in sodium channel conductance in insects, leading to pre-synaptic excitation, increased movement and ultimately paralysis and death. Lepidopteran larvae infected with AcMNPV AaIT exhibited a 50% reduction in feeding damage, and 25% reduction in lethal time, relative to larvae infected with wildtype virus (Stewart et al. 1991). Cessation of feeding and paralysis occur before death. Recombinant baculoviruses expressing insect-specific toxins with increased insecticidal activity have also been produced using the gene for the toxin TxP-1 from the straw-itch mite *Pyemotes tritici* Lagreze-Fosso and Montane (Tomalski & Miller 1991; Tomalski & Miller 1992), the gene for the toxin LqhIT2 from the Israeli yellow scorpion *Leiurus quinquestriatus hebraeus* (Birula) (Zlotkin et al. 1991), and various other insect-specific neurotoxin genes isolated from spiders (Hughes et al. 1997) and sea anemones (Prikhod'ko et al. 1996). Baculoviruses expressing insect-specific neurotoxins have been some of the most effective recombinant viruses developed to date, typically causing infected larvae to die in half the time taken by wildtype viruses (Treacy et al. 2000). For this reason, commercial interest in recombinant baculoviruses has been centered on these toxin-expressing constructs (Treacy & All 1996; Smith et al. 2000a; Treacy et al. 2000). American Cyanamid developed
recombinant forms of AcMNPV and HzSNPV which had deletions in the *egt* gene and contained a gene for AaIT. In laboratory bioassays, LT$_{50}$ estimates for AcMNPV AaIT in *H. virescens* and *T. ni* were 51% and 66% of those for AcMNPV respectively (Treacy & All 1996). The LT$_{50}$ estimates for HzSNPV AaIT in *H. virescens* and *H. zea* were 49% and 41% those for HzSNPV respectively (Treacy et al. 2000). Three field trials carried out in USA in 1998 showed that control of *H. virescens* and *H. zea* on cotton by HzSNPV AaIT was better than that achieved with *Bacillus thuringiensis* Berliner, equal to the biological insecticide spinosad and only slightly worse than with pyrethroid and carbamate insecticides (Treacy et al. 2000). DuPont Agricultural Products developed recombinant forms of AcMNPV and HzSNPV containing a gene for the scorpion toxin LqhIT2. Field trials carried out in 1997 with these viruses on cotton, showed that appropriately timed applications of HzSNPV LqhIT2 protected cotton from damage significantly better than applications of wildtype virus and as well as a chemical insecticide control (Smith et al. 2000a).

Recently a group of recombinant baculoviruses with a novel mode of action has been developed (Harrison & Bonning 2001). For some time evidence has suggested that the basement membranes that surround the tissues of all insects, act as a barrier to the spread of infections within insect hosts (Hess & Falcon 1987). It follows that if basement membranes do serve to restrict the spread of infection within insect hosts, then recombinant baculoviruses expressing enzymes which degrade basement membrane, might allow a more rapid spread of infection, leading to reductions in time to death. Six recombinant AcMNPV baculoviruses were constructed incorporating genes for three proteases known to degrade basement membrane (Harrison & Bonning 2001). Three of these recombinant viruses caused infected *H. virescens* larvae to melanize prior to death, but only one virus, AcMLF9.ScathL, exhibited significant improvements in insecticidal efficacy. AcMLF9.ScathL expresses a gene for a protease called cathepsin L (Scath L) isolated from the flesh fly, *Sarcophaga perigrina* Robineau-Desvoidy, under the control of the p6.9 baculovirus late promoter. LC$_{50}$ estimates for AcMLF9.ScathL in
first instar *H. virescens* were not significantly different from those of wildtype AcMNPV. However LT$_{50}$ estimates for AcMLF9.ScathL were only 49% those of wildtype AcMNPV, and were also lower than LT$_{50}$ estimates for recombinant viruses expressing AaIT and LqhIT2. In laboratory feeding trials on lettuce, second instar *H. virescens* infected with AcMLF9.ScathL, consumed similar amounts to larvae infected with a recombinant virus expressing LqhIT2, and 5-fold less than larvae infected with wildtype virus. These improvements in speed of kill and reductions in feeding damage observed with AcMLF9.ScathL are similar to those observed with the toxin-expressing recombinant baculoviruses developed by American Cyanamid and DuPont, which are among the most effective viruses produced to date.

**Risk assessment**

Recent progress with baculovirus research has improved the prospects of using baculoviruses to manage insect pests. Owing to their high specificity, the use of wildtype and recombinant baculoviruses will result in fewer deleterious effects on organisms in natural systems than the use of broad-spectrum chemical insecticides. Nevertheless, this does not mean that the use of baculoviruses poses no threat to the organisms present in natural systems. A theoretical framework was recently proposed which can be used to assess the risks posed to organisms in natural systems by the use of baculoviruses (Richards et al. 1998). They propose that in order to arrive at an accurate assessment of the risk posed to natural systems by the use of baculoviruses, consideration must be given to two separate issues: (1) impact evaluation and (2) exposure identification. Impact evaluation involves studies to determine the direct and indirect effects on organisms in the environment arising from contact with baculoviruses. Meanwhile, exposure identification examines the ways in which organisms in the environment might come into contact with baculoviruses. This framework recognizes the fact that in order for there to be a risk posed to organisms in the environment from the use of baculoviruses,
there must be both a negative impact on susceptible organisms and also some likelihood of exposure of susceptible organisms to the baculovirus.

Impact evaluation studies seek to determine whether or not there are likely to be detrimental effects on nontarget organisms present within agricultural systems arising from the use of baculoviruses. The nontarget species that have typically been examined play beneficial roles within agricultural systems, such as insect predators, parasitoids and pollinators. Pollinators, predators and parasitoids are not susceptible to infection by NPVs. However the possibility exists that insect predators and parasitoids that come into contact with lepidopteran larvae infected with recombinant baculoviruses, may suffer adverse effects as a consequence of contact with the foreign protein expressed by the virus.

Although a study on the hemipteran predator *Nabis roseipennis* Reuter (Ruberson et al. 1991) documented adverse effects arising from consumption of prey infected with wildtype baculoviruses relative to consumption of uninfected prey, the vast majority of studies on insect predators have failed to document any adverse effects on predator life history traits arising from consumption of baculovirus-infected prey (Young & Hamm 1985a; Young & Hamm 1985b; Vinson 1990). Studies done to date have failed to document any detrimental effects on insect predators arising from consumption of prey infected with recombinant baculoviruses relative to consumption of prey infected with wildtype baculoviruses. Consumption of *H. virescens* larvae infected with AcMNPV AaIT had no detrimental effects on adults of the insidious flower bug, *Orius insidiosus* (Say), or larvae of the green lacewing, *Chrysoperla carnea* Stephens, relative to consumption of wildtype virus-infected larvae (Heinz et al. 1995). In addition, injection of the recombinant virus into honey bees, *Apis mellifera* L. failed to cause adverse effects (Heinz et al. 1995). The social wasp, *Polistes metricus* Say, is a generalist predator in many ecosystems. Studies in which *P. metricus* adults were fed *S. frugiperda* larvae infected with recombinant baculoviruses expressing the mite neurotoxin TnP-1 or the scorpion toxin AaIT, failed to show any detrimental effects on wasp life history traits (McNitt et al. 1995). A
marker protein chloramphenicol acetyltransferase (CAT) was detected in *P. metricus* fed *S. frugiperda* larvae infected with a recombinant baculovirus in which the gene for CAT was under control of the *Drosophila* HSP70 promoter, which is a general promoter active in cells both permissive and non-permissive to baculovirus infections. However, CAT was not detected in wasps fed *S. frugiperda* larvae infected with a recombinant baculovirus in which the gene for CAT was under control of a baculovirus very late promoter, which is only active in cells permissive to baculovirus infection. Studies with five recombinant AcMNPV and HzSNPV viruses expressing AaIT or LqhIT2 failed to show any adverse effects on the adults of three generalist predators (Li et al. 1999). *H. virescens* larvae infected with these recombinant baculoviruses were fed to red imported fire ants, *Solenopsis invicta* Buren, big-eyed bugs, *Geocoris punctipes* (Say), and convergent ladybird beetles, *Hippodamia convergens* (Guérin-Méneville), but no impacts on survival, fecundity, travel speed or rate of food consumption were detected relative to predators fed *H. virescens* infected with wildtype virus. Field trials in cotton, in which predator densities were investigated following applications of AcMNPV AalT and HzSNPV AalT to control *H. virescens* and *H. zea*, failed to detect any differences in predator densities or diversity between recombinant and wildtype virus treatments (Smith et al. 2000a).

In contrast to the situation with most predators, impact evaluation studies have generally documented adverse effects of baculoviruses on parasitoids parasitizing the same host. These deleterious effects are not due to infection of parasitoids by baculoviruses, but arise from the fact that the parasitoid and virus compete for host resources. In addition if a host dies prematurely due to virus infection, so does the developing parasitoid larva. The issue of whether or not a developing parasitoid can survive baculovirus infection of the host is typically dependent upon the period of time that elapses between parasitization and virus infection of the host. If parasitization occurs two or three days before infection, the parasitoid larva will often survive. However if the gap is less, parasitoid survival is low. Two studies have looked at the
effect of recombinant baculovirus infection of a host on parasitoid survival. Although development time and adult size of *Microplitis croceipes* (Cresson) were reduced in wasps parasitizing *H. virescens* larvae infected with AcMNPV AaIT or a recombinant JHE mutant virus, parasitoid survival was not significantly different to that seen in hosts infected with wildtype virus (McCutchen et al. 1996). Survival, adult size and sex were recorded for *M. croceipes* parasitizing *H. virescens* larvae that were exposed at 2 or 4 days post-parasitization to field application rates of AcMNPV LqhIT2 and HzSNPV LqhIT2 or wildtype virus controls (Smith et al. 2000b). No differences were seen in emergence and sex ratios between parasitoids among recombinant, wildtype and control treatments. Significantly more parasitoids emerged from hosts parasitized four days before virus infection, but these wasps were significantly smaller than wasps from the two-day cohort, regardless of treatment.

Exposure identification studies have been concerned primarily with aspects of baculovirus ecology, namely replication, stability and dispersal because these factors determine the likelihood of exposure of susceptible organisms to baculoviruses. Studies have shown that baculoviruses can persist for many years in soil (Jaques 1985), but they are rapidly degraded upon exposure to UV radiation (Hunter-Fujita et al. 1998e). Baculoviruses in the environment are dispersed primarily by abiotic factors such as rain (Entwistle 1986), but may also be dispersed by biotic factors such as birds and grazing mammals (Fuxa 1991b; Entwistle et al. 1993), and insect predators, parasitoids and scavengers (Young & Yearian 1989; Vinson 1990; Vasconcelos et al. 1996; Li et al. 1999). Wildtype baculoviruses are ubiquitous in natural systems. Nevertheless widespread use of baculoviruses with broad host ranges such as AcMNPV, *Rachiplusia ou* MNPV or *Mamestra brassicae* MNPV may be avoided in areas with populations of ecologically sensitive Lepidoptera. Concerns have been raised about the possibility of genetic engineering causing significant changes in the biological characteristics of recombinant baculoviruses. Several exposure identification studies have attempted to address this issue, and also whether such changes are likely to influence the exposure of susceptible
organisms to the virus. For example, several studies of recombinant baculoviruses have documented lower production of polyhedra relative to the wildtype viruses, typically due to earlier death of the host (Cory et al. 1994; Kunimi et al. 1994). Studies have also documented delays in the lysis of the cuticles of hosts killed by recombinant baculoviruses (Fuxa et al. 1998), and this together with lower polyhedral production, reduces the spread of virus and likelihood of exposure of nontarget organisms to the recombinant baculoviruses.

Thus risk assessment, or environmental impact evaluation, for wildtype and recombinant baculoviruses must incorporate impact evaluation and exposure identification in order to accurately gauge whether the use of a baculovirus poses a threat to nontarget organisms in a given agricultural system.

Dissertation Organization

Chapters 2-4 of this dissertation contain three manuscripts. Chapter 2 is entitled "Characterization of a Nucleopolyhedrovirus from the Black Cutworm, Agrotis ipsilon (Lepidoptera: Noctuidae)". This manuscript was published in the Journal of Invertebrate Pathology (Volume 74, 289-294, 1999) and is co-authored by Robert L. Harrison, Leslie C. Lewis and Bryony C. Bonning. Dr. Harrison's contribution to the paper is the restriction enzyme digest shown in Fig. 1. Dr. Lewis provided the initial sample of AgipMNPV and comments on the manuscript. The manuscript in Chapter 3, "Potential of Agrotis ipsilon Nucleopolyhedrovirus for Suppression of the Black Cutworm (Lepidoptera: Noctuidae) and Effect of an Optical Brightener on Virus Efficacy", has been accepted, subject to minor revisions, for publication in the Journal of Economic Entomology. This paper is co-authored by Leslie C. Lewis and Bryony C. Bonning. Dr. Lewis provided extensive advice on the design and execution of the greenhouse and field trials described in this paper. The studies in Chapter 4, describe the effects of a new class of protease-expressing recombinant baculovirus on life history traits of the predator Coleomegilla maculata (Coleoptera: Coccinellidae). As my
major professor, Dr. Bonning has been involved in the design of all the experiments described in these manuscripts, and with the interpretation of results. Dr. Bonning also provided editorial comments during the preparation of these manuscripts. In addition to the manuscripts, this dissertation also contains a General Introduction (Chapter 1) and General Conclusion (Chapter 5). References are cited at the end of each chapter.

References


Shapiro, M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol. 85: 1682-1686.


Sixth Report of the International Committee on Taxonomy of Viruses. 10. Springer-Verlag, Vienna.


Zou, Y. & S. Y. Young. 1996. Use of fluorescent brightener to improve *Pseudoplusia includens* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus activity in the laboratory and field. J. Econ. Entomol. 89: 92-96.
CHAPTER 2. CHARACTERIZATION OF A NUCLEOPOLYHEDROVIRUS FROM THE BLACK CUTWORM, AGROTIS IPSILON (LEPIDOPTERA: NOCTUIDAE)

A paper published in the Journal of Invertebrate Pathology

Anthony J. Boughton, Robert L. Harrison, Leslie C. Lewis and Bryony C. Bonning

ABSTRACT

The black cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), is a serious localized pest of vegetable and field crops. We have characterized a newly discovered baculovirus, the Agrotis ipsilon multicapsid nucleopolyhedrovirus (AgipMNPV), that was isolated from A. ipsilon in Illinois. Restriction enzyme fragment profiles of AgipMNPV DNA were distinct from those of previously described nucleopolyhedroviruses. Electron microscopy of AgipMNPV-infected tissues indicated that nucleocapsids of this virus are multiply enveloped. A. ipsilon was highly susceptible to infection by AgipMNPV and significantly more susceptible to infection by AgipMNPV than by Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV). Host range studies showed that Heliothis virescens and Helicoverpa zea were moderately susceptible to infection; Pseudaletia unipuncta and Spodoptera frugiperda were only partially susceptible, and Anticarsia gemmatalis, Spodoptera exigua, Trichoplusia ni, and Ostrinia nubilalis were not susceptible to infection by AgipMNPV.

1 Reprinted from Journal of Invertebrate Pathology, Volume 74, 289-294 copyright © 1999 by Academic Press, reprinted by permission of the publisher.
2 Department of Entomology, Iowa State University, Ames, Iowa 50011
3 USDA-ARS Corn Insects and Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, IA 50011.
Because of its high virulence, AgipMNPV has potential as an alternative to chemical insecticides for control of *A. ipsilon*.

**INTRODUCTION**

The Baculoviridae is a large family of entomopathogenic viruses. Within the family, two genera of viruses are recognized, the nucleopolyhedroviruses (NPVs) and the granuloviruses (GVs) (Volkman et al., 1995). Baculoviruses have a narrow host-range, are highly pathogenic, and have occlusion bodies which make them more environmentally stable than some other families of entomopathogenic virus (Entwistle and Evans, 1985). These characteristics enable baculoviruses to be used as microbial control agents against insect pests, and there are many examples of the successful use of baculoviruses in this role (Entwistle, 1998; Moscardi, 1999).

We have isolated a new NPV from the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). *A. ipsilon* is a worldwide pest of over 30 important crops including tobacco, cotton, tomato, potato, cabbage, barley, and oats (Rings et al., 1975). In the US corn belt, *A. ipsilon* can be a serious localized pest of field corn (Clement and McCartney, 1982; Engelken et al., 1990). All instars of *A. ipsilon* feed on the leaves of corn seedlings, but the most serious damage results from leaf and stem cutting by the late instars (Clement and McCartney, 1982). Present management of *A. ipsilon* is based on population monitoring and rescue applications of chemical insecticide against damaging larval populations (Stockdale, 1977). Several Microsporidia, bacteria, fungi and viruses infect *A. ipsilon*, and amongst these pathogens, baculoviruses appear to have the most potential as alternatives to chemical insecticides (Ignoffo and Garcia, 1979).

In this paper we describe characterization of a new NPV, *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV). We present restriction enzyme profiles, ultrastructural
morphology, and lethal concentration data for nine species of agriculturally important Lepidoptera.

**MATERIALS AND METHODS**

*Insects*

Nine species of Lepidoptera were used in the study; eight from the family Noctuidae and one species, *Ostrinia nubilalis* (Hübner), from the family Crambidae. *A. ipsilon*, *Pseudaletia unipuncta* (Haworth), and *O. nubilalis* were obtained from USDA-ARS Corn Insects & Crop Genetics Research Unit, Ames, IA. *Helicoverpa zea* (Boddie) was obtained from a colony maintained by Dr. T. C. Baker, Entomology Department, Iowa State University, Ames, IA. *Spodoptera frugiperda* (J. E. Smith) was obtained from a colony maintained by Dr. F. Davis, USDA-ARS, Crop Research Laboratory, Mississippi State University, Starkville, MS. *Heliothis virescens* (Fabricius), *Spodoptera exigua* (Hübner), and *Anticarsia gemmatalis* Hübner, were obtained from Southern Insect Management Laboratory, Stoneville, MS. *Trichoplusia ni* (Hübner) was obtained from a colony maintained by Dr. T. Coudron, USDA-ARS, Biocontrol of Insects Laboratory, Columbia, MO. All insects were reared from eggs at 27°C with a photoperiod of 12 h light, 12 h dark. *A. ipsilon, P. unipuncta, H. zea, and A. virescens* were reared on pinto bean diet (Hendrix III et al., 1991), *O. nubilalis* was reared on wheatgerm diet (Guthrie, 1987). *S. frugiperda, S. exigua, and A. gemmatalis* were reared on general purpose soyflour diet (Southland Products, Lake Village, AR). *T. ni* was reared on Insecta-diet (Bio-Serv, Frenchtown, NJ).

*Viruses*

The original isolate of AgipMNPy was obtained from infected *A. ipsilon* larvae collected in Illinois by Dr. J. Maddox, Illinois Natural History Survey, Champaign. AcMNPy clone C6 (Possee, 1986) and *Rachiplusia oz* multicapsid nucleopolyhedrovirus (RoMNPy)
clone R1 (Smith and Summers, 1980) were used for comparison in the restriction enzyme studies.

*Virus amplification and purification*

Amplification of AgipMNPV was performed in fifth instar *A. ipsilon*, and virus concentrations were quantified with a phase contrast microscope and a Neubauer bright-line hemocytometer (Fisher Scientific, Pittsburg, PA). Larvae were placed in individual 17-ml jelly cups (Fill-Rite Inc., Newark, NJ), and starved overnight. One 3 mm cube of diet inoculated with 800,000 polyhedral occlusion bodies (POBs) of AgipMNPV, was added to each cup. Larvae that consumed the inoculated diet cube were supplied with additional diet and maintained at 27°C. Virus infections were confirmed by the presence of POBs in the hemolymph, when viewed under phase contrast microscopy. Subsequent amplification of virus was accomplished by injection of hemolymph (5 μl) from infected larvae, into uninfected sixth instar *A. ipsilon*, which were maintained on diet until death. For purification of viral POBs, cadavers were homogenized using a 30-ml dounce homogenizer (Wheaton Scientific Products Inc., Millville, NJ) for 10 min in 0.1% SDS (1 ml per cadaver), and filtered through five layers of cheese cloth. POBs were pelleted by centrifugation at 3,600g for 10 min at room temperature in 42-ml glass centrifuge tubes. The pellet was resuspended in 0.5% SDS, and centrifugation and resuspension repeated with 0.5 M NaCl, before final resuspension of POBs in distilled water (O'Reilly et al., 1992). POBs were mixed with 0.01 M phosphate buffered saline (PBS) pH 7.4, loaded (2 ml per tube) onto 34-ml linear sucrose gradients (40-65%) in 0.01 M PBS, and centrifuged at 93,000g for 3 h at room temperature in SW28 ultracentrifuge tubes (Beckman Inc., Palo Alto, CA). The bands of viral occlusions were recovered with a tissue culture pipette and diluted five fold in distilled water. The resulting mixture was centrifuged at 900g for 10 min at room temperature in 42-ml glass centrifuge tubes, and the
pelleted POBs resuspended in a small volume of distilled water. AgipMNPV used in all subsequent experiments came from this stock.

**Extraction of viral DNA**

Virus purified by sucrose gradient centrifugation, was diluted to $1 \times 10^6$ POBs/μl, and sodium carbonate added to a final concentration of 0.1 M. This mixture was incubated at room temperature for 30 min, and then Tris HCl was added to a final concentration of 0.1 M (O'Reilly et al., 1992). The resulting suspension was centrifuged at 3,600g for 5 min at room temperature in 50-ml tissue culture tubes (Fisher Scientific, Pittsburg, PA). The supernatant, containing the released virions, was then layered onto 3-ml cushions of 25% sucrose in 5 mM NaCl, 10 mM EDTA, and made up to a final volume of 35 ml with 0.01 M PBS, and centrifuged at 76,000g for 75 min at 4°C in SW28 ultracentrifuge tubes. The supernatant and underlying sucrose cushion were decanted, and the virion pellets resuspended in 0.5 ml of TE (10 mM Tris, 1 mM EDTA, pH 8). Virions and nucleocapsids were digested by incubation with 1% SDS and 1/20 vol. protease-k (200-400 units/ml), for 1 h at 56°C. DNA was extracted by a series of organic extractions using phenol, 1:1 phenol: chloroform-isoamyl alcohol, and 24:1 chloroform: isoamyl alcohol. DNA was precipitated with 0.3 M sodium acetate and 95% ethanol, and following overnight incubation at 4°C, was pelleted by centrifugation at 16,000g for 15 min. The resulting DNA pellets were washed in 70% ethanol, dried, and then resuspended in a small volume of TE.

**Restriction enzyme digests**

Viral DNA (3 μg) from AgipMNPV, AcMNPV C6 and RoMNPV R1 was digested with 25 units of Hind III or Eco RI, for 3 h at 37°C. Digested DNA, together with 1-kb ladder DNA (0.5 μg) and Lambda-Hind III marker DNA (0.5 μg), were loaded onto a 20 x 25-cm
0.8% agarose gel, and run at 75 V for 14 h. The gel was stained for 30 min in ethidium bromide solution (0.5 μg/ml), and then photographed under ultraviolet light. AgipMNPV DNA was also digested with 11 other restriction enzymes, and run out on an agarose gel, under similar reaction conditions. Restriction enzyme (RE) fragment patterns from digests of AgipMNPV DNA were then compared with published RE digest profiles of DNA from 28 other NPVs.

**Ultrastuctural studies**

Sixth instar *A. ipsilon* were fed 3-mm cubes of diet inoculated with $6 \times 10^6$ POBs of AgipMNPV, and subsequently maintained at 27°C. Hemolymph was collected six days post infection from anaesthetized larvae (chilled to 4°C), by making a small incision at the base of one of the abdominal prolegs, and collecting hemolymph in an eppendorf tube on ice. Dithiothreitol was immediately added to the hemolymph to a final concentration of 1 mM, to inhibit the phenol oxidase cascade (O'Reilly et al., 1992). Virus infections were confirmed by examining larval hemolymph for the presence of POBs within hemocytes, using phase contrast microscopy. Hemolymph was centrifuged at 400g for 4 min at 4°C, to pellet hemocytes. Fat body tissue was dissected from infected larvae bathed in 1% electron microscopy (EM) grade glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA). Hemocyte pellets and fat body tissue were fixed overnight at 4°C, in 1% EM grade glutaraldehyde. Following fixation, samples were washed three times in 0.01 M PBS, and postfixed in 1% osmium tetroxide in PBS, for 1 h at room temperature. Samples were dehydrated through a standard ethanol series (25-100% ethanol, 15 min per step), and cleared with three washes in acetone. Tissues were infiltrated with EMBED 812 epoxy resin (Electron Microscopy Sciences) in a stepwise fashion with ratios of acetone to EMBED of 3:1, 1:1, 1:3, and finally several changes of pure resin. Resin blocks were dried in an oven at 60°C for 48 h. Thin silver interference sections (50-80 nm thick) were cut with a Reichert Ultracut S ultramicrotome, using a diatome
Diamond knife, and collected on 200-mesh copper grids. Thin sections were counterstained with 10% methanolic uranyl acetate for 15 min, followed by Sato-S lead acetate stain for 10 min. Sections were examined with a Jeol 1200 EX scanning/transmission electron microscope at 80 KV.

Bioassays

Lethal concentration bioassays were carried out with first instar *A. ipsilon*, using a droplet feeding technique (Hughes and Wood, 1981). First instars were allowed to drink from suspensions of distilled water containing 1/25 vol. blue food coloring dye, and one of six concentrations of virus (0 (control), 200, 1000, 5000, 25,000, 125,000 POBs/µl), in 60-mm petri dishes. After 15 min, larvae that had ingested these suspensions were moved into 24-well tissue culture plates (Fisher Scientific) containing diet, using paint brushes. Treatments were checked after 24 h for deaths resulting from handling, and were scored for mortality at 10 days post infection, and subsequently until no further mortality occurred. *A. ipsilon*, *H. virescens*, *H. zea*, *S. frugiperda*, *P. unipuncta*, *A. gemmatalis*, *S. exigua*, *T. ni*, and *O. nubilalis* were tested for susceptibility to infection by AgipMNPV. Thirty five larvae were used per dose, and bioassays in each species were replicated at least twice. For comparative purposes, bioassays in *A. ipsilon* were also performed using AcMNPV C6 and RoMNPV R1. Data were subjected to probit analysis (Russell et al., 1977) and the assumptions of the models verified (Robertson and Preisler, 1992).

RESULTS

Restriction Enzyme Digests

Restriction enzyme digest profiles of AgipMNPV DNA lacked submolar bands. AgipMNPV DNA digested with *Hind* III and *Eco* R1 yielded fragment patterns distinct from those produced by digestion of AcMNPV C6 and RoMNPV R1 DNA (Fig. 1). Digests of
AgipMNPV DNA carried out with 11 additional restriction enzymes (data not shown), yielded restriction enzyme profiles distinct from those of 28 NPVs in the literature.

**Ultrastructural Studies**

Electron microscopy of tissues from insects infected with AgipMNPV, revealed multiple POBs within the nuclei of infected cells (Fig. 2a). Regions of virogenic stroma, were visible in the nuclei of infected host cells. POBs were seen to contain many virions, and multiple nucleocapsids were encapsulated within the membrane of each virion (Fig. 2b). Within the nuclei of some cells, nucleocapsids were stacked along sheets of membrane. Fibrillar bodies were also apparent in the nuclei of infected cells (Fig. 2c).

**Bioassays**

Preliminary data indicated that susceptibility to infection by AgipMNPV varied greatly among the lepidopteran species used in the host range studies (Table 1). First instar *A. ipsilon* were the most susceptible, with an LC$_{50}$ of 269 POBs/µl. *H. virescens* and *H. zea* were moderately susceptible to infection, while *P. unipuncta* and *S. frugiperda* were only semi-permissive to infection. *A. gemmatalis, S. exigua, T. ni,* and *O. nubilalis* were not susceptible to infection by AgipMNPV at the concentrations used in this study. *A. ipsilon* was susceptible to AcMNPV C6 and RoMNPV R1, with LC$_{50}$ values of 1,478 and 27,626 POBs/µl respectively.

**DISCUSSION**

Ultrastructural studies of infected insect tissue, showed AgipMNPV to have a typical baculovirus morphology. POBs were present in the nuclei of infected cells, confirming that this new virus is an NPV, and multiple nucleocapsids were seen within each virion, indicating that this virus is a multicapsid NPV (Volkman et al., 1995). Restriction digest fragment patterns of
AgipMNPV DNA were distinct from published fragment patterns from 28 NPVs. It was confirmed that restriction fragment profiles of AgipMNPV were different from the fragment profiles of Spodoptera frugiperda MNPV (Shapiro et al., 1991) and Anticarsia gemmatalis MNPV (Johnson and Maruniak, 1989), which are both common throughout the geographic range of A. ipsilon. The restriction fragment profiles of AgipMNPV were also determined to be distinct from the fragment profiles of Agrotis segetum MNPV (AsMNPV) (Allaway and Payne, 1983). The 4.2 Kb Hind III K fragment of the AgipMNPV genome was sequenced, and yielded a nucleotide sequence unlike that of any previously published baculovirus nucleotide sequence (data not shown). Although no submolar bands were detected in the restriction enzyme digests, the possibility of a small amount of contaminating virus in the stock suspension, cannot be excluded.

Preliminary bioassay data indicated that A.ipsilon was the most susceptible species tested, although H. virescens and H. zea were also susceptible to AgipMNPV. Other species were not susceptible, or were only semi-permissive to infection by AgipMNPV even at the highest infection concentration of 125,000 POBs/μl. In addition to differences in susceptibility between different host species, the slopes of the dose mortality curves differed markedly. Steep slopes indicate that mortality levels change significantly in response to relatively small changes in virus dose. The volumes of fluid ingested by first instars of several noctuid species during droplet feeding bioassays have been quantified (Hughes et al., 1986; Van Beek and Hughes, 1986; Kunimi and Fuxa, 1996). In all cases, larvae ingested 6-11 nl of fluid. Assuming that first instar A.ipsilon ingest an average of 11 nl of virus suspension during droplet feeding bioassays, a conservative estimate for the LD₅₀ of AgipMNPV, is 3 POBs per larva. This LD₅₀ is similar to that of AcMNPV against a susceptible host, such as T. ni (Hughes et al., 1986).

The high susceptibility of A.ipsilon to AgipMNPV suggests that this virus will be more effective for A.ipsilon control than other viruses that have been tested, such as Agrotis
segetum granulovirus, AcMNPV C6 and RoMNPV R1 (Johnson and Lewis, 1982; Zethner and Ogaard, 1982; Allaway and Payne, 1984).

In summary, AgipMNPV is a new baculovirus isolated from *A. ipsilon*. AgipMNPV has high virulence against *A. ipsilon* and appears to have greater potential as a microbial control agent for use against this insect than other baculoviruses investigated to date. Greenhouse and field trials are underway to assess the potential of AgipMNPV for control of *A. ipsilon*.

ACKNOWLEDGMENTS

AcMNPV clone C6 and RoMNPV clone R1 were obtained from Dr. R. D. Possee (Institute of Virology and Environmental Microbiology, Oxford, UK) and Dr. M. Summers (Texas A&M, TX) respectively. We would also like to thank Drs. Joel Coats, Diane Debinski and John Obrycki for comments on the manuscript. This is a joint contribution from the USDA, Agricultural Research Service, and the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project 3301, as Journal Paper J-18422. Names are necessary to report factually on available data; however, neither the USDA nor Iowa State University guarantees or warrants the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may be suitable.

REFERENCES


**FIGURE LEGENDS**

**FIG. 1.** Comparison of restriction enzyme digest profiles of AgipMNPV DNA (Ai<sub>1</sub> and Ai<sub>2</sub>), with those of AcMNPV C6 DNA (Ac) and RoMNPV RI DNA (Ro). Purified DNA was digested with *Hind* III or *Eco* RI and electrophoresed on 0.8% agarose gel for 14 h at 75 V. 1-kb DNA ladder (1Kb) and λDNA *Hind* III (λHIII) size standards are shown.

**FIG. 2.** Electron micrographs of AgipMNPV in tissue samples from *Agrotis ipsilon*. (a) Nucleus of infected hemocyte showing polyhedral occlusion bodies (POB) and virogenic stroma (VS). Nuclear membrane (NM). Bar = 2 μm. (b) POB in nucleus of fat body cell, showing virions (V) in longitudinal and transverse section. Each virion contains multiple nucleocapsids (NC). Bar = 200 nm. (c) Nucleus of fat body cell showing fibrillar bodies (FB). Nucleocapsids (NC) can be seen associating with *de novo* synthesized membrane (M) prior to packaging into mature virions. Bar = 500 nm.
TABLE 1

Preliminary Lethal Concentration Data For AgipMNPV Against First Instar Lepidoptera

<table>
<thead>
<tr>
<th>Host species</th>
<th>LC$_{50}$ b (POBs/µl)</th>
<th>95% Confidence interval</th>
<th>Slope</th>
<th>Heterogeneity (χ²/D.F.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrotis ipsilon</em></td>
<td>269</td>
<td>(145-407)</td>
<td>1.77</td>
<td>0.26</td>
</tr>
<tr>
<td><em>Heliothis virescens</em></td>
<td>797</td>
<td>(324-1,533)</td>
<td>0.78</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Helicoverpa zea</em></td>
<td>7,083</td>
<td>(3,359-15,788)</td>
<td>0.86</td>
<td>0.34</td>
</tr>
<tr>
<td><em>Spodoptera frugiperda</em></td>
<td>108,260</td>
<td>(44,647-550,820)</td>
<td>0.69</td>
<td>0.96</td>
</tr>
<tr>
<td><em>Pseudalita unipuncta</em></td>
<td>125,370</td>
<td>(36,639-694,510)</td>
<td>1.03</td>
<td>1.88</td>
</tr>
<tr>
<td><em>Anticarsia gemmatalis</em></td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Spodoptera exigua</em></td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Trichoplusia ni</em></td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Ostrinia nubilalis</em></td>
<td>NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note. NS, not susceptible to AgipMNPV at the doses used in this study.

a 35 larvae were exposed per virus concentration. Bioassays were replicated twice for each species, and data reported are not significantly different from the second replicate for that species.

b Data analyzed by probit analysis (Russell et al., 1977). Probit-dose relationship linear at α=0.05 confidence level. Data fit probit model by χ² test at α=0.05.
FIG. 1.
CHAPTER 3. POTENTIAL OF *AGROTIS IPSILON* NUCLEOPOLYHEDROVIRUS FOR SUPPRESSION OF THE BLACK CUTWORM (LEPIDOPTERA: NOCTUIDAE) AND EFFECT OF AN OPTICAL BRIGHTENER ON VIRUS EFFICACY

A paper submitted to Journal of Economic Entomology

Anthony J. Boughton¹, Leslie C. Lewis¹,² and Bryony C. Bonning¹

Abstract

Studies were performed in the laboratory, greenhouse and field to assess the potential of *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV) and a viral enhancing agent, M2R, for suppression of *Agrotis ipsilon* (Hufnagel). In laboratory droplet feeding bioassays, AgipMNPV was shown to be highly active against third-instar *A. ipsilon*. The optical brightener M2R significantly reduced LD₅₀ estimates by approximately 160-fold, but had no direct effect on survival time estimates. In greenhouse trials, spray and bait formulations of AgipMNPV significantly reduced feeding damage to corn seedlings caused by third-instar *A. ipsilon*. In two sets of replicated field trials, bait formulations of AgipMNPV significantly reduced feeding damage to corn seedlings by third-instar *A. ipsilon*. However there were no beneficial effects attributable to the inclusion of M2R in AgipMNPV formulations under greenhouse or field conditions. It seems likely that in an appropriately designed pest management program AgipMNPV could be used to suppress field populations of early and mid-instar *A. ipsilon*.

¹Department of Entomology, Iowa State University, Ames, Iowa 50011
²USDA-ARS Corn Insects & Crop Genetics Research Unit, Genetics Laboratory, Iowa State University, Ames, IA 50011.
Introduction

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a worldwide pest of over 30 important crops (Rings et al. 1975), and in the US corn belt, *A. ipsilon* can be a serious localized pest of field corn (Clement & McCartney 1982; Engelken et al. 1990). All instars of *A. ipsilon* feed on the leaves of corn seedlings, but the most serious damage results from leaf and stem cutting by late instars (Clement & McCartney 1982). Present management of *A. ipsilon* is based on population monitoring and rescue applications of chemical insecticide against damaging larval populations (Stockdale 1977).

A baculovirus, *Agrotis ipsilon* multicapsid nucleopolyhedrovirus (AgipMNPV) with potential for use in managing *A. ipsilon* was recently characterized (Boughton et al. 1999). However to date, baculoviruses have not been widely used as microbial insecticides for the management of insect pests, due primarily to four main factors limiting their use (Moscardi 1999): (1) high production costs, (2) low biological activity (slow to kill), (3) low activity against late instars, and (4) low persistence due to degradation by ultraviolet (UV) light. A range of compounds have been evaluated as potential adjuvants for inclusion in baculovirus formulations to overcome some of these inherent biological limitations. Amongst these compounds a group of stilbene disulfonic acid derivatives known as optical or fluorescent brighteners have shown particular promise (Shapiro 1992). Optical brighteners absorb radiation in the non-visible UV part of the electromagnetic spectrum and then re-emit this radiation as visible light in the blue part of the spectrum. These compounds have long been used in the textile and detergent industries where their fluorescence activity makes clothes appear whiter and brighter.

Shapiro initially investigated optical brighteners as possible UV protectants for inclusion in formulations of the gypsy moth nucleopolyhedrovirus (LdMNPV) (Shapiro 1992). Subsequently it was found that not only did these compounds offer protection against UV light, but they also enhanced virus-induced mortality in gypsy moth, *Lymantria dispar* (L.)
larvae (Shapiro & Robertson 1992). When second-instar *L. dispar* were fed artificial diet laced with LdMNPV and the optical brightener M2R (Calcofluor white M2R, Tinopal LPW, fluorescent brightener 28), lethal concentrations were reduced 529-1670 fold relative to the virus alone, and the time taken for 50% of exposed larvae to die from virus infection (LT$_{50}$) was lowered from 11.2 days for the virus alone to 6.0 days for the virus and brightener (Shapiro & Robertson 1992). In addition, optical brighteners were also found to greatly enhance the activity of LdMNPV against later instar gypsy moth larvae (Webb et al. 1994a).

Since then, studies in a range of baculovirus-host systems have shown optical brighteners to function as enhancers of viral activity (Hamm & Shapiro 1992; Farrar et al. 1995; Vail et al. 1996; Zou & Young 1996; Argauer & Shapiro 1997; Li & Otvos 1999). Several studies have also shown that optical brighteners can act as enhancers of baculovirus activity under field conditions, either by elevating pest mortality relative to treatment with virus alone (Hamm et al. 1994; Li & Otvos 1999; Vail et al. 1999) or by allowing similar levels of mortality to be achieved using lower application rates of virus (Webb et al. 1994a; Thorpe et al. 1998).

In this paper we report the results of laboratory bioassays to evaluate enhancement of AgipMNPV activity by the optical brightener M2R, against *A. ipsilon* larvae. We also present the results of greenhouse and field trials to evaluate the potential of AgipMNPV and M2R for suppression of *A. ipsilon*.

**Materials and Methods**

**Insects.** *A. ipsilon* eggs were obtained from a colony maintained by USDA-ARS Corn Insect and Crop Genetics Research Unit (Ames, IA). Eggs were placed in an incubator at 28°C with a photoperiod of 12 h light, 12 h dark, and following emergence, groups of 50 newly emerged first-instar larvae were transferred to 237-ml ice cream cups containing artificial pinto bean diet (Hendrix III et al. 1991). Larvae were reared to third-instar, and were either used at this developmental stage, or were transferred individually into 17-ml jelly cups (Fill-
Rite Inc., Newark, NJ) containing diet, and maintained at 28°C until they reached the desired developmental stage.

**Virus.** AgipMNPV used in the lethal dose and lethal time bioassays originated from the same virus stock used for the initial host range screening studies (Boughton et al. 1999), which was produced by a single amplification passage through *A. ipsilon* larvae. The AgipMNPV used in the greenhouse and field studies was produced by a second amplification passage through *A. ipsilon* larvae. Virus concentrations were quantified with a phase contrast microscope and a Neubauer bright-line hemocytometer (Fisher Scientific, Pittsburg, PA). Three hundred fourth-instar *A. ipsilon* were starved overnight and inoculated orally with a solution of AgipMNPV stock, using a modified droplet feeding technique (Hughes & Wood 1981). A 10-μl micropipette (Rainin Instrument Company, Inc., Woburn, MA) was used to pipette 2 μl of a 500,000 polyhedral occlusion body (POB) per microliter solution of AgipMNPV, containing 4% Schilling blue food coloring dye (McCormick & Co., Inc., Hunt Valley, MD), directly in front of the starved larva in each jelly cup, and the larva allowed to drink. Larvae that fully ingested the droplet of virus solution were provided with artificial diet, and maintained at 28°C. Larvae that failed to ingest the entire droplet were discarded. Larvae were monitored daily from four days post inoculation, and following death, cadavers were frozen and polyhedra extracted as described previously (Boughton et al. 1999). Sodium azide was added to polyhedral preparations to a final concentration of 0.02% to prevent bacterial growth, and virus stocks were stored at 4°C.

**Lethal dose bioassays.** Third-instar *A. ipsilon* housed individually in 17-ml jelly cups were starved overnight and inoculated with solutions of AgipMNPV in water or AgipMNPV in 0.5% (W:V) M2R. For bioassays of AgipMNPV in water concentrations of 0, 250, 750, 1000, 1500 and 3000 POBs/μl were used, while for bioassays of AgipMNPV in 0.5% M2R, concentrations of 0, 1, 3, 9, 27 and 81 POBs/μl were used. All solutions contained 4% blue food coloring dye, and larvae were inoculated with a 1-μl droplet of virus.
solution, using the modified droplet feeding technique described above. Larvae that failed to ingest the entire droplet of fluid were discarded. Sufficient larvae were inoculated at each dose to obtain 35 larvae that had ingested the entire droplet of solution. Following inoculation, cubes of diet were introduced into each jelly cup and the larvae subsequently maintained at 28°C. Bioassays were replicated three times. Mortality was scored at nine days post infection. Data were subjected to probit analysis (Russell et al. 1977) and the assumptions of the models verified (Robertson & Preisler 1992b).

**Survival time bioassays.** The bioassays used to evaluate the mortality-time relationship between AgipMNPV and *A. ipsilon* were such that larvae were exposed to virus for only a short period of time (≈ 40 min), as opposed to continual exposure in diet contamination studies, and as such these bioassays estimated median survival time (ST) rather than median lethal time (Farrar & Ridgway 1998). Third-instar *A. ipsilon* in separate 17-ml jelly cups were starved overnight and inoculated with solutions of AgipMNPV in water with or without 0.5% M2R using the droplet feeding technique described above. In each survival time bioassay, larvae were inoculated with AgipMNPV in water and AgipMNPV in 0.5% M2R at LD₅₀ and LD₉₀ doses. For AgipMNPV in water, LD₅₀ and LD₉₀ doses consisted of 1-μl droplets of solutions of 433 POBs/μl and 11,918 POBs/μl, respectively. For AgipMNPV in 0.5% M2R, LD₅₀ and LD₉₀ doses consisted of 1-μl droplets of solutions of 3 POBs/μl and 27 POBs/μl respectively. Larvae that failed to ingest the entire 1-μl droplet were discarded. Sufficient larvae were inoculated with both virus treatments to obtain 40 and 80 larvae at the LD₅₀ and LD₉₀ doses, respectively. In each bioassay, a batch of larvae was first inoculated with the LD₅₀ dose of AgipMNPV, then one hour later another batch of larvae was inoculated with the LD₅₀ dose of AgipMNPV in 0.5% M2R. At one-hour intervals subsequently, batches of larvae were inoculated with the LD₅₀ dose of AgipMNPV and finally the LD₉₀ dose of AgipMNPV in 0.5% M2R. Controls consisting of 20 larvae inoculated with water and 4% food dye with or without 0.5% M2R were set up with each bioassay to monitor for non-viral
mortality. Following inoculation, cubes of diet were introduced into each jelly cup and the larvae maintained at 28°C. Mortality was scored at 55 hours post inoculation and every 4-6 hours until there was no further mortality. Bioassays were replicated twice. Data were analyzed using the Kaplan-Meier product-limit estimator method (Kabfleisch & Prentice 1980). Median ST$_{50}$ estimates were compared using log-rank tests (Kabfleisch & Prentice 1980).

**Greenhouse studies.** Greenhouse studies were carried out to assess the potential of AgipMNPV and M2R for reducing feeding damage to corn seedlings caused by *A. ipsilon* larvae. Eight treatments were used in the greenhouse studies. Four treatments were formulated on a wheatgerm bait, while the other four treatments were applied as aqueous solutions. The four bait treatments consisted of wheatgerm bait which had been sprayed with (1) distilled water, (2) a solution of 0.5% M2R, (3) AgipMNPV in distilled water, or (4) AgipMNPV in 0.5% M2R. Solutions were sprayed on to the bait at a rate of 0.5 ml/g of bait, using two plant misters, one of which was used exclusively for virus solutions and another of which was used exclusively for non-virus solutions. Virus concentrations were adjusted to obtain rates of $3.13 \times 10^8$ POBs/g of wheatgerm bait. Baits were allowed to dry and stored at room temperature. The four treatments applied as aqueous solutions consisted of (1) distilled water, (2) a solution of 0.5% M2R, (3) AgipMNPV in distilled water, and (4) AgipMNPV in 0.5% M2R.

Trays (36 x 51 x 10 cm) filled to a depth of 8 cm with sieved soil were planted with Pioneer 3489 corn in two rows, with five seeds per row. The seeds were planted at 5-cm intervals with 15 cm between the rows. Strips of aluminum lawn edging 16 x 180 cm were pushed into the soil around the edges of each tray, to form a continuous barrier, and the tops of these barriers were smeared with Vaseline® to prevent larval escapes. The trays were watered daily and maintained in a greenhouse with a maximum temperature of 26°C and minimum of 18°C and a photoperiod of 16 h light, 8 h dark. Treatments were randomly assigned to trays and were applied when seedlings reached the two-leaf stage. Baits were sprinkled on to the
surface of the soil in each tray at a rate of 2.24 g/m² which gave a virus application rate of 7 \times 10^8 \text{ POBs/m}^2. Ten ml of each aqueous solution was sprayed onto each soil flat, and concentrations of AgipMNPV were adjusted to obtain application rates of 7 \times 10^8 \text{ POBs/m}^2. Trays that were to be treated with aqueous solutions were removed from the greenhouse during the application process to avoid spray-drift contamination of other trays. Once all the trays had been treated, 15 third-instar \textit{A. ipsilon} were released into the center of each tray. Trays were subsequently watered daily and feeding damage was scored seven days after treatment. The 10 seedlings in each tray were each assigned a damage rating on a five-point scale, 1 for undamaged seedlings, 2 for seedlings with shot-holes in the leaves, 3 for seedlings with chewed leaf edges, 4 for seedlings with leaves chewed off, and 5 for seedlings with stems cut through. These 10 damage ratings were then averaged to obtain a mean damage rating for that treatment. The greenhouse trials were replicated four times using fresh baits, soil and trays for each trial. All bait preparations were bioassayed in third-instar \textit{A. ipsilon} to assess mortality. Larvae were placed in separate jelly cups and allowed to feed overnight on an excess (approximately 0.03 g) of each wheatgerm bait. Twenty-five larvae were used for each bait treatment and after feeding, larvae were moved into clean jelly cups containing cubes of fresh diet. Larvae were maintained at 28°C and mortality scored after eight days.

Mean feeding damage ratings for each treatment from the four replicates were \log_{10} transformed and analyzed by one-way analysis of variance using Tukey's test to separate means, and subsequently by factorial analysis of variance using the General Linear Model. Data sets satisfied the assumptions of normality of error and homogeneity of variance.

**Field trials.** Field trials were carried out during May 2000 to assess the effectiveness of bait formulations of AgipMNPV with or without M2R, at reducing feeding damage to corn seedlings caused by \textit{A. ipsilon} larvae. Trials were conducted at an Iowa State University research site, Johnson Farm, Ames, IA. A field was machine planted with Garst 8543 corn, using a row spacing of 76 cm, on 2 May 2000. Following emergence of the corn, barriers
consisting of lawn edging (610 x 13 and 381 x 13 cm) (Suncast Corporation, Batavia, IL) were dug into the ground to a depth of 5 cm around five adjacent rows of corn, to form blocks 610 x 381 cm in size. Four additional barriers (610 x 13) were then dug into the ground within each block, between the rows of corn seedlings. Each block thus contained five treatment plots, and each plot consisted of a row of approximately 30 corn seedlings, fully enclosed by barriers, to prevent larvae escapes. The trials were set up according to a randomized complete block design, with four blocks. The greenhouse trials revealed no differences in the effectiveness of spray and bait formulations of AgipMNPV, but because of their ease of handling, bait treatments were selected for use in the field studies. Treatments consisted of the same four baits used in the greenhouse studies plus an untreated control, and were randomly assigned to plots when seedlings reached the two-leaf stage. The baits were produced as described for the greenhouse studies, except that wheatbran was used in place of wheatgerm. Wheatbran proved to be less sticky when damp than wheatgerm, and consequently wheatbran baits were easier to produce and gave better coverage in the field than wheatgerm. Baits were produced immediately prior to application and were applied to plots just before sunset at the same application rates as were used in the greenhouse studies. Following application of treatments, plots were infested with third-instar *A. ipsilon* by sprinkling larvae along the center line of each row at a rate of 3 larvae per seedling. Feeding damage was scored seven days after application of treatments using the same five-point damage rating scale used in the greenhouse studies. Damage ratings for each of the seedlings in a row were averaged to obtain a mean damage rating for that treatment. This was done for each treatment in each of the four blocks. All bait preparations were bioassayed in third-instar *A. ipsilon* as described for the greenhouse studies. The trials were repeated a second time in a different area of the field on corn that was planted on 16 May 2000. A second batch of baits was prepared, but all other aspects of the experimental design were the same as for the first set of field trials.
For both field trials, mean feeding damage ratings for each treatment from the four blocks were log_{10}-transformed and analyzed by factorial analysis of variance using the General Linear Model. Data sets satisfied the assumptions of normality of error and homogeneity of variance.

Results

Lethal dose bioassays. AgipMNPV proved to be highly active against third-instar *A. ipsilon*. LD_{50} estimates for the three replicates with AgipMNPV suspended in water ranged from 226 to 496 POBs per larva, while for the three replicates with AgipMNPV suspended in 0.5% M2R, LD_{50} estimates ranged from 1.3 to 3.0 POBs per larva (Table 1; Fig 1). For both treatments, data from the three replicates showed equivalent dose-mortality responses by likelihood ratio tests of equality (Robertson & Preisler 1992a), so the data were pooled (Table 2). Comparisons were made between the dose-response curves for AgipMNPV in water and AgipMNPV in M2R using likelihood ratio tests of equality on the pooled data. For the AgipMNPV in M2R treatment, the intercept was significantly lower and the slope of the dose-response curve was significantly higher than for the AgipMNPV in water treatment. *A. ipsilon* was significantly more susceptible to infection by AgipMNPV in M2R (i.e., significantly lower LD_{50} estimate) and significantly greater mortality occurred per unit increase in virus dose when AgipMNPV was suspended in M2R, than when AgipMNPV was suspended in water (Robertson & Preisler 1992b).

Survival time bioassays. ST_{50} estimates for both virus treatments were between four and five days in both bioassay replicates (Table 3). Comparisons made at the LD_{50} dose level, showed that there were no significant differences between ST_{50} estimates for AgipMNPV and AgipMNPV in 0.5% M2R. There were also no significant differences at the LD_{99} level between ST_{50} estimates for AgipMNPV and those for AgipMNPV in 0.5% M2R. As expected
for both AgipMNPV and AgipMNPV in 0.5% M2R, ST₅₀ estimates were significantly lower at the LD₉₉ level than at the LD₅₀ level (p < 0.05).

**Greenhouse studies.** One-way analysis of variance showed that treatment explained a significant amount of the variation seen in feeding damage in the greenhouse (F = 21.09; df = 7, 24; p = 0.000). Mean damage ratings for the four treatments containing AgipMNPV were not significantly different from each other, nor were the mean damage ratings for the four non-AgipMNPV treatments (Fig. 2). Trays treated with formulations containing AgipMNPV exhibited significantly lower feeding damage ratings than did trays receiving non-virus treatments. Typical levels of feeding damage observed in trays receiving different treatments are shown in Figure 3. Photographs (A), (B), (C) and (D) show examples of feeding damage observed in trays that received non-virus treatments. Photographs (E), (F), (G) and (H) show the lower levels of feeding damage typical of trays that received virus treatments containing virus.

In the initial factorial analysis of variance, all possible interactions between virus, brightener and formulation were included in the model, but none of these terms proved to be significant. In a subsequent analysis that excluded interaction terms, virus was the most significant factor explaining differences in observed feeding damage, although replicate and brightener also accounted for significant amounts of variation in feeding damage (Table 4). Laboratory bioassays of baits from the greenhouse trials resulted in 100% mortality of larvae fed baits containing AgipMNPV but no mortality in larvae fed non-AgipMNPV baits.

**Field trials.** Mean feeding damage ratings for each of the five treatments were averaged across the four blocks for both the first and second field trials (Fig. 4). Factorial analysis of variance failed to show a significant interaction between virus and brightener in either the first or second field trial. In subsequent analyses that excluded interaction terms, virus was the only factor explaining significant amounts of variation in feeding damage in both the first (F = 14.60; df = 1, 13; p = 0.002) and second field trial (F = 79.73, df = 1, 13; p =
Replicate, bait and brightener did not have significant effects on feeding damage. Laboratory bioassays of baits from both field trials resulted in 100% mortality of larvae fed baits containing AgipMNPV but no mortality in larvae fed non-AgipMNPV baits.

**Discussion**

Laboratory bioassays showed AgipMNPV to have high activity against *A. ipsilon* larvae. The LD$_{50}$ estimate for AgipMNPV in third-instar *A. ipsilon* (pooled data - 330 POBs/larva) was much lower than published LD$_{50}$ estimates in second-instar *A. ipsilon* (Abol et al. 1989) for *Agrotis segetum* MNPV (44,000 POBs/larva) and *Autographa californica* MNPV (670,000 POBs/larva), but were similar to those for *Heliothis armigera* NPV (105 POBs/larva) in second-instar *A. ipsilon* (Abol et al. 1989). The addition of 0.5% M2R to AgipMNPV treatments significantly reduced LD$_{50}$ estimates in third-instar *A. ipsilon* by about 160-fold to approximately 2 POBs per larva. This magnitude of viral enhancement by an optical brightener is somewhat lower than those of 1690-fold seen in *L. dispar* (Shapiro & Robertson 1992; Shapiro & Argauer 1995; Argauer & Shapiro 1997), 1584-fold seen in *Pseudoplusia inclndens* (Walker) (Zou & Young 1996) and 303,000-fold seen in *Spodoptera frugiperda* (J. E. Smith) (Hamm & Shapiro 1992), but is similar to enhancements of 3.3-fold in *Choristoneura occidentalis* Freeman (Li & Otvos 1999) and 215-fold in *L. dispar* (Farrar et al. 1995). It seems likely that at least some of these differences in reported enhancement rates are due to differences between the lethal concentration and lethal dose bioassays used to calculate the enhancement estimates. The studies of optical brighteners that documented the highest enhancements in activity (Hamm & Shapiro 1992; Shapiro & Robertson 1992; Shapiro & Argauer 1995; Zou & Young 1996; Argauer & Shapiro 1997) used lethal concentration bioassays in which larvae were allowed to feed for the duration of the study on diet contaminated with virus and brightener. Meanwhile, brightener studies documenting lower levels of enhancement used lethal dose bioassays in which larvae were
exposed to virus and brightener for short periods of time prior to transfer of larvae to uncontaminated diet (Farrar et al. 1995; Li & Otvos 1999). The significance of these differences in methodology is that extended exposure to brighteners in diet contamination studies has substantial deleterious effects on larval survival and development (Wang & Granados 2000). These deleterious brightener effects on host physiology in diet contamination studies could easily stress larvae, leading to inflated mortality and overestimation of viral enhancement. This would explain higher estimates of virus enhancement by brighteners in studies using diet contamination bioassays relative to enhancements calculated in studies using lethal dose bioassays. This was found with LdMNPV and brighteners in gypsy moth larvae where brightener enhancement estimates were much higher from diet contamination studies (Shapiro & Robertson 1992; Shapiro & Argauer 1995; Argauer & Shapiro 1997) than when enhancement estimates were calculated from lethal dose studies (Farrar et al. 1995).

In contrast to most previous studies, our results showed no direct effect of M2R on survival times. In previous studies, lethal times were determined for the same virus dose in the presence or absence of optical brightener, and any reductions in LT50 estimates for the virus-brightener treatment were attributed to effects of brightener. However, because optical brighteners greatly reduce lethal doses, the virus-brightener treatments cause higher mortality than virus alone. Virus-brightener treatments thus constitute a greater relative viral dose than treatments consisting of the same dose of virus alone (despite the same absolute number of POBs), and since it is already known that increases in virus dose cause reductions in lethal time (Van Beek et al. 1988), the finding of lower LT50 estimates for virus-brightener treatments would be expected. In studies designed in this way it is thus impossible to determine whether reductions in lethal times are due to brightener effects, dose effects, or a combination of both. An additional problem with some of these studies is that survivors were not excluded from the analysis of the lethal time data. If survivors are not excluded from the analysis, LT50 estimates from different treatments should only be compared if both treatments cause similar levels of
mortality in the test subjects (Farrar & Ridgway 1998). If LT$_{50}$ estimates are compared between treatments which cause significantly different levels of mortality, and survivors are not excluded from the analysis, LT$_{50}$ estimates are heavily inflated in treatments that cause lower levels of mortality. Our results show that when mortality levels are matched for virus vs. virus-brightener treatments and survivors are excluded from the analysis, survival times are not significantly affected by brightener, although they are significantly affected by virus dose. We suggest this is also likely to be the case in other virus-brightener systems.

Greenhouse studies showed that AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon* larvae. Seedlings that received treatments containing AgipMNPV remained relatively intact, while seedlings receiving non-virus treatments were almost completely consumed. M2R caused small but significant reductions in feeding damage which were independent of any effects attributable to virus, and could have resulted from the feeding deterrent effects of stilbene optical brighteners (Farrar et al. 1995; Farrar & Ridgway 1997; Vail et al. 1999). The interaction between virus and brightener was not significant, indicating that the presence of M2R in treatments did not lower feeding damage below that observed with virus alone. Thus at the virus doses used in these greenhouse studies, there was no beneficial effect of M2R as a UV protectant or as a viral enhancer. The absence of any UV protectant benefits may be explained by the fact that greenhouse glass filters out much of the UV radiation in sunlight, while the absence of viral enhancing effects may be explained by the fact that even AgipMNPV treatments lacking M2R were more than potent enough to cause very high levels of mortality. Indeed all larvae in virus-treated trays were dead by five days post treatment.

In both replicated field trials, AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon*. In contrast to the greenhouse studies, where seedlings in non-virus treatments were almost completely destroyed, seedlings in non-virus plots in the field merely exhibited higher levels of leaf feeding damage. There was little cutting damage to seedlings in the field trials. Chemical insecticides are usually applied against *A. ipsilon* populations in the
field when 3% or more of corn seedlings are cut by fifth or earlier instar larvae (Rice 1999). This level of damage was not observed in either of the AgipMNPV field trials. Evidently the lower seedling density in the field plots relative to the greenhouse trials, coupled with environmental mortality factors such as weather and predation by natural enemies, greatly reduced the feeding pressure on the corn seedlings, despite a larval infestation rate of twice that used in the greenhouse. Greater differences in feeding damage would have been apparent between AgipMNPV and non-virus treatments had feeding pressure been higher in the field trials. Field trials by Johnson and Lewis (1982) demonstrated significant reductions in feeding damage to corn seedlings by *A. ipsilon* following applications of AcMNPV and *Rachiplusia ou* MNPV (Harrison & Bonning 1999) baits. These viruses were highly effective at reducing damage by first and second-instar, but were much less effective against third-instar, and laboratory bioassays of AcMNPV and RoMNPV baits caused only 55% mortality in third-instar *A. ipsilon* after 14 days (Johnson & Lewis 1982). In contrast, laboratory bioassays of the baits from the AgipMNPV field trials caused 100% mortality after only eight days. Data from both sets of field trials failed to show significant interactions between AgipMNPV and M2R.

Despite the apparent promise shown by M2R as a viral enhancer in laboratory studies, there was no evidence that M2R acted as a UV protectant or viral enhancer under greenhouse or field conditions. Although previous studies have documented a beneficial effect of the inclusion of optical brighteners in baculovirus formulations, these studies tended to be in systems where the plant canopy offered significant protection from sunlight (Webb et al. 1994a; Webb et al. 1994b; Zou & Young 1996). In crop systems that are more open, such as cotton, the benefits of including optical brighteners in formulations appear to be more marginal, because the brightener itself is degraded by UV light within a few days (Vail et al. 1999). The high efficacy of AgipMNPV baits that did not contain M2R, may also have obscured potential beneficial effects of the brightener. The real utility of M2R may lie in obtaining similar levels of pest
suppression at lower virus application rates, rather than from enhancement of viral activity at more conventional application rates of $10^{12}$ POBs/ha.

In summary AgipMNPV is highly active against third-instar *A. ipsilon*. The optical brightener M2R significantly reduced the LD$_{50}$ by approximately 160-fold, but had no direct effect on ST$_{50}$ estimates. AgipMNPV significantly reduced feeding damage to corn seedlings by *A. ipsilon* in greenhouse and field studies, although the addition of M2R produced no improvements in virus performance at the virus application rates used in this study. In an appropriately designed pest management program it seems likely that AgipMNPV could be used to suppress populations of early and middle-instar *A. ipsilon*, although slow speed of kill and high production costs continue to make it difficult for baculoviruses such as AgipMNPV to compete with chemical insecticides (Moscardi 1999). Nevertheless, the soaring cost of developing new chemical insecticides, coupled with increasing public concern about pesticide residues on food, may lead to greater interest in using baculoviruses for pest management in the future.

**Acknowledgments**

We thank Bob Gunnarson and the staff of the USDA ARS Corn Insect and Crop Genetics Research Unit for assistance with the greenhouse and field studies. Journal Paper No. J-19098 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3301, and supported by Hatch Act and State of Iowa funds. This work was supported in part by the United States Department of Agriculture, Iowa State University Center for Advanced Technology Development, and Iowa State University Research Foundation. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the Iowa State University or USDA for its use.
References Cited

Abol, E. S., M. Khattab & N. S. El. 1989. Susceptibility of *Agrotis ipsilon* (Hfn.) and *Agrotis segetum* (Schiff) to certain nuclear polyhedroses viruses. Bulletin of Faculty of Agriculture, University of Cairo 40: 703-718.


Shapiro, M. 1992. Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol. 85: 1682-1686.


Zou, Y. & S. Y. Young. 1996. Use of fluorescent brightener to improve Pseudoplusia includens (Lepidoptera: Noctuidae) nuclear polyhedrosis virus activity in the laboratory and field. J. Econ. Entomol. 89: 92-96.

**Figure Legends**

Fig. 1. Percent mortality resulting from various doses of AgipMNPV in water (Agip) or AgipMNPV in 0.5% M2R (Agip M2R), as predicted by probit analysis of LD₉₀ data. Agip data plotted with solid symbols, Agip M2R data plotted with open symbols.
Fig. 2. Back-transformed mean feeding damage ratings averaged across four replicates, for the eight treatments used in the greenhouse studies. Damage rating of 5 corresponds to cut seedlings while 1 corresponds to undamaged seedlings (see Methods). Bars show back-transformed standard errors. Columns with the same letter do not differ at $p = 0.05$ significance level by Tukey's means separation test. Columns with different letters are different at $p = 0.05$ significance level.

Fig. 3. Trays of corn seedlings from the first replicate of the greenhouse studies, showing typical feeding damage observed after seven days for trays receiving different treatments. Treatments were as follows: (A) water only, (B) 0.5% M2R, (C) bait only, (D) bait + 0.5% M2R, (E) AgipMNPV in water, (F) AgipMNPV in 0.5% M2R, (G) bait + AgipMNPV, (H) bait + AgipMNPV in 0.5% M2R.

Fig. 4. Back-transformed mean feeding damage ratings averaged across blocks for the five treatments used in the first and second field trials. Damage rating of 5 corresponds to cut seedlings while 1 corresponds to undamaged seedlings (see Methods). Bars show back-transformed standard errors.
Table 1. Lethal dose data for AgipMNPV in 3rd-instar *A. ipsilon*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate</th>
<th>n</th>
<th>LD$_{50}$ (95% CL)</th>
<th>Slope ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agip</td>
<td>1</td>
<td>210</td>
<td>226 (73-370)</td>
<td>1.38 ± 0.32</td>
</tr>
<tr>
<td>Agip</td>
<td>2</td>
<td>210</td>
<td>496 (148-816)</td>
<td>1.61 ± 0.30</td>
</tr>
<tr>
<td>Agip</td>
<td>3</td>
<td>210</td>
<td>369 (202-520)</td>
<td>1.62 ± 0.31</td>
</tr>
<tr>
<td>Agip M2R</td>
<td>1</td>
<td>210</td>
<td>3.0 (2.2-4.0)</td>
<td>2.24 ± 0.31</td>
</tr>
<tr>
<td>Agip M2R</td>
<td>2</td>
<td>210</td>
<td>2.5 (1.9-3.2)</td>
<td>2.51 ± 0.37</td>
</tr>
<tr>
<td>Agip M2R</td>
<td>3</td>
<td>210</td>
<td>1.3 (1.0-1.6)</td>
<td>3.11 ± 0.62</td>
</tr>
</tbody>
</table>

* Agip, AgipMNPV; Agip M2R, AgipMNPV + 0.5% M2R.

* Six virus doses used per replicate. 35 larvae per dose.

* Dose, POBs per larva. Data analyzed by probit analysis (Russell et al. 1977). Probit-dose relationship linear at p = 0.05 confidence level. Data fit probit model by χ$^2$ test at p = 0.05 (Robertson & Preisler 1992b).
Table 2. Pooled lethal dose data for AgipMNPV in 3rd-instar *A. ipsilon*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>LD$_{50}$ (95% CL)</th>
<th>Slope ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agip</td>
<td>630</td>
<td>330 (83-548)</td>
<td>1.57 ± 0.18</td>
</tr>
<tr>
<td>Agip M2R</td>
<td>630</td>
<td>2.1 (1.8-2.5)</td>
<td>2.28 ± 0.20</td>
</tr>
</tbody>
</table>

*a Agip, AgipMNPV; Agip M2R, AgipMNPV + 0.5% M2R.

*b For both treatments, data from three replicates indicated equivalent dose-mortality, by likelihood ratio test of equality (Robertson & Preisler 1992a), so data were pooled. Six virus doses per replicate, with 35 larvae per dose.

*c Dose, POBs per larva. Data analyzed by probit analysis (Russell et al. 1977). Probit-dose relationship linear at p = 0.05 confidence level. Data fit probit model by $\chi^2$ test at p = 0.05. (Robertson & Preisler 1992b).

Comparison of treatments by $\chi^2$ tests on slopes and intercepts indicates that the LD$_{50}$ for AgipMNPV+0.5% M2R is significantly lower and that the slope is significantly greater than for AgipMNPV alone at the p = 0.05 significance level (Robertson & Preisler 1992a).
Table 3. Survival time data for AgipMNPV in 3rd-instar *A. ipsilon*

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>ST&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; dose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>LD&lt;sub&gt;99&lt;/sub&gt; dose</td>
</tr>
<tr>
<td>Agip</td>
<td>123aA</td>
<td>101aB</td>
</tr>
<tr>
<td>Agip M2R</td>
<td>127aA</td>
<td>108aB</td>
</tr>
</tbody>
</table>

<sup>a</sup> Agip, AgipMNPV; Agip M2R, AgipMNPV + 0.5% M2R.

<sup>b</sup> Survival time, hours. Estimates obtained using the Kaplan-Meier product-limit estimator method (Kaufman & Prentice 1980). Survivors were excluded from analyses.

<sup>c</sup> Replicates used 80 larvae at the LD<sub>50</sub> dose level and 40 larvae at the LD<sub>99</sub> dose. Within replicates, ST<sub>50</sub> estimates for different doses and treatments were compared by log-rank tests (Kaufman & Prentice 1980). ST<sub>50</sub> estimates in the same column, followed by the same lower-case letter do not differ significantly at P=0.05 level. ST<sub>90</sub> estimates in the same row, followed by different upper-case letters differ significantly at p = 0.05 level.
Table 4. Factorial analysis of variance showing the factors explaining significant amounts of variation in feeding damage under greenhouse conditions

<table>
<thead>
<tr>
<th>Factor</th>
<th>F ratio (df)</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate</td>
<td>3.20 (3)</td>
<td>0.040</td>
</tr>
<tr>
<td>Virus</td>
<td>170.91 (1)</td>
<td>0.000</td>
</tr>
<tr>
<td>Brightener</td>
<td>4.79 (1)</td>
<td>0.038</td>
</tr>
<tr>
<td>Formulation</td>
<td>0.39 (1)</td>
<td>0.537</td>
</tr>
</tbody>
</table>

Damage data were log<sub>10</sub> transformed and subjected to factorial analysis of variance using the General Linear Model. Data satisfied assumptions of normality of error and homogeneity of variance. Analysis based on treatment means from four replicates.

<sup>a</sup> All possible interaction terms were included in the initial model, but none were significant. Results shown are from a subsequent analysis that did not contain interaction terms.

<sup>b</sup> Factors explaining significant amounts of variation in feeding damage indicated by p-values < 0.05.
Fig. 1.
Fig. 4.
CHAPTER 4. EFFECTS OF A NEW CLASS OF PROTEASE-EXPRESSING RECOMBINANT BACULOVIRUS ON LIFE HISTORY TRAITS OF THE PREDATOR COLEOMEGILLA MACULATA (COLEOPTERA: COCCINELLIDAE)

Abstract

Laboratory studies were conducted to assess potential negative impacts on life history traits of Coleomegilla maculata (Degeer), arising from consumption of Heliothis virescens F. larvae infected with the recombinant baculovirus AcMLF9.ScathL. This virus expresses a basement membrane degrading protease, cathepsin L, derived from Sarcophaga perigrina Robineau-Desvoidy. Control groups of C. maculata were fed H. virescens infected with the wildtype virus AcMNPV C6, mock-inoculated H. virescens, or a mixture of European corn borer (ECB), Ostrinia nubilalis (Hübner), eggs and green peach aphids, Myzus persicae (Sulzer). C. maculata larvae fed on the three H. virescens regimes exhibited significantly lower survival to the adult stage than C. maculata fed on ECB eggs and aphids. There was no significant difference in C. maculata survival between the three H. virescens feeding regimes. Mean survival time of C. maculata larvae fed on mock-inoculated H. virescens, was significantly longer than C. maculata fed on virus-infected H. virescens, possibly due to lower nutritional quality of virus-infected prey. There were no significant differences in survival times between C. maculata fed H. virescens infected with AcMLF9.ScathL or AcMNPV C6. Overall the data suggest that the use of AcMLF9.ScathL as a microbial insecticide in agricultural systems would pose no greater threat to C. maculata than would the use of AcMNPV C6. The effects of both viruses on predator populations would be significantly less than those arising from the use of chemical insecticides.
Introduction

The Baculoviridae are a large family of double stranded DNA viruses, with rod-shaped virions occluded in a protective protein occlusion body (Volkman et al. 1995). Baculoviruses are arthropod-specific and do not infect vertebrates or plants. They are highly pathogenic, causing acute infections in their hosts which cause death in 4-14 days. Baculoviruses are active primarily against insects in the order Lepidoptera, and there are many examples of the successful use of wildtype baculoviruses in insect pest management (Entwistle & Evans 1985; Fuxa 1990; Moscardi 1999).

Advances in genetic engineering have made it possible to genetically manipulate wildtype baculoviruses to make them more effective insect pest management agents (Harrison & Bonning 2000). Genes coding for neurotoxins, enzymes and insect peptide hormones have been engineered into baculovirus genomes to produce recombinant baculoviruses with increased speed of kill, that reduce feeding damage caused by insect pests. Some of the most effective recombinant baculoviruses developed to date have incorporated genes for insect-specific neurotoxins (Smith et al. 2000; Treacy et al. 2000). In susceptible hosts, these neurotoxin viruses reduce feeding damage and LT_{50} estimates by 25-50% relative to larvae infected with wildtype viruses (McCutch en et al. 1991; Stewart et al. 1991; Tomaiski & Miller 1992; Cory et al. 1994; Treacy & All 1996).

Impact evaluation studies with a variety of recombinant baculoviruses have been performed to address whether recombinant baculoviruses have adverse effects on nontarget organisms in the environment such as predators, parasitoids and pollinators (Richards et al. 1998). Owing to the host range of baculoviruses, adverse effects on predators, parasitoids and pollinators will not result from virus infection of the nontarget organism, but adverse effects may occur from contact of the nontarget organisms with the recombinant protein. Studies to date have failed to document adverse effects on insect predators from consumption of lepidopteran larvae infected with recombinant baculoviruses expressing proteins that are active
inside the hemocoel, such as neurotoxins or modified juvenile hormone esterases (Heinz et al. 1995; McNitt et al. 1995; Li et al. 1999; Smith et al. 2000).

Recently a new group of recombinant baculoviruses utilizing a novel mode of action have been developed (Harrison & Bonning 2001). These recombinant viruses express proteases that degrade basement membranes. Basement membranes surround the tissues of all insects and vertebrates, providing a filtration function as well as a layer for cell attachment (Rohrback & Rohrback 1993). Evidence suggests that basement membranes limit the movement of virions within insects and as such constitute barriers to the dissemination of baculovirus infection within a host (Hess & Falcon 1987). By producing recombinant baculoviruses that express proteases that degrade basement membranes it was hypothesized that systemic virus infections would be established more quickly within infected hosts, leading to increased rates of kill. The NPV of the alfalfa looper, Autographa californica (Speyer), (AcMNPV) was engineered to express basement membrane-degrading proteases. The most effective of these recombinant constructs contained a gene for a protease called cathepsin L (Scath L) isolated from the flesh fly Sarcophaga perigrina Robineau-Desvoidy. The protease gene was placed under the control of the baculovirus p6.9 late promoter and the virus was designated AcMLF9.ScathL. Heliothis virescens F. (Lepidoptera: Noctuidae) larvae infected with AcMLF9.ScathL melanize prior to death. The lethal dose of AcMLF9.ScathL was not significantly different from that of the parental virus AcMNPV C6, but LT₅₀ estimates and feeding damage ratings for AcMLF9.ScathL were 49% and 20% respectively of those for AcMNPV C6 (Harrison & Bonning 2001).

Infection of nontarget organisms by AcMLF9.ScathL will not occur and use of a baculovirus late promoter ensures that the protease gene will only be expressed in cells that are fully permissive to baculovirus infections, i.e. lepidopteran cells (McNitt et al. 1995). However there may be adverse effects on nontarget organisms associated with contact with the Scath L protease following consumption or parasitization of lepidopteran larvae infected with
AcMLF9.ScathL. This paper examines the effects of consumption of *H. virescens* larvae infected with AcMLF9.ScathL or AcMNPV C6 on life history traits (survival and development) of larvae of a common insect predator, the twelve-spotted lady beetle, *Coleomegilla maculata* (Degeer) (Coleoptera: Coccinellidae).

**Materials and Methods**

**Viruses.** The recombinant virus AcMLF9.ScathL was constructed to express the protease Scath L as previously described (Harrison & Bonning 2001). AcMNPV clone C6 (Possee 1986) was the parental virus from which AcMLF9.ScathL was constructed, and was used as the wildtype control in virus control treatments.

**Virus amplification and purification.** AcMLF9.ScathL was amplified in fifth instar *H. virescens*. Virus concentrations were quantified with a phase contrast microscope and a Neubauer bright-line hemocytometer (Fisher Scientific, Pittsburg, PA). Thirty larvae were placed in individual 17-ml jelly cups (Fill-Rite Inc., Newark, NJ), and starved overnight. One 3-mm cube of diet inoculated with $1 \times 10^5$ polyhedral occlusion bodies (POBs) of tissue culture derived AcMLF9.ScathL was added to each cup. Larvae that consumed the inoculated cube were provided with additional diet and maintained at 27°C until death. For purification of POBs, cadavers were homogenized using a 30-ml dounce homogenizer (Wheaton Scientific Products Inc., Millville, NJ) for 10 min in 0.1% SDS (1 ml per cadaver), and filtered through five layers of cheese cloth. POBs were pelleted by centrifugation at 3,600 g for 10 min at room temperature in 42-ml glass centrifuge tubes. The pellet was resuspended in 0.5% SDS, and centrifugation and resuspension repeated with 0.5 M NaCl, before final resuspension of POBs in distilled water (O'Reilly et al. 1992). Sodium azide was added to this master stock of AcMLF9.ScathL to a final concentration of 0.02% to inhibit bacterial growth. From this master stock of AcMLF9.ScathL, the amplification procedure was repeated with 70 fifth instar *H. virescens* and an inoculation dose
of 250,000 POBs per diet cube. Larvae were maintained at 27°C until death and viral POBs purified and resuspended as described above, to produce a working stock of AcMLF9.ScathL (6 ml). This working stock of AcMLF9.ScathL was used for all subsequent experiments.

**Insects.** Second instar *C. maculata* were obtained from Rincon Vitova Insectaries (Ventura, CA). Larvae were moved into individual 11-ml glass vials (1.5 cm diameter × 9.0 cm) stoppered with clean cotton, and maintained at 28°C, 80% RH with a photoperiod of 14 h light, 10 h dark on a diet of green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and eggs of the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) for three days prior to the start of the survival studies. Peach aphids were obtained from a colony maintained in the lab on Chinese cabbage plants. ECB eggs were obtained from USDA-ARS Corn Insects & Crop Genetics Research Unit, Ames, IA. Tobacco budworm, *H. virescens*, eggs were obtained from a colony maintained by Dr. T. C. Baker, Entomology Department, Iowa State University, Ames, IA and from USDA-ARS Southern Insect Management Laboratory, Stoneville, MS. *H. virescens* were reared from eggs at 27°C, 60% RH, with a photoperiod of 14 h light, 10 h dark on *H. virescens* soy flour diet (Southland Products, Lake Village, AR).

**Survival studies.** Following a three day acclimatization period on a diet of peach aphids and ECB eggs, all remaining aphids and ECB eggs were removed from the glass vials and *C. maculata* larvae were assigned to one of four feeding regimes: (1) Aphids and ECB eggs; (2) mock-inoculated *H. virescens* larvae; (3) *H. virescens* larvae infected with AcMNPV C6; (4) *H. virescens* larvae infected with AcMLF9.ScathL. Glass vials assigned to the different feeding regimes were labeled and survival and development of individual *C. maculata* larvae was monitored. *C. maculata* larvae assigned to the different feeding regimes were fed as follows:
Aphids & ECB eggs: Every three days, *C. maculata* larvae in each vial were provided with two or three ECB egg masses and a small piece of Chinese cabbage leaf on which were 7-10 peach aphids.

*H. virescens* treatments: Every day, *C. maculata* larvae were provided with first instar *H. virescens* that had been infected 24 hours previously using a droplet feeding technique (Hughes & Wood 1981). Larvae were infected with AcMNPV C6 or AcMLF9.ScathL, or were mock-inoculated. First instar larvae were allowed to drink from suspensions of distilled water and 4% Schilling blue food coloring dye (McCormick & Co., Inc., Hunt Valley, MD), containing 100 × LC₅₀ doses of AcMNPV C6 (112,00 POBs/μl) or AcMLF9.ScathL (133,000 POBs/μl), in 90-mm petri dishes. Mock inoculations were carried out with distilled water and food coloring dye alone. After 15 min larvae that had ingested the colored suspensions were moved using paintbrushes into 240-ml ice cream tubs containing artificial diet, and maintained at 27°C for 24 hours.

In the first replicate of the survival studies 40 *C. maculata* larvae were used per feeding regime, and *C. maculata* larvae in the *H. virescens* treatments were fed 2-4 mock-inoculated, AcMNPV C6-infected, or AcMLF9.ScathL-infected *H. virescens* larvae per day. However, virus infection retarded growth of *H. virescens* larvae, such that 24 hours post infection (hpi), mock-inoculated *H. virescens* larvae were larger than AcMNPV C6-infected larvae, which were larger than AcMLF9.ScathL-infected larvae. To increase overall prey quantities, only 20 *C. maculata* larvae were used per feeding regime in the second replicate of the survival studies. Meanwhile in an attempt to compensate for differential *H. virescens* sizes, *C. maculata* larvae in the mock-inoculated, AcMNPV C6-infected and AcMLF9.ScathL-infected treatments were fed 5-7, 6-8, and 7-9 *H. virescens* larvae per day respectively. Development and survival of *C. maculata* larvae in the different feeding regimes were monitored daily and both replicates of the trials were continued until all *C. maculata* larvae had either died or emerged as adult beetles.
Statistical analyses. Differences in the response variables (proportion *C. maculata* surviving to the adult stage, and time to death) were examined between replicates and across feeding regimes using analysis of variance (GLM or ANOVA) (SAS-Institute 1990). To normalize the data and stabilize the variance, the response variables were square-root transformed before analysis. Reported means and standard errors of the mean (SEM) were back-transformed following analysis. Differences in mean survival and mean time to death between the feeding regimes, were examined using Tukey's means separation test.

Results

In both replicates of the feeding trials, *C. maculata* larvae fed on a mixture of ECB eggs and aphids, developed faster and exhibited significantly higher rates of survival to the adult stage, than *C. maculata* larvae fed on any of the three *H. virescens* feeding regimes (Fig. 1 & 2). All *C. maculata* larvae fed on ECB eggs and aphids had either died or emerged as adults by three weeks into the trials, whereas *C. maculata* larvae fed on the three *H. virescens* regimes took up to five weeks to reach the adult stage.

The proportions of *C. maculata* larvae surviving to the adult stage on the different feeding regimes, was not significantly different between replicates (F = 2.98; df = 1, 3; p = 0.183). Therefore survival data from the two replicates were pooled. One-way ANOVA indicated that feeding regime explained a significant amount of the variation seen in survival (F = 24.99; df = 3, 4; p = 0.005). Tukey's means separation test indicated that survival to the adult stage was significantly higher in *C. maculata* larvae fed on the ECB eggs and aphids, than in *C. maculata* fed on the three *H. virescens* regimes. There were no significant differences in survival to adult between *C. maculata* larvae fed *H. virescens* infected with AcMNPV C6 or AcMLF9.ScathL, relative to *C. maculata* fed mock-inoculated *H. virescens*. No significant differences in survival were seen between *C. maculata* larvae fed *H. virescens*
infected with AcMLF9.ScathL compared to *C. maculata* fed *H. virescens* infected with AcMNPV C6 (Table 1).

Few *C. maculata* larvae fed on the *H. virescens* regimes survived to the adult stage (2 of 180), so times to death, rather than survival times were examined. Relatively few *C. maculata* larvae fed on ECB eggs and aphids died (10 of 60), so comparisons of time to death were restricted to *C. maculata* larvae fed on the three *H. virescens* regimes. The mean times to death for the three *H. virescens* regimes did not differ between replicates (F = 2.27; df = 1, 174; p = 0.133), so the time to death data were pooled. One-way ANOVA indicated that feeding regime explained a significant amount of the variation seen in time to death between the three *H. virescens* feeding regimes (F = 21.86; df = 2, 175; p = 0.000). Tukey's means separation test indicated that mean time to death was significantly shorter in *C. maculata* larvae fed *H. virescens* infected with AcMLF9.ScathL (mean = 12.3 d) or AcMNPV C6 (mean = 13.3 d) relative to *C. maculata* fed on mock-inoculated *H. virescens* (mean = 18.8 d). However there were no significant differences in mean time to death between *C. maculata* larvae fed *H. virescens* infected with either AcMNPV C6 or AcMLF9.ScathL (Table 1).

**Discussion**

The studies detailed in this paper satisfy the impact evaluation studies for one nontarget predatory arthropod required by the Environmental Protection Agency (EPA) prior to approval of a genetically modified organism (GMO) for field release. As per EPA guidelines, the selected nontarget organism, *C. maculata* was fed on pest individuals infected with a 100 × LC₉₀ dose of AcMLF9.ScathL. The LT₅₀ for AcMLF9.ScathL in first instar *H. virescens* is approximately 48 hours (Harrison & Bonning 2001). *H. virescens* were used at 24 hpi in the feeding studies so that AcMLF9.ScathL-infected *H. virescens* would still be alive. Use of *H. virescens* larvae infected with a high dose of AcMLF9.ScathL in no-choice
feeding studies, simulated a worst case scenario in which *C. maculata* were exposed to maximum possible levels of the recombinant protein Scath L.

Survival of *C. maculata* larvae fed on a control diet of ECB eggs and aphids was good, with 98% of larvae in the first replicate reaching the adult stage. The reason why survival of *C. maculata* on ECB eggs and aphids dropped to 55% in the second replicate is unclear. All *C. maculata* larvae fed on ECB eggs and aphids completed development to the adult stage or died in less than three weeks. In contrast, 99% of *C. maculata* larvae fed on the *H. virescens* regimes died as larvae, although many survived for longer than three weeks and some lasted four and a half weeks. This extended period of survival suggests that *C. maculata* fed on *H. virescens* did not die of starvation, but that a diet composed exclusively of *H. virescens* larvae may be nutritionally unsuitable to support survival and development of *C. maculata* larvae. This suggestion is supported by data from previous impact evaluation studies in which the ladybird beetle, *Hippodamia convergens* was fed on mock-inoculated *H. virescens*, or *H. virescens* larvae infected with wildtype or recombinant baculoviruses (Li et al. 1999). In these studies, survival curves for *H. convergens* adults declined over time and were similar to the survival curves obtained in this study.

*C. maculata* larvae fed virus-infected *H. virescens* died significantly sooner than *C. maculata* fed mock-inoculated *H. virescens*. Larvae infected with AcMNPV C6 or AcMLF9.ScathL were smaller at 24 hpi than mock-inoculated larvae. Attempts were made to compensate for these differences in *H. virescens* larval sizes in the second replicate of the feeding trials, but *C. maculata* larvae still died sooner when fed virus-infected prey. Because it does not seem likely that food limitation was the cause of the earlier death of *C. maculata* fed virus-infected *H. virescens*, it is suggested that earlier death may have resulted from lower nutritional quality of virus-infected prey. Adverse effects such as reduced adult longevity, reduced fecundity and an increase in the pre-ovipositional period were observed in the predator *Nabis roseipennis* Reuter when individuals were fed lepidopteran larvae infected with wildtype
baculovirus, relative to *N. roseipennis* fed on a control diet of mock-inoculated lepidopteran larvae (Ruberson et al. 1991).

Late instar *H. virescens* infected with AcMLF9.ScathL, undergo premature melanization prior to death, and their cuticles harden and are less likely to lyse following death than cadavers of larvae killed by wildtype virus (Harrison & Bonning 2001). Since melanin is toxic, this raised the question of whether or not *H. virescens* larvae infected with AcMLF9.ScathL would be as palatable to predators as *H. virescens* that were infected with AcMNPV C6 or that were uninfected. During the course of the experiments detailed in this paper, it was determined that first instar *H. virescens* infected with AcMLF9.ScathL do not melanize prior to death, but that the resulting cadavers are smaller and exhibit a reduced tendency to lyse, relative to cadavers of AcMNPV C6-killed hosts. Thus it did not seem likely that reduced palatability to predators of AcMLF9.ScathL-infected *H. virescens* relative to AcMNPV C6-infected *H. virescens* would be an issue. This assumption was supported by the observation that *C. maculata* larvae attacked *H. virescens* immediately following introduction into the rearing vials and always consumed all available prey within 24 hours, regardless of whether or not they were infected with AcMLF9.ScathL. However it is likely that hosts infected with AcMLF9.ScathL will produce fewer polyhedra than hosts infected with AcMNPV C6 because of reduced survival time, and that fewer of these polyhedra will be released into the environment, owing to reduced lysis of AcMLF9.ScathL cadavers. Reduced production and dissemination of AcMLF9.ScathL relative to wildtype virus are desirable traits from a risk assessment perspective because they result in lower exposure of nontarget organisms to the GMO.

In summary, no significant differences in mean survival or time to death were detected between *C. maculata* fed AcMLF9.ScathL-infected or AcMNPV C6-infected prey. Although it is possible that different predators might be more sensitive to AcMLF9.ScathL and Scath L than *C. maculata*, previous impact evaluation studies have consistently failed to document
adverse effects of neurotoxin and JHE recombinant viruses, regardless of the species of predator used (Heinz et al. 1995; McNitt et al. 1995; Li et al. 1999; Smith et al. 2000). Small differences in time to death were detected in these no-choice studies between *C. maculata* fed virus-infected and mock-inoculated *H. virescens*. However, in an agricultural system where a predator would typically feed on a diversity of prey, it is unlikely that the consumption of virus-infected *H. virescens* as part of a mixed diet, would have any detectable impact on *C. maculata* survival or development. Thus it seems likely that under field conditions there would be no greater impact on *C. maculata* in agricultural systems from the use of AcMLF9.ScathL than from use of AcMNPV C6, and that any adverse effects attributable to the use of AcMLF9.ScathL or AcMNPV C6 would be mild relative to the impacts of broad-spectrum chemical insecticides.

**Acknowledgments**

This material is based upon work supported by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, under Agreement No. 00-39210-9772.

**References**


Treacy, M. F. & J. N. All (1996). Impact of insect-specific AaHIT gene insertion on inherent bioactivity of baculovirus against tobacco budworm, Heliothis virescens, and
cabbage looper *Trichoplusia ni.* Proceedings of The Beltwide Cotton Conference, Nashville, TN.


**Figure Legends**

**Fig. 1.** Impact of AcMLF9.ScathL on *C. maculata* survival. Survival over time of *C. maculata* larvae fed on four different feeding regimes. Data for *C. maculata* larvae fed on ECB eggs and aphids are presented until the point of adult emergence only, when feeding was discontinued. Data from first replicate of survival studies. 40 *C. maculata* larvae per treatment.

**Fig. 2.** Impact of AcMLF9.ScathL on *C. maculata* survival. Survival over time of *C. maculata* larvae fed on four different feeding regimes. Data for *C. maculata* larvae fed on ECB eggs and aphids are presented until the point of adult emergence only, when feeding was discontinued. Data from second replicate of survival studies. 20 *C. maculata* larvae per treatment.
Table 1. Time to death and survival proportions for *C. maculata* larvae fed on different feeding regimes

<table>
<thead>
<tr>
<th>Feeding regime</th>
<th>Time to death, d</th>
<th>Proportion surviving</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECB &amp; aphids</td>
<td>NA</td>
<td>0.75 ± 0.02a</td>
<td>(60)</td>
</tr>
<tr>
<td>*H. virescens/*mock-inoculated</td>
<td>18.8 ± 0.00a</td>
<td>0.01 ± 0.01b</td>
<td>(58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(60)</td>
</tr>
<tr>
<td>*H. virescens/*AcNPV C6</td>
<td>13.3 ± 0.01b</td>
<td>0.00 ± 0.00b</td>
<td>(60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(60)</td>
</tr>
<tr>
<td>*H. virescens/*AcNPV Scath L</td>
<td>12.3 ± 0.01b</td>
<td>0.00 ± 0.00b</td>
<td>(60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(60)</td>
</tr>
</tbody>
</table>

*a* Means and SEMs were calculated from pooled data from two replicates and are back-transformed from square root transformed data. Means in column followed by the same letter are not significantly different by Tukey’s means separation test (*p* < 0.05).

*b* Mean proportion of larvae surviving to adult stage.
Fig. 1.
ECB & aphids
H. virescens/mock-inoculated
H. virescens/AcNPV C6
H. virescens/AcNPV Scath L

Fig. 2.
CHAPTER 5. GENERAL CONCLUSIONS

Summary

AgipMNPV is a new multicapsid nucleopolyhedrovirus with high activity against *A. ipsilon*. AgipMNPV was also active to a lesser degree against four of seven other noctuid species tested. AgipMNPV was highly active against third instar *A. ipsilon*, and killed 50% of infected third instar *A. ipsilon* in approximately five days, which is typical for a virulent baculovirus in a susceptible host. Spray and bait formulations of AgipMNPV applied to corn seedlings in greenhouse feeding studies, offered significant protection from feeding damage by third instar *A. ipsilon*. The optical brightener M2R significantly reduced lethal doses in third instar *A. ipsilon* by about 160-fold, but had no effect on survival time estimates. In greenhouse and field trials, the brightener failed to generate detectable enhancements in virus performance.

Impact evaluation studies suggest that adverse effects on the predator *C. maculata* arising from contact with baculovirus-infected lepidopteran larvae are mild, and that the effects of the recombinant virus AcMLF9.ScathL are not significantly different to those of AcMNPV C6.

Further Research

Studies showed that AgipMNPV is sufficiently active to be used in the field to suppress populations of *A. ipsilon*. There are two possible ways in which AgipMNPV could be used in pest management programs against *A. ipsilon*. The most cost effective way to use baculoviruses in pest management is by introduction-establishment, because once established in an area, the virus regulates populations of the host without further management intervention and thus without incurring further costs (Fuxa 1990). This strategy might work for management of *A. ipsilon* in corn. If spray applications of AgipMNPV could establish a
sufficient reservoir of virus inoculum in the soil, then it is possible that rain splash would transfer sufficient virus from the soil onto newly emerged corn seedlings, to cause mortality of leaf feeding early instar *A. ipsilon*. This hypothesis could be tested in appropriately designed studies. The other possible strategy by which AgipMNPV could be used to manage *A. ipsilon* in corn, would be to make some form of augmentative applications similar to those used in the initial field studies. Since, *A. ipsilon* is only a pest of corn until seedlings reach the four-leaf stage (Showers et al. 1983), season long control is not necessary. Thus AgipMNPV would be used in inundative augmentative applications as a microbial insecticide. Current management recommendations for *A. ipsilon*, call for rescue applications of chemical insecticides against economically damaging larval populations (when more than 3% of seedlings have their stems cut) (Rice 1999). However given that baculoviruses have a relatively slow speed of kill and are most effective against first and second instar larvae, it would be desirable to make AgipMNPV applications earlier, against populations of first or second instar *A. ipsilon*. According to the current management plan, following significant pheromone trap captures of male *A. ipsilon* moths, a degree day model is used to predict a date by which fourth instar larvae will be present in the field. Scouting is then started on this date, and if cutting damage exceeding the economic injury level is observed, applications of chemical insecticides are subsequently made. By adapting the current degree day model, it might be possible to make applications of AgipMNPV against first or second instar *A. ipsilon* larvae, which are the most susceptible developmental stages. However this approach would involve making virus applications before any significant feeding damage to corn seedlings had occurred. The goal of integrated pest management is to avoid making unnecessary applications of insecticidal compounds against non-economic pest populations, and thus further work may need to be done to improve understanding of the relationship between the number of adult *A. ipsilon* moths caught in light traps and the likelihood that these captures will subsequently translate into economically damaging larval populations.
Despite the dramatic enhancement in AgipMNPV activity brought about by M2R in lethal dose bioassays, the brightener failed to generate detectable enhancements in virus performance in greenhouse and field studies. The reason for this is unclear. The AgipMNPV application rates used in the greenhouse and field trials were high at $1 \times 10^{12}$ POBs/ha, but were not different from rates used in many field trials (Bourmer et al. 1992; Cory et al. 1994; Thorpe et al. 1998; Williams et al. 1999; Smith et al. 2000; Treacy et al. 2000). Given the high application rates and the fact that AgipMNPV alone is highly virulent, it is possible that A. ipsilon larvae in the greenhouse and field trials received quantities of AgipMNPV that were already sufficient to kill 100% of exposed larvae. If this were the case, inclusion of optical brightener in virus formulations would not significantly improve virus performance. This may in fact have occurred because it was found that AgipMNPV-only baits used in the greenhouse and field trials contained sufficient AgipMNPV to kill 100% of exposed A. ipsilon larvae in bait bioassays. In addition, all A. ipsilon larvae in AgipMNPV-only treatments in the greenhouse trials were dead by five days post treatment. An alternative explanation for the failure of the optical brightener to enhance viral activity in the field is that it was degraded by exposure to sunlight. This degradation of M2R occurred in field trials with AfMNPV on cotton (Vail et al. 1999). Three days after application of brightener, only 33% remained on cotton leaves as indicated by fluorescence spectroscopy. A final possible explanation for the failure of M2R to enhance viral efficacy in the greenhouse and field is that there may simply have been insufficient brightener deposited on the corn seedlings or released from the baits, to bring about enhancement. These questions relating to why M2R failed to produce detectable enhancements in virus activity in the greenhouse and field trials could be answered by further studies. Questions about possible degradation of M2R in the field could be answered using fluorescence spectroscopy. Brighteners could be sprayed onto corn seedlings in the field, and leaves collected at various time points post application. These leaves could then be washed and the resulting rinse solutions analyzed for residual brightener levels (Vail et al. 1999).
Meanwhile, repeating the greenhouse and field trials with lower AgipMNPV application levels, and including some higher rates of M2R, would make it possible to determine whether M2R was causing viral enhancement.

Impact evaluation studies suggest that adverse effects on the predator *C. maculata* arising from contact with baculovirus-infected lepidopteran larvae are mild, and that the effects of AcMLF9.ScathL are not significantly different to those of AcMNPV C6. Although there are no reasons to believe that other predators will respond differently to consumption of lepidopteran larvae infected with AcMLF9.ScathL than did *C. maculata*, additional impact evaluation studies are planned using the green lacewing, *Chrysoperla carnea* Stephens. Further work could be done with AcMLF9.ScathL to address issues relating to exposure identification. Although preliminary observations suggest that production and dissemination of AcMLF9.ScathL from infected hosts will be lower than for AcMNPV C6, these issues need to be addressed.

**General Discussion**

Baculoviruses have a long history of use in insect pest management and their continued use will remain a viable option for certain pests for the foreseeable future. In modern intensive agriculture systems, with demands for high yields and blemish-free produce, it will continue to be difficult for wildtype baculoviruses to be used in inundative augmentation programs, where they come into direct competition with fast-acting chemical insecticides. Even in situations in which wildtype baculoviruses are sufficiently biologically active to manage populations of the pest, other factors influence the decision of whether the virus will ultimately be used or not. An example of this is provided by AgipMNPV. Although AgipMNPV is sufficiently biologically active that it could be used to suppress populations of first and second instar *A. ipsilon*, the likelihood of AgipMNPV being used in an augmentative fashion as a microbial insecticide is remote. The high species specificity of AgipMNPV makes it ineffective against other pests, and
the fact that *A. ipsilon* is only a sporadic pest, means that stocks of AgipMNPV would likely sit in storage for years between uses. Rather than invest money in a pest management product that will only be used infrequently, farmers are much more likely to opt for a chemical insecticide with broader activity, which would likely see some use each growing season. Recent government regulation, in the form of the Food Quality Protection Act (FQPA), has begun to restrict the use of highly toxic organophosphate insecticides, and this has provided an incentive to use biologically-based insecticides. However, several synthetic pyrethroids with low toxicity are available for control of *A. ipsilon*, and so increased government regulation is unlikely to increase interest in the use of AgipMNPV as a microbial insecticide in the new future.

The prospects for the use of wildtype baculoviruses are much better in production systems where the crop can tolerate a degree of injury without sustaining economic damage. In these situations, baculoviruses can be used according to an introduction-establishment or inoculative augmentation strategy. For example there would likely be much more interest in using AgipMNPV according to an introduction-establishment strategy, if this was feasible, rather than as a microbial insecticide. Use of AgipMNPV according to an introduction-establishment strategy to manage *A. ipsilon* would be cheap, because following initial spray applications to establish the virus in the environment, regulation or suppression of *A. ipsilon* populations would occur without further intervention. Wildtype baculoviruses will continue to offer viable pest management solutions in developing countries, where shortage of hard currency often reduce availability of modern chemical insecticides. The increased availability of cheap labor can also facilitate cost effective *in vivo* production of baculoviruses, which is often not financially viable in western countries.

Many studies have investigated optical brighteners for inclusion in baculovirus formulations as potential UV protectants and viral enhancers. Studies in this dissertation show enhanced AgipMNPV activity in the laboratory attributable to an optical brightener, but failed to
detect viral enhancement by the brightener in greenhouse and field trials. However, other studies have shown improvements in baculovirus activity attributable to optical brighteners, which has raised the question of whether optical brighteners can be used to reduce the quantity of virus needed to achieve pest control in the field. Some previous studies have suggested that brighteners might allow this (Webb et al. 1994; Thorpe et al. 1998). Use of brighteners to reduce virus applications rates would be a valuable strategy for improving the prospects for using baculoviruses in pest management, because present problems with baculovirus production and high costs are significant limiting factors (Moscardi 1999). By reducing virus application rates, logistical problems of baculovirus production would be reduced, as would the per-hectare treatment costs. This approach does raise some additional considerations relating to the cost of optical brighteners and environmental safety concerns. It has been suggested that the cost of brighteners might make their inclusion in baculovirus formulations financially unfeasible (Martinez et al. 2000). Meanwhile a recent study raised the question of possible effects of optical brighteners on pollinating insects. Bees rely on UV reflectance and special UV absorbing areas, termed nectar guides on flowers, to lead them to the nectaries of flowers. Optical brighteners absorb UV radiation and hence disrupt patterns of UV reflectance on flowers. A recent study showed that application of optical brighteners to flowers reduced the number of visits made by honey bees (Goulson et al. 2000).

Genetic engineering has allowed the construction of recombinant baculoviruses with vastly improved insecticidal properties compared to wildtype viruses. The neurotoxin-expressing constructs are the most effective recombinant baculoviruses designed to date, and they offer control of *H. virescens* in cotton, equivalent to that achieved with chemical insecticides (Smith et al. 2000; Treacy et al. 2000). Reductions in lethal times and feeding damage of larvae infected with the new recombinant baculovirus AcMLF9.ScathL, are comparable to those achieved by the neurotoxin-expressing viruses. In addition to achieving levels of pest control comparable to that of chemical insecticides, recombinant baculoviruses
are safe to humans and their use in agricultural systems will not cause devastation of natural
enemy populations. A risk assessment evaluation for AcMLF9.ScathL suggests that impacts on
predators are not likely to differ from those of wildtype AcMNPV C6, while exposure of
nontarget organisms to AcMLF9.ScathL is likely to be lower than would occur with AcMNPV
C6. Thus on the basis of comparisons with studies using neurotoxin-expressing viruses, it
seems likely that AcMLF9.ScathL will offer control of *H. virescens* in cotton equivalent to that
offered by chemical insecticides, without the serious adverse effects on nontarget organisms
that occur with broad-spectrum chemical insecticides.

Despite the appealing characteristics of recombinant baculoviruses, it does not seem
likely they will be widely used in the near future. Although the research and development costs
associated with the development of a recombinant baculovirus are less than those for a new
chemical insecticide, there are other factors that limit commercial interest. Production of
baculoviruses is still somewhat problematic, and at present per-hectare treatment costs for a
baculovirus still exceed those for chemical insecticides. In addition, the high specificity of
baculoviruses which is an advantage from an integrated pest management perspective, because
of low impacts on nontarget organisms, is viewed as something of a disadvantage from the
point of view of a farmer or industry, because a viral insecticide will only control one or a
limited number of pest species. In the case of large agrochemical companies which often have
minimum sales earnings requirements for their products, the high pest specificity of
baculoviruses translates into a low market share and low revenue, even if the baculovirus is
competitive with rival products. This may not be such a concern for smaller pest management
companies, but it is another factor limiting commercialization of baculoviruses. Future
advances in genetic engineering allowing manipulation of baculovirus host range, and
improved virus production in cell culture should improve the prospects for the use of
recombinant baculoviruses, as will further government regulation of chemical insecticides.
References


Rice, M. 1999. Scout for cutworms in seedling corn, pp. 70-71. In Integrated Crop Management Newsletter. Iowa State University,


ACKNOWLEDGMENTS

I would like to thank those who have helped during my graduate career. First, very special thanks to my major professor Dr. Bryony Bonning for her help and advice throughout the course of my graduate studies. I would also like to thank Dr. Robert Harrison for his help and for his opinions on the many areas of research we discussed during the course of my graduate training. I also appreciate the help and guidance I received from members of my POS committee: Dr. Joel Coats, Dr. Diane Debinski, Dr. Leslie Lewis and Dr. John Obrycki.