NN08201, 2937

Stellingen

1. Procesmodellen bieden een gedetailleerder en beter toepasbaar inzicht in biologische systemen dan gangbare analytische modellen.
   Dit proefschrift.

2. Het tijdstip van toepassing van baculovirussen is van groter belang voor een succesvolle bestrijding van insecten dan de werkingssnelheid van deze virussen.
   Dit proefschrift.

3. De gangbare wijze van het analyseren van tijd-reponsdata in de baculovirologie is niet-wetenschappelijk.
   Vistat, 1990.

4. De term "virulent" is een verwarrende uitdrukking voor de biologische activiteit van baculovirussen.
   Myers and Rothman, 1995.
   Fuxa and Richter, 1992.

5. De voortgang van de wetenschap wordt belemmerd doordat artikelen die strijdig zijn met gangbare theorieën moeilijker worden geaccepteerd dan artikelen die deze theorieën bevestigen.

6. Voor de toelating van genetisch gemodificeerde organismen geldt: 'om risico's te kunnen beoordelen, moet je ze kunnen kwantificeren'.

7. Waar de commercie in de sport begint, stopt de liefde voor de sport.

8. Een 'win-win situatie' is een hedendaags eufemisme voor het aloude gezegde 'gedeelde smart is halve smart'.

Stellingen behorende bij het proefschrift
'Process-based modeling of the control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouse chrysanthemum'
Felix J.J.A. Bianchi, Wageningen, 9 februari 2001

Process-based modeling of the control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouse chrysanthemum

Felix J.J.A. Bianchi

CENTRALE LANDBOUWCATALOGUS
0000 0872 2601

Promotoren: prof. dr. ir. R. Rabbinge
Hoogleraar Plantaardige Productiesystemen

prof. dr. J.M. Vlak
Persoonlijk Hoogleraar bij de Leerstoelgroep Virologie

Co-promotor: dr. ir. W. van der Werf
Universitair docent bij de Leerstoelgroep Gewas- en Onkruidecologie

Samenstelling promotiecommissie:
dr. J.S. Cory (Centre for Ecology and Hydrology, NERC, UK)
prof. dr. M. Jeger (University of London, UK)
prof. dr. J.C. van Lenteren (Wageningen Universiteit)
dr. P.H. Smits (Plant Research International, Wageningen)

NN08201,2937

Process-based modeling of the control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouse chrysanthemum

Felix J.J.A. Bianchi

proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit
prof. dr.ir. L. Speelman,
in het openbaar te verdedigen
op vrijdag 9 februari 2001
des namiddags te 13.30 uur in de Aula

1605101

The work presented in this thesis was carried out at the Laboratory of Virology and the Laboratory of Theoretical Production Ecology of Wageningen University. It was financially supported by the Foundation of Technical Sciences (STW), Dutch Organization of Scientific Research (NWO).

Bianchi, Felix J.J.A.

Process-based modeling of the control of beet armyworm,
Spodoptera exigua, with baculoviruses in greenhouse chrysanthemum
Thesis Wageningen University – with ref. – with summary in Dutch
ISBN 90-6464-862-x
Subject headings: Beet armyworm, baculovirus, biological control

What the caterpillar calls the end,
The rest of the world calls a butterfly

La O-Tze

Aan mijn ouders

Contents

Chapter		Page
1	General Introduction	1
2	Biological activity of SeMNPV, AcMNPV and three deletion mutants of AcMNPV against *Spodoptera exigua* larvae (Lepidoptera: Noctuidae)	15
3	The polyhedral membrane does not protect polyhedra of AcMNPV against inactivation on greenhouse chrysanthemum	27
4	The influence of greenhouse chrysanthemum on the interaction between the beet armyworm, *Spodoptera exigua*, and the baculovirus SeMNPV: parameter quantification for a process-based simulation model	33
5	Transmission dynamics of the multicapsid nucleopolyhedrovirus SeMNPV in *Spodoptera exigua* populations in greenhouse chrysanthemum	45
6	Greenhouse evaluation of dose- and time-mortality relationships of two nucleopolyhedroviruses for the control of beet armyworm, *Spodoptera exigua*, on chrysanthemum	55
7	Description of a process-based model for the biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses	67
8	Validation of a process-based model for the biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses	87
9	Scenario studies with a process-based model for the biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses	103
10	General discussion	115

References	124
Summary	133
Samenvatting Nederlands	136
Nawoord	139
Curriculum Vitae	141
List of publications	142

1

General introduction

1.1 Control of pest insects

There is an increasing demand from society for sustainable agricultural production for a growing world population in an environmentally safe, biologically sound and ethically acceptable manner. Losses due to the activity of insect pests are a major constraint for the achievement of optimal productivity of agricultural crops. The development of pesticides that control pests is an important factor that enabled the massive increase in yield that has been achieved during the twentieth century. In 1991 the worldwide sales of insecticides was 8 billion US $, of which chemical insecticides constituted 99% (Georgis, 1997).

Although chemical insecticides often provide adequate control, are reliable and require simple application methods, their use may be detrimental for man and environment in the long run (Carson, 1964; Jansma *et al.*, 1993) and may lead to the development of resistance in insects (e.g. Hung and Sun, 1989; Cheng *et al.*, 1990). In addition, the capital investment and time involved to bring new chemical insecticides to the market are a major constraint for the development of chemical insecticides. These environmental and economical constraints triggered the search of alternative methods of pest control such as integrated pest management and biological control.

Biological control is an important alternative to chemical insecticides, where predators, parasitoids and pathogens are used to control insect pests (Albajes *et al.*, 1999). In 1991 biological control agents constituted only a modest part of 1% of the world pesticide market, which was 80 million US $. In 1995 the market for biological control agents was 380 million US $ including 3-4 million US $ for the use of viruses. By 2000 the market for biological control agents is predicted to have increased to 500-520 million US $, with viruses making up 5-6 million $ of the total (Georgis, 1997). Thus, biological control agents are a small but growing market.

Baculoviruses are naturally occurring insect pathogens that are used as biological control agents of pest insects (Black *et al.*, 1997; Moscardi, 1999). Their infectivity, specificity and safety to non-target organism make them promising candidates to replace chemical insecticides. For insect species that have developed resistance to chemical insecticides, such as the beet armyworm *Spodoptera exigua*, the use of baculoviruses is one of the few options left for control (Brewer and Tumble, 1989). Examples of successful introductions of baculoviruses as biological pesticides are *Anticarsia gemmatalis* multicapsid nucleopolyhedrovirus (AgMNPV) against *A. gemmatalis* in soybean, *Lymantria dispar* MNPV against *L. dispar* in forests and *Spodoptera exigua* MNPV against *S. exigua* in

Table 1.1 *Examples of baculoviruses developed as commercial microbial control agents for Lepidoptera.*

Pest insect	Baculovirus	Crops	Commercial name	Country
Adoxophyes orana	GV	apple	Capex	Switzerland
Anticarsia gemmatalis	NPV	soybean	Baculoviron	Brazil
Autographa californica	MNPV	cotton, ornamentals	VPN 80	Guatemala
Cydia pomonella	GV	apple, pears	Madex	Switzerland
			Carpovirusine	France
			Cyd-X	USA
			Granusal	Germany
			Virin-GyAp	Russia
Heliothis virescens	MNPV	cotton	Elcar	USA
			Gemstar	USA
Helicoverpa armigera	SNPV	cotton, tomato	-	China
			Virin-Hs	Russia
Lymantria dispar	MNPV	forests	Gypchek	USA
			Dispavirus	Canada
			Virin-ENSH	Russia
Mammestra brassicae	MNPV	cabbage	Mamestrin	France
			Virin-EKS	Russia
Neodiprion sertifer	SNPV	pine	Monisärmiövirus	Finland
			Virox	UK
Spodoptera exigua	MNPV	ornamentals	Spod-X	The Netherlands
		vegetables	Spod-X	USA
		vegetables	Spod-X	Thailand
Spodoptera littoralis	MNPV	cotton	Spodopterin	France (Africa)

Data: Huber (1998) and Moscardi (1999).

ornamentals and vegetables (Moscardi, 1999). An overview of a selection of commercialized virus preparations is given in Table 1.1.

In 1991 the Dutch government started the "Multi-Year Plan Crop Protection" which intended to reduce the use of agrochemicals in The Netherlands by 50% in the year 2000 as compared to the level of 1991 (MJP-G, 1991). This policy stimulated the quest and demand for biological control agents that can replace chemical insecticides. The baculovirus *S. exigua* MNPV is the first baculovirus on the market to control pest insects in the Netherlands. SeMNPV has been registered in 1993 (Smits and Vlak, 1994) and used against larvae of the beet armyworm in greenhouse ornamentals since the beginning of 1994 (Brinkman, 1993).

The work presented in this thesis focuses on the control of pest insects with baculoviruses. The control of beet armyworm, *S. exigua*, in greenhouse chrysanthemum with baculoviruses is chosen as model system because this system is well studied (Smits, 1987).

1.2 Chrysanthemum cropping system

In the Netherlands chrysanthemum is grown year-round in greenhouses. The crop is harvested in 9 to 13 weeks after planting in summer and winter, respectively. Plant density varies from 640,000 plants ha^{-1} in summer to 400,000 plants ha^{-1} in winter. Chrysanthemum is an important crop in Dutch greenhouse industry. Although the total surface of chrysanthemum cultivation was only 757 ha in 1998 (CBS, 1999), in 1999 the sales of cut chrysanthemum amounted to 300 million US $ with an average price of 0.20 US $ per plant (De Veld, 2000). The combination of the high value of the chrysanthemum crop with a low threshold for cosmetic damage underscores the importance of adequate control of pests and diseases. In 1998 the average chemical pesticide input per hectare in chrysanthemum was 40 kilogram (active component) ha^{-1}, which makes chrysanthemum after lily and rose the ornamental crop with the third highest pesticide input (CBS, 2000). The closed compartment of greenhouses and the ability to regulate environmental conditions offer advantages for the implementation of biological control methods (Albajes *et al.*, 1999). Greenhouses have an additional advantage for the use of microbial control agents because the glass filters UV-radiation, which is an important factor in the inactivation of baculoviruses (Jones *et al.*, 1993, McLeod *et al.*, 1982).

1.3 *Spodoptera exigua*

The beet armyworm, *S. exigua*, is a lepidopterous species belonging to the family Noctuidae. Originating from Southeast Asia, *S. exigua* is reported to be a pest in the USA, Mexico, Spain, Thailand and The Netherlands (Moscardi, 1999; Kolodny-Hirsch *et al.*, 1993; Alvaraso-Rodriguez, 1987; Caballero *et al.*, 1992; Kolodny-Hirsch *et al.*, 1997; Smits, 1987). In the Netherlands *S. exigua* occurs in greenhouses where climatic conditions are favorable and crops are cultivated throughout the year. Here, infestations are mainly found in a large number of horticultural crops, including chrysanthemum (Smits, 1987). The life cycle of *S. exigua* consists of an egg stage, five larval stadia, a pupal and an adult stadium (Figure 1.1) (Fye and McAda, 1972; Lee *et al.*, 1991a,b; Ali and Gaylor, 1992). Females of *S. exigua*

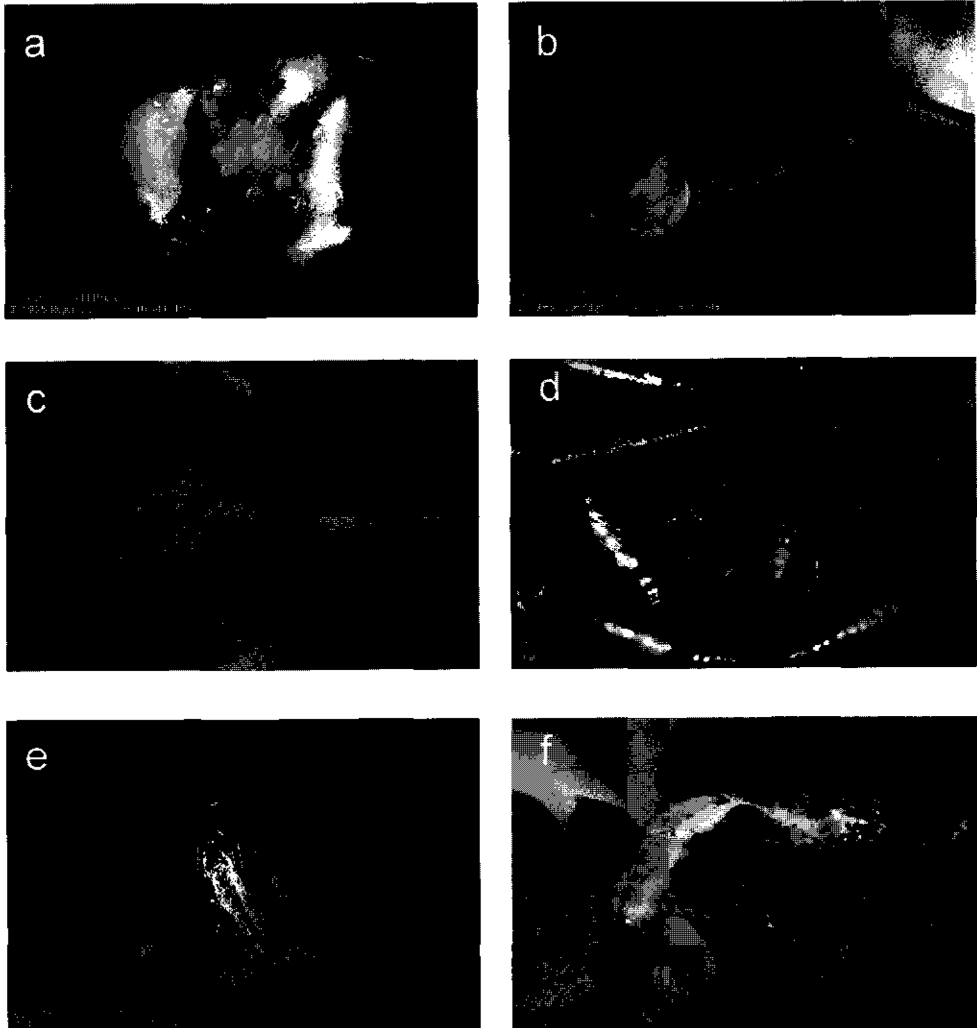

Figure 1.1 *Various stages of S. exigua. Egg batch (a), third instar larvae (b) fifth instar larva (c), pupa (d), adult (e) and virus-killed larvae (f). (Photos kindly supplied by Joyce Strand, University of California.)*

deposit egg batches on the underside of chrysanthemum leaves near the ground, preferably in young crops (Smits *et al.*, 1986). The first instar larvae are not very mobile and stay near the plant on which the eggs were laid. First instar larvae stay at the lower part of the plants. During their larval development the foraging domain increases and larvae tend to feed on the upper parts of the plants (Smits *et al.*, 1987a). Only the larvae cause host plant injury. The daily amount of leaf material eaten increases with larval instar: the first three instars cause only 5% of the crop injury, while 75% of the crop injury is caused by the fifth instar larvae. Young larvae mainly feed on the abaxial side of the leaves, leaving the cuticle of the adaxial side of the leaf intact, whereas older *S. exigua* larvae perforate leaves (Smits *et al.*, 1987a). After the fifth instar the larvae pupate in the soil. From the pupae new adults emerge. The life cycle parameters, such as developmental times at different temperatures, fecundity, egg batch size, sex ratio and background mortality have been determined under laboratory conditions by Fye and McAda (1972), Lee *et al.* (1991a,b) and Ali and Gaylor (1992).

1.4 Baculoviruses

Baculovirus structure and infection cycle

Baculoviruses are large DNA viruses that occur only in arthropods. The baculoviridae comprise two genera: the nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) (Murphy *et al.*, 1995). In the environment baculoviruses occur in the form of occlusion bodies, which are protein structures that contain one or more virions. The occlusion bodies of GVs generally contain a single virion with one genome (nucleocapsid). The occlusion bodies of polyhedroviruses may contain several hundreds of virions that may contain one (SNPV) or multiply (MNPV) nucleocapsids (Figure 1.2). Nucleocapsids are the elementary genetic units of baculoviruses and contain a large double-stranded DNA ranging in size between 100-160 kilo base pairs. Occlusion bodies or polyhedra are one of the two distinct morphological forms of baculoviruses. Polyhedra are infectious for insects and are responsible for the transmission of baculovirus infections in insect populations. The other morphological form of baculoviruses is the budded virus that is responsible for the spread of virus through a larval body (Federici, 1997).

The polyhedra are taken up orally by the larvae together with leaf or soil material. After ingestion the polyhedra dissolve under the alkaline conditions in the midgut. The nucleocapsids are liberated and infect the midgut epithelial cells. In the nucleus of these cells

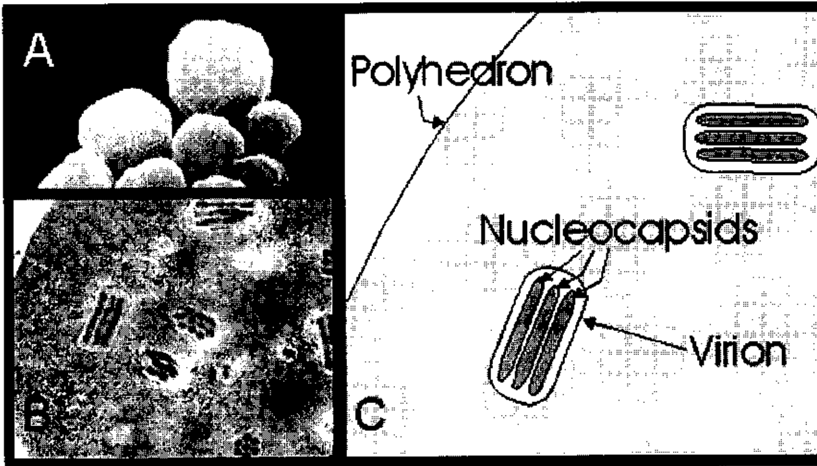

Figure 1.2 *Scanning electron micrograph of polyhedra of LdMNPV (a), transmission micrograph of a polyhedron (b), and schematic representation of a polyhedron (c). (Graphic kindly supplied by Vince D'Amico.)*

virus replication takes place and budded viruses are produced that leave the infected cells and infect other larval tissues, such as the haemocoel and the fat body. In secondarily infected cells new polyhedra and budded viruses are produced. The larvae generally die when most tissues have been infected and a massive amount of new polyhedra have been generated.

Baculovirus properties

Baculoviruses mainly have been reported in insects (Granados and Federici, 1986). Most baculoviruses occur in the insect orders *Lepidoptera*, *Hymenoptera* and *Diptera* (Martignoni and Iwai, 1986; Adams and McClintock, 1991). In general, baculovirus species have a limited host range (Table 1.2). For example, the baculoviruses of *S. exigua* (SeMNPV) and *L. dispar* (LdMNPV) are restricted to a single host species. The baculoviruses of *Autographa californica* (AcMNPV) and *Mamestra brassicae* (MbMNPV) are located at the other end of the host range spectrum and can infect dozens of insect species (Cory *et al.*, 1997). From an environmental and safety standpoint a limited host range has the advantage that the control agent is restricted to a single pest and has no direct detrimental effects on any other organism. On the other hand, a limited host range may be a drawback when more than one pest insect species is present and various baculoviruses have to be used for the different pests, rather than one virus with a broad-spectrum activity.

Table 1.2 *Host range of baculoviruses in Lepidoptera.*

Pest insect	*Helicoverpa zea*	*Heliothis virescens*	*Spodoptera eridania*	*Spodoptera exigua*	*Spodoptera frugiperda*	*Trichoplusia ni*
A. californica MNPV	+	++++	++	++++	++	++++
H. virescens MNPV	++	++++	+	+++	++	++++
H. armigera SNPV	++++	-	-	+++	-	++
H. zea SNPV	++++	++++	-	-	-	+
S. exigua MNPV	-	-	-	++++	-	-
T. ni MNPV	-	++	-	+++	-	++++

Susceptibility is reflected in a semi-quantitative scale that runs from "-" (nonpermissive to infection) to "++++" (higly permissive to infection). Data: Black *et al.* (1997).

Ingestion of polyhedra is the normal route of baculovirus entry into the host. The lethal dose of the host generally increases with larval instar, as for example in the insect-virus systems of *S. exigua*-SeMNPV, *Mamestra brassicae*-MbMNPV, *Anticarsia gemmatalis*-AgMNPV and *Heliothis punctiger*-HzSNPV (Smits and Vlak, 1988a; Evans, 1981; Boucias *et al.*, 1980; Teakle *et al.*, 1986). Kirkpatrick *et al.* (1998) suggested that this increasing resistance is related to changes in midgut physiology and/or tracheation. The time to death of larvae that ingested a lethal dose of polyhedra depends on temperature and the virus-host system, and takes typically 3 to 8 days (Stairs, 1978; Boucias *et al*, 1980; Smits and Vlak, 1988a; Tuan *et al.*, 1989). During infection of SeMNPV, up to 10^9 polyhedra can be produced in a single fifth instar *S. exigua* larva (Smits and Vlak, 1988c). The bodies of larvae that die of baculovirus infection disintegrate and the body contents of the cadavers containing the polyhedra are spilled on the plant and soil. These polyhedra can be inactivated by several factors (Hunter-Fujita *et al.*, 1998). UV-radiation is the major inactivating factor in the field (McLeod *et al.*, 1982; Richards and Payne, 1982; Jones *et al.*, 1993). Ignoffo *et al.* (1997) reported a half-life value of 4.9 hour for polyhedra of *Helicoverpa/Heliothis* NPV that were continuously exposed to natural sunlight. UV radiation may induce molecular changes in baculovirus DNA resulting in a block of DNA synthesis, a high mutation rate (Harm, 1980) or the generation of highly active radicals that degrade baculovirus DNA (Ignoffo and Garcia, 1994). Another factor that can induce polyhedra inactivation are plant exudates. Excreted compounds, such as Mg^{2+} and Ca^{2+}, tannins, phenols, peroxidases, rutin and chlorogenic acid have been reported to inactivate polyhedra (Elleman and Entwistle, 1985; Felton *et al.*, 1987; Hoover *et al.*, 1998a,b; Keating *et al.*, 1988; 1990).

The maintenance of baculoviruses in insect populations requires transmission of the virus from infected to uninfected individuals. Larvae may become infected by ingestion of polyhedra from the environment, which is referred to as horizontal transmission. Polyhedra produced in infected larvae contaminate leaves and soil and can cause new infections when ingested by uninfected larvae. These polyhedra may further be distributed by excrements of infected larvae (Vasconcelos, 1996), rain (Kaupp, 1981) and predators, such as birds (Entwistle *et al.*, 1993). Baculoviruses can also be released in the environment by human activity, for example by the application of baculovirus sprays. An alternative route of infection is vertical transmission, which is the direct transfer of virus from parents to their own offspring. Larvae exposed to polyhedra during their larval stage may develop into sublethally infected moths that are able to transmit the virus to part of their offspring (Hamm and Young, 1974; Smits and Vlak, 1988a; Young, 1990; Fuxa and Richter, 1992).

The biological properties of baculoviruses, such as their host range, infectivity, speed of action, inactivation rate and to a lesser extent their horizontal and vertical transmission dynamics are key factors that determine their agronomic efficacy as biological control agents.

Baculovirus recombinants

Although baculoviruses are effective biological insecticides, their relatively low speed of action is a factor that limits their use in practice because larvae continue feeding after they have been infected (Moscardi, 1999). With technical improvements these drawbacks may be overcome. For example, genetically modification techniques enable the construction of baculoviruses with an increased speed of action (Black *et al.*, 1997; van Beek and Hughes, 1998). One genetic engineering strategy is the deletion of nonessential baculoviruses genes that prolong the life of the infected host. This has been achieved for the baculovirus *egt* gene (O'Reilly and Miller, 1989). This gene encodes an enzyme that inactivates insect ecdysteroid hormones. Therefore, presence of the EGT protein delays or prevents molting of infected host insects. This leads to an extended period for the virus to multiply in the host and an increased virus yield. Deletion of the *egt* gene results in reductions in larval survival times with up to 30% (O'Reilly and Miller, 1991; Flipsen *et al.*, 1995; Treacy *et al.*, 1997; Dai *et al.*, 2000; Chen *et al.*, 2000). This egt effect has been reported for a number of insect-virus systems, but Slavicek *et al.* (1999) observed it only in fifth instar *Lymantria dispar* larvae infected with LdMNPV, and not in first to fourth instar larvae. Other genes of interest are the *pp34* and *p10* genes. The *pp34* gene encodes a polyhedral membrane protein (Gombart *et al.*, 1989). An AcMNPV *pp34* deletion mutant has been reported to have an increased infectivity (Ignoffo *et*

al., 1995). The *p10* gene encodes fibrillin forming fibrillar structures in the nucleus and cytoplasm of infected cells (van Oers and Vlak, 1997). These structures are thought to play a role in the disintegration of infected cells and may affect the horizontal transmission process.

Another genetic engineering strategy is the insertion of foreign genes in the baculovirus genome that encode gene products that interfere with insect physiology or development. Baculoviruses with increased speed of action have been constructed that express juvenile hormone esterase (Hammock *et al.*, 1990), insect-specific neurotoxins (Stewart *et al.*, 1991; Maeda *et al.*, 1991; McCutchen *et al.*, 1991; Tomalski and Miller, 1991; Tomalski *et al.*, 1993; Prikhod'ko *et al.*, 1996; Chen *et al.*, 2000) or a maize mitochondrial gene (Korth and Levings, 1993). Time to kill the host has been reduced by 25-50% by the insertion of these foreign genes. Recombinant baculoviruses expressing neurotoxins are promising candidates as improved biological insect control agents (Cory *et al.*, 1994; Black *et al.*, 1997).

UV protection formulations

The rapid inactivation of baculoviruses in field situations is, besides their low speed of action, a constraint for their use in practice (Moscardi, 1999). The persistence of baculoviruses has been prolonged by the addition of substances that protect polyhedra against UV-radiation, such as lignosulfates, carbon, gelatin and titanium dioxide (Black *et al.*, 1997). The mode of action of these substances is based on reflection or absorption of UV-radiation. Besides these traditional formulations that reduce polyhedra inactivation, stilbene optical brighteners have been found to provide protection against UV-radiation and enhance the infectivity and speed of action of baculoviruses (Shapiro and Robertson, 1992). Optical brighteners have been reported to reduce LD_{50} values substantially in a number of insect-virus systems (Dougherty *et al.*, 1996; Vail *et al.*, 1996) and this was confirmed in field situations (Webb *et al.*, 1994, Cunningham *et al.*, 1997). The action mechanism of optical brighteners is not fully understood. It may involve an effect on the perithrophic membrane which is the main mechanical barrier protecting the midgut cells from baculoviruses (Shapiro, 1995).

1.5 Objective and approach of the study

Baculovirus genetics and pathogenesis are relatively well studied. Mechanisms of DNA replication and gene expression have been intensively studied, and numerous studies investigated interactions between baculoviruses and insects (Federici, 1997; Lu *et al.*, 1997).

However, insight in the dynamics of the control of insect pests in field situations is still limited. This is reflected in the strategy that is used to determine optimal baculovirus spraying regimes in field situations. Baculovirus efficacy is generally assessed in a 'trial and error' approach by the application of different baculovirus dosages and by the determination of the level of control in extensive field testing programs (e.g. Smits *et al.*, 1987b; Kolodny-Hirsch *et al.*, 1993; 1997; Hunter-Fujita *et al.*, 1998). This field testing strategy has also been used for the determination of the commercial potential of new recombinant baculoviruses and new formulations (e.g. Jacques, 1972; Cory *et al.*, 1994). Given the rapid increasing number of recombinants and formulations (Black *et al.*, 1997; van Beek and Hughes, 1998), there is a need for methods that can support and guide these field testing programs. Support systems for the assessment of the field efficacy of baculoviruses may contribute to improved understanding of the biological control and improved field testing procedures, with a minimum of field experiments.

The control of insect pests with baculoviruses in an agricultural crop involves the management of a highly complex system. Mathematical models have been used to study and gain insight in such systems. Parameter-sparse *analytical* models have been developed for epidemics of baculoviruses in insect populations and have successfully been used to study the long term dynamics and stability of insect-virus systems (Anderson and May, 1981; Dwyer, 1992; Dwyer and Elkinton, 1993; Dwyer *et al.*, 1997; 2000). Because these models only contain limited detail, they are less suitable for precise, short-term predictions of the agronomic efficacy of baculoviruses as biocontrol agents. An alternative modeling approach that entails a detailed description of the biological system is that of *numerical* simulation models. These models describe the biological system at the process level and integrate information of the different processes. This approach may be suitable to describe biological control of pest insects with baculoviruses with the level of detail needed for the evaluation of (genetically modified) baculoviruses and formulations.

The objective of this study is to evaluate the potential of baculoviruses as biological control agents of pest insects by the development and analysis of an explanatory simulation model that describes the dynamics of the crop-insect-virus system. The model applies to AcMNPV and SeMNPV, two viruses with distinct biological properties, in populations of *S. exigua* larvae in greenhouse chrysanthemum. The development of this model occurs in four steps (Rabbinge and de Wit, 1989). The first step is the quantification of the processes that determine the control of baculoviruses in insect populations in crops, such as the life cycle and population dynamics of insects, plant growth characteristics, spray deposition, the baculovirus

infection cycle and transmission routes. The second step is the development of a simulation model that integrates these processes and describes the control of baculoviruses in insect populations at a field scale. The third step is the validation of this model with independent data of baculovirus efficacy under field conditions. When model simulations are in line with validation experiments there will be growing confidence that the model accurately describes the underlying processes of the crop-insect-virus system. When the model simulations are not in line with validation experiments this is an indication of incomplete understanding and/or poor system predictability. In this case steps one to three should be reexamined. The fourth step is the generation and analysis of scenario studies. This information provides quantitative understanding of the functioning of the system. This insight may contribute to the identification and prediction of effective application regimes for (genetically modified) baculoviruses.

1.6 Outline of the thesis

This thesis describes a feasibility study on the control of *S. exigua* with baculoviruses. A process-based simulation model is developed and validated that can be used to evaluate the potential of baculoviruses as biological control agents of pest insects. The biological properties of SeMNPV and AcMNPV and deletion mutants of AcMNPV have been quantified in studies presented in chapter 2 and 3. In chapter 2 the infectivity and speed of action of AcMNPV and SeMNPV and *egt*, *pp34* and *p10* deletion mutants of AcMNPV have been quantified in laboratory experiments. The inactivation of AcMNPV and an AcMNPV *pp34* deletion mutant have been studied on chrysanthemum in small-scale greenhouse experiments (chapter 3). These studies provide quantitative information about the baculovirus infection and inactivation processes. This information will be used for the model description and parameter estimation of these processes. In chapter 4 interactions between *S. exigua*, SeMNPV and greenhouse chrysanthemum have been quantified. Understanding of these interactions is essential for the correct extrapolation of processes quantified under laboratory conditions to a field situation. Chapter 5 describes the quantification of the horizontal transmission process of SeMNPV in *S. exigua* populations in greenhouse chrysanthemum and vertical transmission of SeMNPV by sublethally infected *S. exigua* moths to their progeny. The efficacy of SeMNPV and AcMNPV applications in terms of mortality, time to kill and crop injury of *S. exigua* populations in chrysanthemum has been determined in a series of greenhouse experiments (chapter 6). These data will be used for the validation of the simulation model

BACSIM. The description of the simulation model BACSIM, which integrates the processes quantified in chapters 2 to 5 is presented in chapter 7. The validation of BACSIM with independent data of SeMNPV and AcMNPV applications against *S. exigua* populations in chrysanthemum (as described in chapter 6) is given in chapter 8. Finally, scenario studies generated with BACSIM are presented in chapter 9. Scenario studies include the timing, dosage and spraying interval of SeMNPV applications, the evaluation of the efficacy of UV-protection agents that reduce polyhedra inactivation, as well as the comparison of the efficacy of wild-type AcMNPV and AcMNPV recombinants that have an increased speed of action. The study is concluded with a general discussion (chapter 10), which focuses on the scientific and practical implications of this study.

2

Biological activity of SeMNPV, AcMNPV and three deletion mutants of AcMNPV against *Spodoptera exigua* larvae (Lepidoptera: Noctuidae)

Virulence and speed of action, as related to dose, are important effectiveness-determining properties of insect-pathogenic biocontrol agents. We used the droplet-feeding bioassay to compare dose responses between two wild-type baculoviruses, Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and Spodoptera exigua MNPV (SeMNPV), and three deletion mutants of AcMNPV in S. exigua larvae. In each mutant one gene was deleted by genetic engineering: pp34, coding for the polyhedral membrane, egt, coding for ecdysteroid UDP-glucosyltransferase, or p10, coding for fibrillar structures in infected insect cells. SeMNPV had the lowest median lethal dose (LD_{50}) as well as the highest speed of action (LT_{50}) of all viruses investigated. In our comparative bioassays the only significant effect of gene deletions in AcMNPV was a slightly lower speed of action for the p10 deletion mutant. Otherwise, wild-type and recombinant AcMNPVs had similar biological activities. Our results suggest, in contrast to what is generally assumed, that gene deletions in AcMNPV for improved insecticidal activity should be critically assessed in each host system prior to further implementation as a control agent. Insertion of foreign genes coding for entomo-toxins is less questionable and more promising in this respect.

This chapter has been published as:
Bianchi, F.J.J.A., Snoeijing, I., van der Werf, W., Mans, R.M.W., Smits, P.H., and Vlak, J.M. 2000.
J. Invertebr. Pathol. **75**, 28-35.

2.1 Introduction

In the recent past *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) recombinants with gene deletions have been constructed and the effect of these deletions on viral characteristics have been studied (Vlak *et al.*, 1988; Zuidema *et al.*, 1989; O'Reilly and Miller, 1991; Ignoffo *et al.* 1995). These studies indicate that the deletion of single genes from the AcMNPV genome can alter the biological properties of the virus. A well-known example is the deletion of the ecdysteroid UDP-glucosyltransferase (*egt*) gene that enhances the speed of action of the virus and the virus yield (O'Reilly and Miller, 1989; 1991). The *egt* gene encodes an enzyme that inactivates insect ecdysteroid hormones. Presence of sufficient quantities of the EGT protein delays or prevents molting of infected host insects, leading to an extended period for the virus to multiply in the host and an increased virus yield. Deletion of the *egt* gene resulted in an increased speed of action in *Spodoptera frugiperda* larvae (O'Reilly and Miller, 1991), most likely through an effect on the Malpighian tubules (Flipsen *et al.*, 1995). The AcMNPV *pp34* gene encodes a polyhedral membrane protein (Gombart *et al.*, 1989). When this gene is deleted, polyhedra lack a protective calyx, which results in a higher sensitivity of polyhedra to weak alkali (Zuidema *et al.*, 1989). Ignoffo *et al.* (1995) found that AcMNPV *pp34* deletion mutants have an increased infectivity in *Trichoplusia ni* larvae. The increased infectivity of AcMNPV-Δ*pp34* could be explained by the efficient release of occlusion-derived virions (ODV) from the polyhedra in the midgut to start the infection process. A third gene, AcMNPV *p10*, encodes fibrillin forming fibrillar structures in the nucleus and cytoplasm of infected cells (van Oers and Vlak, 1997). These structures are thought to play a role in the disintegration of infected cells. Deletion of the AcMNPV *p10* gene resulted in polyhedra with slightly increased infectivity in second instar *Spodoptera exigua* (Vlak *et al.*, 1988). The role of fibrillin in this respect is unknown.

In the studies of Ignoffo *et al.* (1995) and Vlak *et al.* (1988) the *pp34* and *p10* deletion mutants contained β-galactosidase as a marker gene whereas wild-type AcMNPV did not contain this marker. It is therefore uncertain whether the differences found in these studies are entirely due to gene deletions or that the marker gene may also have had an impact. Although β-galactosidase is generally considered neutral, Bonning *et al.* (1992) and Wood *et al.* (1993) clearly demonstrated that this may not always be the case. In a study of Bonning *et al.* (1992) an AcMNPV recombinant containing β-galactosidase had a 7 times higher LD_{50} value as the wild-type AcMNPV in *Heliothis virescens* larvae. Wood *et al.* (1993) found that a polyhedrin negative AcMNPV recombinant expressing β-galactosidase had a 17 hour increase in survival

time against neonate *T. ni* larvae compared to a polyhedrin negative AcMNPV recombinant without β-galactosidase.

Thus, for a proper evaluation of the effect of a gene deletion on viral characteristics, deletion mutants without a foreign genetic element should be studied. For this purpose AcMNPV *egt*, *pp34* and *p10* deletion mutants have been constructed without a marker gene. The infectivity and speed of action of these deletion mutants were compared to wild-type AcMNPV and SeMNPV in five larval instars of the beet armyworm, *S. exigua*. The aim of the study is to assess and quantify the effect of the *egt*, *pp34* and *p10* genes on AcMNPV infectivity and speed of action in this insect. In addition, the effect of virus dose on the molting behavior of *S. exigua* is studied in presence (AcMNPV) and absence of *egt* (AcMNPV-Δ*egt*) is studied.

2.2 Material and methods

Insects

Laboratory colonies of *S. exigua* were maintained as described by Smits *et al.* (1986). Larvae for bioassay were reared from surface sterilized eggs and incubated at 27°C, 70-80% relative humidity and a 16 h photoperiod.

Viruses

The control viruses were the E2 clone of *A. californica* MNPV and a US isolate of *S. exigua* MNPV (Hunter and Hall, 1968). Three recombinant viruses were constructed. A *pp34*-negative mutant, AcMNPV-RM3, was made taking a β-galactosidase positive mutant, AcMNPV-DZ5 (Zuidema *et al.*, 1989), as a start. The AcMNPV fragment BamHI-D was cloned into pTZ to give pAcRM0. The ATG start codon and the TAA stop coding of the *pp34* gene were mutagenized by site-directed mutagenesis into *Bgl*II sites. After cleavage with *Bgl*II and ligation of the ends a *pp34* deletion was obtained (pAcRM3). This transfer vector was co-transfected with AcMNPV-DZ5 DNA (β-galactosidase +) into *Sf*21 cells and AcMNPV-RM3 (β-galactosidase -) was plaque-purified. The second recombinant was the *p10*-negative mutant AcMNPV-MO21 (Vlak *et al.*, 1988; Martens *et al.*, 1995) which has a deletion of the *p10* open reading frame. The third recombinant was the *egt*-negative mutant AcMNPV-RM2, a β-galactosidase negative mutant of AcMNPV-RM1 (Flipsen *et al.*, 1995). This mutant has a natural *Xba*I site located 160 nucleotides upstream from the 3' end of the

egt gene, and is constructed by insertion of a *Xba*I site by mutagenesis at the transcriptional start site of the *egt* gene (Figure 2.1).

The five viruses AcMNPV, SeMNPV, AcMNPV-RM3 (AcMNPV-Δ*pp34*), AcMNPV-MO21 (AcMNPV-Δ*p10*) and AcMNPV-RM2 (AcMNPV-Δ*egt*) were propagated by infection of fourth instar *S. exigua* larvae via surface contamination of semi-synthetic diet (Smits *et al.*, 1988). Virus was purified by grinding deceased larvae, filtering through a double layer of cheese-cloth and two centrifugation steps. The polyhedra were resuspended and stored in a glycerol/water (1:1) solution in the dark at 4°C.

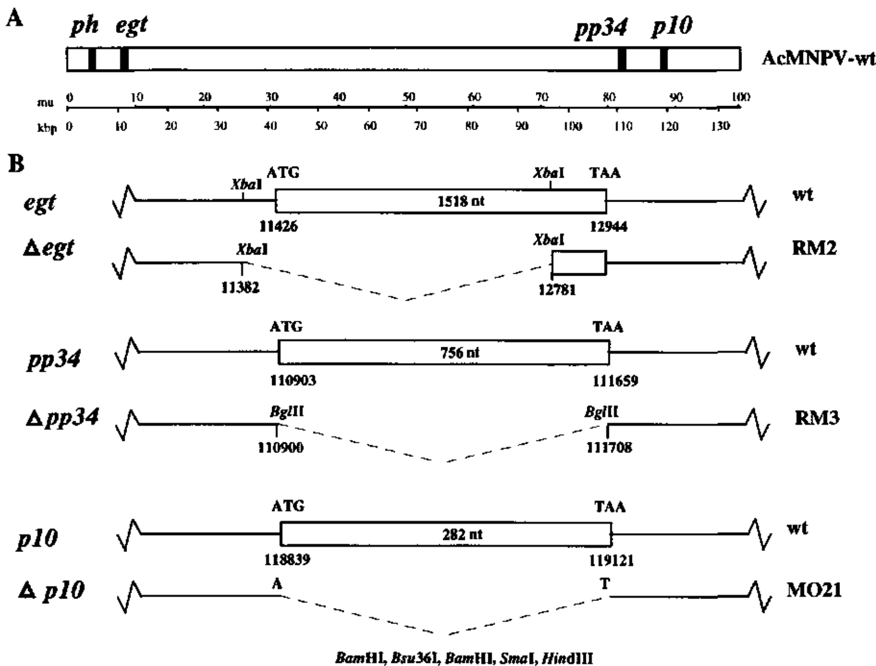

Figure 2.1 *Schematic representation of the locations of the polyhedrin (ph), ecdysteroid UDP-glucosyltransferase (egt), polyhedral membrane protein (pp34) and fibrillin (p10) genes (A) and their deletions (B) in the AcMNPV genome. Nucleotide positions are according to Ayres et al. (1994).*

Virus identification

Fourth instar *S. exigua* larvae were infected by surface contamination of semi-synthetic diet with freshly produced AcMNPV, AcMNPV-Δ*pp34*, AcMNPV-Δ*p10* and AcMNPV-Δ*egt*. After four days, haemolymph of the infected larvae was taken. One percent phenylthio-urea was added to prevent the action of tyrosinases. The haemolymph containing budded virions

(BV) was used to infect Sf21-cells. BVs were obtained from the cell culture supernatant and the DNA was isolated for further analyses (Caballero *et al.*, 1992). Viral DNA was identified by restriction enzyme analysis.

Bioassays

Dose-mortality and dose-lethal time relationships were determined using the modified droplet-feeding method (Hughes and Wood, 1981). Larvae were starved for 16 h (for all instars) at 27°C and were allowed to molt overnight. Stock suspensions of polyhedra were diluted, using a 1% PBS-solution with 10% sucrose. Polyhedra were counted using a haemocytometer. When the appropriate dilution was obtained, säure-blau (food-coloring) was added until a deep blue solution was obtained. Newly molted larvae were allowed to drink from polyhedron suspensions of known concentrations for 10 minutes. The suspensions were offered in small droplets, applied in a circle on a layer of parafilm placed on the bottom of a Petri dish. Larvae that ingested polyhedron suspension, as judged by the uptake of blue stain, were transferred to individual wells of a 12-well tissue culture plate with fresh artificial diet. The wells were covered with two layers of paper tissue and a lid. Larvae were reared at 27°C and mortality was recorded twice per day until all larvae had either pupated or died.

In a first set of assays, dose-mortality and dose-lethal time relationships of second and fourth instar larvae were determined for all five viruses, whereas for neonate, third and fifth instar larvae these relationships were determined for wild-type AcMNPV and AcMNPV-Δ*pp34* only. For each virus five different polyhedra concentrations were used, with 36 larvae per concentration. In a second set of assays, aimed at comparison of lethal times, second instar larvae were inoculated with 10^6 and 10^9 polyhedra ml^{-1} suspensions of wild-type AcMNPV and AcMNPV-Δ*egt*. The larval stage and mortality was recorded twice per day. Bioassays of both experiments were replicated three times.

Measurement of volumes ingested:

The volume ingested by larvae was measured by feeding 16 h starved larvae the standard PBS solution with 10% sucrose, spiked with a known concentration of ^{32}P (Hughes *et al.*, 1984; Smits and Vlak, 1998a). After ingestion, the larvae were placed individually in counting vials and frozen at -20°C. The isotope uptake was determined using Čerenkov radiation in a scintillation counter. Scintillation counts from individual larvae were converted to volumes ingested solution.

Statistical analyses

The dose-mortality data were analyzed by probit analysis, using the computer program POLO (Russell *et al.*, 1977). Median lethal times (LT_{50}) were calculated according to Snedecor and Cochran (1989). Median lethal times, log LD_{50} and slopes of probit regression lines of the first experiment were analyzed in two subsets. One subset consisted of data of all five viruses for second and fourth instar larvae. A second subset consisted of data of AcMNPV and AcMNPV-Δ*pp34* for all five larval instars. The data sets were analyzed using regression analysis and t-tests of pairwise differences using Genstat (Payne *et al.*, 1993).

2.3 Results

The cumulative frequency distributions of the volumes ingested by *S. exigua* are presented in Figure 2.2. The median volumes ingested were 0.013, 0.09, 0.55, 1.7 and 6.6 μl for first to fifth instar larvae, respectively. The distribution of ingested volumes was skewed, which resulted in lower median than mean ingested volumes. For the calculation of the ingested dose median ingested volumes were used.

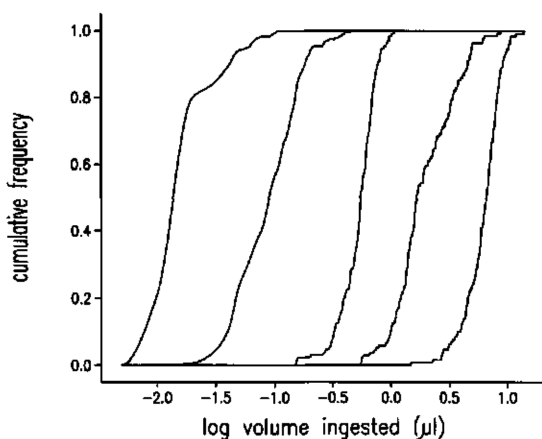

Figure 2.2 *Cumulative frequency distributions of the volumes ingested by five larval instars S. exigua.*

Estimated parameters (LD_{50} and slope) and associated statistics of probit regression lines describing dose-mortality relationships are shown in Table 2.1. The LD_{50} values increased rapidly from the first to fifth larval instar. AcMNPV LD_{50} values for second and fourth instar were approximately 13 and 600 polyhedra, respectively. SeMNPV LD_{50} values

for these larval instars were 3 and 44 polyhedra, respectively, which were significantly lower than those for AcMNPV ($P < 0.001$). The slopes of AcMNPV and SeMNPV probit lines were not significantly different, indicating a similar variation in susceptibility of the insect population to these two viruses.

The deletion of single *pp34*-, *p10*- and *egt*-genes had little or no effect on the dose-mortality relationships (Table 2.1). AcMNPV and AcMNPV-Δ*pp34* log LD_{50} values were not significantly different for five larval instars of *S. exigua* ($P = 0.143$). Log LD_{50} values for AcMNPV-Δ*p10* and AcMNPV-Δ*egt* in second and fourth instar larvae did not differ significantly from wild-type AcMNPV. None of the AcMNPV deletion mutants had a significantly different slope as compared to wild-type AcMNPV.

Table 2.1 *Summary of probit analyses of dose-mortality data obtained for SeMNPV, AcMNPV and three recombinants of AcMNPV using the droplet-feeding method.*

Instar	Virus	log LD_{50}[†]	LD_{50}[†]	Slope ± SD	χ^2/df
1	AcMNPV	$0.554^a \pm 0.04$	3.6	$1.59^a \pm 0.44$	11.4/3, 1.6/3, 2.8/3
1	AcMNPV-Δ*pp34*	$0.596^a \pm 0.15$	3.9	$1.41^a \pm 0.42$	11.8/3, 3.9/3, 2.2/3
2	AcMNPV	$1.13^a \pm 0.27$	13.5	$1.50^a \pm 0.42$	0.7/3, 9.1/3, 3.5/3
2	AcMNPV-Δ*pp34*	$1.03^a \pm 0.39$	10.7	$1.26^a \pm 0.13$	1.5/3, 3.8/3, 3.3/3
2	AcMNPV-Δ*egt*	$1.06^a \pm 0.35$	11.5	$1.88^a \pm 0.33$	10.5/3, 5.2/2, 0.9/3
2	AcMNPV-Δ*p10*	$1.35^a \pm 0.65$	22.2	$1.00^a \pm 0.38$	7.5/3, 1.6/3, 0.9/3
2	SeMNPV	$0.47^b \pm 0.22$	2.9	$1.55^a \pm 0.10$	30.0/3, 10.0/3, 11.3/3
3	AcMNPV	$2.33^a \pm 0.29$	214	$1.03^a \pm 0.21$	8.7/3, 6.6/3, 4.0/3
3	AcMNPV-Δ*pp34*	$2.28^a \pm 0.14$	190	$1.08^a \pm 0.14$	7.0/3, 1.9/3, 2.3/3
4	AcMNPV	$2.81^a \pm 0.16$	639	$0.94^a \pm 0.12$	2.9/3, 10.5/3, 2.4/3
4	AcMNPV-Δ*pp34*	$3.19^a \pm 0.31$	1562	$1.12^a \pm 0.14$	11.5/3, 7.1/3, 5.1/3
4	AcMNPV-Δ*egt*	$3.17^a \pm 0.03$	1486	$0.84^a \pm 0.10$	1.6/3, 5.0/3, 4.1/3
4	AcMNPV-Δ*p10*	$2.78^a \pm 0.36$	605	$0.95^a \pm 0.15$	8.3/3, 3.7/3, 1.1/3
4	SeMNPV	$1.64^b \pm 0.61$	44	$0.99^a \pm 0.26$	5.7/3, 7.3/3, 7.6/3
5	AcMNPV	$6.8^a \pm 1.2$	7.0×10^6	$1.26^a \pm 0.75$	1.2/3, 5.8/3, 2.3/3
5	AcMNPV-Δ*pp34*	$7.9^a \pm 0.0$	7.8×10^7	$0.70^a \pm 0.23$	8.8/3, 0.0/3, 0.9/3

[†] Log LD_{50} and LD_{50} calculations based on median values of ingested volume.

Superscript letters indicate significant differences compared to wild-type AcMNPV for each instar.

Table 2.2 *Summary of LT_{50} data obtained for SeMNPV, AcMNPV and three recombinants of AcMNPV using the droplet-feeding method.*

	conc. †	L1	L2	L3	L4	L5
AcMNPV	1	185.5 ± 0.0 §	91.3 ± 0.7 §	106.2 ± 20.3 §	104.5 ± 10.5	122.7 ± 0.0 §
	2	77.9 ± 5.8 §	93.7 ± 7.5	96.7 ± 12.0	100.1 ± 4.9	124.0 ± 0.0 §
	3	89.5 ± 5.6	88.7 ± 2.0	87.4 ± 2.8	96.4 ± 3.6	86.5 ± 0.0 §
	4	87.8 ± 0.9	88.3 ± 0.6	89.0 ± 4.9	91.6 ± 2.0	110.7 ± 0.4 §
	5	87.0 ± 4.6	88.0 ± 0.3	87.3 ± 1.4	91.5 ± 1.2	110.5 ± 0.3 §
AcMNPV-Δ*pp34*	1	89.3 ± 7.4 §	94.7 ± 5.9	99.2 ± 18.6 §	106.3 ± 12.9	nd
	2	85.4 ± 17.1 §	92.9 ± 5.6	93.7 ± 5.0	102.0 ± 7.2	124.0 ± 0.0 §
	3	85.8 ± 11.1	93.3 ± 3.5	95.2 ± 4.8	102.1 ± 7.2	124.0 ± 0.0 §
	4	91.7 ± 4.6	91.4 ± 6.7	91.9 ± 7.9	90.7 ± 7.2	92.2 ± 4.2 §
	5	85.3 ± 1.7	87.8 ± 2.6	88.9 ± 6.0	91.0 ± 0.8	129.8 ± 8.1 §
AcMNPV-Δ*egt*	1		88.2 ± 4.2 §		102.7 ± 3.8	
	2		86.1 ± 1.7		100.2 ± 5.7	
	3		86.7 ± 2.9		101.3 ± 11.5	
	4		87.3 ± 2.8		95.8 ± 9.6	
	5		86.2 ± 1.3		93.4 ± 7.5	
AcMNPV-Δ*p10*	1		98.6 ± 11.5		122.9 ± 17.6	
	2		98.0 ± 4.0		119.4 ± 13.0	
	3		99.2 ± 1.0		111.0 ± 11.9	
	4		99.5 ± 2.8		105.4 ± 4.9	
	5		94.2 ± 2.7		101.3 ± 2.0	
SeMNPV‡	1		70.8 ± 1.4 §		87.6 ± 7.3	
	2		74.0 ± 0.0 §		91.4 ± 8.6	
	3		69.1 ± 4.9		87.2 ± 6.6	
	4		72.1 ± 4.2		83.5 ± 4.0	
	5		71.1 ± 3.1		79.0 ± 8.6	

† polyhedra concentrations used for first to third instar larvae: 10^4 (1), $3x0^4$ (2), 10^5 (3), $3x10^5$ (4), 10^6 (5), fourth instar larvae 10^5 (1), $3x10^5$ (2), 10^6 (3), $3x10^6$ (4), 10^7 (5) and fifth instar larvae 10^6 (1), $3x10^6$ (2), 10^7 (3), $3x10^7$ (4), 10^8 (5) (polyhedra ml^{-1}).

‡ polyhedron concentrations for SeMNPV were ten times lower than for wild-type AcMNPV and AcMNPV recombinants

§ LT_{50} values based on few observations

The median lethal times (LT_{50}) for AcMNPV, SeMNPV, AcMNPV-Δ*pp34*, AcMNPV-Δ*p10* and AcMNPV-Δ*egt* are presented in Table 2.2. The LT_{50} decreased with dose and increased with instar. SeMNPV, with typical LT_{50} values of 72 and 84 hour, respectively, for the second and fourth instar at concentrations 4 and 5, had a higher speed of action than AcMNPV, with typical LT_{50} values of 88 and 92 hour for the same instar/concentration combinations ($P < 0.001$). AcMNPV-Δ*p10* had significantly higher LT_{50} values than wild-type AcMNPV with approximately 97 and 103 hour for second and fourth instar larvae at concentrations 4 and 5 ($P < 0.001$). The speed of action of AcMNPV-Δ*pp34* did not differ from that of wild-type AcMNPV ($P = 0.303$). Likewise, AcMNPV-Δ*egt*, which was expected to have an increased speed of action on the basis of previous observations (O'Reilly and Miller, 1991; Flipsen *et al.*, 1995), had a similar speed of action as AcMNPV in our assay system ($P = 0.776$).

The speed of action of wild-type AcMNPV and AcMNPV-Δ*egt* was investigated in a further experiment. The LT_{50} values and the instar at death of *S. exigua* larvae infected at second instar with 10^6 and 10^9 polyhedra ml^{-1} suspensions are presented in Table 2.3.

Table 2.3. *Summary of LT_{50} data and molting behavior obtained for AcMNPV wild-type and AcMNPV-Δegt using the droplet-feeding method.*

Instar	Virus	conc.	LT_{50}	instar at death
2	AcMNPV	10^6	95.3^a ± 1.5	L3
2	AcMNPV-Δ*egt*	10^6	93.7^a ± 1.2	L3
2	AcMNPV	10^9	69.0^b ± 1.1	L2
2	AcMNPV-Δ*egt*	10^9	69.9^b ± 0.8	L3

Superscript letters indicate significant differences compared to wild-type AcMNPV for each instar.

The LT_{50} values of wild-type AcMNPV and AcMNPV-Δ*egt* decreased with dose, but did not significantly differ. Larvae infected with the low dose of wild-type AcMNPV molted to the third instar before they died, whereas larvae infected with the high dose died as second instars. Larvae treated with both the low and high dose AcMNPV-Δ*egt* all died as third instar. A small fraction of larvae that were infected with the high dose of AcMNPV-Δ*egt* died during their molt into the third instar (0.11, 0.17 and 0.24 per repetition). These individuals died 40 to 50 hours after infection, whereas those, which survived the molt, died 60-90 hours after infection. Peak mortality of larvae infected with the high dose AcMNPV-Δ*egt* occurred somewhat later than in wild-type AcMNPV, resulting in a slightly higher LT_{50} value of

AcMNPV-Δ*egt* despite of the early death of larvae which died during their molt. Third instar larvae infected with the high dose AcMNPV-Δ*egt* often had difficulty to shed their cuticle, which resulted in an abnormal development.

2.4 Discussion

In this study we compared the biological activity of wild-type AcMNPV and three AcMNPV deletion mutants in absence of a marker gene such as β-galactosidase. In earlier studies deletion mutants often contained such a genetic marker that may give rise to erratic results (Bonning *et al.*, 1992; Wood *et al.*, 1993). For an appropriate evaluation of the effect of a gene deletion on biological activity, a selection of AcMNPV deletion mutants was constructed with a single deletion only, and compared with wild-type AcMNPV. In this way the effects of a monofactorial difference can be determined.

Information on the volumes ingested is critical for an accurate calculation of the actual virus dose. The *S. exigua* larvae ingested volumes were 30 to 70 % lower than the values found by Smits and Vlak (1988a) but corresponded well with the findings of Caballero *et al.* (1992) for second instar *S. exigua*. This suggests that the ingested volumes vary and may need to be determined for each experiment. The distribution of the ingested volume is skewed, with a lower median as mean value. The choice of either median or mean ingested volume to calculate the ingested dose affects the LD_{50} value. This value becomes higher when the mean ingested volume is used than when the median volume is used. There are no fundamental reasons why one choice would be better than the other, as long as it is consistently used. We used the median ingested volume.

The LD_{50} values of the wild-type AcMNPV and SeMNPV in this study are in the same range as reported by Smits and Vlak (1988 a,b,c) and Caballero *et al.* (1992). Smits *et al.* (1988a) conducted the experiments with the same source of SeMNPV and the same insect population, but ten years earlier. Apparently, the susceptibility of the laboratory insects and the intrinsic biological activity of the virus remained unchanged during this period.

AcMNPV-Δ*p10* and wild-type AcMNPV did not differ in infectivity for second and fourth instar *S. exigua* larvae. Previously, Vlak *et al.* (1988) found a slightly lower LD_{50} of an AcMNPV-Δ*p10* expressing β-galactosidase (4.2) as compared to wild-type AcMNPV (11.3) for second instar *S. exigua* in a similar bioassay. These LD_{50} values were based on a single bioassay and the difference in infectivity between AcMNPV-Δ*p10* and wild-type AcMNPV may fall within the power of discernment. The similarity of dose-mortality relationships of wild-type AcMNPV and the polyhedral membrane deletion mutant AcMNPV-Δ*pp34* found

for all five larval stadia of *S. exigua* is not in accordance with observations of Ignoffo *et al.* (1995). In the latter a six times lower LD_{50} value for an AcMNPV-Δ*pp34* mutant was reported in first instar of *T. ni*, but this mutant expressed β-galactosidase. In contrast to the present experiments, first instar larvae of *T. ni* were reared on contaminated diet and thus were continuously exposed to the virus. Alternatively, the difference in results may be attributed to a difference in species response and not to the presence of β-galactosidase. Bonning *et al.* (1992) found that recombinants expressing β-galactosidase had higher rather than lower LD_{50} values than wild-type AcMNPV. It seems that β-galactosidase thus has an erratic behavior. As expected (O'Reilly and Miller, 1991), LD_{50} values of AcMNPV-Δ*egt* and wild-type AcMNPV were not significantly different in our experiments.

The infectivity of a virus depends on its ability to release virions from the polyhedra, overcome resistances in the larval midgut (e.g. the peritrophic membrane), to attach to susceptible cells, and to penetrate and replicate in these cells. Our findings indicate that polyhedra formation in absence of fibrillar structures (Δ*p10*), the polyhedral membrane (Δ*pp34*) or ecdysteroid UDP-glucosyltransferase (Δ*egt*) in infected cells does not substantially alter the infectivity of AcMNPV. In the case of AcMNPV-Δ*pp34*, which has an increased capacity to release ODVs in a weak alkaline environment (Zuidema *et al.*, 1989), the data suggest that the efficiency of polyhedron release in the larval midgut is not a limiting factor in the infection process.

The LT_{50} values for AcMNPV and SeMNPV in *S. exigua* were in general somewhat lower than those reported by Vlak *et al.* (1988) and Smits and Vlak (1988a), but resemble values of Caballero *et al.* (1992) in that SeMNPV kills about a day faster than AcMNPV. The deletion of the *egt* gene did not lead to an increased speed of action of AcMNPV in *S. exigua* (Table 2.2). This is in contrast to findings of O'Reilly and Miller (1991) who found that deletion of the *egt* gene resulted in an increased speed of action in *S. frugiperda.* In our study clear differences in molting behavior between larvae infected with wild-type AcMNPV and AcMNPV-Δ*egt* were observed (Table 2.2). Larvae inoculated with 10^6 polyhedra ml^{-1} wild-type AcMNPV molted into the third larval stage before they died whereas larvae exposed to 10^9 polyhedra ml^{-1} died in the second instar. When larvae were infected with a high dose wild-type AcMNPV ecdysteroids are apparently efficiently conjugated, leading to a developmental arrest as described by O'Reilly and Miller (1989). This arrest does not take place when larvae were infected with lower doses, suggesting that the EGT titer in larvae at the molt is not high enough to conjugate all larval ecdysteroids. In this case, the number of initially infected cells may not be sufficient to produce enough EGT. Larvae infected with

AcMNPV-Δ*egt* always molted into the third larval stage except for a small fraction of the larvae inoculated with 10^9 polyhedra ml^{-1} that died during their molt. Nevertheless, this fraction was too small to affect the average LT_{50}.

Flipsen *et al.* (1995) demonstrated a 30-h reduction in LT_{50} of an AcMNPV-Δ*egt* expressing β-galactosidase as compared to AcMNPV/LacZ with second instar *S. exigua* inoculated with a 10^7 polyhedra ml^{-1} suspension. Although no differences in molting patterns between mock-infected, wild-type AcMNPV and AcMNPV-Δ*egt* infected larvae were then observed, the *egt* deletion mutant killed considerably faster. An early degeneration of Malpighian tubules in the AcMNPV-Δ*egt*-infected larvae was held responsible for the reduction in LT_{50}. In our study the ingestion of high doses AcMNPV-Δ*egt* or wild-type AcMNPV polyhedra induced, as expected (O'Reilly and Miller, 1991), a molt or a development arrest in infected larvae, respectively. However, in our case a difference in speed of action was not observed. This may be due to the absence of β-galactosidase in the present construct AcMNPV-Δ*egt*. To test this possibility a comparative bioassay using the AcMNPV-Δ*egt* mutant expressing β-galactosidase (Flipsen *et al.*, 1995) and the AcMNPV-Δ*egt* mutant used in this paper showed similar LT_{50} values. This suggests that changes in the insect population over time or the synchrony of larval development might explain the discrepancy between the present and previous results rather than the absence/presence of β-galactosidase.

The occurrence of the molt upon infection with our AcMNPV-Δ*egt* recombinant is clearly the result of the absence of the *egt*-gene. Our results thus reveal that the improved insecticidal properties of AcMNPV-Δ*egt* against *S. exigua* larvae are at least ambiguous and suggest that the suitability of AcMNPV-Δ*egt* as improved crop protection agents needs to be evaluated case-by-case (Slavicek *et al.*, 1999). The LT_{50} values for AcMNPV-Δ*p10* were higher than for AcMNPV wild-type. Possibly, the absence of the fibrillar structures in AcMNPV-Δ*p10*-infected cells (van Oers and Vlak, 1997) impairs the disintegration of the tissues and hence the spread of the virus in the whole organism.

Extensive bioassays revealed that AcMNPV and SeMNPV had clear differences in infectivity and speed of action, but that deletion of single *pp34* and *egt* genes of AcMNPV had hardly any effect on these viral characteristics. For significant improvement of the speed of action of AcMNPV and other baculoviruses the insertion of entomotoxic genes such as AaIT holds more promise (Stewart *et al.*, 1991; McCutchen *et al.*, 1991; Cory *et al.*, 1994; Black *et al.*, 1997).

3

The polyhedral membrane does not protect polyhedra of AcMNPV against inactivation on greenhouse chrysanthemum

Polyhedral inactivation of wild-type Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and an AcMNPV mutant lacking the gene for the polyhedral membrane protein (AcMNPV-Δpp34) was studied on greenhouse chrysanthemum. It was anticipated that polyhedra without a polyhedral membrane might be more susceptible to inactivation on plants. The density of infectious polyhedra of both viruses on the leaf surface decreased in time. It appeared that there were two distinct fractions of polyhedra with clear differences in persistence. One fraction of polyhedra is not inactivated at all, whereas the other fraction is inactivated in an exponential fashion. Relative inactivation rates of the inactivated polyhedra fraction for wild-type AcMNPV and AcMNPV-Δpp34 were 0.16 and 0.13 day^{-1}*, respectively, which is not significantly different. After 28 days on leaves in a greenhouse, both viruses still showed residual infectivity. The fraction of residual infectious polyhedra were not significantly different and amounted to approximately 20 % of the original density for both wild-type AcMNPV and AcMNPV-Δpp34. Therefore, the polyhedral membrane does not protect polyhedra against inactivation on greenhouse chrysanthemum.*

This chapter has been published as:
Bianchi, F.J.A., Joosten, N.N., Gutiérrez, S., Reijnen, T.M., van der Werf, W., and Vlak, J.M. 2000. *Biocontr. Science Technol.* 9, 523-527.

3.1 Introduction

Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) is the baculovirus type species (Murphy *et al.*, 1995). The viral genome has been sequenced and several genes have been functionally characterized (Ayres *et al.*, 1994; Miller, 1997). Deletion mutagenesis proved to be a useful strategy to study the function of specific genes in baculovirus replication, pathogenesis and environmental behavior (Miller, 1997).

Zuidema *et al.* (1989) and Ignoffo *et al.* (1995) reported that an AcMNPV mutant with an insertion in the *pp34* gene (AcMNPV-Δ*pp34*) had altered polyhedral characteristics. When the *pp34* gene is inactivated, polyhedra lack the polyhedral membrane or calyx, which makes such polyhedra more sensitive to weak alkali (Zuidema *et al.*, 1989). Ignoffo *et al.* (1995) reported for *Trichoplusia ni* larvae that polyhedra of an AcMNPV-Δ*pp34* insertion mutant had a six times higher infectivity than wild-type AcMNPV. In contrast, we found that an AcMNPV-Δ*pp34* deletion mutant that lacked the complete *pp34* gene had the same infectivity as wild-type AcMNPV for *Spodoptera exigua* larvae (chapter 2).

Wild-type AcMNPV and AcMNPV-Δ*pp34* polyhedra had similar inactivation curves when exposed to simulated sunlight-ultraviolet (UV) radiation (Ignoffo *et al.*, 1995). Although the polyhedral membrane does not seem to be involved in the inactivation of polyhedra by this type of radiation, its presence may still be important for the persistence of polyhedra on plants and in the environment. Besides UV radiation, polyhedra on plants are exposed to other potential inactivation factors, such as plant exudates and dew (Elleman and Entwistle, 1985; Tuan *et al.*, 1989). AcMNPV-Δ*pp34* polyhedra might be more susceptible to inactivation by these factors than wild-type AcMNPV because of their increased sensitivity to weak alkali.

Greenhouse chrysanthemum is an economically important ornamental in the Netherlands that is severely infested by beet armyworm, *S. exigua*. Baculoviruses are often used as biological control agents to control this pest insect. In addition, growth characteristics of greenhouse chrysanthemum, *S. exigua* behavior as well as baculovirus epidemiology are well studied, allowing computer modeling of this system. In this study we quantified the inactivation rates of wild-type AcMNPV and AcMNPV-Δ*pp34* polyhedra on chrysanthemum in a greenhouse.

3.2 Material and methods

Insects

Laboratory colonies of *S. exigua* were maintained as described by Smits *et al.* (1986). Larvae were reared from sterilized eggs and incubated at 27°C, 70-80% relative humidity and a 16 h photoperiod.

Viruses

In the AcMNPV-*Δpp34* deletion mutant the *pp34* gene was deleted from the ATG to the TAA (Figure 3.1). The E2 clone of AcMNPV was used as control virus (Summers and Smith, 1987). The viruses were propagated in fourth instar *S. exigua* larvae via surface contamination of semi-synthetic diet (Smits and Vlak, 1988a). Virus was purified by grinding deceased larvae, filtering through a double layer of cheese-cloth and two centrifugation steps. The polyhedra were resuspended and stored in a glycerol/water (1:1) solution in the dark at 4°C. Suspensions of wild-type AcMNPV and AcMNPV-*Δpp34* (1.67×10^6 polyhedra ml^{-1}) were prepared in a 1 % PBS solution and the polyhedra concentrations were measured with a haemocytometer. These polyhedra suspensions were stored in the dark at 4° C during the experiment.

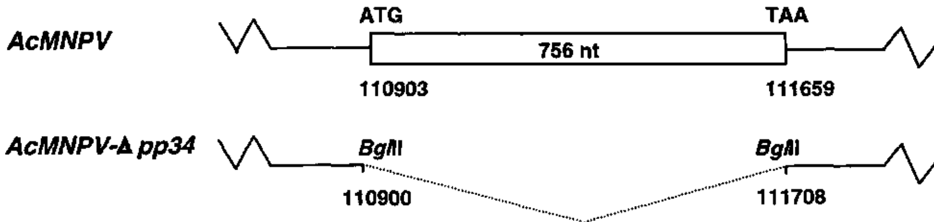

Figure 3.1 *Schematic representation of wild-type AcMNPV and the AcMNPV-Δpp34 deletion mutant. Nucleotide positions are according to Ayres et al. (1994).*

Bioassays

The inactivation of wild-type AcMNPV and AcMNPV-*Δpp34* polyhedra on plants was studied under greenhouse conditions. At weekly intervals, 3 μl droplets of polyhedra suspensions were applied to the upper side of chrysanthemum leaves. Twenty-eight days after the first set of droplets had been applied, all treated leaves were collected, and discs prepared

using a 10 mm diameter cork bore. Care was taken to include all applied polyhedra on the leaf discs by marking the sites with a Tipp ex (Citius) ring prior to virus application.

The infectivity of the exposed polyhedra on the leaf discs was determined in a leaf disc bioassay. Leaf discs were placed individually in wells of a 12-well tissue culture plate containing 1 ml 1.5 % agar. One third-instar *S. exigua* larva was added per well. The wells were covered with two layers of paper tissue and a lid. Larvae that ingested the entire disc within 24 h were transferred to individual wells of a 12-well tissue culture plate with fresh artificial diet. Larvae were reared at 27°C and mortality was recorded after all larvae had either pupated or died. To construct dose-mortality reference lines, additional leaf disc bioassays were conducted with five known doses of each virus and using third instar *S. exigua* larvae. For each leaf disc bioassay 36 larvae were used. The experiment was repeated three times.

Statistical analysis

Logit regression lines were fitted to dose-mortality data with the computer program POLO (Russell *et al.*, 1977) and these lines were used as dose-mortality reference lines. Mortality of larvae that ingested leaf discs with exposed polyhedra were converted to number of infectious polyhedra using the dose-mortality reference lines for each virus. Polyhedra inactivation was described by a model that distinguished two fractions of polyhedra: a residual fraction of infectious polyhedra that is not inactivated and a fraction of infectious polyhedra that is inactivated in an exponential fashion. The parameters of this model are the relative inactivation rate of the inactivated polyhedra fraction and the number of polyhedra in the residual fraction. The number of polyhedra in the inactivated fraction is calculated as the number of infectious polyhedra measured in the unexposed sample minus the number of polyhedra in the residual fraction. Inactivation curves were fitted to polyhedra inactivation data. Fitted parameters for wild-type AcMNPV and AcMNPV-Δ*pp34* were compared with the Wilcoxon rank-sum test using Genstat (Payne *et al.*, 1993).

3.3 Results and discussion

The inactivation of polyhedra of wild-type AcMNPV and AcMNPV-Δ*pp34* on chrysanthemum was studied under greenhouse conditions. The decrease in the number of infectious polyhedra was high during the first 7 days after which it gradually decreased. In Table 3.1, fitted relative inactivation rates and residual number of infectious polyhedra are

given. The relative inactivation rates of wild-type AcMNPV and AcMNPV-Δ*pp34* were not significantly different ($P < 0.20$) with mean relative inactivation rates of 0.16 and 0.13 polyhedra day^{-1}, respectively. After 28 days exposure a considerable fraction of infectious polyhedra (20 %) was still present on the leaves. The mean residual number of infectious polyhedra of wild-type AcMNPV and AcMNPV-Δ*pp34* were 860 and 810 out of the original 4000. These residual number of infectious polyhedra were also not significantly different ($P < 0.35$). In Figure 3.2, measured inactivation curves of wild-type AcMNPV and AcMNPV-Δ*pp34* polyhedra and inactivation curves with mean relative inactivation rates and residual

Table 3.1 *Fitted relative inactivation rates of polyhedra (rir, day^{-1}), residual number of infectious polyhedra (rip) and goodness of fit of inactivation curves for wild-type AcMNPV and AcMNPV-Δpp34 exposed to greenhouse chrysanthemum for 28 days.*

	repetition	rir (d^{-1})	rip (-)	R^2
AcMNPV	1	0.1873	744	0.993
	2	0.1151	974	0.994
	3	0.1762	865	0.960
AcMNPV-Δ*pp34*	1	0.0964	314	0.995
	2	0.1346	1047	0.871
	3	0.1528	1061	0.891

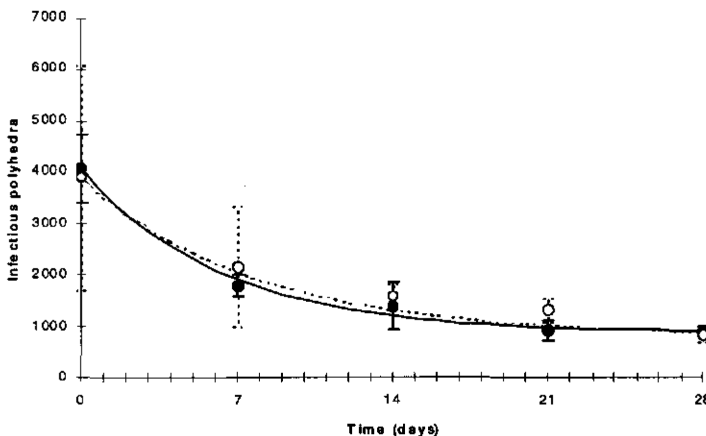

Figure 3.2 *Measured inactivation curves of wild-type AcMNPV (●) and AcMNPV-Δpp34 polyhedra (○) on greenhouse chrysanthemum. Exponential inactivation curves with mean relative inactivation rates and residual numbers of infectious polyhedra for wild-type AcMNPV and AcMNPV-Δpp34 are represented by solid and dashed lines, respectively.*

number of infectious polyhedra (Table 3.1) are presented. Polyhedra inactivation curves in our study had similar shapes to those reported for *Cydia pomonella* GV and *Helicoverpa/Heliothis* NPV (Jaques *et al.*, 1987; Ignoffo *et al.*, 1997). However, the half-life of the original-activity remaining of AcMNPV polyhedra exposed to greenhouse conditions in our study (1-2 weeks) were much higher than reported values of 4.9 hour for *Helicoverpa/Heliothis* NPV polyhedra that were continuous exposed to natural sunlight (Ignoffo *et al.*, 1997). The lower polyhedra inactivation rates in our study are almost certainly explained by the filtering-out of UV radiation by the glass of the greenhouse.

After 28 days exposure, approximately 20% of the initial number of polyhedra was still infectious. Jones *et al.* (1993) and Brassel and Benz (1979) also found a residual fraction of infectious polyhedra of *S. littoralis* NPV and *C. pomonella* GV when exposed to UV radiation. The presence of this residual fraction may be explained by the presence of different viral genotypes that are inactivated at different rates (Brassel and Benz, 1979; Shapiro and Bell, 1984).

Our finding that polyhedra of wild-type AcMNPV and AcMNPV-Δ*pp34* had similar inactivation curves showed that under the chosen conditions the membrane surrounding the polyhedra does not contribute to the protection of AcMNPV polyhedra. In an earlier study it was shown that wild-type AcMNPV and AcMNPV-Δ*pp34* had a similar infectivity and speed of action (chapter 2). This suggests that deletion of the polyhedral membrane has little or no effect on the properties of AcMNPV polyhedra outside the host (similar infectivity, speed of action and polyhedra persistence). It is possible, however, that the polyhedral membrane plays a role in the long term persistence in the environment, for example in the soil. It has been observed (D. Zuidema, personal communication) that AcMNPV-Δ*pp34* polyhedra quickly aggregate upon freezing, underscoring the difference in biophysical properties of polyhedra with and without a polyhedral membrane. This may affect the spatial distribution of these polyhedra as compared to wild-type polyhedra in the environment. The function of the polyhedral membrane in field persistence is being further investigated.

4

The influence of greenhouse chrysanthemum on the interaction between the beet armyworm, *Spodoptera exigua*, and the baculovirus SeMNPV: parameter quantification for a process-based simulation model

*During the process of building a process-based simulation model for the epidemiology of SeMNPV in populations of Spodoptera exigua in greenhouse chrysanthemums, we discovered that the effect of host plant had been underrated. "Missing links" included **(i)** the "natural" background mortality of larvae of S. exigua in practical cropping conditions; **(ii)** the developmental rate of larvae of S. exigua on plant substrate in a glasshouse as compared to artificial medium in the laboratory; **(iii)** the validity of the results of dose-mortality and time-mortality bioassays conducted on artificial medium as compared to natural plant substrate; **(iv)** the distribution of inoculum released from deceased caterpillars over chrysanthemum leaves; and **(v)** the leaf visit rate of healthy caterpillars (as it affects horizontal transmission). Experiments were done to quantify these processes. Developmental rates of S. exigua larvae on greenhouse chrysanthemum were 36% lower than on artificial diet. The fraction survival during the first second and fourth instar S. exigua larvae in greenhouse chrysanthemum was 0.60, 0.80, 0.88 and 0.95, respectively. Forty percent of the first instar larvae reached the fifth larval stage. Second instar S. exigua larvae reared on chrysanthemum were significantly more susceptible to S. exigua multicapsid nucleopolyhedrovirus (SeMNPV) than larvae reared on artificial diet. The food source had no effect on the time to kill S. exigua larvae. Cadavers of second, third and fourth instar S. exigua larvae contaminated on average 1.4, 2.5 and 3.3 chrysanthemum leaves. Second to fourth instar S. exigua larvae visited 2 to 3 leaves per day and spent 15 to 55% of the time on the under side of leaves. The above information is of critical importance for a trustworthy simulation of the epidemiology of SeMNPV in chrysanthemums.*

This chapter has been submitted as:
Bianchi, F.J.J.A., Joosten, N.N., Vlak, J.M. and van der Werf, W.

4.1 Introduction

Beet armyworm, *Spodoptera exigua*, is a pest insect that infests a wide range of agricultural crops all over the world. *S. exigua* causes serious damage in greenhouse crops in The Netherlands, such as chrysanthemum and gerbera. In 1999 the sales of chrysanthemum in The Netherlands was 600 million Dutch guilders, which is approximately 300 million US $ (de Veld, 2000). Chrysanthemum has a low threshold for cosmetic damage and therefore even small feeding marks can cause economic loss. The control of *S. exigua* is problematic because the insect has become resistant to most insecticides (Brewer and Tumble, 1989). The multicapsid nucleopolyhedrovirus of *S. exigua* (SeMNPV) is successfully used to control beet armyworm (Kolodny-Hirsch *et al.*, 1997). The virus is commercially available in the USA, Thailand, Spain and The Netherlands (Moscardi, 1999).

In most laboratory studies on virus-insect interactions insects are reared and tested on artificial diet for reasons of convenience and standardization. However, the validity of the results for the field situation is generally not ascertained. Care is needed in the extrapolation of laboratory based data to the greenhouse, because host plants may affect larval susceptibility to baculoviruses (Richter *et al.*, 1987; Keating *et al.*, 1988; Forschler *et al.*, 1992; Santiago-Alvarez and Ortiz-Garcia, 1992; Hoover *et al.*, 1998a,b), larval developmental rates (Broadway and Duffey, 1986; Bloem and Duffey, 1990; Felton *et al.*, 1992) and larval foraging behavior (Schmidt *et al.*, 1987). The effect of food source on SeMNPV infectivity in *S. exigua* has not been investigated before.

We have constructed a comprehensive, process-based simulation model for the biological control of *S. exigua* with SeMNPV in greenhouse chrysanthemum (chapter 7). During the development of this model we discovered that *S. exigua* foraging behavior, background mortality (attrition) in chrysanthemum, as well as the spread of polyhedra from SeMNPV-killed cadavers have not been determined. Furthermore, when model simulations were compared with experimental data, consistent discrepancies were observed between developmental rates and dose-mortality relationships that were simulated and those that were observed in the greenhouse. The quantification of these effects of the host plant chrysanthemum on the interactions between *S. exigua* and SeMNPV is essential for a valid simulation of the biological control of *S. exigua* with SeMNPV.

This paper describes (**i**) larval attrition rates and (**ii**) larval developmental rates on chrysanthemum plants, (**iii**) dose- and (**iv**) time-mortality relationships of SeMNPV in *S. exigua* larvae reared on artificial diet or chrysanthemum, (**v**) the spread of polyhedra from

SeMNPV-killed cadavers, and (**vi**) *S. exigua* foraging behavior on chrysanthemum plants. The aim of these experiments is to quantify each of these processes in such a way that they are representative for the situation in greenhouses and can be implemented in the above-mentioned simulation model for biological control.

4.2 Material and methods

Insects, virus and plant material

Laboratory colonies of *S. exigua* were maintained as described by Smits *et al.* (1986). In short, larvae were reared from surface sterilized eggs at 27°C and a 16 h photoperiod. The larvae used for experiments were either reared on artificial diet or on fresh chrysanthemum leaves, until the appropriate instar was reached. The SeMNPV US1 isolate (Hunter and Hall, 1968) was used and was propagated as described in chapter 3. Chrysanthemum plants (cultivar Tiger) used for laboratory experiments were grown in pots with day/night temperatures of 16/18°C and a 19/5 h light/dark period in a greenhouse, Wageningen, The Netherlands. Three to six week old plants were used for the experiments.

S. exigua attrition rates in greenhouse chrysanthemum

The experiment was conducted in a greenhouse (10 x 30 m) from April 19 to 29, 1999, in Wageningen, The Netherlands. Twenty-four plots with insecticide-free chrysanthemum plants were prepared. Each plot had an area of 1 m^2 and consisted of 64 plants that were planted in a square pattern at a mutual distance of 12.5 cm. The plots were surrounded by 40 cm-high transparent plastic that was dug into the soil and covered with tanglefoot at the top to prevent larvae from escaping the plot. Minimum night temperatures in the greenhouse were 18°C and the daytime ventilation set-point was 29°C. Hourly temperatures were recorded using a datalogger (Depex datataker 600) with seven thermocouples that were located in the canopy. When the plants were 3 weeks old, 100 first instar *S. exigua* larvae were released in each plot by brushing them gently onto the plants. At 2, 4, 6, 8, 9, and 10 days after the introduction of the larvae, four plots were destructively sampled and the number of recovered larvae determined. The number of recovered larvae reflects the attrition dynamics of *S. exigua* populations in greenhouse chrysanthemum.

S. exigua development rates in greenhouse chrysanthemum

Developmental rates of *S. exigua* in greenhouse chrysanthemum were derived from the greenhouse experiment described in the previous section. The developmental stage of the recovered larvae 2, 4, 6, 8, 9, and 10 days after the introduction of 100 first instar larvae were recorded. The developmental stage of the recovered larvae from the plots was compared with developmental times of *S. exigua* reared on Lima bean medium (Fye and McAda, 1972). Expected larval development during the greenhouse experiment was simulated with the BACSIM model (chapter 7). The model used the larval developmental rates of *S. exigua* on artificial diet at 20, 25, 30 and 33°C reported by Fye and McAda (1972), as well as the hourly measured greenhouse temperatures as input. *S. exigua* developmental rates were interpolated linearly between 20, 25, 30 and 33°C. A scaling factor was introduced in the model to calibrate the development rate based on the data of Fye and McAda (1972) to the observations in the greenhouse, such that simulated larval development corresponded with the actual measurements. The scaling factor represents the effect of the host plant on the development rate.

Effect of diet on susceptibility of S. exigua larvae for SeMNPV

Second and third instar *S. exigua* larvae that were reared on artificial diet or chrysanthemum leaves were used for a leaf-disc bioassay with 5 doses of SeMNPV as described in chapter 3. For second instar larvae the doses were 0 (control), 3, 9, 30, 90 and 300 polyhedra per leaf disc, whereas for third instar larvae 0, 9, 30, 90, 300, 900 polyhedra were used. Larvae that ingested the entire leaf disc were further reared on artificial diet. Mortality was recorded after all larvae either had pupated or died by SeMNPV infection. Each bioassay included 36 larvae and the experiments were replicated three times in total. Larval populations reared on artificial diet and chrysanthemum were synchronized by using three and five days old larvae since egg hatch, respectively. When both treatment groups had reached the second larval stage, they were simultaneously used in the bioassay. For third instar larvae the bioassay with the larvae reared on chrysanthemum was conducted two days later than that of the artificial diet treatment. The objective of using differently aged larvae for the two treatment groups in the bioassay was to have comparable stage and weight in the two treatment groups. Ten batches of 10 third instar larvae of both treatment groups were weighed to verify the efficacy of the synchronization procedure.

Effect of diet on speed of action of SeMNPV in S. exigua larvae

Third instar *S. exigua* larvae reared on artificial diet or chrysanthemum leaves were allowed to ingest a 1 mm^3 diet plug or a 12.6 mm^2 leaf disc, respectively, within 4 hours. To obtain similar mortality levels for both diet treatments, 3000 polyhedra SeMNPV were applied per diet plug and 300 polyhedra SeMNPV per leaf disc. A ten times higher SeMNPV dose was administered to the diet plugs to compensate for the lower larval feeding rate on artificial diet than on plant material. A polyhedra-free control was included for both diet treatments. After 4 hours all larvae were transferred to clean 12-well tissue culture plates and reared further on the diet type on which they had been reared before the bioassay. Each diet treatment included 36 larvae and the experiment was replicated three times in total. Larvae were checked twice a day for mortality until all larvae had either died by SeMNPV infection or pupated. The synchronization procedure in this experiment was identical to the synchronization procedure for third instar larvae described in the previous section.

Foliage contamination by SeMNPV-killed S. exigua larvae

S. exigua larvae were reared on chrysanthemum leaves until they reached the second (L2), third (L3) or fourth instar (L4). A series of experiments was conducted with *S. exigua* larvae that were infected with 300 (L2), 900 (L3) or 3000 (L4) polyhedra of SeMNPV, using the leaf-disc bioassay (chapter 3). These larvae were placed individually on the top of chrysanthemum plants with 8 to 12 leaves in a climate room at 27°C and a 16 h photoperiod. For second, third and fourth instar larvae, five, eight and seven plants were included, respectively. The plants were placed in trays at a mutual distance of 20 cm and water contact with the leaves was avoided. There was no leaf contact between plants and larvae remained at the plant on which they were released. Two mock-infected larvae (which had ingested leaf discs with no polyhedra) on chrysanthemum plants were included as a control. After four days the SeMNPV infected larvae had died and four samples of 64 mm^2 were taken with cork borers from all leaves of the plants. Cork borers were disinfected after each sampling by boiling them for 10 minutes in 0.2 M NaOH. The four leaf discs covered approximately 25% of the area of a leaf. The leaf discs were tested for the presence of SeMNPV polyhedra by a leaf-disc bioassay using third instar *S. exigua* larvae.

S. exigua foraging behavior

Ten single neonate *S. exigua* larvae were placed on the top of chrysanthemum plants in a climate room with a 16 h photoperiod at 27°C. The instar and location on the plant of second

to fifth instar larvae were recorded nine times per day with hourly intervals. Larvae were observed during periods of four days. The following foraging sites were distinguished: adaxial side of leaf, abaxial side of leaf, leaf edge, petiole, internode, axil leaf and bud. The location of feeding marks were recorded daily. Larvae that died during the observation period were replaced with larvae of the same instar from the chrysanthemum culture. The experiment was repeated three times in total.

Statistical analysis

The dose-mortality data were analyzed by probit analysis, using the computer program POLO (Russell *et al.,* 1977). Survival time (ST_{50}) values of time-mortality data were determined using the ViStat program (version 2.1; Boyce Thompson Institute, Cornell University, Ithaca, New York). LogLD_{50} values and ST_{50} values were analyzed by regression analysis and t-tests of pairwise differences between treatments using Genstat (Payne *et al.*, 1993). The number of contaminated leaf segments and leaves that contained at least one contaminated leaf segment by SeMNPV-infected cadavers were compared with the Wilcoxon rank-sum test using Genstat.

4.3 Results and discussion

S. exigua attrition rates in greenhouse chrysanthemum

The survival of groups of 100 first instar *S. exigua* larvae during 10 days in greenhouse chrysanthemum is presented in Figure 4.1. The attrition rates were maximal during the first instar and gradually decreased in the following instars. The fractions surviving larvae during the first through fourth larval stages were 0.60, 0.80, 0.88 and 0.95, respectively. Forty percent of the first instar larvae reached the fifth larval stage. Attrition rates of *S. exigua* larvae are generally determined under controlled conditions (Lee *et al.*, 1991; Ali and Gaylor, 1992). However, these attrition rates are not representative for the situation in a greenhouse crop where environmental conditions and food quality fluctuate, and predators and pathogens may be present. Observed larval survival in this study corresponds with an other study where 43% of the released first instar larvae reached the fifth stage in a chrysanthemum crop (chapter 5). Survival of *S. exigua* larvae in greenhouse chrysanthemum is clearly higher than in cotton in California, USA, where only 1.4% of the first instar larvae reached the pupal stage (Hogg and Gutierrez, 1980). This difference may well be due to a higher natural enemy impact and a harsher abiotic environment in the open field.

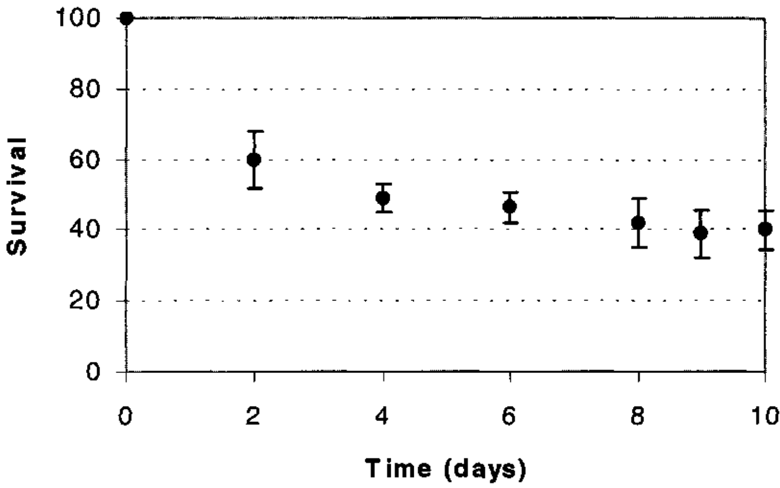

Figure 4.1 *Survival of S. exigua larvae at 0, 2, 4, 6, 8, 9 and 10 days after the introduction of 100 first instar larvae in greenhouse chrysanthemum plots. Error bars represent standard error of the mean.*

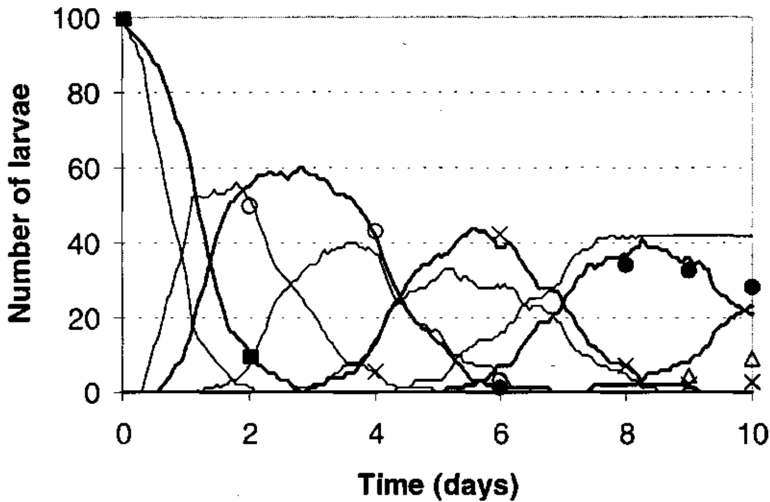

Figure 4.2 *Observed development of first (■), second (○), third (×), fourth (•) and fifth instar (Δ) S. exigua larvae in greenhouse chrysanthemum. Simulations of S. exigua development with scaling factors of 1 and 0.64 for the conversion of larval developmental rates from artificial diet to chrysanthemum are indicated with thin and thick lines, respectively.*

S. exigua developmental rates in greenhouse chrysanthemum

Developmental rates of *S. exigua* larvae in greenhouse chrysanthemum were determined and appeared clearly lower than developmental rates reported on Lima bean medium (Fye and McAda, 1972). Simulated *S. exigua* development based on larval developmental rates reported by Fye and McAda (1972) corresponded with the development of *S. exigua* larvae in greenhouse chrysanthemum when a conversion factor for the larval developmental rate from artificial diet to chrysanthemum of 0.64 was included (Figure 4.2). Ali and Gaylor (1992) also reported reduced developmental rates of *S. exigua* larvae reared on pigweed and cotton as compared to artificial diet. *S. exigua* developmental rates are highly correlated with the nutritional value of the food source (Broadway and Duffey, 1986; Bloem and Duffey, 1990; Felton *et al.*, 1992) and this may explain the lower developmental rate on chrysanthemum as compared to medium.

Effect of diet on susceptibility of S. exigua larvae for SeMNPV

SeMNPV $LogLD_{50}$ values of second and third instar *S. exigua* larvae reared on chrysanthemum were consistently lower than those for larvae reared on artificial diet. This difference was significant for second instar larvae ($P < 0.05$; Table 4.1) but not for third instar larvae ($P = 0.12$). *S. exigua* populations reared on artificial diet and chrysanthemum were synchronized before they were subjected to bioassays to eliminate possible effects of differences in larval development and growth on different food sources. The appropriateness of the synchronization procedure was confirmed by similar weights of third instar larvae reared on both food sources: $3.2\ 10^{-3} \pm 0.5\ 10^{-3}$ and $3.3\ 10^{-3} \pm 0.8\ 10^{-3}$ g (mean and SD) for artificial diet and chrysanthemum, respectively.

In most studies on virus-plant interactions reduced larval susceptibilities to baculoviruses induced by host plants have been reported (Richter *et al.*, 1987; Keating *et al.*, 1988; Forschler *et al.*, 1992; Santiago-Alvarez and Ortiz-Garcia, 1992; Hoover *et al.*, 1998a). In several instances it has been found that plant chemicals reduce larval susceptibility to baculoviruses (Keating *et al.*, 1988; Hoover *et al.*, 1998b). In contrast, in our study we observed that the susceptibility of second instar *S. exigua* larvae reared on chrysanthemum was significantly higher as compared to larvae reared on artificial diet. Increased susceptibilities have previously been reported for *Heliothis zea* larvae infected with HzSNPV reared on tomato as compared to artificial diet (Forschler *et al.*, 1992; Hoover *et al.*, 1998a). The reason for this increased susceptibility is unknown. Our results strongly suggest that a case-by-case investigation is necessary.

Table 4.1 *LogLD_{50} (mean and SEM) and LD_{50} values of SeMNPV against second and third instar S. exigua larvae that were reared on artificial diet or chrysanthemum.*

instar	LogLD_{50}		LD_{50}	
	artificial diet	chrysanthemum	artificial diet	chrysanthemum
L2	3.84 ± 0.36^a	2.71 ± 0.20^b	46	15
L3	4.98 ± 0.85^a	4.45 ± 0.64^a	145	86

Superscript letters indicate significant differences between treatments for each instar ($P < 0.05$).

Table 4.2 *Number of contaminated leaf segments and leaves (mean and SEM) by SeMNPV-killed S. exigua cadavers that were infected as second, third, or fourth instar larvae.*

Instar	contaminated leaf segments	contaminated leaves
L2	2.2 ± 1.0^a	1.4 ± 0.6^a
L3	3.6 ± 1.4^a	2.5 ± 0.7^a
L4	5.1 ± 1.3^a	3.3 ± 0.7^a

Superscript letters indicate significant differences between instars ($P < 0.05$).

Table 4.3 *Time allocation and leaf visit rate (d^{-1}) of second, third, fourth and fifth instar S. exigua larvae on chrysanthemum.*

location	2nd instar	3rd instar	4th instar	5th instar
adaxial side of leaves	0.23	0.21	0.24	0.18
abaxial side of leaf	0.15	0.41	0.39	0.56
leaf edges	0.06	0.10	0.08	0.07
bud	0.32	0.14	0.05	0.06
petiole	0.07	0.05	0.10	0.07
axil	0.13	0.05	0.08	0.02
internode	0.04	0.04	0.06	0.04
leaf visit rate	2.0	2.0	2.9	1.5

Effect of diet on speed of action of SeMNPV in S. exigua larvae

ST_{50} values of third instar *S. exigua* larvae infected with SeMNPV and reared on artificial diet or chrysanthemum were 86.2 ± 1.2 h. and 88.7 ± 1.9 h. (mean and SEM), respectively, which was not a significant difference ($P < 0.05$). The SeMNPV doses caused mortality levels of 90% for both diet treatments. Mean weights of third instar larvae used for ST_{50} determination were $2.9\ 10^{-3} \pm 0.4\ 10^{-3}$ and $2.6\ 10^{-3} \pm 0.4\ 10^{-3}$ g (mean and SD) for artificial diet and chrysanthemum. Thus, in contrast to LD_{50} values, the type of diet on which the larvae were reared did not affect ST_{50} values. A longer survival time might have been anticipated, given the slower development of larvae on plants, but this was not observed. Apparently, the progression of the virus infection in the larvae is not associated with the growth rate of the larvae.

Foliage contamination by SeMNPV-killed S. exigua larvae

The mean number of contaminated chrysanthemum leaf segments (leaf discs) and leaves that contained at least one leaf segment contaminated by cadavers of second, third, or fourth instar *S. exigua* larvae are presented in Table 4.2. Cadavers of second, third and fourth instar *S. exigua* larvae contaminated an increasing number of leaf segments and leaves, but the differences were not significant ($P < 0.05$). To our knowledge the spread of polyhedra from virus-killed cadavers onto foliage has not been quantified before. SeMNPV-killed *S. exigua* cadavers contaminated in most cases more than one leaf, which indicates that the polyhedra are also spread onto other leaves than the one on which the cadaver died. Possibly, polyhedra from cadavers may have dripped onto other leaves lower in the canopy. Alternatively, polyhedra may have been spread by excrements of infected larvae (Vasconcelos, 1996). The release of polyhedra from cadavers appears to be very efficient, even in the case of small cadavers of second instar larvae.

S. exigua foraging behavior

The foraging behavior of *S. exigua* larvae on chrysanthemum was characterized by time allocation of larvae on various plant parts (Table 4.3). The mean leaf visit rate of *S. exigua* larvae increased from 2.0 d^{-1} for second and third instar larvae to 2.9 d^{-1} of fourth instar larvae. Fifth instar larvae were less mobile and consumed large parts of leaves without changing leaf. *S. exigua* larvae spent increasing time budgets on the abaxial side of leaves during their development, up to 55% for fifth instar larvae. Since the deposition of polyhedra on the abaxial side of chrysanthemum leaves is negligible in chrysanthemum cultivation

practice, this implies that the polyhedron intake of larvae feeding on the abaxial side of leaves is much lower than those that feed on other plant parts. The abaxial sides of leaves may function as a refuge for larvae and this may have serious consequences for the efficacy of baculovirus applications.

Conclusion

The host plant chrysanthemum affected many processes that are relevant for the epidemiology of SeMNPV in *S. exigua* populations. Therefore, virus-insect interactions generated in laboratory bioassays can not directly be extrapolated to field situations. For the extrapolation of *S. exigua* developmental rates and SeMNPV infectivity determined on artificial diet in a laboratory situation to chrysanthemum in a greenhouse situation conversion factors of 0.64 and 2 should be incorporated, respectively. The speed of action of SeMNPV in *S. exigua* larvae determined on artificial diet can directly be applied to populations *S. exigua* in chrysanthemum. The conversion factors and detailed information of *S. exigua* attrition rates, foraging behavior and spread of polyhedra from SeMNPV-killed cadavers fill a gap in our knowledge of the chrysanthemum-*S. exigua*-SeMNPV system. This information contributes to an improved understanding of this tri-trophic system.

5

Transmission dynamics of the multicapsid nucleopolyhedrovirus SeMNPV in *Spodoptera exigua* populations in greenhouse chrysanthemum

The multicapsid nucleopolyhedrovirus of Spodoptera exigua, SeMNPV, is naturally maintained in S. exigua populations by vertical transmission from females to eggs and by horizontal transmission from cadavers of caterpillars to other caterpillars. Laboratory and greenhouse experiments were conducted to quantify transmission rates and provide baseline data to assess the potential of this virus to maintain itself naturally in pest populations after application in biocontrol programs. As to vertical transmission, 18% of the first instar larvae originating from egg batches produced by infected moths contracted SeMNPV, while 34% of the egg batches gave rise to one or more infected first instar larvae. As only few large egg batches were virus free, seventy percent of all uninfected larvae hatched in egg batches that contained one or more infected larvae. Substantial horizontal transmission was observed under greenhouse conditions. Presence of 0, 1 or 10 infected first instar larvae in an egg batch of 100 first instar larvae resulted in survival percentages to the fifth instar of 41, 30 and 20%, respectively. However, the effect on crop injury was marginal. The observed high transmission rates favor the maintenance of SeMNPV in S. exigua populations in greenhouses.

This chapter has been accepted for publication as:
Bianchi, F.J.J.A., van Essen, P.H.A., van der Werf, W., and Smits, P.H. in *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society.*

5.1 Introduction

Baculoviruses are pathogens that cause epidemics in insect populations. They are used as biological agents to control pest insect populations such as beet armyworm (*Spodoptera exigua*), gypsy moth (*Lymantria dispar*) and tobacco budworm (*Heliothis virescens*) (Moscardi, 1999). Baculovirus populations are maintained in insect populations by transmission of the virus from infected to susceptible hosts. There are two major transmission routes: vertical and horizontal transmission. Vertical transmission is the direct transfer of virus from parents to their own offspring whereas horizontal transmission is the infection of susceptible individuals by ingestion of virus from the environment.

Vertical transmission has been studied in several virus-insect systems (Smits and Vlak, 1988a; Hamm and Young, 1974; Young, 1990; Fuxa and Richter, 1992). In these studies, vertical transmission was quantified by the proportion of eggs, laid by sublethally infected moths, that hatched into infected larvae. However, this characterization of vertical transmission is incomplete. It is epidemiologically significant to know how the infected individuals are distributed over egg batches (Anderson and May, 1981). Larvae that die of a vertically transmitted baculovirus infection spill newly produced virus on the foliage, hence new infections can occur when this is ingested by uninfected larvae. The number of encounters between vertically infected and uninfected hosts is greater when infected individuals are evenly distributed over all egg batches than when all infected individuals are deposited within a single egg batch. To understand baculovirus epidemics in insect populations, quantitative information of both vertical and horizontal transmission dynamics (including the distribution of infected larvae over egg batches) is needed.

Aim of this study is to quantify the vertical transmission rate of *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) by studying the distribution of infected larvae over egg batches of sublethally infected *S. exigua* moths. In addition, the rate of the subsequent horizontal transmission is determined in chrysanthemums in the greenhouse.

5.2 Material and methods

Insects and virus

Laboratory colonies of *S. exigua* were maintained as described by Smits *et al.* (1986). Larvae were reared from surface sterilized eggs and incubated at 25°C, 70-80% relative humidity and a 16 h photoperiod. The SeMNPV US isolate was used (Hunter and Hall, 1968) and was

propagated in fourth instar *S. exigua* larvae via surface contamination of semi-synthetic diet (Smits and Vlak, 1988c). The virus was purified by grinding deceased larvae, filtering through a double layer of cheese-cloth and two centrifugation steps. The polyhedra were resuspended and stored in a glycerol/water (1:1) solution in the dark at 4°C.

Vertical transmission

Two-hundred fifth instar larvae were allowed to ingest SeMNPV surface contaminated semi-synthetic medium with a blue stained $1x10^3$ polyhedra mm^{-2} solution, whereas a control group was reared in absence of virus. The larvae were reared at 25°C without light until pupation. Pupae were collected and transferred to an oviposition cylinder. The cylinder contained vermiculite and two layers of paper on the wall for egg deposition. After four and six days a paper with egg batches was removed from the cylinder. Neither pupae nor egg batches were surface-decontaminated in order to allow potential vertical transmission through external virus. The egg batches were reared individually and the egg batch size, the number of hatched larvae and the number of virus-killed larvae were recorded after four days of incubation in a stove at 25°C without light. Egg batch size, hatching and the number of infected larvae were analyzed using the Wilcoxon rank-sum test with Genstat (Payne *et al.*, 1993).

Horizontal transmission

The experiment was conducted in a greenhouse from April 6 to May 16 1994 in Wageningen, The Netherlands. Temperatures ranged from 20-30°C in the daytime, and were kept at 20°C during the night. A total of 48 plots with chrysanthemum plants were constructed in the greenhouse. Each plot consisted of 54 plants, which were planted in eight rows at distances of 10 cm. At the start of the experiment the plants were 8 weeks old and 100 cm high. The plots were surrounded by sticky tape to prevent larvae from escaping.

The treatment factors of the experiment were two chrysanthemum cultivars, 'Tiger' and 'Tigerrag', and three density levels of primary infected larvae (PIL). The PIL density levels were: 0, 1 and 10 infected larvae per 100 first instar *S. exigua* larvae. The two chrysanthemum cultivars were assigned to the 48 plots in alternating design. The three PIL density levels were assigned to the plots in a completely randomized design.

Virus infection was obtained by allowing newly hatched larvae to ingest an LD_{100} of surface contaminated semi-synthetic medium ($1x10^3$ polyhedra mm^{-2}) with a blue stained virus solution. After 24 hours, larvae were selected for the experiment by their blue color. Primarily infected and uninfected larvae were released in the plots by placing petri dishes

with a total of 100 first instar larvae upside down on top of a plant in the middle of the plot, allowing the larvae to descend into the crop. The number of larvae not leaving the petri dish was noted. The experiment was terminated when fifth instar larvae were first observed in the crop (22 and 23 days after the release of the larvae). Destructive sampling was carried out by dividing the plants in three foliage strata with an equal number of leaves. All larvae in each stratum were collected and counted. The total number of virus-killed and recaptured larvae, their instar, and the number of larvae recaptured on tape were recorded per plot. Recaptured larvae were individually reared on semi-synthetic diet until the larvae had either pupated or died. The number of virus-killed larvae was calculated as the sum of recaptured virus-killed larvae in plots and larvae that died of SeMNPV infection during the incubation period. These larvae were assumed to be secondarily infected larvae since the primarily infected first instar larvae disintegrate rapidly and are unlikely to be found. Crop injury was recorded by counting the number of damaged leaves. Larval survival and virus-induced mortality were analyzed by ANOVA with Genstat.

5.3 Results

Vertical transmission

The ingestion of SeMNPV surface contaminated diet by fifth instar larvae in the virus treatment caused 67% larval mortality and 42 larvae developed into adults. The first paper layer in the oviposition cylinder was removed four days after the first adults had emerged and contained 52 egg batches. A second group of 54 egg batches was collected two days later, after which the moths rapidly died without producing any more eggs. The size of the egg batches ranged from 5 to 175 eggs per batch and the 106 batches added up to a total of 3179 eggs. After four days of incubation, 95 egg batches contained hatched larvae and 64% of the deposited eggs hatched. Thirty-two egg batches contained virus-killed larvae, which is 34% of the hatched egg batches. The total number of progeny that developed SeMNPV infection was 367, which is 18% of the total number of hatched larvae. In Figure 5.1 the mean number of eggs, hatched larvae, and infected L1 larvae in egg batches of the first four days and the consecutive two days are given. The first group contained a higher number of eggs, hatched larvae, and infected L1 larvae per egg batch than the second group, although these differences only were significant for the number of eggs and the number of infected larvae ($P < 0.05$). The cumulative frequency distributions of the number of eggs, the number of hatched larvae

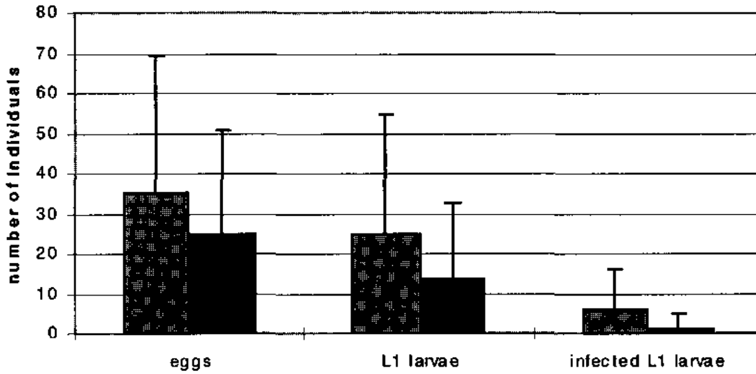

Figure 5.1 *Number of eggs, number of hatched larvae, and number of infected L1 larvae in egg batches laid by sublethally infected S. exigua during the first four days after emergence (light bars) and during the consecutive two days (dark bars). Bars indicate mean and standard deviation.*

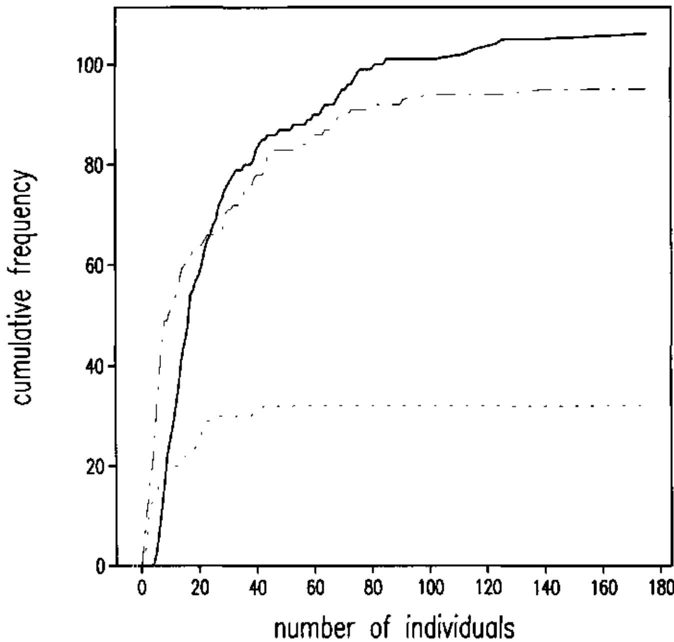

Figure 5.2 *Cumulative frequency distributions of the number of eggs (solid line), number of hatched larvae (dashed line), and number of infected L1 larvae (dotted line) in egg batches laid by sublethally infected S. exigua after six days.*

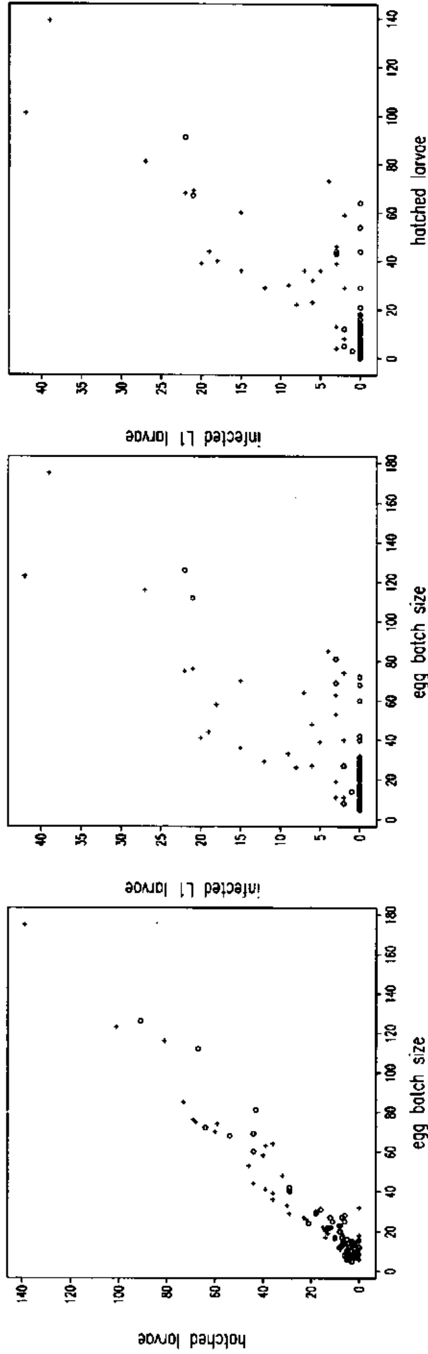

Figure 5.3. *Relation between the egg batch size, the number of hatched larvae and the number of infected L1 larvae in egg batches deposited by sublethally infected S. exigua. Egg batches laid during the first four days are indicated as '+' and egg batches laid during the consecutive two days are indicated as 'o'.*

and the number of infected L1 larvae are presented in Figure 5.2. The number of eggs and the number of hatched larvae were highly correlated ($R^2 = 0.939$, $P < 0.001$) (Figure 5.3a). The relations between the number of infected L1 larvae and the egg batch size and the number of hatched larvae are presented in figures 3b and 3c. The number of infected L1 larvae per egg batch was positively correlated with the egg batch size ($R^2 = 0.631$, $P < 0.001$) and the number of hatched larvae ($R^2 = 0.684$, $P < 0.001$). As a consequence, 80% of the egg batches larger than 40 eggs contained infected larvae. Seventy percent of all uninfected larvae hatched in egg batches that contained one or more infected larvae.

Horizontal transmission

The survival and SeMNPV induced mortality in populations of *S. exigua* larvae with 0, 1 and 10 primary infected larvae are shown in Table 5.1. Almost 80% of the recaptured larvae in the control plots had reached the fifth instar and the rest was still in the fourth instar. The chrysanthemum cultivar had no effect on the number of surviving ($P = 0.13$) and virus-killed larvae ($P = 0.38$). The PIL density levels significantly affected the number of *S. exigua* larvae that survived in the plots and the subsequent incubation period ($P < 0.001$). Survival of larvae of all three PIL density levels with 0, 1 and 10 SeMNPV-infected larvae differed significantly ($P < 0.05$) with 41, 30 and 20% survival. The number of virus-killed larvae of the two PIL density levels with 1 and 10 infected larvae were significantly higher than the control, but the difference between these two density levels was not significant ($P = 0.20$). The proportion of damaged leaves was 29%, 25% and 22.5% for treatments with respectively 0, 1 and 10 infected larvae.

Table 5.1 *Mean and SEM of the fate of populations of 100 S. exigua larvae in greenhouse chrysanthemums with 0, 1 and 10 primary infected larvae.*

	PIL treatment		
	0	1	10
survival	41.2 ± 2.7	29.9 ± 3.6	19.6 ± 2.9
primarily infected	0 ± 0	1 ± 0	10 ± 0
secondarily infected	0 ± 0	6.7 ± 1.4	9.1 ± 1.1
background mortality	52.2 ± 2.9	56.9 ± 3.0	56.4 ± 2.8
caught on tape	0.9 ± 0.3	1.1 ± 0.3	1.3 ± 0.3
unreleased	5.7 ± 3.1	4.4 ± 2.5	3.6 ± 2.5

5.4 Discussion

S. exigua moths that were treated with SeMNPV as fifth instar larvae transmitted the virus to 18% to their progeny. Reported vertical transmission rates of SeMNPV through sublethally infected moths vary. Young (1990) and Goulson and Cory (1995) found no or minimal vertical transmission in *S. ornithogalli* and *Mamestra brassicae* whereas Young and Yearian (1982) and Smits and Vlak (1988a) found vertical transmission rates of 8 to 28% in *Pseudoplusia includens* and *S. exigua.*

Thirty-four percent of the hatched egg batches contained infected larvae. In general, these contaminated egg batches contained high numbers of eggs and were typically laid within four days after emergence of the adults. It is well established that the rate of vertical transmission of moths exposed to polyhedra declines in time (Hamm and Young, 1974; Elnagar *et al.*, 1982). This suggests that the first egg batches deposited, which are generally larger, have a higher probability to be contaminated with polyhedra. In contrast, smaller egg batches, which are deposited later, may be almost free of polyhedra because the majority already has been deposited with previous egg batches.

The number of *S. exigua* larvae recovered in the horizontal transmission experiment was much lower than the number released. Overall mortality in the control plots was almost 60% and are in accordance with observations in chapter 4 and Smits *et al.* (1987a) who found similar percentages of background mortality in greenhouse chrysanthemum. The survival of groups of 100 *S. exigua* larvae was significantly reduced by the release of 1 or 10 SeMNPV-infected larvae, indicating that horizontal transmission impacted survival. The number of surviving larvae was negatively correlated with the number of primary infected larvae released into the plots. On the other hand, the number of recaptured SeMNPV killed larvae in plots with 1 and 10 initially infected larvae were not significantly different with average values of 7 and 9 larvae, respectively. These high horizontal transmission rates suggest that uninfected larvae may be attracted to virus-killed larvae, or that virus-killed larvae contaminate many leaves. Although larval survival was reduced at increasing PIL densities, crop injury was hardly reduced in plots with such PIL densities.

The results of this study indicate that sublethally infected *S. exigua* moths are efficient transmitters of SeMNPV to their progeny. Because the highest numbers of infected larvae tend to be deposited in early laid, large egg batches, the resulting horizontal transmission is likely to infect a substantial part of the larval population. Therefore, vertical transmission may play an important role in maintaining the baculovirus epidemic within the insect

population. Comprehensive simulation models can be used to evaluate the consequences of these transmission dynamics at the population level (van der Werf *et al.*, 1991).

6

Greenhouse evaluation of dose- and time-mortality relationships of two nucleopolyhedroviruses for the control of beet armyworm, *Spodoptera exigua*, on chrysanthemum

Dose- and time-mortality relationships of baculoviruses in pest insects are important for the determination of effective spraying regimes. A series of experiments with Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and Spodoptera exigua MNPV (SeMNPV) against synchronized populations of S. exigua larvae in greenhouse chrysanthemum was conducted. Dose- and time-mortality relationships of different virus concentrations and S. exigua target stages were determined and the area foliage consumption measured. Crop injury was greatly reduced when S. exigua were controlled as second or third instar larvae, whereas virus applications against fourth instar larvae could not prevent considerable crop injury, even at high concentrations. SeMNPV was approximately 10 times as infectious as AcMNPV when applied in greenhouse chrysanthemum. The relative virulence of AcMNPV and SeMNPV corresponded reasonably well with previously published laboratory bioassay data. SeMNPV killed second and fourth instar S. exigua larvae approximately 12 h faster as AcMNPV in chrysanthemum, but no difference in speed of action was found for third instar larvae. The relative speed of action of AcMNPV and SeMNPV determined in chrysanthemum and in laboratory bioassays did not correspond for third instar S. exigua larvae; laboratory bioassay data can therefore not simply be extrapolated to the crop level.

This chapter has been published as:
Bianchi, F.J.J.A., Joosten, N.N., Vlak, J.M., and van der Werf, W. 2000. *Biol. Control* **19**, 252-258.

6.1 Introduction

Baculoviruses are naturally occurring pathogens that infect a wide range of lepidopteran pest insect species. These viruses are environmentally sound alternatives for chemical insecticides and are used as biological control agents of insect pests in agriculture and forestry (Black *et al.*, 1997; Moscardi, 1999). The *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) provides good control of beet armyworm (*S. exigua*) larvae and is commercially available in the USA, Thailand and The Netherlands. The MNPV of *Autographa californica* (AcMNPV) can also infect *S. exigua* larvae and is registered in the USA (Moscardi, 1999). A genetically engineered AcMNPV with improved insecticidal characteristics is awaiting registration (Cory *et al.*, 1994; Black *et al.*, 1997).

The virulence and speed of action of AcMNPV and SeMNPV are well studied and are generally determined in bioassays under controlled laboratory conditions. Results of such bioassays revealed that (i) the LD_{50} value of SeMNPV is about tenfold lower than that of AcMNPV for *S. exigua* larvae, (ii) the LD_{50} values of both AcMNPV and SeMNPV increase significantly with instar stage and (iii) SeMNPV kills *S. exigua* larvae 0.5-1 day faster than AcMNPV (chapter 2; Smits and Vlak, 1988a). The question is whether the results of these laboratory bioassays can be extrapolated to the crop level. The situation in a greenhouse crop, for example, is much more complex because of fluctuating environmental factors and interactions among the crop, insects, virus and the abiotic environment. However, when the efficacy of baculoviruses in a greenhouse situation is consistent with results from laboratory bioassays, virulence and speed of action determined in laboratory bioassays may be used as indicators for baculovirus efficacy at the crop level. The efficacy of baculoviruses in crops is generally determined in costly field or greenhouse testing programs (Smits *et al.*, 1987b; Kolodny-Hirsch *et al.*, 1993; 1997; Cory *et al.*, 1994; Treacy *et al.*, 1997). When the efficacy of baculoviruses may (in part) be predicted and extrapolated from laboratory bioassay data, the number or size of field experiments may be reduced.

In this paper we describe a series of experiments with AcMNPV and SeMNPV applications against synchronized populations of *S. exigua* larvae in greenhouse chrysanthemum. The aim of this study is to quantify dose- and time-mortality relationships of various virus applications of AcMNPV and SeMNPV in larval populations that develop in greenhouse chrysanthemum. In addition, the foliage area consumed by the larvae is quantified for the different virus applications as a measure of efficacy. The potential of laboratory bioassay data as indicators for baculovirus efficacy at the crop level will be evaluated by the comparison

of AcMNPV and SeMNPV virulence and speed of action in greenhouse chrysanthemum with earlier published laboratory bioassay data.

6.2 Material and methods

Insects and virus

Laboratory colonies of *S. exigua* were maintained as described by Smits *et al.* (1986). In short, larvae were reared from surface-sterilized eggs and incubated at 25°C, 70-80% relative humidity and a 16 h photoperiod. The viruses used were the E2 clone of AcMNPV (Summers and Smith, 1987) and the US1 isolate of *S. exigua* MNPV (Hunter and Hall, 1968). The viruses were propagated as described in chapter 2 and the polyhedra were stored in a glycerol/water solution (1:1) in the dark at 4°C until use.

Greenhouse experiments

Ten experiments were conducted in a greenhouse (10 x 30 m) in Wageningen, The Netherlands, from July 1998 to October 1999. An overview of the experiments is given in Table 6.1. In experiments #1 to #6 dose-mortality relationships were studied, whereas experiments #7 to #10 aimed at the quantification of time-mortality relationships in the crop-insect-virus system. For each experiment 20 or 24 plots with insecticide-free chrysanthemum plants (cultivar Tiger) were used (Table 6.1). The plants were a gift from Brinkman BV, 's Gravezande, The Netherlands. Each plot had an area of 1 m^2 and consisted of 64 plants that were planted in a square pattern at a mutual distance of 12.5 cm. In all experiments the plants were 3 weeks old and approximately 30 cm high at the start of the experiment. The plots were surrounded by 40 cm-high transparent plastic that was dug into the soil and covered with tanglefoot at the top to prevent larvae from escaping. Greenhouse temperatures were kept at 23°C during the night and daytime ventilation set-point was 29°C and 30°C. Hourly temperatures were recorded using a datalogger (Depex datataker 600, De Bilt, The Netherlands) with seven thermocouples that were located in the canopy.

In all experiments synchronized populations of first or second instar *S. exigua* larvae were released in the chrysanthemum plots by brushing the larvae gently from petri dishes onto the top of the plants. When the larvae reached the appropriate instar (second, third or fourth instar), control plots were sprayed with 300 ml 1% phosphate buffered saline (PBS) solution using a simple handsprayer. Virus-treated plots were sprayed using an AZO pressure air handsprayer (4 atmosphere) with four helicon safrei nozzles no. 120 (Birchmeier, Stetten,

Table 6.1 *Overview of the instar used, Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) and Autographa californica MNPV (AcMNPV) concentrations (polyhedra m^{-2}), number of initially released larvae, number of repetitions, number of plots and harvest dates of 10 greenhouse experiments.*

exp #	instar	SeMNPV	AcMNPV	larvae/plot	reps	plots	assessment on day[†]
1	L2	0, $3x10^5$, $3x10^6$, $3x10^7$	-	100	6	24	7 - 8
2	L3	0, $3x10^5$, $3x10^6$, $3x10^7$	-	100	6	24	3
3	L4	0, $3x10^5$, $3x10^6$, $3x10^7$	-	100	6	24	4 - 5
4	L2	0, $3x10^5$, $3x10^6$	$3x10^6$, $3x10^7$	100	4	20	6 - 7
5	L3	0, $3x10^5$, $3x10^6$	$3x10^6$, $3x10^7$	80	4	20	3 - 4
6	L4	0, $3x10^5$, $3x10^6$	$3x10^6$, $3x10^7$	80	4	20	4 - 5
7	L2	0, $3x10^7$	$3x10^8$	80	8 (4)[‡]	24	3 & 6
8	L3	0, $3x10^7$	$3x10^8$	80	8 (4)[‡]	24	3 & 6
9	L3	0, $3x10^7$	$3x10^9$	80	8 (4)[‡]	24	3 & 6
10	L4	0, $3x10^7$	$3x10^8$	80	8 (4)[‡]	24	3 & 5

[†] In the dose-mortality trials 1-6, all plots were harvested during a one- or two day period. In time-mortality trials 7-10 there were two times for destructive harvest: 4 plots per treatment were harvested 3 days after application, and another 4 plots per treatment 2 or 3 days later.

[‡] Eight repetitions per treatment and four repetitions per observation moment per treatment.

Switzerland). Virus applications consisted of 300 ml 1% PBS solution containing the appropriate polyhedral concentration. Before treatment, the plots were shielded with polystyrene foam plates (experiments #1 to #3) or the plots were covered with transparent plastic (experiments #4 to #10) to prevent drift to neighboring plots.

Measurements

Destructive harvests of the plots generally took 2 days, except for experiment #2 that was harvested in 1 day (Table 6.1). All plants per plot were cut into a top, middle and bottom section of equal size. From each section the foliage consumption and the number of living and virus-killed larvae in each instar were recorded. Foliage consumption was estimated using a transparency with a dot grid (4 dots cm^{-2}). The transparency was put on top of leaves with feeding marks and the number of dots overlapping with feeding marks were counted. Dot scores were converted to cm^2 foliage consumption. Recovered living larvae were transferred to 12-well plates filled with artificial diet and reared at 27°C. Larval mortality was recorded when all larvae had either pupated or died of baculovirus infection (experiments #1 to #6) or twice per day until all larvae pupated or died (experiments #7 to #10).

Experimental design

In all experiments a complete randomized block design was used. Experiments #1 to #3 consisted of six blocks that contained a control and three SeMNPV concentration treatments. In experiments #4 to #6 both AcMNPV and SeMNPV were included and the experiment consisted of four blocks of five treatments: one control and two concentrations per virus. Experiments #7 to #10 consisted of four blocks, each containing two control, two AcMNPV and two SeMNPV treatments. Experiments #7, #8 and #9 were harvested 3 or 6 days after application, whereas experiment #10 was harvested after 3 and 5 days.

Statistical analysis

In experiments #1 to #6 the number of virus-killed larvae was calculated as the sum of recaptured virus-killed larvae in the plots and those that died from viral infection in the 12-well plates. In experiments #7 to #10 the time- and dose-mortality relationships were based only on the larvae that were recovered alive in the plots and that were subsequently incubated in the 12-well plates. It is unlikely that in experiments #7 to #10 virus-induced mortality took place before the larvae were recovered in the first harvest, which took place 3 days after application (Table 6.1). Survival time (ST_{50}) values and slopes of time-mortality data were determined using the ViStat program (version 2.1; Boyce Thompson Institute, Cornell University, Ithaca, NY). Larval recovery, mortality, ST_{50} values, slopes of time-mortality relationships and foliage consumption were analyzed by regression analysis and t-tests of pairwise differences between treatments with Genstat (Payne *et al.*, 1993). Larval recovery and mortality were analyzed as binomially distributed variables with the dispersion factor estimated from residual deviance. Log foliage consumption values of experiments #1-3, experiments #4-6 and experiments #7-10 have been lumped and analyzed by regression analysis.

6.3 Results

Dose-mortality relationships of synchronized populations of second, third and fourth instar *S. exigua* larvae in chrysanthemum that were treated with SeMNPV are presented in Table 6.2. Applications of the fixed SeMNPV concentrations (3×10^5, 3×10^6 and 3×10^7 polyhedra m^{-2}) resulted in similar mortality patterns in populations of second, third and fourth instar *S. exigua* larvae. Larval mortality increased significantly with increasing SeMNPV

Table 6.2 *Instar, concentration (polyhedra m^{-2}), recovery (%), mortality (%) and foliage consumption (cm^2) with standard error of the mean of Spodoptera exigua larvae treated with Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV) in greenhouse chrysanthemum.*

exp	instar	concentration	% recovery[†]	% mortality[‡]	foliage consumption
1	L2	0	23 ± 2[a]	9 ± 4[a]	243 ± 48[a]
		3x10^5	21 ± 1[a]	31 ± 2[b]	176 ± 26[a,b]
		3x10^6	12 ± 3[b]	68 ± 7[c]	125 ± 30[b]
		3x10^7	4 ± 2[c]	100 ± 0[d]	100 ± 38[b]
2	L3	0	73 ± 5[a]	11 ± 3[a]	299 ± 31[a]
		3x10^5	68 ± 5[a]	23 ± 3[b]	192 ± 39[a]
		3x10^6	66 ± 3[a]	65 ± 3[c]	267 ± 59[a]
		3x10^7	61 ± 5[a]	92 ± 3[d]	309 ± 66[a]
3	L4	0	84 ± 7[a]	13 ± 2[a]	1117 ± 59[a]
		3x10^5	78 ± 6[a]	47 ± 7[b]	935 ± 41[a]
		3x10^6	68 ± 10[a]	79 ± 5[c]	849 ± 134[a]
		3x10^7	81 ± 6[a]	99 ± 1[d]	988 ± 198[a]

Superscript letters indicate significant differences among treatments for each experiment (P = 0.05).

[†] Recovery from plots calculated as: ((recovered living larvae + recovered virus-killed larvae) / released larvae) x 100%.

[‡] Mortality is calculated as: ((recovered virus-killed larvae in plots + virus-killed larvae in well-plates) / recovered larvae) x 100%.

concentrations for second ($F = 37.63$; d.f. 3, 12; $P < 0.001$), third ($F = 87.75$; d.f. 3, 15; $P < 0.001$), and fourth instar larvae ($F = 55.84$; d.f. 3, 11; $P < 0.001$), and mortality levels of 90-100% were reached for the highest SeMNPV concentration. Log foliage consumption values increased significantly with instar stage treated ($F = 79.77$; d.f. 2, 62; $P < 0.001$). Reduced foliage consumption with increasing SeMNPV concentration was only observed in experiment #1 when larvae were recovered and injury was estimated 7 and 8 days after virus application, but not in experiments #2 and #3 when the assessments were made 3 to 5 days after application. In experiment #1 the larvae died of SeMNPV infection in the chrysanthemum plots, whereas in experiments #2 and #3 the majority of the infected larvae were still alive at the time of recovery.

Larval mortality of second, third and fourth instar *S. exigua* larvae, treated with AcMNPV and SeMNPV, ranged between 5 and 80 % (Table 6.3). Mortality levels increased significantly with increasing AcMNPV and SeMNPV concentration for second ($F = 47.13$;

Table 6.3 *Instar, concentration (polyhedra m^{-2}), recovery (%), mortality (%) and foliage consumption (cm^2) with standard error of the mean of Spodoptera exigua larvae treated with Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and Spodoptera exigua MNPV (SeMNPV) in greenhouse chrysanthemum.*

exp	instar	virus	concentration	% recovery	% mortality‡	foliage consumption
4	L2	control	0	$50 \pm 1^{a,b}$	4 ± 3^{a}	186 ± 22^{a}
		AcMNPV	$3x10^6$	$48 \pm 6^{a,b}$	$14 \pm 3^{a,b}$	236 ± 113^{a}
		AcMNPV	$3x10^7$	$34 \pm 2^{b,c,d}$	60 ± 6^{c}	121 ± 20^{a}
		SeMNPV	$3x10^5$	$45 \pm 3^{a,b,c}$	17 ± 3^{b}	156 ± 20^{a}
		SeMNPV	$3x10^6$	$27 \pm 6^{c,d}$	67 ± 4^{c}	129 ± 23^{a}
5	L3	control	0	$74 \pm 7^{a,b}$	2 ± 1^{a}	122 ± 40^{a}
		AcMNPV	$3x10^6$	82 ± 5^{a}	44 ± 3^{b}	139 ± 31^{a}
		AcMNPV	$3x10^7$	59 ± 2^{b}	79 ± 1^{c}	94 ± 12^{a}
		SeMNPV	$3x10^5$	$78 \pm 8^{a,b}$	22 ± 3^{d}	88 ± 19^{a}
		SeMNPV	$3x10^6$	$67 \pm 1^{a,b}$	73 ± 5^{c}	87 ± 6^{a}
6	L4	control	0	60 ± 10^{a}	3 ± 2^{a}	$1395 \pm 313^{a,b}$
		AcMNPV	$3x10^6$	57 ± 2^{a}	5 ± 2^{a}	$1326 \pm 388^{a,b,c}$
		AcMNPV	$3x10^7$	49 ± 7^{a}	48 ± 3^{b}	$799 \pm 200^{b,c,d}$
		SeMNPV	$3x10^5$	49 ± 3^{a}	22 ± 3^{c}	$763 \pm 159^{b,c,d}$
		SeMNPV	$3x10^6$	54 ± 4^{a}	76 ± 4^{d}	$644 \pm 130^{c,d}$

Superscript letters indicate significant differences among treatments for each experiment (P = 0.05).

† Recovery from plots calculated as: ((recovered living larvae + recovered virus-killed larvae) / released larvae) x 100%.

‡ Mortality is calculated as: ((recovered virus-killed larvae in plots + virus-killed larvae in well-plates) / recovered larvae) x 100%.

d.f. 2, 14; $P < 0.001$), third ($F = 67.10$; d.f. 2, 14; $P < 0.001$), and fourth instar larvae ($F = 32.63$; d.f. 2, 14; $P < 0.001$). SeMNPV was approximately 10 times as virulent as AcMNPV. Log foliage consumption values increased significantly with instar ($F = 135.62$; d.f. 2, 55; $P < 0.001$). Foliage consumption was significantly reduced with increasing concentration in experiment #6, whereas in experiments #4 and #5 the reduction in foliage consumption was not significant. Time-mortality relationships of synchronized populations of second, third and fourth instar *S. exigua* larvae controlled with AcMNPV and SeMNPV are presented in Table 6.4. In all experiments larvae were recovered from the plots after 3 and 6 days, except in experiment #10 where the larvae were recovered at 3 and 5 days after application. Log foliage consumption values increased significantly with instar ($F = 506.27$; d.f. 2, 76;

Table 6.4 *Instar, treatment, concentration (polyhedra m^{-2}), recovery (%), mortality (%), survival time (h), slopes of time-mortality curves and foliage consumption (cm^2) with standard error of the mean of second, third and fourth instar Spodoptera exigua larvae treated with Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and Spodoptera exigua MNPV (SeMNPV), recovered after 3 and 5 or 6 days from chrysanthemum plants.*

				3 days after application					5 or 6 days after application*				
exp	instar	virus	conc.	%rec†	% M§‡	ST_{50} §ʃ	slope§	fol. cons. §	%rec†	% M§‡	ST_{50} §ʃ	slope§	fol. cons. §
7	L2	control	0	50 ± 5^a	10 ± 3^a	- ± -	- ± -	10 ± 1^a	62 ± 5^a	8 ± 3^a	- ± -	- ± -	147 ± 18^a
		AcMNPV	$3x10^8$	62 ± 1^a	79 ± 6^b	124.7 ± 1.0^a	11.4 ± 1.2^a	9 ± 1^a	47 ± 4^b	79 ± 3^b	170.8 ± 2.0^a	8.3 ± 0.6^a	106 ± 5^b
		SeMNPV	$3x10^7$	51 ± 3^a	77 ± 2^b	113.9 ± 2.4^b	9.0 ± 0.6^a	7 ± 1^a	22 ± 2^b	75 ± 9^b	177.6 ± 3.1^a	10.1 ± 0.3^a	50 ± 5^c
8	L3	control	0	73 ± 9^a	2 ± 0^a	- ± -	- ± -	123 ± 7^a	73 ± 2^a	1 ± 1^a	- ± -	- ± -	519 ± 59^a
		AcMNPV	$3x10^9$	89 ± 4^a	99 ± 1^b	123.8 ± 1.4^a	18.1 ± 1.3^a	133 ± 12^a	26 ± 7^b	99 ± 1^b	158.6 ± 4.4^a	23.5 ± 1.7^a	308 ± 29^b
		SeMNPV	$3x10^7$	72 ± 7^a	88 ± 2^c	120.8 ± 2.7^a	10.9 ± 0.7^b	$96 \pm 10^{a,b}$	35 ± 5^b	86 ± 1^c	171.1 ± 5.3^a	14.7 ± 2.3^a	258 ± 23^b
9	L3	control	0	39 ± 1^a	2 ± 1^a	- ± -	- ± -	84 ± 9^a	43 ± 7^a	1 ± 1^a	- ± -	- ± -	526 ± 105^a
		AcMNPV	$3x10^8$	38 ± 8^a	91 ± 2^b	117.5 ± 0.9^a	11.6 ± 0.2^a	106 ± 28^a	7 ± 1^b	77 ± 8^b	175.0 ± 5.1^{nd}	9.7 ± 0.1^{nd}	156 ± 14^b
		SeMNPV	$3x10^7$	33 ± 5^a	94 ± 1^b	116.5 ± 2.4^a	7.7 ± 1.1^b	$45 \pm 8^{a,b}$	4 ± 1^b	85 ± 10^b	175.8 ± 1.2^{nd}	8.2 ± 1.6^{nd}	170 ± 32^b
10	L4	control	0	54 ± 4^a	1 ± 1^a	- ± -	- ± -	634 ± 65^a	18 ± 10^a	1 ± 1^a	- ± -	- ± -	580 ± 81^a
		AcMNPV	$3x10^8$	37 ± 12^a	65 ± 5^b	151.0 ± 1.0^a	10.9 ± 2.5^a	$493 \pm 45^{a,b}$	38 ± 16^a	79 ± 11^b	172.3 ± 11.3^a	12.6 ± 1.5^a	773 ± 273^a
		SeMNPV	$3x10^7$	31 ± 5^a	84 ± 9^b	138.0 ± 0.9^b	9.2 ± 0.4^a	366 ± 33^b	25 ± 4^b	83 ± 6^b	157.4 ± 3.6^a	12.5 ± 2.3^a	510 ± 63^a

* Experiments 7, 8 and 9 were harvested at 6 days after application, experiment 10 was harvested at 5 days after application.

† Recovery from plots calculated as: (recovered living larvae / released larvae) x 100%. Superscript letters indicate significant differences between harvests for each experiment (P = 0.05).

§ Superscript letters indicate significant differences between AcMNPV and SeMNPV for each experiment (P = 0.05).

‡ Mortality is calculated as: (virus-killed larvae in well-plates / recovered living larvae from plots) x 100%.

ʃ ST_{50} values based on recovered living larvae that died of virus-infection during incubation in well-plates.

P < 0.001) and harvest date ($F = 119.16$; d.f. 1, 76; $P < 0.001$). Second and fourth instar larvae that were recovered 3 days after virus application were killed approximately 12 h faster by SeMNPV than by AcMNPV. ST_{50} values of larvae recovered after 3 days and treated with SeMNPV were always lower than those treated with AcMNPV, but this difference was only statistically significant for second ($F = 23.62$; d.f. 1, 3; $P < 0.05$) and fourth instar larvae ($F = 350.67$; d.f. 1, 3; $P < 0.01$). Figure 6.1 presents a typical time-mortality relationship of recovered second instar larvae infected with AcMNPV and SeMNPV. Time-mortality relationships of larvae treated with AcMNPV tended to be steeper than those of SeMNPV, indicating a relatively small variation in time to kill for AcMNPV. The differences in ST_{50} values and slopes of time-mortality relationships of larvae that were recovered after 5 or 6 days were not significant. The number of larvae recovered after 6 days was always significantly lower than the number of recovered larvae after 3 days, except for fourth instar larvae treated with AcMNPV.

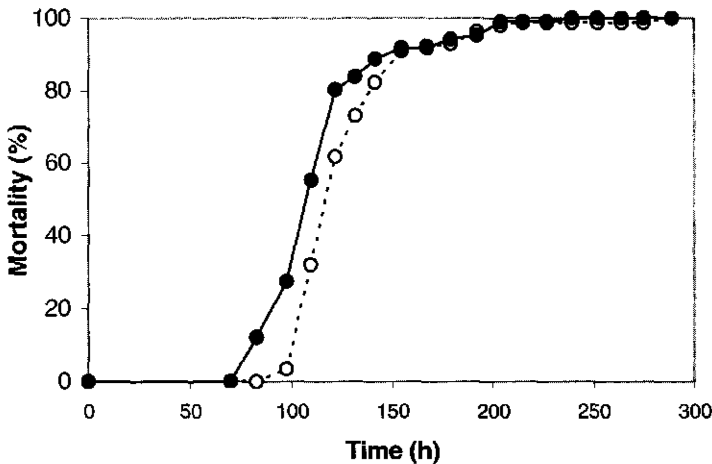

Figure 6.1 *Time-mortality relationship of second instar Spodoptera exigua larvae recovered from chrysanthemum plots 3 days after treatment with $3x10^8$ polyhedra m^{-2} AcMNPV (○) and $3x10^7$ polyhedra/m^2 SeMNPV (●). The larvae were reared on artificial diet at 27 °C.*

6.4 Discussion

In this study dose- and time-mortality relationships of AcMNPV and SeMNPV applications in larval populations of *S. exigua* in greenhouse chrysanthemum were determined. Applications of $3x10^7$ polyhedra SeMNPV m^{-2} caused in general mortality levels of approximately 90 % in populations of second, third and fourth instar larvae. This finding is in agreement with a study of Smits *et al.* (1987b) who reported mortality levels of 99.5 % for first and second instar *S. exigua* larvae treated with $2.5x10^7$ polyhedra SeMNPV m^{-2} in a 30 cm-high chrysanthemum crop in a greenhouse. The same treatment caused mortality levels of 91.5 % in populations of third and fourth instar *S. exigua* larvae. Applications of $3x10^7$ polyhedra SeMNPV m^{-2} are equivalent to $3x10^{11}$ polyhedra SeMNPV ha^{-1} which was the lowest concentration tested by Kolodny-Hirsch *et al.* (1993; 1997) on field crops. The latter studies showed that repeated applications of $3x10^{11}$ polyhedra SeMNPV ha^{-1} provided good control of *S. exigua* on garden pea, grape, tomato, pepper and garbanzo beans but was less effective on Chinese kale and shallot.

In experiments #1, 2, 3 and 7 mortality levels of approximately 10% in control plots were found. Despite our effort to screen control plots, polyhedra drift from neighboring plots is likely to have occurred. This mortality may have reduced larval recovery and foliage consumption. For experiments with a duration period that exceeded 4-5 days the secondary inoculum of virus-killed larvae may have contributed to a further increase of the mortality levels. However, the amount of polyhedra drift is small as compared to the applied virus dosages.

AcMNPV and SeMNPV virulence in *S. exigua* larvae has been intensively studied under laboratory conditions (chapter 2; Smits and Vlak, 1988a). These laboratory studies indicated that LD_{50} values of SeMNPV are about tenfold lower than those of AcMNPV. We would then expect that SeMNPV and a tenfold higher concentration of AcMNPV would cause similar mortality levels. Under greenhouse conditions, SeMNPV and a tenfold higher AcMNPV concentrations resulted generally in comparable mortality levels for various instars *S. exigua* larvae in a chrysanthemum crop (Table 6.3 and 6.4). Thus, the relative virulence of AcMNPV and SeMNPV in *S. exigua* determined in laboratory bioassays remained unchanged at the crop level. This finding suggests that viral virulence derived from laboratory bioassays can be used as a good indicator of baculovirus virulence in greenhouse chrysanthemum.

The laboratory bioassays of chapter 2 and Smits and Vlak (1988a) further indicated that LD_{50} values of both AcMNPV and SeMNPV in *S. exigua* larvae strongly increase with

instar. Under greenhouse conditions, however, AcMNPV and SeMNPV caused generally comparable mortality levels in populations of synchronized second, third and fourth instar larvae (Tables 6.2 and 6.3). The increased resistance of later instar larvae, as measured in laboratory bioassays, was not observed in a greenhouse crop.

Similar mortality levels in populations of synchronized second, third and fourth instar *S. exigua* larvae treated with AcMNPV and SeMNPV can be explained by the increase in foliage consumption of second to fourth instar larvae (Tables 6.2 and 6.3) (Evans, 1981). When the appropriate adjustments are taken into account for termination dates of different experiments, there was a clear tendency for an increase in foliage consumption by second, third and fourth instar larvae. Apparently, the increased foliage consumption rate (and polyhedra uptake) of later instar larvae compensates for their reduced susceptibility to AcMNPV and SeMNPV in the greenhouse situation. Furthermore, the increase in foliage consumption with instar underscores the importance of timing of baculovirus applications to minimize crop injury caused by *S. exigua* larvae. Crop injury was reduced to a large extent when larvae were controlled as second or third instar larvae, whereas virus applications against fourth instar larvae resulted in crop injury that clearly exceeded the damage threshold level for chrysanthemum sale, even at high virus concentrations.

In laboratory bioassays it was shown that SeMNPV killed *S. exigua* larvae 0.5 - 1 day faster than AcMNPV on artificial diet at a constant temperature of 27°C (chapter 2). When there are no interactions with the crop, we would expect a similar difference in killing time for AcMNPV and SeMNPV under greenhouse conditions. The ST_{50} values of second and fourth instar larvae recovered 3 days after virus application from chrysanthemum plots and reared further on artificial diet at 27°C were about 12 h lower for SeMNPV than for AcMNPV. This is consistent with laboratory bioassays (chapter 2). However, for third instar larvae the difference in ST_{50} value for AcMNPV and SeMNPV was marginal, and this was consistent in two experiments. An increased speed of action for AcMNPV in experiment #8 (Table 6.4) may be explained by a concentration effect. In this experiment $3x10^9$ polyhedra m^{-2} AcMNPV was applied, whereas SeMNPV was applied at a rate of $3x10^7$ polyhedra m^{-2}. However, when AcMNPV was applied at the standard rate of $3x10^8$ polyhedra m^{-2} (experiment #9), similar results were obtained (Table 6.4). Hence, viral speed of action values derived from laboratory bioassays cannot always be extrapolated to the situation on a greenhouse crop.

Various factors may affect the moment of uptake of a lethal dose of polyhedra and the subsequent virus incubation period, e.g. larval feeding location, larval feeding rate, larval

susceptibility to the virus and greenhouse temperature. In this context we observed that *S. exigua* larvae feed preferably on the underside of chrysanthemum leaves without perforating the leaf (chapter 4). Because the crop was sprayed from the top and chrysanthemum leaves are placed horizontally on the stem, virtually no polyhedra are deposited at the underside of leaves. This implies that the underside of leaves may function as a polyhedra-free refuge for larvae and this may interfere with the time-mortality relationship between virus and *S. exigua* larvae in crop situations. Possibly, simulation models that are based on a detailed description of the crop-insect-virus system can be used to obtain a more accurate upscaling of laboratory generated data to the crop level (van der Werf *et al.*, 1991).

7

Description of a process-based model for the biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses

We describe a comprehensive simulation model of the epidemiology and agronomic efficacy of a baculovirus used as a biological control agent for the beet armyworm, Spodoptera exigua, on greenhouse chrysanthemum. The model is based on a detailed quantitative description of the population dynamics of insects, plant growth characteristics, spray deposition, the baculovirus infection cycle and transmission routes. The model is parameterized for S. exigua multicapsid nucleopolyhedrovirus (SeMNPV) and its validity verified for this crop-insect-virus system. Simulations of the model components for chrysanthemum leaf area index growth, S. exigua larval development, SeMNPV infectivity, speed of viral action and viral inactivation, vertical and horizontal transmissions were in line with measurements. The model can be used to simulate the population dynamics of S. exigua and wild-type or genetically-modified baculoviruses under various conditions.

This chapter has been submitted as:
Bianchi, F.J.J.A., Vlak, J.M., Rabbinge, R. and van der Werf, W.

7.1 Introduction

Baculoviruses are pathogens that can cause lethal infections in many insect species, mostly lepidoptera. In nature they may have large impacts on the dynamics of host populations. In agriculture and forestry these viruses find application as biological control agents of insect pests (Black *et al.*, 1997; Moscardi, 1999). Unlike most conventional pesticides baculoviruses are generally host-specific and not harmful for non-target insects or other organisms. For insect species that have developed resistance to insecticides, such as the beet armyworm, *Spodoptera exigua*, the use of baculoviruses is one of the few options left for control (Brewer and Tumble, 1989).

A major drawback of the use of baculoviruses as crop protection agents is their high production costs, relatively slow speed of action, narrow host range and high inactivation rate compared to chemical insecticides (Moscardi, 1999). Recently, recombinant baculoviruses have been constructed with improved insecticidal properties (Black *et al.*, 1997; van Beek and Hughes, 1998). In addition, chemical synergists such as optical brighteners have been developed that can improve baculovirus efficacy as biocontrol agents (Dougherty *et al.*, 1996). The determination of the commercial potential of new recombinant baculoviruses and new formulations involve costly field-testing programs. In the case of recombinant baculoviruses, these experiments are subject to strict regulations and require costly facilities and extensive procedures.

Computer simulation of the impact of baculovirus applications on insect and baculovirus population dynamics can provide fast and extensive information on the agricultural effectiveness of viruses with altered insecticidal properties and formulations that improve baculovirus efficacy. Such simulations may be used for the initial screening of the agricultural effectiveness of baculoviruses, as a tool to set up field experiments, and may reduce the number of field experiments when model simulations are in line with field experiments. In addition, simulations can assist in the determination of effective spraying regimes of baculoviruses in varying crop development stages and at different temperatures for cultivation practice (de Moed *et al.*, 1990; van der Werf *et al.*, 1991). Finally, simulation models can be used to gain insights in the insect-baculovirus-crop system and the relative importance of viral characteristics for crop protection purposes. This knowledge may be used to direct future genetic engineering strategies to obtain baculoviruses with improved insecticidal characteristics.

Parameter-sparse analytical models have been developed to study epidemics of baculoviruses in insect populations (Anderson and May, 1981; Dwyer, 1992; Dwyer and Elkinton, 1993; Dwyer *et al.*, 1997). These analytical models are based on far reaching simplifications regarding the underlying ecology (Onstad *et al.*, 1990). Although they are useful for addressing theoretical issues related to the long term dynamics and stability of insect-virus systems, these models are less suitable for answering most of the short term questions that emanate from practical application of insect viruses as biocontrol agents, e.g. the optimal timing and doses of baculovirus applications. Numerical simulation models that describe the system at the process level and integrate information of the different model components allow a detailed description of the biological system. This simulation approach has successfully been applied in a number of insect-parasitoid and insect-predator systems (van Roermund, 1995; de Kraker, 1996; Xia, 1997). Validation experiments under different conditions revealed that these simulations were in general in close agreement with reality (de Kraker, 1996; van Roermund, 1997; Xia, 1997).

We have developed a spatially explicit simulation model (BACSIM) to simulate the control of the pest, *S. exigua*, with baculoviruses in greenhouse chrysanthemum. The model is based on a detailed quantitative description of the population dynamics of insects, plant growth characteristics, spray deposition, the baculovirus infection cycle and transmission routes. The model can be used to simulate the population dynamics of *S. exigua* and (genetically-modified) baculoviruses under various conditions. This paper describes the simulation model BACSIM and the verification of the model components of leaf area index (LAI) increment of greenhouse chrysanthemum, *S. exigua* larval development, baculovirus infectivity, speed of action and inactivation, and vertical and horizontal transmission at the process level. The verification of the model components ensures an appropriate model concept, statistical procedure (curve fitting) and implementation of the model components in the model. The validation of the model, which is the comparison of simulation results with independent experimental data, is described in chapter 8.

7.2 Modeling concepts

BACSIM is an extremely comprehensive model written in FORTRAN-90. Because of its complexity it is impossible to give a complete explanation of the model. The description of the model given below aims to explain the basic assumptions of the model, rather than to give

a complete outline of the model. A full description of the model, including the FORTRAN code, is available on request (Bianchi and van der Werf, 2000).

Levels of spatial detail

BACSIM is a spatially explicit simulation model with four spatial scales: the greenhouse, chrysanthemum bed, patch and leaf. A patch is defined as a group of plants over which larvae that originate from a single egg batch forage. Its borders are set by the foraging domain of larvae in the final larval stage. An overview of the spatial scales is given in Figure 7.1a. The locations of insects at the various scales are characterized in the model by co-ordinates. These co-ordinates are the bed number, row number, plant number within a row and leaf number on a chrysanthemum plant. In addition, the crop is vertically divided into two types of layers: canopy layers and LAI strata. Canopy layers are used to simulate the foraging behavior of larvae, whereas LAI strata are used to simulate the profile of sprayed polyhedra within the canopy (Figure 7.1b). All processes are simulated with a time step of integration of 0.05 day.

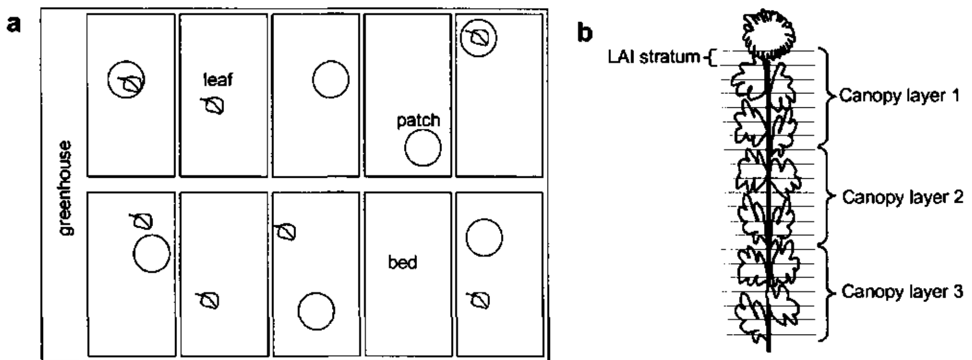

Figure 7.1 *Schematic representation of the four levels of spatial detail in BACSIM: the greenhouse, chrysanthemum bed, patch and leaf scale (a). Schematic representation of a chrysanthemum crop divided into LAI strata and canopy layers (b).*

Greenhouse description and crop growth

The greenhouse is considered as a compartment with plants in growing beds. Moths emerging in the greenhouse remain in the compartment, but inflight of moths into the greenhouse is possible. Each bed consists of plants of the same age and plant characteristics. Crop growth is characterized by LAI and the number of leaves per plant. LAI and number of leaves per plant

increment are described as logistic growth curves that start with an initial LAI and number of leaves of newly planted cuttings:

$$LAI = LAI_0 + (LAI_{max} - LAI_0)/(1+e^{-rgr \times (t-m)}) \quad (1)$$

where LAI_0 is the initial and LAI_{max} is the maximum LAI (m^2m^{-2}), rgr is the relative growth rate (d^{-1}), t is time (d) and m is the inflection point of the curve (d). Parameter values of equation 1 are given in Table 7.1 (Crop growth). No effects of radiation, temperature or larval feeding are incorporated in the crop model. The leaf canopy is divided into a number of LAI strata. The number of LAI strata is not constant but increase with increasing LAI. At planting, a chrysanthemum crop consists of only 3 LAI strata, whereas old chrysanthemums can have up to 60 LAI strata. The thickness of an LAI stratum is calculated as the maximum LAI (7.0 m^2 m^{-2}) divided by 60 (i.e. each LAI stratum represents 7.0/60 m^2 m^{-2}). The LAI strata are clustered into an upper, middle and lower canopy layer (Figure 7.1b). Each canopy layer contains (approximately) one-third of the actual LAI layers. The LAI strata are used to store information of the densities of sprayed polyhedra, whereas the canopy layers are used to account for the foraging behavior of the different larval instars. The beds are harvested when a certain LAI is reached and replaced by cuttings.

Development of S. exigua

The development, variation in development and attrition of *S. exigua* is simulated by an adapted version of the "fractional boxcar train", which is a methodology for the simulation of stage structured population dynamics (Goudriaan and van Roermund, 1993). Attrition is defined as mortality by any cause except baculovirus infection. In fractional boxcar trains, the total development duration of one stage is distributed over a number of classes or boxcars, each representing a constant proportion of development time. Development is simulated by shifting a fraction of the population from one boxcar to the next after a certain time interval. The time between shifts and the fraction shifted is determined by the rate of development and the dispersion in development time that is to be simulated. Attrition is simulated by removing individuals from the boxcar train. At the end of a boxcar train, individuals can be shifted to another boxcar train, representing the next development stage. In the model described here, insect development and population dynamics are simulated as discrete numbers instead of continuous numbers to enable the allocation of a discrete number of cadavers to leaves.

Table 7.1 *Overview of a selection of parameters for S. exigua and SeMNPV.*

process	parameter	value	L1	L2	L3	L4	L5	unit	reference
Crop growth	minimum LAI	0.3						m^2m^{-2}	Heuvelink and Lee, 1998
	maximum LAI	7.0						m^2m^{-2}	Heuvelink and Lee, 1998
	relative LAI growth rate	0.129						d^{-1}	Heuvelink and Lee, 1998
	inflection point of LAI growth curve	33.9						d	Heuvelink and Lee, 1998
S. exigua development	developmental time[†‡]		3.0	1.7	1.5	1.9	3.7	d	Fye and McAda, 1972
	standard deviation of development time[†‡]		0.5	0.5	0.5	0.6	0.8	d	Fye and McAda, 1972
	conversion factor artificial diet/plant	0.64						-	chapter 4
	attrition		0.40	0.20	0.12	0.05	0.00	-	chapter 4
	larval consumption		0.12	0.32	1.2	8.2	20.	cm^2	Bianchi, unpublished data
	sex ratio	0.5						-	Lee *et al.*, 1991
	number of egg batches per female	13						-	Smits, unpublished data
	mean egg batch size	35						-	Smits *et al.*, 1986
	dispersion parameter for egg batch size	2.89						-	derived from Smits *et al.*, 1986
Spatial distribution	number of plants in foraging domains		9	30	42	72	100	-	derived from Smits *et al.*, 1987a
	fraction in top canopy stratum		0.25	0.25	0.5	0.5	0.5	-	derived from Smits *et al.*, 1987a
	fraction in middle canopy stratum		0.25	0.25	0.33	0.33	0.33	-	derived from Smits *et al.*, 1987a
	fraction in bottom canopy stratum		0.5	0.5	0.17	0.17	0.17	-	derived from Smits *et al.*, 1987a
Spray deposition	extinction factor spray	0.8						-	Bianchi, unpublished data
Polyhedron inactivation	relative polyhedron inactivation rate	0.10						d^{-1}	present paper
	residual density of polyhedra	$8x10^5$						m^{-2}	present paper
Infection dynamics	infection chance per ingested polyhedron[§]		$2.9x10^{-2}$	$2.6x10^{-2}$	$2.5x10^{-2}$	$2.6x10^{-3}$	$8.5x10^{-5}$	-	chapter 8
			-	-	$4.3x10^{-3}$	$1.7x10^{-3}$	$1.9x10^{-5}$	-	chapter 8
	SeMNPV incubation time[‡]		3.0[∫]	3.0	3.0[∫]	3.3	3.3[∫]	d	chapter 2
	sd of SeMNPV incubation time[‡]		0.1[∫]	0.1	0.1[∫]	0.4	0.4[∫]	d	chapter 2
Horizontal transmission	leaf visit rate		1.0	2.0	2.0	2.9	1.5	d^{-1}	chapter 4

[†] parameter values for 27°C

[‡] interpolated value between 25°C and 30°C

[§] upper and lower line represent infection chance per ingested polyhedron of relatively susceptible and resistant sub-populations, respectively.

[∫] extrapolated from values of second and fourth instar *S. exigua* larvae

S. exigua passes several stages during its development from egg to adult. The relational diagram of the life cycle of immature *S. exigua* is presented in Figure 7.2. *S. exigua* has an egg stage, five larval stages, a pupal and an adult stage. All insect stages have specific development times, dispersion and attrition rates that are affected by temperature (Fye and McAda, 1972; Lee *et al.*, 1991). Each stage is described by a "fractional integer boxcar train". The development of third, fourth and fifth instar larvae is simulated in two parallel boxcar trains for relatively susceptible and resistant larval populations. Half of the second instar larvae develop into relatively susceptible third instar larvae whereas the other half develop into relatively resistant third instar larvae. Susceptible third instar larvae remain susceptible in the following larval instars, while the resistant third instar larvae develop into resistant fourth and fifth instar larvae. All stages have four boxcars, except for the adult males and females which each have two. The input data for the boxcar train of each stage are development rate and its standard deviation as a function of temperature, as well as the relative attrition rate (Table 7.1, *S. exigua* development). Hatched larvae pass through five larval stages, pupate and emerge as adults. The fraction of eggs or larvae that develop normally are shifted to the boxcar train of the next stage, a fraction may die by attrition and (only for the larvae) a fraction may become infected.

For each patch another set of boxcar trains simulates the development and attrition of contaminated eggs and infected larvae. Larvae that become infected are transferred from the boxcar trains of uninfected larvae to the boxcar trains for infected larvae. A fraction of infected larvae may die by attrition and the remainder develops into cadavers containing infectious polyhedra. Bodies of infected larvae that die by attrition are not considered infectious.

The delay between infection and death of infected larvae is the virus' incubation period, which is affected by temperature and may differ for different larval instars (Table 7.1, Infection dynamics). The mean and standard deviation of time to kill of the virus, both functions of temperature, are input data for the boxcar trains for infected larvae.

Uninfected L4 larvae that develop normally are shifted to a boxcar train that simulates the development of L5 larvae. A fraction of L5 larvae develops into pupae, whereas other fractions may die by attrition, or may become lethally or sublethally infected. The development of insects in these various stages are simulated in sets of boxcar trains for uninfected pupae, lethally infected L5 larvae, sublethally infected L5 larvae and sublethally infected pupae. The sex ratio determines the fraction of pupae that develop into male or female moths, respectively. All new emerging female moths are assumed to mate with male

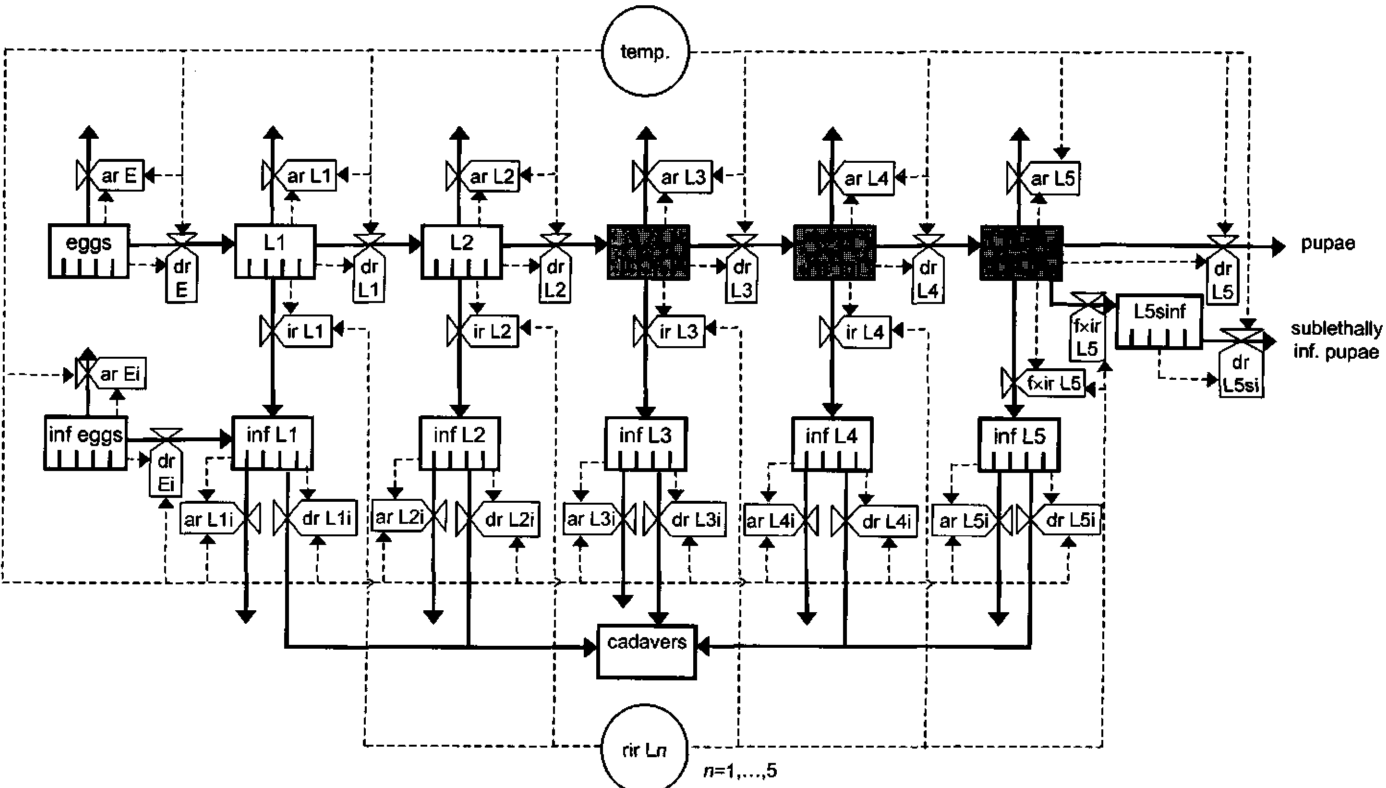

Figure 7.2 *Relational diagram of the development of eggs and larvae of S. exigua at the patch scale. The diagram consists of a flow of uninfected insects (top) and infected insects (down). Legend: dr E = development rate of eggs; dr Ei = development rate of contaminated eggs; dr Ln = development rate of Ln larvae; dr Lni = development rate of infected Ln larvae; dr L5si = development rate of sublethally infected L5 larvae; ar E = attrition rate of eggs; ar Ei = attrition rate of contaminated eggs; ar Ln = attrition rate of Ln larvae; ar Lni = attrition rate of infected Ln larvae; ir Ln = infection rate of Ln larvae; rir Ln = relative infection rate of Ln larvae; f = fraction; n=1,..,5. The development of third, fourth and fifth instar larvae (gray boxcars) are simulated in two parallel boxcar trains for relatively susceptible and resistant sub-populations.*

moths. Fertilized female moths deposit a fixed number of egg batches that vary in size. The number of eggs per egg batch is drawn from a negative binomial distribution with a mean of 35 eggs per egg batch and a dispersion parameter of 2.89 (derived from Smits *et al.*, 1986). Each egg batch results in a cluster of larvae that feed on the crop.

BACSIM contains stochastic elements. The following processes are simulated using a random generator: mating of sublethally infected and uninfected moths, the simulation of egg batch sizes and their location in the crop, the number of contaminated eggs in egg batches laid by sublethally infected moths, the location of cadavers in the crop and the rounding off of population numbers to discrete numbers during insect development.

Spatial distribution of S. exigua

Female moths prefer young plants for egg deposition. In the model, egg batches are deposited randomly in chrysanthemum beds with an LAI lower than 3.5 (derived from Smits *et al.*, 1986). When no chrysanthemum beds with an LAI less than 3.5 are available, the egg batches are deposited randomly over all beds. The locations of egg batches in the crop are recorded using the bed number, row number and the number of the plant in the row. From egg batches new patches will develop, characterized by the co-ordinates of the plant on which the eggs were laid.

The model does not simulate the foraging behavior of individual larvae, but defines foraging domains (for movement in the horizontal plane) and canopy layers (for movement in the vertical plane) in which larvae of specific stages forage. It is further assumed that the larvae are distributed homogeneously within each of these compartments. The foraging domain of each larval instar consists of a fixed number of plants (Table 7.1, Spatial distribution), and the borders are characterized by a number of plants north, west, south and east of the plant on which the eggs were laid. Hence, the larval foraging domains are assumed to be rectangular. The foraging domains of larval instars include an increasing number of plants and cover the foraging domain of the preceding instar. The spatial distribution of patches in the crop will be used for the simulation of horizontal transmission.

The preference for the upper, middle or lower canopy layers by larvae of specific larval instars is simulated by allocating fractions of residence time of these larval instars over the three canopy layers of the plants in their foraging domains (Smits *et al.*, 1987). L1 and L2 larvae forage mainly in the lower and middle canopy layers, whereas the later instar larvae forage mainly in the middle and upper canopy layers (Table 7.1, Spatial distribution).

Spray deposition

The model simulates spray deposition for greenhouse chrysanthemum crops that are sprayed from the top. The simulated polyhedron density on leaves after applications decreases from top to bottom of the plants according to an exponential polyhedron interception profile, which is characterized by an extinction factor k (Table 7.1, Spray deposition). The intercepted polyhedra of an LAI stratum after a virus application are added to the polyhedra, which were still present from previous applications. The number of intercepted polyhedra per LAI stratum is converted to a mean density of sprayed polyhedra per canopy layer.

Polyhedron inactivation

Polyhedra that are sprayed on the crop may be inactivated by UV radiation. Two fractions of polyhedra can be distinguished: a residual fraction of infectious polyhedra that is not inactivated and a fraction of infectious polyhedra that are inactivated in an exponential fashion. Hence, inactivation of sprayed polyhedra can be simulated by assuming a constant relative inactivation rate and a residual density of inactivated polyhedra (chapter 3):

$$PIB = PIB_{res} + (PIB_0 - PIB_{res}) \times e^{(-rir \times t)} \quad (2)$$

where PIB is the density of infectious polyhedra (m^{-2}), PIB_0 is the initial density of infectious polyhedra (m^{-2}), PIB_{res} is the density of residual infectious polyhedra (m^{-2}), rir is the relative polyhedron inactivation rate (d^{-1}) and t is time (d) (Table 7.1, Polyhedron inactivation). Polyhedra produced in cadavers are assumed to be inactivated at the same relative polyhedron inactivation rate as sprayed polyhedra.

Infection by ingestion of sprayed polyhedra

Larvae may become infected after ingestion of polyhedra. The number of larvae that will be infected by ingestion of sprayed polyhedra in a patch can be calculated by the multiplication of the number of larvae that are in a particular instar and the relative infection rate by ingestion of sprayed polyhedra for that instar. The relative infection rate by ingestion of sprayed polyhedra depends on the leaf consumption rate of the larvae, the polyhedron density of the canopy layer and the infection chance per ingested polyhedron for each instar. The susceptibility of populations of first and second instar larvae is characterized as an infection chance per ingested polyhedron which is used for the whole population, whereas for third, fourth and fifth instar larvae the susceptibility is characterized for relatively susceptible and

resistant subpopulations (Table 7.1, Infection dynamics). The relative infection rate by sprayed polyhedra for a specific larval instar can then be calculated from (van der Werf *et al.*, 1991):

$$r_s = C \times PD \times p \qquad (3)$$

where r_s is the relative infection rate by ingestion of sprayed polyhedra (d^{-1}), C the leaf consumption rate ($cm^2\ d^{-1}$), *PD* the polyhedron density (cm^{-2}) and p the infection chance per ingested polyhedron for that larval instar or subpopulation (-).

The susceptibility of *S. exigua* to baculoviruses are generally determined in larvae reared on artificial diet. However, this susceptibility can be affected by the food source on which the larvae are reared. *S. exigua* populations that were reared on chrysanthemum had approximately two times higher infection chances per ingested polyhedron than larvae that were reared on artificial diet (chapter 4). Thus, larvae in greenhouse chrysanthemum are more susceptible than larvae reared on artificial diet in the laboratory. In addition, first to fourth instar *S. exigua* larvae feed partly on the underside of chrysanthemum leaves without perforating the leaf (chapter 4, Smits *et al.*, 1987). Since at this location virtually no polyhedra are deposited, the larval polyhedron intake will be reduced. However, the exact reduction in polyhedron intake by this feeding behavior is difficult to assess.

Hence, there are two processes of the same order of magnitude that affect *S. exigua* susceptibility in opposite directions: larvae feeding on chrysanthemum are more susceptible to polyhedra, but because these larvae are partly feeding on the underside of the leaves, their polyhedron uptake is limited. It is assumed that both processes neutralize each other.

Infection by horizontal transmission

Infected larvae that turn into cadavers spill polyhedra on leaves. Horizontal transmission is defined as the infection of uninfected larvae by ingestion of polyhedra spread by these cadavers. The location of cadavers is simulated at the leaf scale and this is used to calculate the relative infection rate due to horizontal transmission of larvae foraging in patches. The relative infection rate by horizontal transmission is the same as the probability per unit time that a larva consumes part of a contaminated leaf. Each virus-killed insect is assumed to contaminate the area of a whole leaf immediately upon death with a lethal dose of polyhedra. Polyhedra on leaves are assumed to contain infectious virions until the bed is harvested.

For each patch, the fraction of contaminated leaves in the foraging domains of each larval instar are calculated on the basis of the co-ordinates of cadavers, the number of plants in the foraging domain and the number of leaves per plant. The relative infection rate by

horizontal transmission per larval instar is determined by the fraction of contaminated leaves in the larval foraging domain and the number of leaf visits per day of the larvae (Table 7.1, Horizontal transmission). The relative infection rate by horizontal transmission can thus be calculated as:

$$r_{ht} = \Delta t^{-1} \times (1 - (1 - f)^{LV \times \Delta t}) \qquad (4)$$

where r_{ht} is the relative infection rate by horizontal transmission (d^{-1}), Δt the time step of integration (d), f the fraction of contaminated leaves in the larval foraging domain (-) and LV the number of leaf visits per day (d^{-1}).

Infection by vertical transmission

Vertical transmission is the direct transfer of virus from sublethally infected moths to their offspring. Fifth instar larvae exposed to polyhedra may become sublethally infected. These larvae develop into sublethally infected moths, which are able to transmit the virus infection to part of their offspring (chapter 5; Smits and Vlak, 1988a). Uninfected and sublethally infected female moths are assumed to have no mating preference for uninfected or sublethally infected male moths. Assuming that female moths mate only once and males mate as often as is needed to fertilize all females, the chance of an uninfected female mating with a sublethally infected male is equal to the proportion of sublethally infected males. Both sublethally infected female moths and uninfected female moths that mated with a sublethally infected male are assumed to be able to deposit contaminated egg batches. Not all egg batches from these female moths are contaminated. In a study described in chapter 5 using sublethally *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) infected *S. exigua* moths, 70% of the egg batches were virus free, whereas the remaining 30% included at least one contaminated egg. Egg batches containing 40 eggs or more generally gave raise to high proportions of contaminated eggs. In contrast, egg batches containing less than 40 eggs rarely contained contaminated eggs, and if these egg batches contained contaminated eggs, these contaminated eggs only constituted a small proportion of the total number of eggs. Vertical transmission is simulated using a critical egg batch size that determines whether an egg batch is large or small; both types of egg batches are assumed to have a specific chance to contain at least one contaminated egg. The number of contaminated eggs in small and large contaminated egg batches is drawn from a uniform distribution, and both type of egg batches have specific maximum fractions of contaminated eggs per batch. Contaminated eggs develop into infected L1 larvae, which can transmit the virus horizontally when they die.

Crop injury

Crop injury by *S. exigua* larvae is expressed as the area foliage consumption and as the number of damaged plants. The area foliage consumption in the greenhouse is calculated as the sum of the daily foliage consumption (C, $cm^2\ d^{-1}$) in all patches. The foliage consumption of infected larvae is determined by the instar in which the larvae become infected and the incubation time of the virus. As a consequence, larvae infected with a fast killing virus will feed less than larvae infected with a slower killing virus.

The rate at which *S. exigua* larvae infest chrysanthemum plants is derived from the larval leaf visit rate (LV, d^{-1}) and the larval foraging domains. The plant visit rate for each larval instar is calculated as a fraction of the larval leaf visit rate. Larvae are assumed to damage plants within the foraging domain of their instar and to have no preference for feeding on intact or damaged plants. The plant damage caused by infected larvae is determined by instar in which the larvae become infected and the incubation time of the virus. Larvae infected with a fast killing virus will infest a smaller number of plants than larvae infected with a slower killing virus.

7.3 Model parameterization

LAI growth of greenhouse chrysanthemum

LAI growth data of chrysanthemum were obtained from Heuvelink and Lee (1998). Chrysanthemums of cultivar Cassa were grown in greenhouses under normal cultivation practice conditions and in absence of *S. exigua* in the summer of 1997. LAI was measured by destructive sampling. Logistic growth curves were fitted to LAI data using Genstat (Payne *et al.*, 1993). Fitted values for the initial LAI, the maximum LAI, the LAI relative growth rate and the inflection point of the LAI curve were used as parameters for the simulation model.

Development of S. exigua

First instar *S. exigua* larvae were reared until the fifth instar in 12-well tissue culture plates that contained 1 ml 1.5% agar. Fresh chrysanthemum leaf discs (cultivar Tiger) were provided daily. The wells were covered with two layers of paper tissue and a lid. Larvae were reared at 23°C and the instar of each larva was recorded daily until all larvae had pupated. Mean developmental rates and standard deviation of developmental rates were calculated and used as parameters for the simulation model.

Baculovirus infectivity

The SeMNPV dose-mortality relationship was determined in a leaf disc bioassay, as described in chapter 3. Groups of 36 third instar *S. exigua* larvae were allowed to ingest leaf discs containing 6, 17, 60, 170, 600, 1700 and 6000 SeMNPV polyhedra, respectively. Larvae that ingested the entire disc within 24 h were further reared on fresh artificial diet. Larval mortality was recorded after all larvae had either pupated or died. The infection chances per ingested polyhedron were determined by fitting equation 6 to dose-mortality data using Genstat. The fitted infection chances per ingested polyhedron were used as parameters for the simulation model.

For first and second instar larvae the relationship between the dose of ingested polyhedra and larval mortality is described with an exponential model (Peto, 1953; Hughes *et al.*, 1984). This model assumes that each ingested polyhedron has the same chance of causing a lethal infection (p) and that all larvae are equally susceptible to the virus. The exponential model can be noted as:

$$M = 1 - (1-p)^{n} \quad (5)$$

where M is the fraction mortality (-), p is the infection chance per ingested polyhedron (-) and n is the polyhedron dose (-). Since larvae become more resistant to baculovirus infection in their later instars, each instar has a specific infection chance per ingested polyhedron. Populations of third, fourth and fifth instar *S. exigua* larvae generally show variation in susceptibility against baculoviruses, which is reflected in reduced slopes of dose-mortality relationships (chapter 2; Engelhard and Volkman, 1995). Variation in larval susceptibility cannot properly represented by equation 5 and therefore the development of third, fourth and fifth instar larvae is simulated in two parallel boxcar trains for relatively susceptible and resistant sub-populations. The sub-populations have equal sizes and a specific infection chance per ingested polyhedron (Eq. 6):

$$M = 1 - 0.5 \times (1-p_r)^{n} - 0.5 \times (1-p_s)^{n} \quad (6)$$

where M is the fraction mortality (-), p_r and p_s are the infection chances per ingested polyhedron for relatively susceptible and resistant sub-populations (-), and n is the polyhedron dose (-).

Baculovirus speed of action

Time-mortality data of chapter 2 were used. Thirty-six fourth instar *S. exigua* larvae were inoculated with an SeMNPV suspension containing 10^5 polyhedra ml^{-1} and reared on fresh artificial diet at 27°C. Larval mortality was recorded twice per day. Mean time to kill and its standard deviation were calculated and used as parameters for the simulation model.

Polyhedron inactivation

Polyhedron inactivation data of SeMNPV were obtained from a similar experiment as described in chapter 3. Groups of 36 third instar *S. exigua* larvae were allowed to ingest leaf discs containing SeMNPV polyhedra with exposure times of 0, 7, 14, 21 and 28 days on greenhouse chrysanthemum leaves. Three repetitions were included in the experiment. Mortality of larvae that ingested leaf discs with exposed polyhedra was converted to number of infectious polyhedra by the inclusion of a dose-mortality reference line. Polyhedron inactivation curves were fitted to polyhedron inactivation data for each repetition using Genstat. The mean relative inactivation rate and mean density of residual infectious polyhedra were used as parameters for the simulation model.

Vertical transmission

SeMNPV vertical transmission data are obtained from chapter 5. Sublethally infected *S. exigua* moths were allowed to mate and the egg batch size, the number of hatched larvae and the number of first instar larvae that developed SeMNPV infection were determined. The vertical transmission parameters were estimated from the experimental data and used for the simulation of vertical transmission.

Horizontal transmission

The parameters for the horizontal transmission process are based on observations of *S. exigua* foraging behavior. Data from an independent horizontal transmission experiment (chapter 5) were used to test the simulation model. In this study groups of 100 first instar *S. exigua* larvae containing 0, 1, or 10 primarily infected larvae were released in greenhouse chrysanthemum plots. When the larvae reached the fifth instar, the larvae were recovered from the plots and larval survival was determined. The simulation model was initialized with the same larval densities, larval developmental rates and crop characteristics as in the greenhouse study. The attrition rates of the larval stages were calibrated so that simulated survival in the treatment without primarily infected larvae corresponded with measured survival. Ninety-five binomial

confidence intervals of surviving larvae recovered form plots were calculated with Mathcad 7.

7.4 Model verification

The performance of the individual model components for LAI growth, *S. exigua* development, baculovirus infectivity, baculovirus incubation period, polyhedron inactivation, vertical and horizontal transmission were compared to experimental data. The verification procedure consisted of the fitting of the model components to experimental data, which resulted in parameter values for each model component. These parameters were used as input for the model and simulations results were then compared to the original data. The data were therefore dependent for all model components, except for the horizontal transmission component because parameters for this model component were not estimated from this experiment.

The growth of chrysanthemum LAI can be described by a logistic growth curve (R^2 = 0.996; Figure 7.3a). The parameters of the LAI growth curve are presented in Table 7.1 (Crop growth).

The measured and simulated dose-mortality relationships are presented in Figure 7.3b. The fitted infection chances per ingested polyhedron of SeMNPV for the relatively susceptible and resistant sub-populations of third instar *S. exigua* larvae were 0.0203 and 0.00205 (Eq. 6). The simulated dose-mortality relationship using the double exponential model corresponded well with measurements (R^2 = 0.984).

The simulation model reproduced the time-mortality relationships of fourth instar *S. exigua* larvae infected with SeMNPV and the larval development of *S. exigua* in greenhouse chrysanthemum well (Figures 7.3c and e). The measured and simulated time to kill were 3.82 ± 0.67 and 3.85 ± 0.82 days, respectively. The measured and simulated inactivation of SeMNPV polyhedra is given in Figure 7.3d.

Inactivation of SeMNPV polyhedra on chrysanthemum was adequately characterized by an exponential inactivation curve with a mean relative inactivation rate of 0.10 day^{-1} and a density of residual infectious polyhedra of 8 x 10^5 polyhedra m^{-2} leaf surface. The simulated inactivation of SeMNPV polyhedra with these two parameters was well in line with measurements (Figure 7.3d).

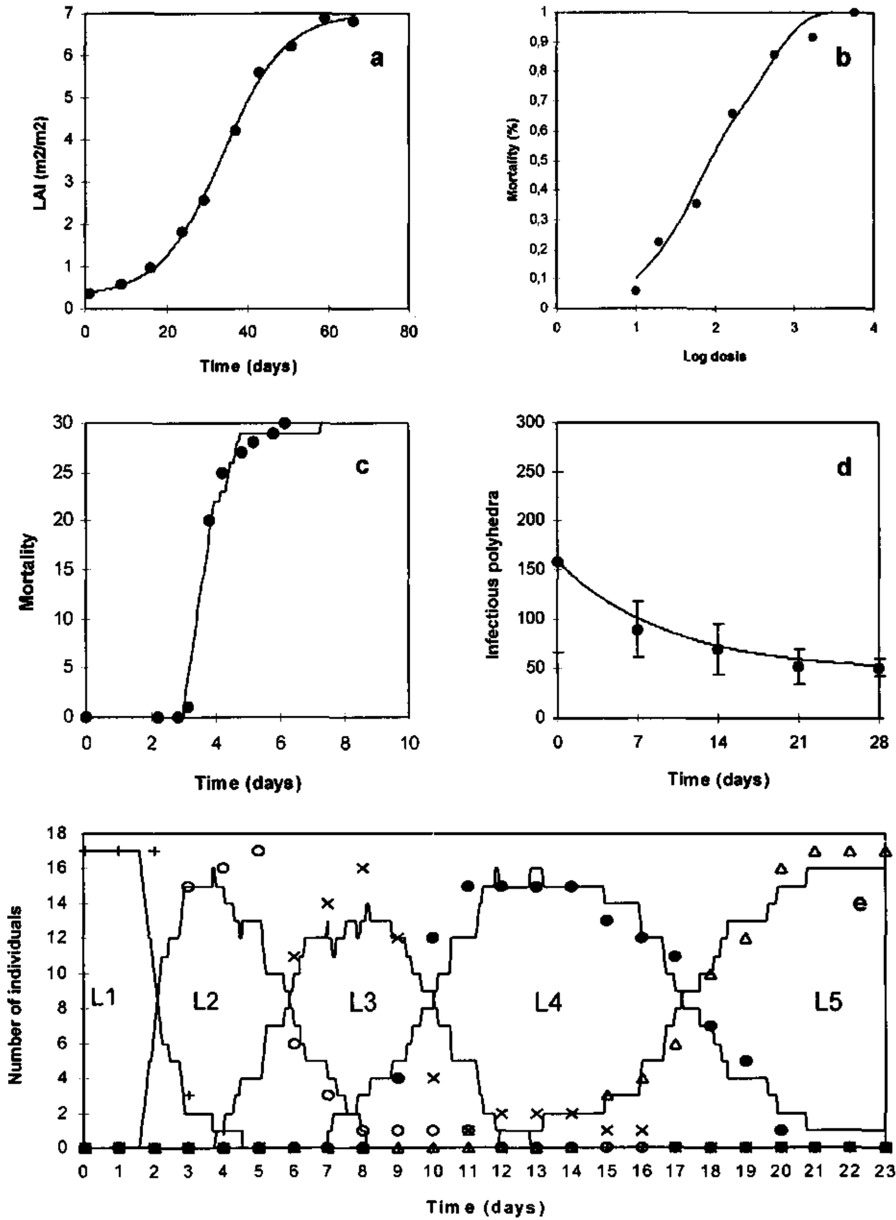

Figure 7.3 *Measured and simulated LAI growth of greenhouse chrysanthemum (a), dose-mortality relationships of third instar S. exigua larvae exposed to 7 doses of SeMNPV polyhedra (b), time-mortality relationships of fourth instar S. exigua larvae infected with a SeMNPV suspension containing* 10^5 *polyhedra* ml^{-1} *(c), inactivation of SeMNPV polyhedra on greenhouse chrysanthemum (d) and development of first (+), second (○), third (×), fourth (●) and fifth instar (Δ) S. exigua larvae on chrysanthemum at 23°C (e).*

The measured and simulated vertical transmission of SeMNPV by sublethally infected *S. exigua* moths to their progeny are given in Figure 7.4. The simulated distribution of infected progeny over egg batches gives a good reflection of the actual measurements. In the experiment 18% of the total progeny was SeMNPV infected and 30% of the egg batches contained one or more infected larvae. In the simulations, this was 7% and 36%, respectively.

Measured and simulated survival in populations of 100 first instar *S. exigua* larvae with 0, 1 or 10 of primarily infected larva are presented in Figure 7.5. The simulations of 99 uninfected first instar in presence of 1 primarily infected larvae overestimated larval survival of fifth instar larvae. However, in treatments with 90 uninfected first instar larvae with 10 primarily infected larvae, simulations were in agreement with measurements, but still overestimated survival.

7.5 Discussion

We developed a spatially explicit, process-based simulation model to simulate the epidemiology of crop-insect-baculovirus systems in greenhouses. This simulation model can be used to evaluate the potential of baculoviruses as biological control agents of pest insects. The basic assumption of the model is that the agronomic efficacy of any virus as a biological control agent can be characterized by the biological characteristics infectivity, incubation period, inactivation and horizontal and vertical transmission dynamics of the virus-insect system. These processes are described in separate model components and integrated in order to simulate the overall dynamics of baculovirus epidemics in insect populations in a crop.

The modeling approach used in this study differs fundamentally from analytical models that describe baculovirus epidemiology in insect populations (Anderson and May, 1981; Dwyer, 1992; Dwyer and Elkinton, 1993; Dwyer *et al.*, 1997). The parameters that are used in these models often aggregate a number of underlying processes into a single parameter, such as the transmission parameter used by Anderson and May (1981). This transmission parameter takes all processes that affect horizontal transmission in insect populations into account (e.g., insect foraging behavior, susceptibility of insects and distribution of virus over the host plants). The transmission parameter summarizes the complex interaction of a number of processes and the parameter itself has no biological meaning. In contrast, the simulation approach describes the crop-insect-virus system at a more detailed level and the

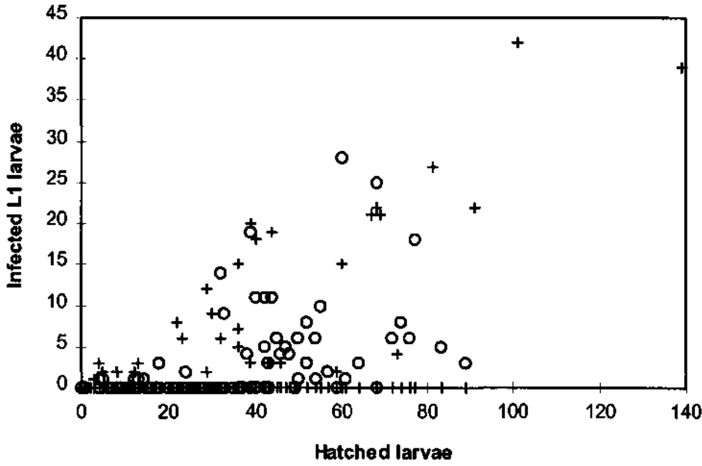

Figure 7.4 *Measured (○) and simulated (+) relation between the number of hatched larvae in egg batches deposited by sublethally infected S. exigua larvae and the number of first instar larvae developing SeMNPV infection.*

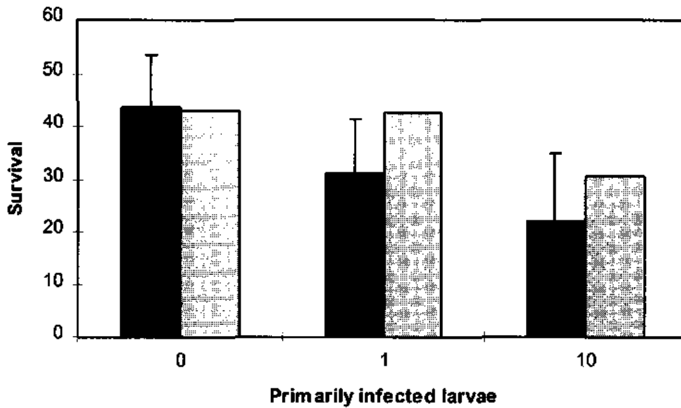

Figure 7.5 *Measured (dark shading) and simulated survival (light shading) of populations of 100 S. exigua larvae containing 0, 1 or 10 primarily infected first instar larvae in greenhouse chrysanthemum. Error bars represent 95%-confidence intervals.*

model components can be studied independently in small scale experiments with measurements on individual insects. The parameters used in BACSIM are generally based on direct measurements on individuals and are biologically meaningful.

The separate model components were verified by comparison of model simulations with experimental data. The model components LAI growth, the infection process of larvae and polyhedron inactivation can well be described with simple equations (Figures 7.3a, b and d).

Larval development and time-mortality relationships are simulated using the boxcar train technique (Goudriaan and van Roermund, 1993) and provide a satisfactory description of the development processes (Figures 7.3c and e). As to vertical transmission, the model underestimated the fraction of contaminated eggs, whereas the fraction contaminated egg batches were in close agreement with measurements (Figure 7.4). The model component that describes vertical transmission contains a number of stochastic elements. The predicted proportion contaminated eggs and proportion contaminated egg batches by the model is strongly affected by this stochasticity. Model simulations of the horizontal transmission process were compared with independent data of a horizontal transmission experiment in greenhouse chrysanthemum (Figure 7.5). Although the survival of fifth instar larvae was slightly overestimated in the situation of 99 uninfected first instar in presence of 1 primarily infected larvae, model simulations were in line with observations in the case of 90 uninfected first instar in presence of 10 primarily infected larvae. In conclusion, the description of the separate model components at the process level was in general in close agreement with measurements.

Although the verification did not produce evidence for major flaws in the model, the proper test for the assessment of the validity of the model is the comparison of model simulations with independent data of the total dynamics of baculovirus epidemics in insect populations in a crop (model validation). The model should be validated for at least two viruses with distinct biological characteristics (e.g. infectivity and incubation period) and a number of spraying regimes and different instars. The validation and sensitivity analysis of model parameters will be presented in chapter 8. The sensitivity analysis is used to analyze the effect of small changes of model parameters on simulation output and reflects the importance of baculovirus characteristics for the agronomic efficacy as a biological control agent. A validated simulation model can be used to predict the efficacy of (genetically engineered) baculoviruses in insect-crop systems for a variety of situations. Scenario studies with the validated model will be presented in chapter 9.

8

Validation of a process-based model for the biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses

This paper describes the validation and sensitivity analysis of a process-based simulation model for the control of beet armyworm, Spodoptera exigua, with baculoviruses (BACSIM). Model predictions are compared to results of independent greenhouse experiments in which second, third or fourth instar larvae of S. exigua in chrysanthemum plots are treated with different concentrations of Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) and S. exigua MNPV (SeMNPV), two viruses with distinct differences in infectivity and speed of action. BACSIM provides robust predictions for the control of S. exigua populations in greenhouse chrysanthemum with both AcMNPV and SeMNPV. Mortality levels of AcMNPV and SeMNPV were generally predicted within a 25% margin of error compared to the observed values. None of the deviations was higher than 40%. All values of simulated foliage consumption, caused by S. exigua populations treated with AcMNPV or SeMNPV applications, fell within 95% confidence intervals of measurements. Simulations of time-mortality relationships were generally shorter than measurements. This discrepancy may be caused by the behavior of S. exigua larvae which feed on the underside of chrysanthemum leaves where they are protected from polyhedra. This suggests that the larval foraging behavior may play an important role in the efficacy of baculovirus applications and should be further studied experimentally. This validated model can be used for the pre-trial evaluation of the efficacy of genetically modified baculoviruses as biological control agents and for the optimization of spraying regimes in chrysanthemum cultivation.

This chapter has been submitted as:
Bianchi, F.J.J.A., Vlak, J.M., and van der Werf, W.

8.1 Introduction

Baculoviruses are insect pathogens that are used as biological control agents in forestry and arable crops (Black *et al.*, 1997; Moscardi, 1999). To evaluate the biological control of *Spodoptera exigua* with (genetically engineered) baculoviruses under glasshouse conditions, we developed a simulation model (BACSIM) (chapter 7). The model is based on a detailed quantitative description of the population dynamics of the insect pest, plant growth characteristics, spray deposition, and on the baculovirus infection cycle and transmission routes. BACSIM is constructed in such a way that the field efficacy of baculoviruses with varying biological properties (e.g. infectivity, speed of action and inactivation rates) and different application strategies can be simulated. Input variables of the model are parameter values describing the biological properties and interactions of the pest, virus and crop, the environmental conditions in the greenhouse, and the timing and dosages of baculovirus applications. Output variables include insect mortality and crop damage. A full description and listing of the model are given in Bianchi and van der Werf (2000).

For the assessment of the applicability of the model and the validity of its predictions simulation results must be compared with independent experimental data of baculovirus efficacy under field conditions. When model simulations are in line with validation experiments, there will be growing confidence that the model can be used to predict the efficacy of genetically engineered baculoviruses and novel application strategies. If the simulations are not in line with field experiments, the discrepancies between simulations and experiments identify areas of incomplete understanding and/or poor system predictability. Validation problems can thus be indicative of overlooked aspects of the system. This role of modeling in the learning process about system behaviour may be as valuable as the predictive capacity of validated models (van der Werf *et al.*, 1999; Rossing *et al.*, 1999).

Although validation is generally considered as an essential step in model development (Rabbinge and de Wit, 1989), only a limited number of models for baculovirus epidemics in insect populations has been validated. Dwyer (1992) and Dwyer and Elkinton (1993) compared outputs from analytical models with independent data on the spatial spread of *Orgyia pseudotsugata* MNPV in *O. pseudotsugata* populations on Douglas-fir seedlings and the dynamics of *Lymantria dispar* MNPV infections in *L. dispar* populations in forest stands.

Sensitivity analysis measures the response of model outputs (e.g., larval mortality, foliage consumption) to changes in inputs (e.g., infectivity, speed of action and inactivation rate), and can be used to scrutinize the performance of the model. Sensitivity analysis could

indicate that a limited number of parameters determines the agronomic efficacy of baculoviruses as biological control agents. This knowledge can direct future biological control and genetic modification strategies to obtain an improved baculovirus control of pest insects.

This paper describes the validation and sensitivity analysis of BACSIM. To test whether BACSIM is applicable for baculoviruses with different biological characteristics, the model is validated for AcMNPV and SeMNPV, two baculoviruses with marked differences in infectivity and speed of action (chapter 2; Smits and Vlak, 1988a). Model predictions are compared with independent data of greenhouse experiments with second, third and fourth instar *S. exigua* larvae treated with different concentrations AcMNPV and SeMNPV in chrysanthemum (chapter 6). In validations as well as in sensitivity analyses, conditions and virus concentrations are chosen to produce intermediate pest mortality, where the model is expected to respond in a sensitive way, as to maximize the chance of finding discrepancies between simulations and measurements. Dose- and time-mortality relationships of AcMNPV and SeMNPV in *S. exigua* populations, as well as predictions of crop damage, are evaluated in validations and sensitivity analyses.

8.2 Material and methods

Greenhouse experiments

To provide test data for model validation, ten factorial experiments were conducted in a greenhouse (10 x 30 m) in Wageningen, The Netherlands, as described in chapter 6. An overview of the experiments and their treatments is given in Table 8.1. In experiments #1 to #6, dose-mortality relationships of SeMNPV and AcMNPV under field conditions were studied, whereas experiments #7 to #10 quantified time-mortality relationships in the crop-insect-virus system. For each experiment, 20 or 24 plots with 64 insecticide-free chrysanthemum plants (cultivar Tiger) were prepared. Each experiment contained 4-8 repetitions (Table 8.1). Experiments started 3 weeks after planting. Hourly temperatures were recorded using a datalogger (Depex datataker 600) with seven thermocouples that were located at a height of 20 cm above soil in the canopy. Minimum night temperatures in the greenhouse were 20°C and the daytime ventilation set point was 29°C.

In all experiments synchronized populations *S. exigua* larvae were released in the chrysanthemum plots and sprayed according to the treatments listed (Table 8.1). Experiments with second and third instar larvae were always sprayed the day after the release of late first

Table 8.1 *Overview of the instar used, SeMNPV and AcMNPV doses (polyhedra m^{-2}), number of initially released larvae, number of treatments, number of repetitions, number of plots, harvest dates, S. exigua attrition during the second to fifth instar and greenhouse temperatures of 10 greenhouse experiments.*

exp #	instar	SeMNPV	AcMNPV	larvae/plot	treatments	reps	plots	harvest dates† (days post treatment)	*S. exigua* attrition				Temperature (mean (min-max))
									L2	L3	L4	L5	
Dose-mortality trials													
1	L2	0, $3x10^5$, $3x10^6$, $3x10^7$	-	100	4	6	24	7-8	0.58	0.40	0.17	0.10	26.5 (23.5 – 36.3)
2	L3	0, $3x10^5$, $3x10^6$, $3x10^7$	-	100	4	6	24	3	-	0.24	0.14	0.10	25.3 (20.8 – 33.3)
3	L4	0, $3x10^5$, $3x10^6$, $3x10^7$	-	100	4	6	24	4-5	-	0.13	0.05	0.05	23.9 (21.2 – 31.9)
4	L2	0, $3x10^5$, $3x10^6$	$3x10^6$, $3x10^7$	100	5	4	20	6-7	0.30	0.25	0.20	0.05	24.4 (21.4 – 32.6)
5	L3	0, $3x10^5$, $3x10^6$	$3x10^6$, $3x10^7$	80	5	4	20	3-4	-	0.23	0.08	0.05	25.8 (22.5 – 32.8)
6	L4	0, $3x10^5$, $3x10^6$	$3x10^6$, $3x10^7$	80	5	4	20	4-5	-	0.31	0.12	0.10	23.3 (19.8 – 31.2)
Time-mortality trials													
7	L2	0, $3x10^7$	$3x10^8$	80	3	8 (4)‡	24	3 & 6	0.22	0.15	0.05	0.05	26.4 (21.6 – 35.0)
8	L3	0, $3x10^7$	$3x10^8$	80	3	8 (4)‡	24	3 & 6	-	0.21	0.08	0.07	24.7 (19.7 – 35.0)
9	L3	0, $3x10^7$	$3x10^9$	80	3	8 (4)‡	24	3 & 6	-	0.41	0.25	0.10	27.2 (23.0 – 35.6)
10	L4	0, $3x10^7$	$3x10^8$	80	3	8 (4)‡	24	3 & 5	-	0.55	0.52	0.20	26.4 (21.3 – 37.1)

† In the dose-mortality trials 1-6, all plots were harvested during a one- or two day period. In time-mortality trials 7-10 there were two times for destructive harvest: 4 plots per treatment were harvested 3 days after application, and another 4 plots per treatment 2 or 3 days later.

‡ Eight repetitions per treatment and four repetitions per observation moment per treatment.

and second instar larvae, whereas in experiments with fourth instar larvae (experiments 3, 6 and 10 ; Table 8.1) late second instar larvae were released and sprayed 2-3 days after the release, such that the larvae were all in the fourth stage at the time of virus application. The experiments #1-6 were destructively harvested 3 to 8 days after virus application in two consectutive days, except for experiment #2 that was harvested in a single day. In experiments #7-10, half of the plots were harvested at 3 days and the other half at 5 or 6 days after virus application. At harvest time, the area of consumed foliage and the number of living and virus-killed larvae in each instar were recorded. Recovered living larvae were transferred to 12-well tissue culture plates filled with artificial diet and reared at 27°C to determine the percentage virus-induced mortality (experiments #1-6) and to quantify survival time (experiments #7-10). Survival was assessed twice per day until all larvae pupated or died.

Model parameterization

Most aspects of the default parameterization of BACSIM for chrysanthemum growth, spray deposition, horizontal transmission, *S. exigua* development and spatial distribution have been described previously (chapter 7). The only difference with these default settings is the fraction background mortality per larval instar (attrition). Attrition in control plots varied in experiments #1-10 and is considered to be an experiment specific factor which is input into the model as measured. Attrition is the only experiment specific factor in the model apart from the spraying schedule, insect introduction and temperature.

The parameters describing the infectivity of AcMNPV and SeMNPV were derived from a substantial number of leaf-disc bioassays with second to fifth instar *S. exigua* larvae that were carried out from 1996 to 1999, as described in chapter 3. The number of bioassays that was carried out per instar-virus combination (n) is indicated in Table 8.2. These bioassays differ in their result, according to uncontrollable variations in susceptibity and experimental conditions. A representative average dose response was determined as follows. For each instar-virus combination, LD_{50} values of all dose-mortality curves were determined using POLO (Russell *et al.*, 1977). The dose-mortality curve with the smallest difference with the mean LD_{50} value, determined by averaging the LD_{50} values of the available bioassays, was selected as the representative dose-mortality curve for that instar-virus combination (repr. LD_{50}; Table 8.2). The parameters of the double exponential model (chapter 7) were then fitted to the representative dose-mortality data of each instar-virus combination using Genstat (Payne *et al.*, 1993). This model assumes that the *S. exigua*

Table 8.2 *Overview of measured and fitted dose-mortality relationships of AcMNPV and SeMNPV in S. exigua larvae. N is the number of dose-response experiments, $logLD_{50}$ and mean LD_{50} represent the mean $logLD_{50}$ value and mean LD_{50} value of series S. exigua populations, respectively, and repr. LD_{50} is the LD_{50} value of the representative dose-mortality curve per instar-virus combination. The parameters p_s and p_r are the fitted infection chances per ingested polyhedron for susceptible and resistant sub-populations of the representative dose-mortality curve and R^2 indicates the goodness of fit of the double exponential model with the fitted parameters p_s and p_r.*

Virus/instar	n	$logLD_{50} \pm SD$	mean LD_{50}†	repr. LD_{50}	p_s	p_r	R^2
AcMNPV							
L2	10	1.90 ± 0.22	80	93	$7.5x10^{-3}$	-	92.4
L3	11	2.72 ± 0.45	515	467	$4.7x10^{-3}$	$3.4x10^{-4}$	99.5
L4	9	3.44 ± 0.16	2757	2657	$6.6x10^{-4}$	$7.9x10^{-5}$	96.3
L5‡	-	5.36	$2.3x10^5$	$2.3x10^5$	$1.2x10^{-5}$	$4.8x10^{-5}$	95.0
SeMNPV							
L2	5	1.32 ± 0.25	21	21	$2.6x10^{-2}$	-	99.0
L3	13	1.76 ± 0.27	58	58	$2.5x10^{-2}$	$4.3x10^{-3}$	99.4
L4	8	2.31 ± 0.30	205	257	$1.7x10^{-3}$	$2.6x10^{-3}$	94.9
L5	1	4.36	$2.3x10^4$	$2.3x10^4$	$8.5x10^{-5}$	$1.9x10^{-5}$	94.2

† Backtransformed from mean LD_{50}

‡ Derived from L5 SeMNPV

population consists of 50% susecptible larvae (with infection chance p_s per polyhedron) and 50% of resistant larvae (with infection chance p_r per polyhedron).

The mean time to kill and the standard deviation of the mean time to kill of AcMNPV and SeMNPV in *S. exigua* larvae were derived from droplet-feeding bioassays (Hughes and Wood, 1981; chapter 2). Third (L3) and fourth (L4) instar *S. exigua* larvae were inoculated with AcMNPV or SeMNPV suspensions that caused mortality levels of approximately 55% (L3) and 90% (L4) and reared on fresh artificial diet at 23, 28 and 33°C. Mean time to kill data of third instar *S. exigua* larvae were also used for second instar larvae, and mean time to kill data of fourth instars were used for fifth instar larvae (Table 8.3).

For AcMNPV and SeMNPV, relative inactivation rates of the polyhedra of 0.10 and 0.16 day^{-1} were used. Residual densities of inactivation-resistant polyhedra of $1.3x10^7$ and $8x10^5$ polyhedra m^{-2} leaf were used, respectively (chapter 3; chapter 7).

Table 8.3 *Instar, mean time to kill (h) and standard deviation of time to kill (h) of AcMNPV and SeMNPV in S. exigua larvae at 23, 28 and 33 °C.*

	SeMNPV			AcMNPV		
Instar	23°C	28°C	33°C	23°C	28°C	33°C
L3	129 ± 13	89 ± 12	64 ± 10	164 ± 11	109 ± 18	90 ± 7
L4	151 ± 38	81 ± 14	64 ± 8	172 ± 18	109 ± 25	104 ± 28

Model initialization and forcing functions

Simulations were initialized with the number and instar of initially released larvae, the concentration and timing of virus applications, *S. exigua* attrition and hourly measurements of greenhouse temperature (Table 8.1). The foraging domains of first to fifth instar larvae were all set equal to the plot size (8 x 8 plants) because the larvae were released randomly over the entire area of the plots. Simulations were repeated fifty times and averaged. No attempt was made to smooth out discrepancies by calibrating the model. The model version validated here is the only version subjected to comparison with these field data.

Sensitivity analysis

The sensitivity of model output to parameters is analyzed for a situation in which a cluster of *S. exigua* larvae originates from an egg batch of 100 eggs in a 3 week old chrysanthemum crop. The crop is sprayed with 1.10^6 polyhedra SeMNPV m^{-2} at 10 days after egg deposition. The model uses hourly temperature measurements of a single day with a mean temperature of 23.8°C and minimumum and maximum temperatures ranging between 18.6 and 31.9°C. These temperature data are repeated for all other days of simulation. The simulation is terminated after all larvae had either died by SeMNPV infection or pupated. The following model parameters were included in the sensitivity analysis: infection chance per ingested polyhedron, virus speed of action (developmental rate of infected larvae), relative inactivation rate of polyhedra and *S. exigua* attrition. Effects of deviations of 10% of these parameter values on larval mortality and foliage consumption were determined. Simulations were repeated a thousand times and averaged.

Statistical analysis

Ninety-five percent binomial confidence intervals of mortality of larvae recovered from plots were calculated with Mathcad 7, whereas 95% confidence intervals of foliage consumption and mean time to kill were calculated using LSD's with Genstat (Payne et al., 1993).

8.3 Results

AcMNPV and SeMNPV infectivity (infection chances per ingested polyhedron) and speed of action (mean time to kill) in *S. exigua* larvae have been determined in laboratory bioassays and are used as input for BACSIM (Tables 8.2 and 8.3). The exponential model provides a good description of the dose-mortality data with R^2 values in all cases above 0.90 (Table 8.2). SeMNPV was more infectious and had a lower mean time to kill *S. exigua* larvae than AcMNPV in each instar tested.

BACSIM was used to simulate the ten independent greenhouse experiments (Table 8.1), using the dose-mortality and time-mortality parameters of Tables 8.2 and 8.3, and experiment-specific inputs for the number and instar of released larvae, dose and timing of virus application, *S. exigua* attrition and temperature. Simulations of the mortality of populations second, third and fourth instar *S. exigua* larvae treated with SeMNPV were generally in close agreement with the actual measurements (Figure 8.1). Simulations of applications of $3x10^6$ polyhedra SeMNPV m^{-2} somewhat overestimated mortality for second (Figures 8.1a and b) and third instar larvae (Figures 8.1c and d). For AcMNPV, simulations of larval mortality corresponded well with measurements for third instar larvae (Figure 8.1d), whereas simulated mortality was overestimated for second and fourth instars (Figures 8.1b and f). For SeMNPV, twelve of the nineteen simulated mortalities differed less than 10% from the observed mortality, three simulated mortalities differed between 10 and 20% from observations, three differed between between 20 and 30%, and one differed between 30 and 40%. For AcMNPV, four of the ten simulated mortalities differed less than 10% from the observed mortality, one simulated mortality differed between 10 and 20% from the observed, three differed between 20 and 30%, and two differed between 30 and 40%. There was no relationship between the size of deviations and the values of the observed mortality (Figure 8.2). As shown in Figures 8.1 and 8.2, the model represents trends in mortality in response to virus species and concentration quite well, although there is some tendency for overestimation of virus impact.

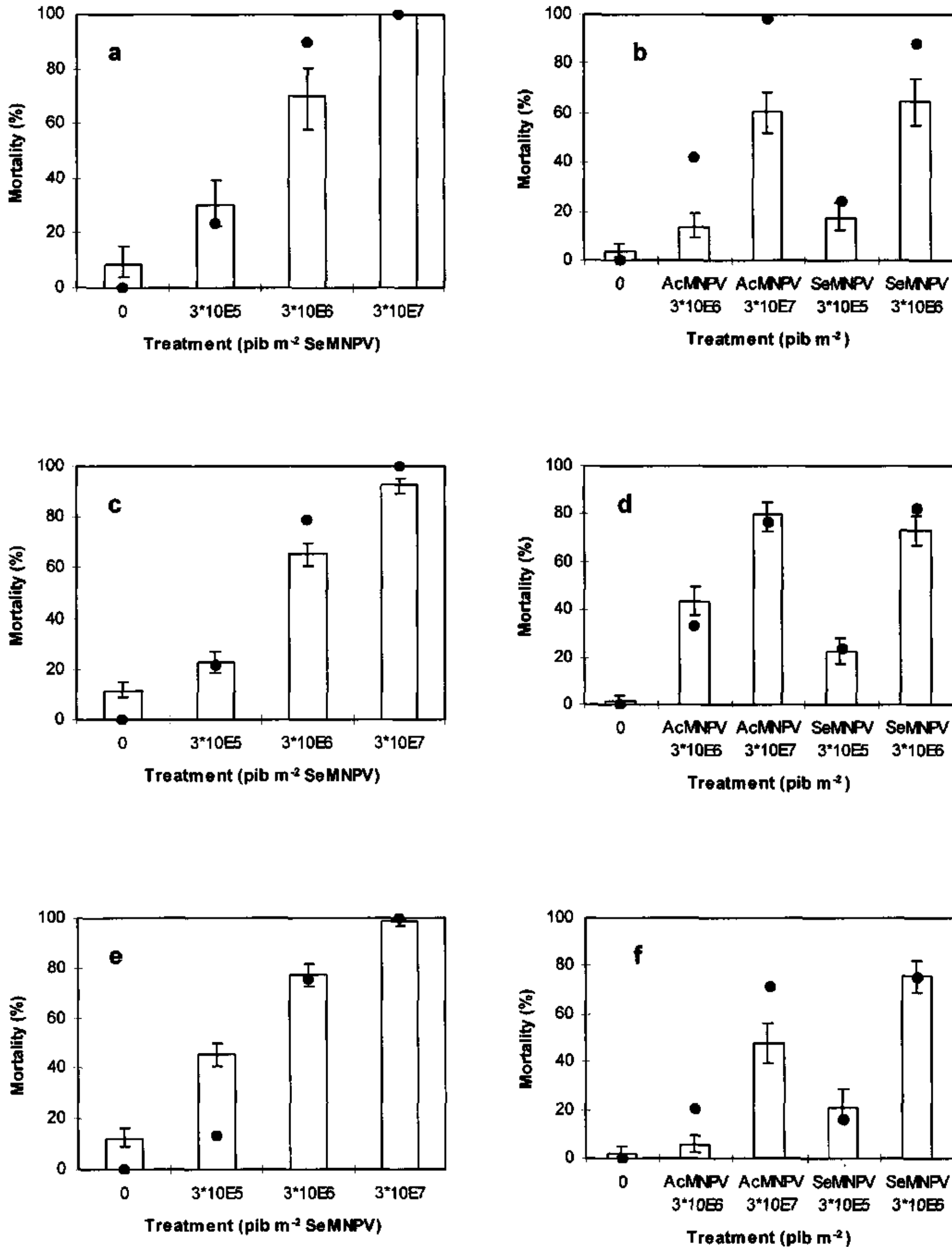

Figure 8.1 *Measured (bars) and simulated (dots) mortality of second (a, b), third (c, d) and fourth instar S. exigua larvae (e, f) treated with AcMNPV and SeMNPV (in pib m^{-2}) in greenhouse chrysanthemum. Error bars indicate 95%-confidence limits.*

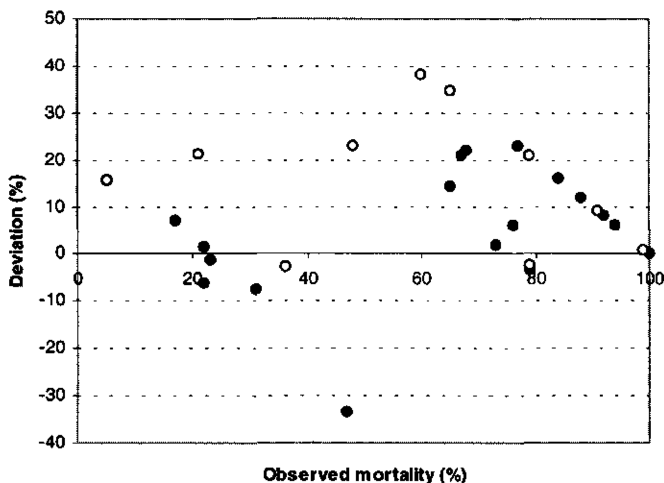

Figure 8.2 *Deviations between simulated and measured mortality of S. exigua larvae treated with AcMNPV (○) and SeMNPV (●) in greenhouse chrysanthemum.*

Measured and simulated foliage consumption of second instar *S. exigua* larvae with AcMNPV and SeMNPV fell in all cases within 95%-confidence intervals of the measurements (Figure 8.3). Thus, the simulated foliage consumption fell within the range in which foliage consumption of new measurements can be anticipated. Simulated and measured foliage consumption of control, AcMNPV and SeMNPV applications was in line with measurements (Figure 8.4). The deviation between simulations and measurements was in general less than 200 cm^2 per plot.

BACSIM generally underestimated AcMNPV and SeMNPV mean time to kill in second (Figure 8.5a), third (Figure 8.5b and c) and fourth instar *S. exigua* larvae (Figure 8.5d). For SeMNPV, the deviations between observed and simulated time to kill ranged between 8 and 45 h., whereas for AcMNPV the deviations were somewhat smaller with maximum deviations of 30 h. (Figure 8.6).

The sensitivity analysis of BACSIM is presented in Table 8.4. The simulation with standard parameter values caused 57% mortality and an area of 836 cm^2 chrysanthemum leaf was consumed by the larvae originating from a single batch of 100 eggs when a spray of 10^6 polyhedra per m^2 was applied 10 days after egg laying. In general, model output was not very sensitive to changes of parameter values. A 10% increase in infection chance per ingested polyhedron of relatively susceptible and resistant sub-populations *S. exigua* (virus infectivity) had a relatively strong impact on larval mortality (6.0%). Ten percent deviations in infection

chance per ingested polyhedron and speed of action caused deviations in foliage consumption around 3 and 5%, respectively. Deviations in the relative inactivation rate of polyhedra of 10% caused less than 1% deviation in simulated foliage consumption, whereas a 10% reduction of larval attrition had a strong effect on foliage consumption (10-11%).

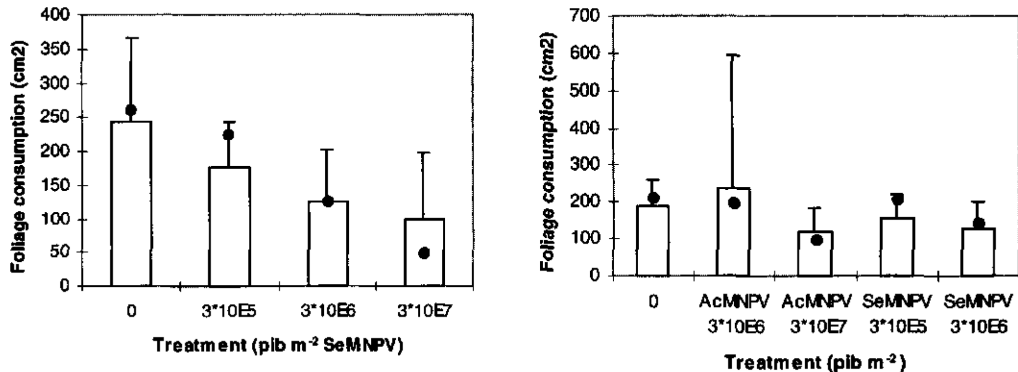

Figure 8.3 *Measured (bar) and simulated (dot) foliage consumption of second instar S. exigua larvae treated with AcMNPV and SeMNPV in greenhouse chrysanthemum. Error bars indicate 95%-confidence limits.*

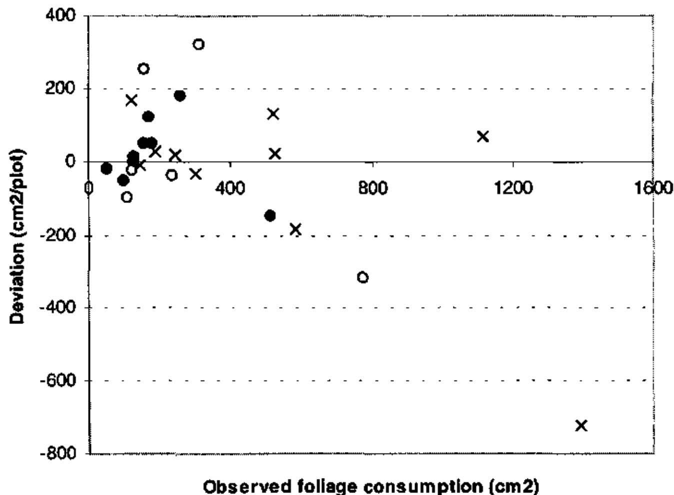

Figure 8.4 *Deviations between simulated and measured foliage consumption of S. exigua larvae after control (×), AcMNPV (○) and SeMNPV (●) treatments in greenhouse chrysanthemum.*

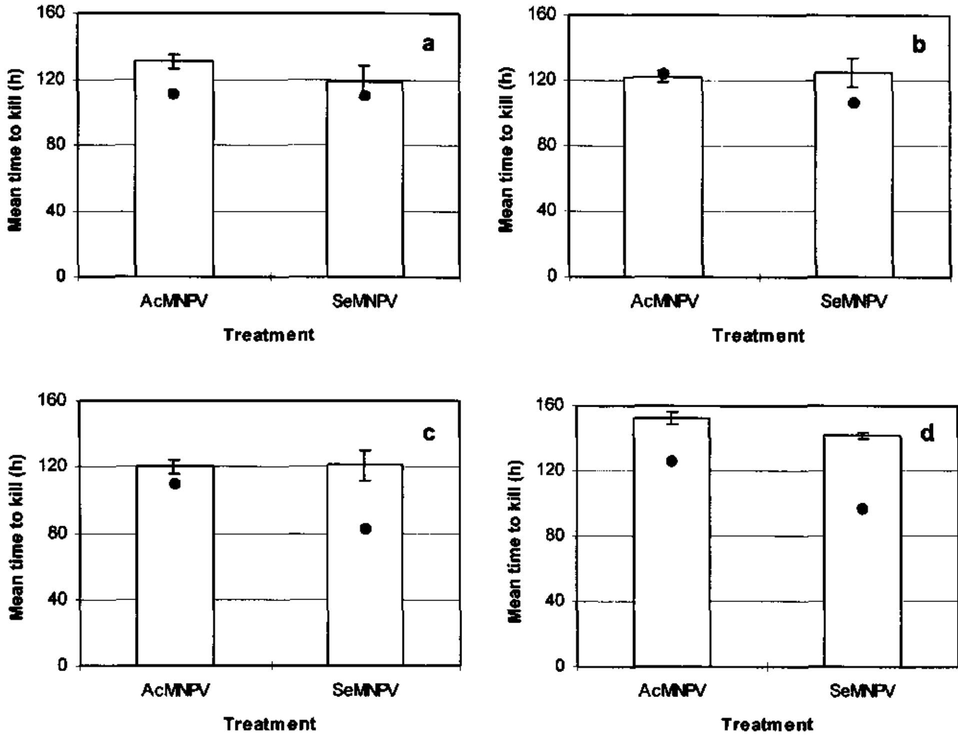

Figure 8.5 *Measured (bar) and simulated (dot) mean time to kill of second (a), third (b, c) and fourth instar S. exigua larvae (d) treated with AcMNPV and SeMNPV in greenhouse chrysanthemum. Error bars indicate 95%-confidence limits.*

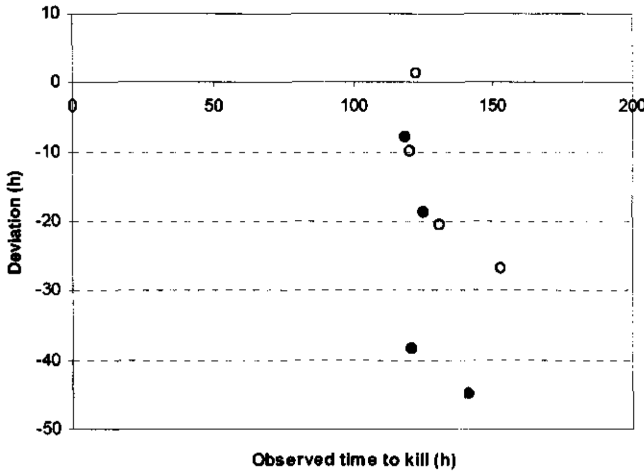

Figure 8.6 *Deviations between simulated and measured mean time to kill of S. exigua larvae treated with AcMNPV (○) and SeMNPV (●) in greenhouse chrysanthemum.*

Table 8.4 *Sensitivity analysis of a selection of parameters on the output variables larval mortality and foliage consumption.*

Parameter	deviation	deviation in larval mortality (%)	deviation in foliage consumption (%)
Infection chance per polyhedron	+ 10%	+ 6.0	- 3.3
	- 10%	- 5.6	+ 2.6
Speed of action	+ 10%	+ 1.2	- 4.7
	- 10%	- 1.9	+ 5.3
Rel. inactivation rate of polyhedra†	+ 10%	- 3.0	+ 0.9
	- 10%	+ 1.8	- 0.5
S. exigua attrition	+ 10%	- 0.8	- 10.2
	- 10%	+ 0.6	+ 11.1

† Residual density of infectious polyhedra set to 0.

8.4 Discussion

We validated a comprehensive simulation model for the control of *S. exigua* with AcMNPV and SeMNPV in chrysanthemum with independent data of greenhouse experiments. For accurate model predictions of dose- and time-mortality relationships reliable estimations of the infection chance per ingested polyhedron and the mean time to kill are needed. AcMNPV and SeMNPV infectivity was determined in a series of leaf-disc bioassays generated from 1996 to 1999 (Table 8.2). Since the dose-mortality relationships varied, a representative dose-mortality relationship was selected for the determination virus infectivity parameters. This variation in dose-mortality relationships between laboratory bioassays sets the base line accuracy of the model predictions because these can never be more accurate than the measured input data.

Approximately ten times lower LD_{50} values for SeMNPV as for AcMNPV (Table 8.2) are in line with previous observations of Smits and Vlak (1988a) and chapter 2. However, the absolute LD_{50} values generated by the leaf-disc method in this study were clearly higher than the LD_{50} values derived from droplet-feeding bioassays (Smits and Vlak, 1988a; chapter 2). The difference in LD_{50} values may be explained by the absence of a starvation period, the ingestion of leaf material together with the polyhedra and by interactions between plant chemicals and the infection process in the leaf-disc bioassay (Hoover *et al.*, 1998a,b). The leaf-disc bioassay is biologically more meaningful than droplet-feeding bioassay, and

therefore the leaf-disc bioassay should always be used for the determination of the infectivity of a baculovirus as a model parameter.

The description of the infection process of larvae by ingestion of sprayed polyhedra used in BACSIM is mechanistically and biologically plausible (chapter 7). The simulation of the larval infection rate takes into account the distribution of larvae over canopy layers, the polyhedra density per canopy layer, the leaf consumption rate of larvae and the infection chance per ingested polyhedron for each instar. Simulated trends of the dose-mortality relationships of AcMNPV and SeMNPV, two viruses with distinct differences in infectivity, were in general in close agreement with actual measurements (Figure 8.1). The implementation of the dose-mortality relationships in BACSIM appeared to be robust and this finding gives confindence that the simulated infection process for other baculoviruses in this system will also be realistic.

Foliage consumption by *S. exigua* larvae can vary considerably within and between greenhouse experiments (chapter 6). For example, populations of 100 and 80 third instar larvae in the control treatment of experiment #2 and #5 consumed 299 and 122 cm^2, respectively. Given this variability, simulations of foliage consumption caused by *S. exigua* populations treated with control, AcMNPV or SeMNPV suspensions corresponded well with the actual measurements (Figure 8.4), except for a single outlier. This outlier underestimated the actual 1400 cm^2 foliage consumption by approximately 700 cm^2 and results from observations from fifth instar larvae in the greenhouse plots and simulations of fourth instar larvae.

The simulated time-mortality relationships in BACSIM result from the simulated moment of infection and the temperature dependency of the virus incubation period in the infected larvae. The relationships that describe the moment of infection are a precise representation of the uptake of polyhedra by caterpillars over time, due to leaf feeding, in combination with the accumulation of a virus dose causing infection. This description time-mortality relationship was able to accurately reproduce measured survival curves of *S. exigua* larvae in droplet-feeding bioassays (chapter 7).

We found that model predictions of AcMNPV and SeMNPV mean time to kill were consistently lower than those observed in greenhouse experiments (Figure 8.6). This discrepancy between measured and simulated mean time to kill indicates that this part of the system is not completely understood and needs further investigation. One hypothesis that may explain the differences between observations and simulations is the fact that *S. exigua* larvae spend up to 50% of the time on the underside of chrysanthemum leaves where

virtually no sprayed polyhedra occur (chapter 4). *S. exigua* larvae may only become infected after a delay period when they leave this refuge area. The foraging behavior of individual larvae is not included in detail in BACSIM. For a detailed description of the foraging behavior of individual larvae an individual-based modeling approach may be advantageous. The discovery of discrepancies in simulated and observed time to kill of *S. exigua* populations in chrysanthemum suggests that the larval foraging behavior may play an important role in the efficacy of baculovirus applications.

The sensitivity analysis of BACSIM revealed that simulated foliage consumption and mortality were not very sensitive to changes of parameter values. A 10% higher infection chance per ingested polyhedron or a 10% increased speed of action for SeMNPV lead to a 3 to 5% reduction in foliage consumption for the tested situation. A 10% reduction of the polyhedron inactivation rate had only a marginal effect on foliage consumption (less than 1%). Thus, the reduction of polyhedron inactivation by the use of formulations is not likely to have an remarkable effect on SeMNPV efficacy in greenhouses, whereas increased SeMNPV infectivity and speed of action will have a higher potential to reduce crop injury.

In general, BACSIM provides robust predictions for the control of *S. exigua* populations in greenhouse chrysanthemum for AcMNPV and SeMNPV. This underscores the validity of our modeling approach in which model parameters (e.g., infectivity and mean time to kill) are quantified at the process level and then integrated in a system model to obtain predictions at the crop level. This approach gave satisfactory results for two baculoviruses with distinct biological properties, and this finding gives confidence that BACSIM may be used for any baculovirus with a specific infectivity, speed of action and polyhedral inactivation rate. With this model we now can simulate the behavior of genetically modified baculoviruses as biological control agents as compared to wild-type baculoviruses and evaluate the efficacy of different spraying regimes for chrysanthemum cultivation practice.

9

Scenario studies with a process-based model for the biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses

Scenario studies were carried out with a process-based model for control of wild-type and genetically modified baculoviruses in populations of Spodoptera exigua in glasshouse chrysanthemum (BACSIM). These scenario studies were used to evaluate the effectiveness of different spraying regimes, concentrations, UV-protection agents and speed of action of viruses in different pest situations. In simulations on dosage and timing of SeMNPV, good control efficacy was obtained with SeMNPV concentrations of $1x10^7$ polyhedra m^{-2} or higher. An early timing of virus applications, soon after egg deposition, is essential for effective control, especially at high temperatures, when the development rate of larvae is high and the window of opportunity for virus control is short. UV-protection agents contribute only marginally to effective biological control in the glasshouse because the decay rates of SeMNPV and AcMNPV already ensure a period of exposure to active virus that is long enough for larvae to acquire a lethal dose under practical conditions. The effect of genetic improvement towards shortening the survival time of infected larvae depends on the situation in which the virus is used. When virus is used in a situation with constant immigration of pest insects, a greater reduction of feeding injury is achieved with a faster killing virus than with a wild-type virus. When, however, immigration of pest insects occurs by way of sudden influxes, resulting in synchronous insect populations in the crop, it appears that early timing of virus applications is more critical to reduce crop injury than using a fast killing virus. In such a situation, good monitoring followed by virus application is indispensable and may even alleviate the need for a fast killing virus.

This chapter has been submitted as:
Bianchi, F.J.A., Vlak, J.M., and van der Werf, W.

9.1 Introduction

Baculoviruses are pathogens that can cause lethal infections in many insect species and there is growing interest for their use as biological control agents against insect pests in agriculture and forestry (Black *et al.*, 1997; Moscardi, 1999). Their infectivity, specificity and safety to non-target organism make them promising candidates to replace chemical insecticides. However, their high production costs, relatively slow speed of action, narrow host range and high inactivation rate compared to chemical insecticides limit their use in practice (Moscardi, 1999). With the implementation of technical improvements some of these difficulties might be overcome.

Recently, recombinant baculoviruses have been engineered with an increased speed of action (Black *et al.*, 1997; van Beek and Hughes, 1998). These recombinants are typically 25-30% faster than wild-type viruses, but also recombinants with reductions of up to 50% have been reported (Tomalski *et al.*, 1993). In addition, chemical synergists such as optical brighteners have been used that improved baculovirus efficacy as biocontrol agents (Dougherty *et al.*, 1996). The determination of the commercial potential of new recombinant baculoviruses and new formulations involve costly field testing programs. In the case of recombinant baculoviruses, these experiments are also subject to strong government regulations and require costly facilities and procedures. Hence, there is a need for the assessment of recombinant baculoviruses using a theoretical approach.

We developed a process-based model (BACSIM) that can be used to simulate the control of beet armyworm, *S. exigua,* with baculoviruses in greenhouses (chapter 7). The model is based on a detailed quantitative description of the behavior and population dynamics of insects, plant growth characteristics, spray deposition, the baculovirus infection cycle and transmission routes. BACSIM is validated for the baculoviruses *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) and *S. exigua* MNPV (SeMNPV) with independent data from greenhouse experiments and provides robust predictions for mortality levels and foliage consumption of *S. exigua* populations in greenhouse chrysanthemum (chapter 8). BACSIM can be used for the evaluation of the agricultural effectiveness of viruses with altered insecticidal properties and formulations that improve baculovirus efficacy. The evaluation of the biological control of insect pests with simulation models offers the possibility to include many treatments and situations, to simulate baculoviruses with extreme biological properties and to include many covariables that may modify the efficacy of baculoviruses in practice, such as crop age and temperature. In addition, this

simulation approach allows optimization procedures that can assess optimal virus properties and spraying regimes under varying conditions (de Moed *et al.*, 1990; van der Werf *et al.*, 1991).

In this paper we evaluate the potential of baculoviruses as biological control agents for *S. exigua* in greenhouse chrysanthemum by analysis of scenario studies of BACSIM. The scenario studies comprise the evaluation of optimal spraying regimes of SeMNPV, the comparison of the efficacy of AcMNPV and SeMNPV wild-type and recombinants with an increased speed of action and the evaluation of the efficacy of UV-protection agents that reduce polyhedron inactivation.

9.2 Material and methods

Model parameterization

The default parameterization of BACSIM for chrysanthemum growth, spray deposition, horizontal transmission, *S. exigua* development and spatial distribution have been described previously (chapter 7). The parameterization of AcMNPV and SeMNPV infectivity, speed of action and polyhedra inactivation is described in chapter 8. The parameterization of AcMNPV and SeMNPV wild-type in the presented scenario studies is the same as in the validation of BACSIM (chapter 8). AcMNPV wild-type has a relatively low mean time to kill (5.7 d at 25°C) as compared to SeMNPV wild-type (4.6 d at 25°C) in first to third instar *S. exigua* larvae (chapter 7). The parameterization of AcMNPV and SeMNPV recombinants with a 25 and 50% reduction in mean time to kill are identical to the parameterization of the wild-type viruses, except for the adjusted speed of action. The two AcMNPV recombinants reflect the speed of action of an AaIT-expressing AcMNPV recombinant reported by Stewart *et al.* (1991), which has a 25% reduction in time to kill, and a neurotoxin 21-A-expressing AcMNPV recombinant (Tomalski *et al.*, 1993) which has a 50% reduction in time to kill.

Model initialization

In all simulations the initial crop age was 21 days and constant temperatures were used. Because BACSIM contains stochastic elements, simulations of single generations *S. exigua* at a patch scale were repeated fifty times and averaged. For the simulation of multiple generations of *S. exigua*, a 1 ha greenhouse with 630,000 chrysanthemum plants, grown in 9 age cohorts of 100 x 700 plants each, was considered. The oldest age class was harvested and replanted at a weekly basis. Inflying moths in the greenhouse were simulated by the

introduction of one fertilized female moth per day that deposited 13 egg batches of 35 eggs each (derived from Smits *et al.*, 1986). Crop injury is in most cases expressed as the number of damaged plants rather than the area of foliage consumption. This is done because even minor feeding marks make chrysanthemum unacceptable for sale and may cause serious growth deformations. These scenario studies apply to situations that resemble cultivation practice and can therefore directly be related to practical situations.

9.3 Results

Spray strategy

The optimal timing and dosage of SeMNPV applications, as well as effects of temperature and chrysanthemum development stages are evaluated by scenario studies at a patch scale using BACSIM. SeMNPV applications with varying concentrations and timing against *S. exigua* larvae originating from egg batches of 35 eggs at 23 and 30°C are presented in Figures 9.1a, b, c and d. Well-timed applications of $1x10^7$, $3x10^7$ and $1x10^8$ polyhedra SeMNPV m^{-2} provide total control of *S. exigua* larvae, whereas applications with lower concentrations ($3x10^5$, $1x10^6$ and $3x10^6$ polyhedra SeMNPV m^{-2}) lead to *S. exigua* survival (Figures 9.1a and b). SeMNPV applications of $1x10^7$, $3x10^7$ and $1x10^8$ polyhedra m^{-2} at 23°C provide good control unless virus is applied more than 15 days after egg deposition (Figure 9.1a). At 30°C the time window in which complete control can be achieved with these concentrations is reduced to the first 9 days after egg deposition (Figure 9.1b). Increasing SeMNPV dosage results in a reduced number of damaged plants, but a small amount of crop injury can not be prevented because larvae feed before they die (Figures 9.1c and d). The differences between SeMNPV applications at 23 and 30°C can be explained by the increasing developmental rates of *S. exigua* larvae at higher temperatures (Figures 9.1e and f). The emergence of *S. exigua* moths after high dosage SeMNPV applications at 23 and 30°C coincide with the appearance of fifth instar larvae in the crop. These larvae are almost resistant to SeMNPV (Smits and Vlak, 1988a).

Crop age has a limited effect on the efficacy of SeMNPV applications in greenhouse chrysanthemum (Figures 9.2a and b). There is a slight increase in the number of emerging *S. exigua* moths after SeMNPV applications in older crops as compared to younger crops (Figure 9.2a). This effect of crop age is more pronounced for the number of damaged plants (Figure 9.2b). Applications of $1x10^8$ polyhedra SeMNPV m^{-2} provide maximal control in all chrysanthemum stages.

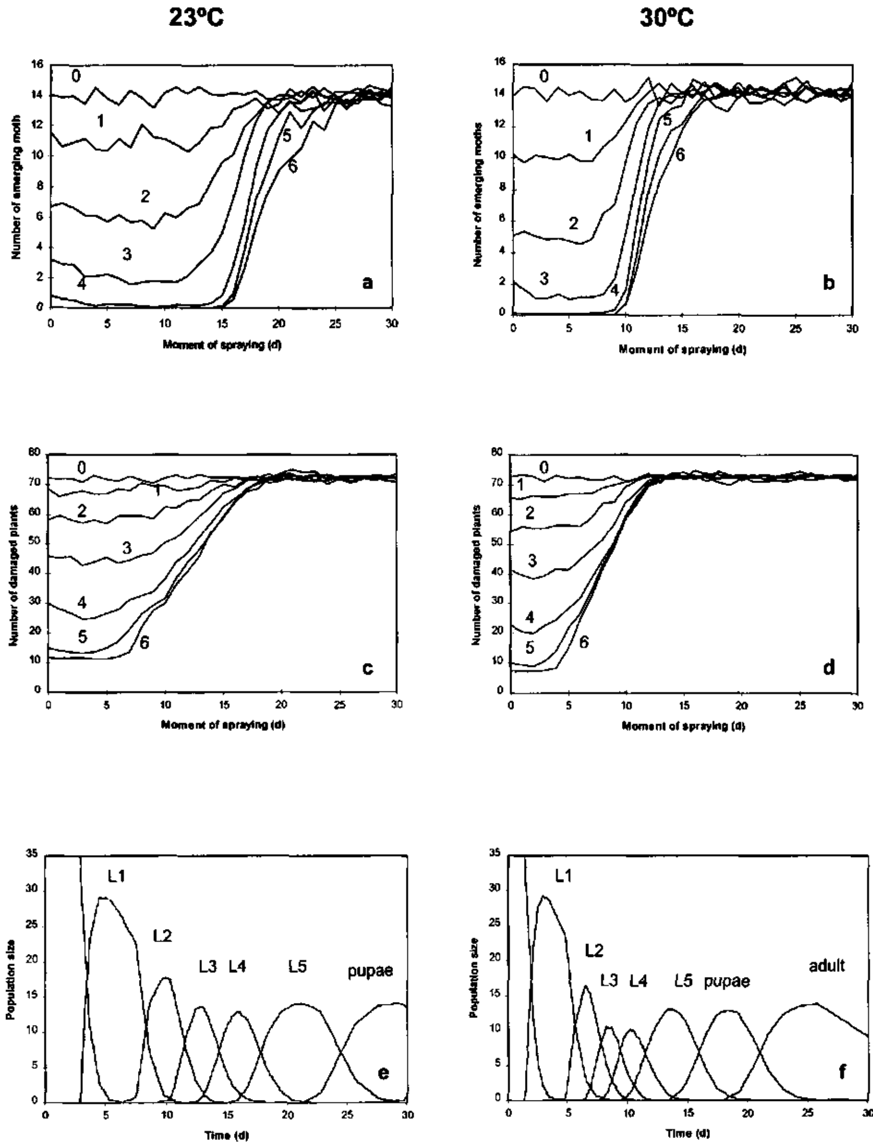

Figure 9.1 *Simulation of the development of S. exigua egg batches in greenhouse chrysanthemum after SeMNPV applications. Emergence of S. exigua moths at 23°C (a) and 30°C (b), and number of damaged plants at 23°C (c) and 30°C (d) after applications of 0 (0), $3x10^5$ (1), $1x10^6$ (2), $3x10^6$ (3), $1x10^7$ (4), $3x10^7$ (5) and $1x10^8$ (6) polyhedra m^{-2} at 0 to 30 days after egg batch deposition. S. exigua phenology at 23°C and 30°C is presented in Figures 9.1e and f, respectively.*

The optimal time interval between SeMNPV applications is evaluated for multiple generations of *S. exigua* at a greenhouse scale. The simulated infestation pressure is one fertilized *S. exigua* moth ha^{-1} day^{-1}. Varying time intervals between applications of $3x10^7$ polyhedra SeMNPV m^{-2} are simulated for a 100-day period at 23 and 30°C (Figure 9.3). Initially, the number of damaged plants increases gradually with increasing time intervals between applications, but the number of damaged plants rapidly increases when these intervals exceed 22 and 12 days at 23 and 30°C, respectively.

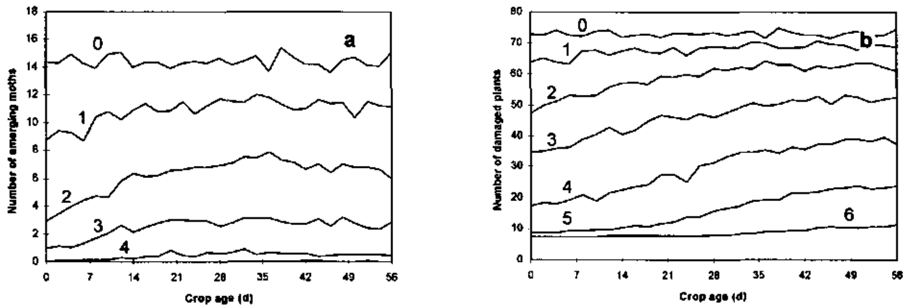

Figure 9.2 *Simulated relationship between crop age and emergence of S. exigua moths (a) and number of damaged plants (b) after SeMNPV applications in greenhouse chrysanthemum at 25°C. Lines represent applications of 0 (0), $3x10^5$ (1), $1x10^6$ (2), $3x10^6$ (3), $1x10^7$ (4), $3x10^7$ (5) and $1x10^8$ (6) polyhedra m^{-2} at the moment of egg batch deposition.*

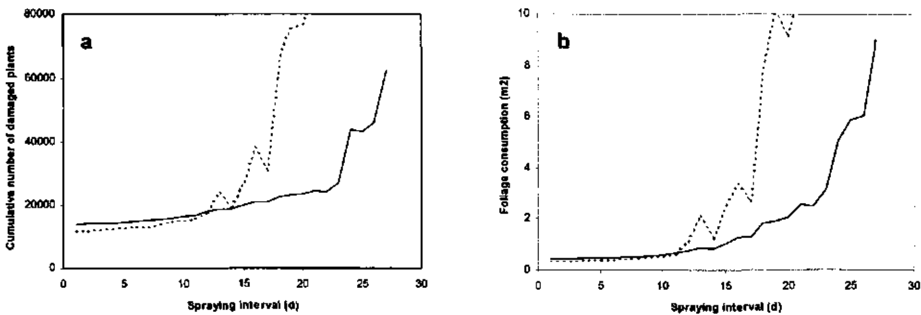

Figure 9.3 *Simulated relationship between the time interval between applications of $3x10^7$ polyhedra m^{-2} SeMNPV and the number of damaged plants (a) and foliage consumption (b). Simulations at 23 and 30°C are represented by solid and dashed lines, respectively.*

AcMNPV and SeMNPV recombinants

The potential of AcMNPV and SeMNPV wild-type and two AcMNPV and SeMNPV recombinants with a 25% (AcMNPV-25%, SeMNPV-25%) and 50% reduction in the mean time to kill (AcMNPV-50%, SeMNPV-50%) are evaluated by scenario studies at the patch scale (Figures 9.4 and 9.5). Applications of $3x10^8$ polyhedra m^{-2} caused 100% mortality for all six viruses. When plants are sprayed with AcMNPV wild-type or one of the two AcMNPV recombinants at the moment of egg batch deposition, there are clear differences in the time-mortality curves of the viruses (Figure 9.4a), as well as in the number of damaged plants (Figure 9.4c). The number of damaged plants after applications of AcMNPV recombinants is approximately half of that of the AcMNPV wild-type application. For SeMNPV there are also clear differences in time-mortality curves of the wild-type and recombinants (Figure 9.4b), but the effect on the number of damaged plants is much smaller than in the case of AcMNPV (Figure 9.4d). The difference in the number of damaged plants between applications of AcMNPV wild-type on the one hand and that of AcMNPV recombinants and SeMNPV on the other, is caused by the fact that the incubation time of AcMNPV wild-type in infected larvae is long enough to allow infected larvae to expand their foraging domain to that of second instar larvae, whereas larvae infected with AcMNPV recombinants or SeMNPV are killed before they infest a larger number of plants.

The relationship between the timing of applications of AcMNPV wild-type, AcMNPV-25% and AcMNPV-50% and the number of damaged plants illustrates that AcMNPV-25% and AcMNPV-50% are more effective than the wild-type virus when virus sprays are applied between 0 and 6 days after the deposition of the egg batch (Figure 9.5a). In contrast, SeMNPV wild-type and recombinants are equally effective in reducing the number of damaged plants when virus sprays are applied in this period (Figure 9.5b). The amount of foliage consumption by *S. exigua* larvae that are controlled by virus applications timed between 0 and 6 days after the deposition of the egg batch is minimal, irrespective of the virus used (Figures 9.5c and d). In applications timed between 6 and 17 days after egg batch deposition both AcMNPV and SeMNPV recombinants are more effective in reducing crop injury than the respective wild-type viruses. However, since crop injury increases rapidly in time, accurate timing of virus applications remains essential for adequate control, even for fast killing viruses.

When a greenhouse is considered with a daily inflight of *S. exigua* moths and ten-day interval applications of $3x10^8$ polyhedra m^{-2} AcMNPV wild-type, AcMNPV-25% and AcMNPV-50%, the recombinants are more effective in reducing crop injury (Figures 9.6a

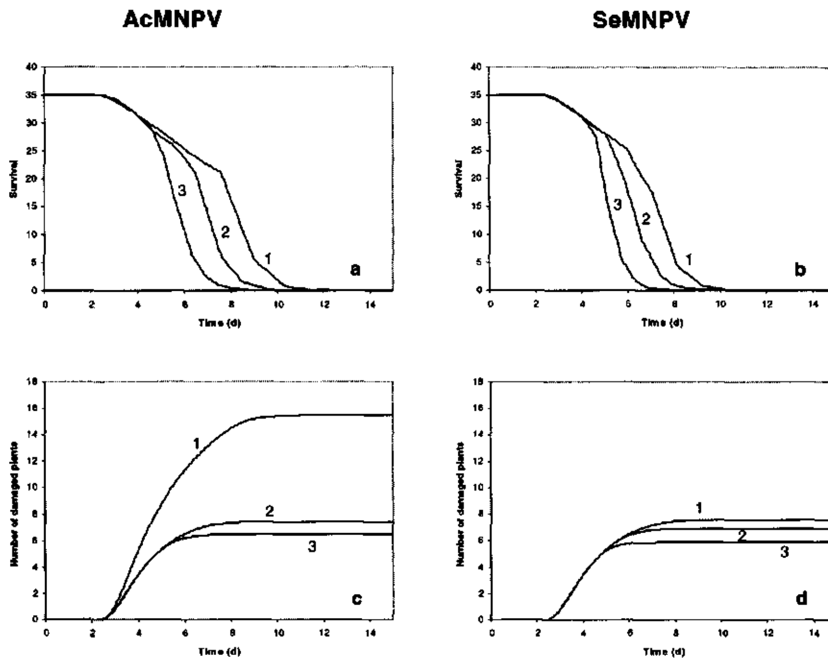

Figure 9.4 *Simulated time-mortality curves and number of damaged plants of applications of AcMNPV (a and c) and SeMNPV (b and d) at the moment of egg batch deposition in greenhouse chrysanthemum at 25°C. Lines represent applications of $3x10^8$ polyhedra m^{-2} wild-type (1) and recombinants with a 25% (2) and 50% reduction in time to kill (3).*

and b). Hence, applications of AcMNPV-25% and AcMNPV-50% result in reductions of 17% and 43% in the number of damaged plants and reductions of 31% and 50% in the cumulative area of foliage consumed as compared to AcMNPV wild-type, respectively.

UV-protection agents

The potential of UV-protection agents to improve the efficacy of SeMNPV in greenhouses is evaluated in a series of simulations at the patch scale. The relationship between the relative inactivation rate of SeMNPV, the survival of *S. exigua* and the number of damaged plants after SeMNPV applications is given in Figures 9.7a and b. SeMNPV applications were timed at the moment of egg batch deposition and the residual density of infectious polyhedra was set to zero. The survival of *S. exigua* and the number of damaged plants increased with higher polyhedra inactivation rates. For SeMNPV concentrations that cause 80-100% mortality, the number of damaged plants responds in a sensitive way to changes of polyhedra inactivation

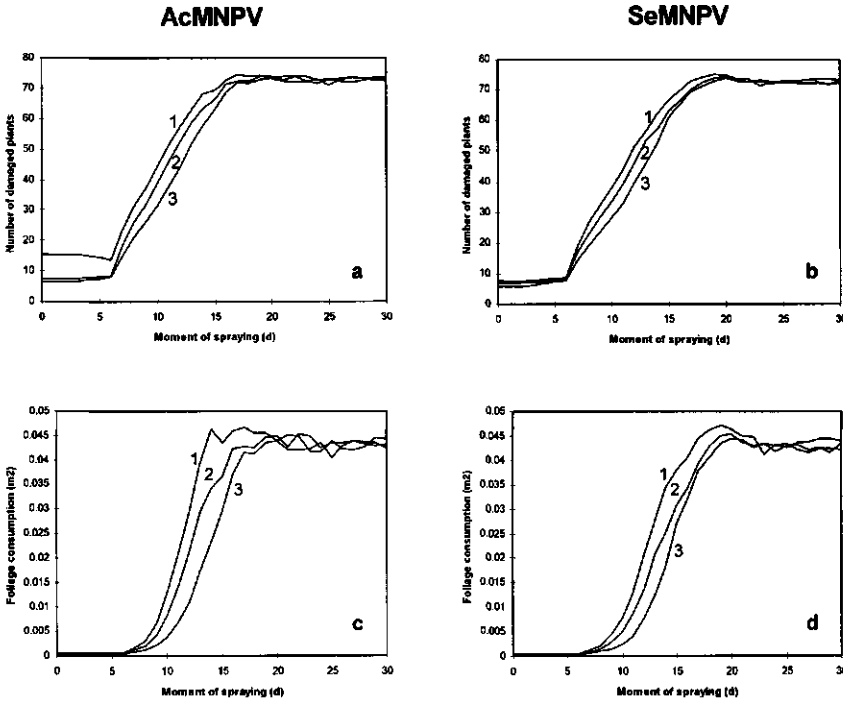

Figure 9.5 *Simulated foliage consumption and number of damaged plants caused by larvae originating from a S. exigua egg batch after AcMNPV (a and c) and SeMNPV (b and d) applications in greenhouse chrysanthemum at 25°C. Lines represent applications of $3x10^8$ polyhedra m^{-2} wild-type (1) and recombinants with a 25% (2) and 50% reduction in time to kill (3) at 0 to 30 days after egg batch deposition.*

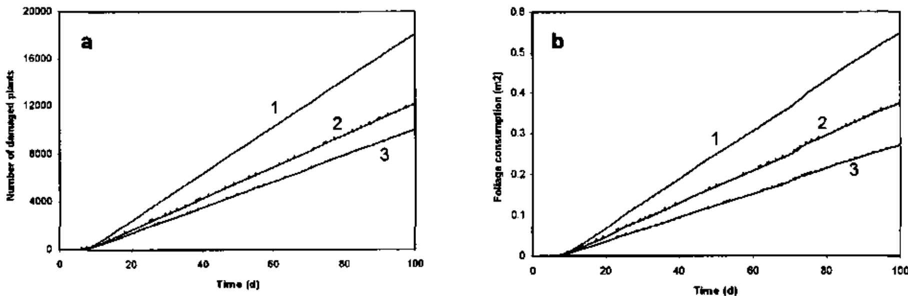

Figure 9.6 *Simulated number of damaged plants (a) and foliage consumption (b) of AcMNPV applications with 10 day time intervals and daily inflying S. exigua moths in greenhouse chrysanthemum at 25°C. Lines represent applications of $3x10^8$ polyhedra m^{-2} AcMNPV wild-type (1) and two recombinants with a 25% (2) and 50% reduction in time to kill (3).*

rates (Figure 9.7b). Thus, for polyhedra concentrations that are hardly sufficient to provide total control of *S. exigua* larvae, a small increase in polyhedra inactivation will result in an increasing larval survival and an accompanying increase of crop injury.

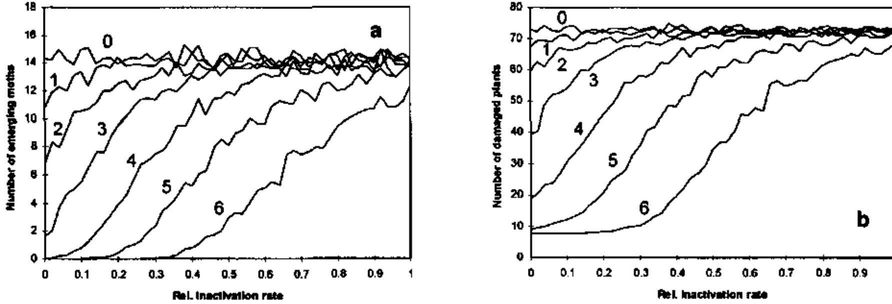

Figure 9.7 *Simulated emergence of S. exigua moths (a) and number of damaged plants (b) after SeMNPV applications in greenhouse chrysanthemum at 25°C. Lines represent applications of 0 (0), $3x10^5$ (1), $1x10^6$ (2), $3x10^6$ (3), $1x10^7$ (4), $3x10^7$ (5) and $1x10^8$ (6) polyhedra m^{-2} with relative polyhedra inactivation rates ranging between 0 and 1 day $^{-1}$.*

9.4 Discussion

The field efficacy of various spraying regimes of wild-type and recombinant baculoviruses has been simulated with the validated, process-based simulation model BACSIM. Simulations of dosage and timing of SeMNPV applications indicate that concentrations of $1x10^7$ polyhedra m^{-2} or higher provide good control of *S. exigua* larvae, given that the application is properly timed (Figures 9.1a and b). This is in agreement with greenhouse experiments where doses of $3x10^7$ polyhedra m^{-2} SeMNPV generally cause 90–100 % mortality in *S. exigua* populations (chapter 6; Smits *et al.*, 1987b). Similar doses provided 75-100% mortality in *S. exigua* populations in garden pea and grape in open field experiments (Kolodny-Hirsch *et al.*, 1997). Since fifth instar larvae are much less sensitive to SeMNPV (chapter 2; Smits and Vlak, 1988a), SeMNPV applications against later instar larvae result in poor control (Figures 9.1a, b, e and f). Therefore, SeMNPV sprays should always be applied before the larvae reach the fifth instar.

Accurate timing of baculovirus applications is essential to minimize crop injury. Best control is obtained when larvae become infected immediately after egg hatch (Figures 9.1c and d). At this time the larvae are small and cause only minor feeding damage on a limited number of plants. The relationship between spraying interval and crop injury depends on

temperature. At low temperatures the generation time of *S. exigua* will be relatively long and spraying interval can be prolonged without a strong increase of crop injury (Figures 9.1e and 3). With increasing temperatures SeMNPV spraying intervals should be shortened to prevent serious crop damage (Figures 9.1f and 9.3).

Simulations indicated that crop age has a limited effect on the efficacy of SeMNPV applications in greenhouse chrysanthemum (Figure 9.2). Smits *et al.* (1988) studied the efficacy of SeMNPV applications in 20 cm and 60 cm-high chrysanthemum crops and concluded that crop height did not greatly influence the level of larval control for high volume applications. Thus, our conclusions based on simulations correspond well with conclusions derived from experimental data.

Cory *et al.* (1994) quantified the area foliage consumption of third instar *Trichoplusia ni* larvae on cabbage controlled with AcMNPV wild-type and a toxin expressing AcMNPV recombinant with a 25% reduction in time to kill. Applications of the recombinant virus resulted in reductions in foliage consumption of 29% and 23% for low and medium/high concentrations, respectively, as compared with AcMNPV wild-type. Although it is difficult to relate these findings to the chrysanthemum-*S. exigua*-AcMNPV system, simulations of the efficacy of AcMNPV wild-type and AcMNPV-25% indicated reductions in foliage consumption in the same order of magnitude as in the study of Cory *et al.* (1994) (Figure 9.5c).

Applications of baculoviruses with an increased speed of action do not in all situations lead to lower crop injury levels than applications of wild-type viruses. In simulations, AcMNPV and SeMNPV and their recombinants reduced foliage consumption to a minimum when applications were timed between 0 and 6 days after egg batch deposition (Figures 9.5b and d). However, a higher number of plants were injured after AcMNPV wild-type applications than after applications of AcMNPV-25% and AcMNPV-50%. Larvae infected with AcMNPV wild-type were able to infest chrysanthemum plants in the foraging domain of second instar larvae while fast acting viruses killed the larvae before they expanded their foraging domain to that of second instar larvae. Recombinant baculoviruses with an increased speed of action were more effective than wild-type viruses when applied against second, third and fourth instar larvae as compared to wild-type viruses (Figure 9.5). Yet, accurate timing of virus applications remains important because crop injury caused by *S. exigua* larvae increases rapidly in time. The use of faster killing viruses prolongs the time-window of detecting and controlling *S. exigua* infestations without serious crop injury.

In a 100-day simulation with daily inflight of *S. exigua* moths applications of AcMNPV-25% and AcMNPV-50% clearly reduced crop injury as compared to AcMNPV wild-type (Figure 9.6). This implies that in a situation of sudden infestations of *S. exigua*, accurate monitoring followed by virus application has a higher potential to reduce crop injury than the use of faster killing baculoviruses without proper monitoring, whereas in a situation of continuous infestations faster killing baculoviruses are effective in reducing crop injury.

Polyhedra that are exposed to natural sunlight have reported half-life values of 4.9 h (Ignoffo *et al.*, 1997), which corresponds with relative inactivation rates of 3.4 day^{-1}. In greenhouses, however, the glass of the greenhouse filters UV-irradiation and therefore polyhedra inactivation in greenhouses is much lower. Relative inactivation rates of AcMNPV and SeMNPV determined on greenhouse chrysanthemum are only 0.16 and 0.10 day^{-1}, respectively (chapter 3 and 7). Since the relative inactivation rate of SeMNPV in greenhouses is low, reducing the SeMNPV inactivation rate even further by adding UV-protection agents would hardly reduce the number of damaged plants (Figures 9.7a and b). UV-protection agents will have higher potential to reduce crop injury in the open field where polyhedra are directly exposed to sunlight.

BACSIM has been used to evaluate the potential of SeMNPV, AcMNPV recombinants and UV-protection agents for a greenhouse situation. However, for the simulations of recombinants with an increased speed of action and formulations that reduce polyhedral inactivation, only the effects of altered larval survival times and polyhedra inactivation rates were taken into account. No interactions between foreign gene products or formulations with the crop-insect-virus system have been incorporated, such as altered behavior of larvae infected with toxin-expressing viruses (Hoover *et al.*, 1995). Presence of such interactions in crop-insect-virus systems should be determined experimentally and incorporated in BACSIM to allowed more refined simulations.

Besides the determination of effective spraying regimes and the evaluation of the potential of recombinants and formulations, BACSIM can further be used for initial screening of the agricultural effectiveness of baculoviruses and as a tool to set up field experiments. In addition, BACSIM may provide insight in the insect-baculovirus-crop system and the relative importance of viral characteristics for crop protection purposes. However, simulations should be accompanied by field experiments to assess the accuracy of model predictions (chapter 8). The information generated by BACSIM may be useful for growers, industry and research.

10

General discussion

In this thesis a study on the control of *S. exigua* with baculoviruses in greenhouse chrysanthemum is described. A process-based simulation model (BACSIM) has been developed to investigate the feasibility of the control of the pest *S. exigua* with baculoviruses. The model has been validated and used to carry out scenario studies that have been exploited for the evaluation of the potential of baculoviruses as biological control agents.

Upscaling baculovirus-host relationships to the crop level

The field of baculovirus pathogenesis is well studied, but at the same time our knowledge of the control of insects with baculoviruses at a field scale is still limited. For example, there is considerable information available on the reduction in the time to kill insects by recombinant baculoviruses as compared to wild-type viruses, but we do not know how effective such recombinants are in reducing crop injury in field situations. Evidently, there are many factors that determine the efficacy of baculoviruses as biological control agents. Existing knowledge of these factors has been integrated in a process-based simulation model for the control of *S. exigua* with baculoviruses in greenhouse chrysanthemum. This knowledge was typically derived from laboratory studies where experiments are conducted under controlled conditions. The synthesis of this knowledge proved a successful strategy for the identification of "white spots" in the chrysanthemum-*S. exigua*-baculovirus system. These areas of incomplete understanding have been studied in a series of experiments presented in chapters 2 to 5. Our simulation approach enabled the integration of all this fragmented knowledge of separate processes in a model that describes the interactions of each of these processes in the chrysanthemum-*S. exigua*-baculovirus system at a field scale. Thus, this simulation approach bridges the gap between knowledge at the individual level and understanding of the chrysanthemum-*S. exigua*-baculovirus system at the field scale.

AcMNPV deletion mutants

Initially, a series of five baculoviruses were included in the study. The biological properties of wild-type AcMNPV and SeMNPV and three AcMNPV deletion mutants that lacked the *egt*, *pp34* and *p10* genes have been quantified (chapter 2). The *egt* gene encodes ecdysteroid UDP-glucosyltransferase, an enzyme that alters the molting process in infected larvae; the *pp34* gene encodes the polyhedral membrane protein and might be involved in the protection of polyhedra against environmental decay (Ignoffo *et al.*, 1995), while the *p10* gene encodes fibrillin, which forms fibrillar structures in infected cells. Deletion of these genes had no or only a marginal impact on the infectivity, speed of action and polyhedron inactivation rate of

AcMNPV (chapters 2 and 3). The absence of an *egt* effect in the *S. exigua*-AcMNPV system corresponds with a study of Slavicek *et al.* (1999) who found that the speed of action of a wild-type and an *egt* deletion mutant of *Lymantria dispar* MNPV were similar in first to fourth instar *L. dispar* larvae. In contrast, a number of studies reported reduced lethal times of larvae infected with *egt* deletion mutants as compared to the wild-type virus (O'Reilly and Miller, 1991; Treacy *et al.*, 1997; Chen *et al.*, 2000). Although these studies showed an increased speed of action of *egt* deletion mutants in the laboratory, the efficacy of *egt* deletion mutants in the field is less than observed under laboratory conditions (Treacy *et al.*, 1996). This implies that the improved insecticidal properties of *egt* deletion mutants need to be evaluated case-by-case for different insect-virus systems. To improve the speed of action of baculoviruses under field conditions significantly the insertion of entomotoxic genes such as AaIT is more promising.

The similarity of biological properties of wild-type AcMNPV and the three AcMNPV deletion mutants nullified the necessity to study all four of the AcMNPV viruses. Therefore, the model system was reduced from five (wild-type AcMNPV and SeMNPV, and three AcMNPV deletion mutants) to two viruses (wild-type AcMNPV and SeMNPV). Wild-type AcMNPV and SeMNPV have clear differences in infectivity and speed of action (chapter 2), but the relative inactivation rate of AcMNPV and SeMNPV polyhedra were not significantly different ($P<0.05$, Wilcoxon rank-sum test).

Validation of BACSIM

The simulation model BACSIM integrates knowledge of crop growth characteristics, baculovirus infectivity, speed of action (chapter 2), polyhedron inactivation (chapter 3), *S. exigua* bionomics (chapter 4) and transmission dynamics (chapter 5). The validation of the simulation model BACSIM (chapter 8) with independent data of AcMNPV and SeMNPV applications in greenhouse chrysanthemum were in general satisfying. The measured control of *S. exigua* with these viruses in the greenhouse corresponded with the outcomes of the simulation model. BACSIM provided robust predictions of AcMNPV and SeMNPV mortality levels and foliage consumption of *S. exigua* populations. However, the time to kill *S. exigua* larvae after AcMNPV and SeMNPV applications, as predicted by BACSIM, were generally lower than the actual measurements. This indicates that this part of the system is not completely understood. One hypothesis that may explain the differences between observations and simulations is the fact that *S. exigua* larvae spend up to 50% of the time at the underside of chrysanthemum leaves (chapter 4, Smits *et al.*, 1987). At this location there are virtually no

sprayed polyhedra present (Bianchi, unpublished data). *S. exigua* larvae may only become infected after they leave this refuge area, which may cause delayed infection under field conditions. This would suggest that the larval foraging behavior plays an important role in the efficacy of baculovirus applications. This issue needs further experimental investigation. Detailed field experiments are needed to collect such information.

Model validation has the function to evaluate the model performance by the comparison of model simulations with independent experimental data. Our study clearly demonstrated the benefit of validation. Validation appeared to be a powerful tool to give at the one hand confidence in the model performance and at the other hand the ability to detect areas of incomplete system understanding. The comparison of model output with independent experiments provides insight in the crop-insect-virus system, irrespective of the results.

Despite the clear benefit of validation, it is striking how few mathematical models models for baculovirus epidemics in insect populations have been validated. Besides models of Dwyer (1992) and Dwyer and Elkinton (1993), to my knowledge no validated model have been reported. Therefore, it is strongly recommend to include validation as a standard procedure in simulation studies (Rabbinge and de Wit, 1989).

Applicability of BACSIM

BACSIM can be used for a variety of purposes. First, the model can be used as a tool to assist in the determination of effective spraying regimes of (genetically modified) baculoviruses or formulations under varying conditions. Simulations of baculoviruses may be used for the initial screening of the agricultural effectiveness of baculoviruses, as a tool to set up field experiments, and to reduce the number of field experiments when model simulations are in line with field experiments. Second, BACSIM can be used to gain insight in the insect-baculovirus-crop system and the relative importance of viral characteristics for crop protection purposes. This knowledge may be used to direct future genetic engineering or formulation strategies to obtain baculoviruses with improved insecticidal characteristics. Third, BACSIM can be used to address fundamental ecological questions. For example, simulations of BACSIM could be used to test the mass action assumption that is often used in analytic models for the description of the horizontal transmission process (Cory *et al.*, 1997). Finally, BACSIM may contribute to the assessment of risks associated with the use of genetically modified baculoviruses by the generation of quantitative information of the behavior of recombinant baculoviruses in crops. BACSIM can be used for the upscaling of biological properties of the recombinant baculoviruses that have been quantified under laboratory conditions to a field scale.

For instance, estimations of polyhedra budgets produced in *S. exigua* larvae by wild-type and recombinant baculoviruses in crops can be made when the polyhedra production in individual larvae has been quantified in the laboratory. The inoculum reproduction rate is an important parameter for the assessment of the competitive capacity of a baculovirus. The model cannot be used to answer qualitative questions related to the risk assessment issues for genetically modified baculoviruses. The assessment of probability and hazard of gene transfer from recombinant baculoviruses to wild populations, the effect of foreign gene products on non-target organisms and the effect of foreign gene expression on baculovirus fitness by e.g. changes in the host range and infectivity have to be determined experimentally.

Restrictions for the use of BACSIM

The use of BACSIM for new crop-insect-virus systems should always be accompanied with field experiments. Accompanying field experiments are needed to assess the accuracy of model predictions for this system and to confirm that there are no interactions in this system that have not been incorporated in the simulation model. Interactions, such as altered larval behavior of larvae infected with toxin expressing baculoviruses (Hoover *et al.*, 1995) may have a clear effect on the epidemiology of baculoviruses. When these effects are not properly included in the model, model simulations may consistently deviate from experimental data. Therefore, conclusions on the agricultural efficacy of baculoviruses can never be based on both model simulations alone.

Future development of BACSIM

Until now the use of BACSIM has been restricted to the control of *S. exigua* populations with AcMNPV and SeMNPV in greenhouse chrysanthemum. It would be desirable to expand the applicability of the model to other systems, e.g. for different insect species, baculoviruses, crops and field situations. However, it is evident that other systems may differ from the system described in this thesis. Therefore, an inventory of the applicability of BACSIM for different biological systems and purposes is given below.

Expanding the insect range: Insect behavior has a strong impact on the epidemiology of baculoviruses in insect populations. Gregarious or solitary feeding behavior of larvae will affect the horizontal transmission process, whereas the exposure of larval feeding sites to sprayed polyhedra is a major factor determining the efficacy of baculovirus applications. When an intended insect species has a clearly different behavior than *S. exigua*, parameter values and/or the structure of the simulation model have to be adjusted.

Expanding the baculovirus range: Changes in baculovirus infectivity, speed of action and inactivation rate can easily be incorporated in the model by adjusting parameter values of the intended virus. However, interactions between formulations or expressed gene products of recombinant baculoviruses with the crop-insect-virus system are not taken into account and have to be quantified experimentally. When these interactions alter the epidemiology of the baculovirus, parameter values and/or the structure of the simulation model have to be adjusted.

Expanding the crop range: Crop architecture and leaf area index will affect the distribution of sprayed polyhedra over the canopy layers. In addition, the distribution of leaf angles will affect the distribution of sprayed polyhedra over the abaxial and adaxial sides of the leaves. Together with the insect bionomics and foraging behavior these factors affect the polyhedra uptake by larvae. A chrysanthemum crop has a homogeneous canopy structure and is therefore simple to model. The interception of sprayed polyhedra in trees or crops with a heterogeneous canopy structure, such as cotton, requires a more extended description of the crop than the present description for chrysanthemum.

Expanding the physical environment: The applicability of BACSIM could substantially be expanded when the model could be applied for open field situations. However, the physical environment in field situations is much more heterogeneous than in greenhouses, since a greenhouse is a closed compartment in which the growing conditions can be controlled. This suggests that in a field situation, processes that are driven by environmental factors may also show increased variability, such as insect development and the speed of action of baculoviruses. Furthermore, presence of e.g. natural enemies will add further complexity to field situations. This increased level of variation and complexity may have serious consequences for the predictability of the plant-insect-baculovirus system in the field. Thus, when the use of BACSIM is extended to field situations an adequate characterization of the spatial heterogeneity of the field, as well as a proper description of additional mortality factors may be necessary. Also, in the field the exposure to UV-radiation is much higher than in greenhouses because the glass of the greenhouse filters UV-radiation. The polyhedra inactivation rate can easily be changed to values representative for a field situation by adjusting polyhedra inactivation parameters. However, for a field situation also the incorporation of a canopy layer dependent polyhedra inactivation should be considered. Polyhedra inactivation rates on sheltered sites are much lower than on sites that are directly exposed to sunlight. The current version of BACSIM does not include canopy layer dependent polyhedra inactivation because of the limited polyhedron inactivation rate in greenhouses. Another factor which makes the open field a more complex environment than greenhouses are polyhedra redistribution processes, such as rain. Although

sprayed polyhedra cannot easily be washed of the leaf surface, rain may cause redistribution of polyhedra produced in virus-killed larvae to other plant parts and increase the area of contaminated leaf in the crop. On the other hand, rain is also likely to wash a fraction of these polyhedra to the soil compartment where the rate of polyhedra uptake by larvae may be reduced. This redistribution is a highly complex process that contains a great deal of stochasticity. However, the first fundamental question that has to be answered is whether a separate description of rain events is needed for accurate model predictions. This should be studied experimentally.

Baculovirus competition: The use of BACSIM could be extended for risk assessment purposes when the competition between two baculoviruses for hosts is included. The first requirement for this is quantitative experimental data on virus genotype fitness in mixed infections of recombinant and wild-type baculoviruses. At the moment these data are not available, but this type of studies are ongoing (Cory *et al.*, 1999). The incorporation of competition between baculoviruses for hosts will require a substantial revision of BACSIM. The model should include a proper description of the infection process, reproduction rate and polyhedra yield of two or more baculovirus genotypes in an infected host.

Practical implications

Scenario studies clearly indicated that timing and dosage are key factors for the efficacy of baculovirus applications against *S. exigua* larvae (chapter 9). Baculovirus sprays should always be applied as early as possible, preferably at egg hatch. Polyhedra doses should be high enough to kill all larvae, but dosages exceeding LD_{100} values still contribute to a reduction of crop injury. When baculovirus applications with an appropriate dose are well-timed, other factors such as crop height, the speed of action of the virus and polyhedra inactivation rates only have a limited effect on the efficacy of the application. Thus, timing and dosage of baculovirus applications are the key factors for an optimal control of *S. exigua*. As a consequence, growers should emphasize monitoring of inflying *S. exigua* moths followed by immediate virus applications with the recommended dose when moths have been observed, rather than to base the spraying regime on observations of feeding marks in the crop.

Implications for use of genetically engineered baculoviruses

There is increasing interest for genetically engineered baculoviruses that can be used as improved biological control agents for insect pests. However, with the release of such

baculoviruses also risks are associated. At present, there is a broad public debate ongoing whether the introduction of genetically modified organisms is desirable.

Scenario studies indicated that the agronomic efficacy of viruses is largely determined by the timing and dose of the application, rather than the virus' speed of action (chapter 9). Crop injury is largely reduced when *S. exigua* is controlled as first or second instar larvae. For baculoviruses with a low speed of action the enhancement of the virus' speed of action by genetic engineering may reduce crop injury of well-timed applications, but the additional reduction in crop injury will be modest. For baculoviruses that have a natural high speed of action, the enhancement of the virus' speed of action does not contribute significantly to a further reduction in crop injury, as long as the application is properly timed. However, when the virus is applied when the larvae already reached the third or later instars, the use of genetically modified baculoviruses will result in reduced crop injury as compared to wild-type viruses. Hence, in situations where it is not possible to control sudden infestations of *S. exigua* with immediate virus applications recombinant baculoviruses may be desirable.

The desirability of baculovirus recombinants should be determined by the deliberations upon the benefit of its use and the associated risks. The introduction of genetically modified baculoviruses may be desirable when the benefits of their use outweigh the risks and when these baculoviruses are more suitable than alternative control methods, such as the use of pathogens, predators, fungi, parasitoids or environmentally safe chemicals.

The recombinant baculovirus that received until now most attention is AcMNPV-AaIT. This AcMNPV recombinant expresses the insect-specific neurotoxin AaIT and kill lepidopteran larvae typically 25% faster than AcMNPV wild-type (van Beek and Hughes, 1998). The field efficacy of AcMNPV-AaIT has been tested in several trials, which confirmed the improved biological properties of this virus in the field (Black *et al.*, 1997). Yet, with the use of such recombinant baculoviruses hazards, such as gene transfer to other organisms or wild populations, adverse effects of foreign gene products on non-target organisms and the effect of foreign gene expression on baculovirus competitive ability and fitness cannot be excluded. These risks are difficult to quantify. Studies that addressed these issues for AcMNPV-AaIT did not report negative effects of the virus nor the expressed AaIT on mammals and nontarget invertebrates (Black *et al.*, 1997, for review). The reduced polyhedron yield in larvae infected with AcMNPV-AaIT suggests that expression of this toxin make these recombinants less competitive than wild-type viruses because of this reduced reproductive capacity. Studies on the competitive ability of AcMNPV-AaIT and wild-type AcMNPV in mixed-infections are ongoing

(Cory *et al.*, 1999). At present there are no indications of unwanted side effects of the use AaIT expressing AcMNPV recombinants, but such effects can never be excluded.

In conclusion, high quality biological control agents may contribute to a further reduction of the use of chemical insecticides. The question whether the introduction of genetically modified baculoviruses is desirable is not easy to answer, but these decisions should be made on a case-by-case basis and should be supported by the general public. Therefore, there is a need for unbiased scientific information and an informed public debate about the benefits and risks associated with the use of genetically modified baculoviruses.

References

Adams, J.R., and McClintock, J.T. 1991. Baculovidae. *In* "Atlas of invertebrate viruses" (Adams, J.R., and McClintock, J.T., Eds.), pp 87-204. CRC Press, Boca Raton, USA

Albajes, R., Lodovica Gullino, M., van Lenteren, J.C., and Elad, Y. (Eds.) 1999. Integrated pest and disease management in greenhouse crops. Kluwer Academic Publishers, Dordrecht. 545 pp.

Ali, A., and Gaylor, M.J. 1992. Effects of temperature and larval diet on development of the beet armyworm (Lepidoptera: Noctuidae). *Environ. Entomol.* **21,** 780-786.

Alvaraso-Rodriguez, B., 1987. Parasites and diseases associated with larvae of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) infesting processing tomatoes in Sinaloa, Mexico. *Florida entomol.* **7**, 444-449.

Anderson, R.M., and May, R.M. 1981. The population dynamics of microparasites and their invertebrate hosts. *Phil. Trans. R. Soc. B.* **291,** 451-524.

Ayres, M.D., Howard, S.C., Kuzio, J., Lopez-Ferber, M., and Possee, R.D. 1994. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. *Virology* **202,** 586-605.

Beek, van, N.A.M., and Hughes, P.R. 1998. The response time of insect larvae infected with recombinant baculoviruses. *J. Invertebr. Pathol.* **72,** 338-347.

Bianchi, F.J.J.A., and van der Werf, W. 2000. Description, parameterization and user guide of BACSIM: an epidemiological model of the control of beet armyworm, *Spodoptera exigua,* with baculoviruses. Quantitative approaches in system analysis. CIP-DATA Koninklijke bibliotheek, The Hague. *In prep.*

Black, B.C., Brennan, L.A., Dierks, P.M., and Gard, I.E. 1997. Commercialization of baculoviral insecticides. *In* "The Baculoviruses" (L.K. Miller, Ed.), pp. 341-387. Plenum Press, New York.

Bloem, K.A., and Duffey, S.S. 1990. Effect of protein type and quantity on growth and developmental of larval *Heliothis zea* and *Spodoptera exigua* and the endoparasitoid *Hyposoter exigua. Entomol. Exp. Appl.* **54,** 141-148.

Bonning, B.C., Hirst, M., Possee, R.D., and Hammock, B.D. 1992. Further development of a recombinant baculovirus insecticide expressing the enzyme juvenile hormone esterase from *Heliothis virescens. Insect Biochem. Molec. Biol.* **22,** 453-458.

Boucias, D.G., Johnson, D.W., and Allan, G.E. 1980. Effects of host age, virus dosage, and temperature on the infectivity of a nucleopolyhedrosis virus against the velvetbean caterpillar, *Anticarsia gemmetalis*, larvae. *Environ. Entomol.* **9**, 59-61.

Brassel, J., and Benz, G. 1979. Selection of a strain of the granulosis virus of the codling moth with improved resistance against artificial ultraviolet radiation and sunlight. *J. Invertebr. Pathol.* **33,** 358-363.

Brewer, M.J., and Tumble, J.T. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **82,** 1520-1526.

Brinkman BV. 1993. Virussen als spuitmiddel tegen insecten toegelaten. Press Release, December 1.

Broadway, R.M., and Duffey, S.S. 1986. Plant proteinase inhibitors: mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua. J. Insect Physiol.* **32,** 827-833.

Caballero, P., Zuidema, D., Santiago-Alvarez, C., and Vlak, J.M. 1992. Biochemical and biological characterization of four isolates of *Spodoptera exigua* nuclear polyhedrosis virus. *Biocontr. Sci. Technol.* **2**, 145-157.

Carson, R. 1964. Silent spring. Fawcett Crest Books, New York.

CBS, 2000. Kencijfers tuinbouwgewassen onder glas. Centraal Bureau voor de statistiek.

CBS, 1999. Kencijfers tuinbouwgewassen onder glas. Centraal Bureau voor de statistiek.

Chen, X., Sun, X., Hu, Z.H., Li, M., O'Reilly, D.R., Zuidema D., and Vlak, J.M. 2000. Genetic engineering of *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus as an improved bioinsecticide. *J. Invertebr. Pathol., in press.*

Cheng, E.Y., Kao, C.H., and Chiu, C.S. 1990. Insecticide resistance study in *Plutella xylostella* L. X. The IGR-resistance and the possible management strategy. *J. Agr. Res. China* **39**, 208-220.

Cory, J.S., Burden, J.P., and Hails, R.S. 1999. Competition between wild-type and recombinant baculoviruses. *In* "Proceedings of the 32th annual meeting of the Society of Invertebrate Pathology", pp 30. Irvine, USA.

Cory, J.S., Hails, R.S., and Sait, S.M. 1997. Baculovirus ecology. *In* "The Baculoviruses" (L.K. Miller, Ed.), pp. 301-339. Plenum Press, New York.

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

Cunningham, J.C., Brown, K.W., Payne, N.J., Mickle, R.E., Grant, G.G., Flemming, R.A., Robinson, A., Curry, R.D., Langevin, D., and Burns, T. 1997. Aerial spray trials in 1992 and 1993 against gypsy moth, *Lymatria dispar* (Lepidoptera: Lymantriidae), using nuclear polyhedrosis virus with and without optical brightener compared to *Bacillus thuringiensis*. *Crop. Protect.* **16**, 15-23.

Dai, X., Hajos, J.P., Joosten, N.N., van Oers, M.M., IJkel, W.F.J., Zuidema, D., Yi, P., and Vlak, J.M. 2000. Isolation of a *Spodoptera exigua* baculovirus recombinant with retained biological activity despite a 10.6 kb deletion. *J. Gen. Virol., in press.*

David, W.A.L., Ellaby, S.J., and Taylor, G. 1971. The stability of a purified granulosis virus of the European cabbageworm, *Pieris brassicae*, in dry deposits of intact capsules. *J. invertebr. Pathol.* **17**, 228-233.

Dougherty, E.M., Guthrie, K.P., and Shapiro, M. 1996. Optical brighteners provide baculovirus activity enhancement and UV radiation protection. *Biol. Control* **7**, 71-74.

Dwyer, G. 1992. On the spatial spread of insect pathogens: theory and experiment. *Ecology* **73**, 479-494.

Dwyer, G., Dushoff, J., Elkinton, J.S., and Levin, S.A. 2000. Pathogen-driven outbreaks in forest defoliators revisited: building models from experimental data. *Am. Nat.* **156**, 105-120.

Dwyer, G., and Elkinton, J.S. 1993. Using simple methods to predict virus epizootics in gypsy moth populations. *J. Anim. Ecol.* **62**, 1-11.

Dwyer, G., Elkinton, J.S., and Buonaccorsi, J.P. 1997. Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. *Am. Nat.* **150**, 685-707.

Elleman, C.J., and Entwistle, P.F. 1985. Inactivation of a nuclear polyhedrosis virus on cotton by the substances produced by the cotton leaf surface glands. *Ann. Appl. Biol.* **106**, 83-92.

Elnagar, S., Tawfik, M.F.S., and Abdelrahman, T. 1982. Transmission of the nuclear polyhedrosis virus (NPV) disease of *Spodoptera littoralis* (Boisd.) via exposure of adults to the virus. *Z. Ang. Entomol.* **94**, 152-156.

References

Engelhard, E.K., and Volkman, L.E. 1995. Developental resistance in fourth instar *Trichoplusia ni* orally inoculated with *Autographa californica* M nuclear polyhedrosis virus. *Virology* **209**, 384-389.

Entwistle, P.F., Forkner, A.C., Green, B.M., and Cory, J.S. 1993. Avian dispersal of nuclear polyhedrosis viruses after induced epizootics in the Pine beauty moth, *Panolis flammea* (Leidoptera: Noctuidae). *Appl. Env. Microbiol.* **45**, 493-501.

Evans, H.F. 1981. Quantitative assessment of the relationships between dosage and response of the nuclear polyhedrosis virus of *Mamestra brassicae. J. Invertebr. Pathol.* **37**, 101-109.

Federici, 1997. Baculovirus pathogenesis. *In* "The Baculoviruses" (L.K. Miller, Ed.), pp. 33-59. Plenum Press, New York.

Felton, G.W., Doanto, K.K., Broadway, R.M., and Duffey, S.S. 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua. J. Insect Physiol.* **38**, 277-285.

Felton, G.W., Duffey, S.S., Vail, P.V., Kaya, H.K., and Manning, J. 1987. Interaction of nuclear polyhedrosis virus with catechols: potential incompatibility for host-plant resistance against noctuid larvae. *J. Chem. Ecol.* **4**, 947-957.

Flipsen, J.T.M., Mans, R.M.W., Kleefsman, A.W.F., Knebel-Mörsdorf, D., and Vlak, J.M. 1995. Deletion of the baculovirus ecdysteroid UDP-glucosyltransferase gene induces early degeneration of Malpighian tubules in infected insects. *J. Virol.* **69**, 4529-4532.

Forschler, B.T., Young, S.Y., and Felton, G.W. 1992. Diet and the susceptability of *Helicoverpa zea* (Noctuidae: Lepidoptera) to a nuclear polyhedrosis virus. *Environ. Entomol.* **21**, 1220-1223.

Fuxa, J.R., and Richter, A.R. 1992. Virulence and multigeneration passage of a nuclear polyhedrosis virus selected for an increased rate of vertical transmission. *Biol. Control* **2**, 171-175.

Fye, R.E., and McAda, W.C. 1972. Laboratory studies on the development, longevity, and fecundity of six lepidopterous pests of cotton in Arizona. *USDA Tech. Bull.* **1454**, 73 pp.

Georgis, R. 1997. Commercial prospects of microbial insecticides in agriculture. *In* "BCPC Symposium Proceedings No. 68: Microbial Insecticides: Novelty or Necessity?", pp. 243-252. Farnham, UK.

Gombart, A.F., Pearson, M.N., Rohrmann, G.F., and Beaudreau, G.S. 1989. A baculovirus polyhedral envelope-associated protein: genetic location, nucleotide sequence, and immunocytochemical characterization. *Virology* **169**, 182-193.

Goudriaan, J., and van Roermund, H.J.W. 1993. Modeling of ageing, development, delays and dispersion. *In* "On systems analysis and simulation of ecological processes" (P.A. Leffelaar Ed.), pp. 89-126. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Goulson, D., and Cory, J.S. 1995. Sublethal effects of baculovirus in the cabbage moth, *Mamestra brassicae. Biol. Control* **5**, 361-367.

Granados, R., and Federici, B. (Eds.) 1986. The biology of baculoviruses. Vol. I. Biological properties and molecular biology. CRC Press, Boca Raton, USA.

Hamm, J.J., and Young, J.R. 1974. Mode of transmission of nuclear polyhedrosis virus to progeny of adult *Heliothis zea. J. Invertebr. Pathol.* **24**, 70-81.

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

Harm, W. 1980. Biological effects of Ultra Violet-radiation. Cambridge. Cambridge University Press.

Heuvelink, E., and Lee, J.H. 1998. Effects of plant density and length of the long-day period on dry matter production in cut chrysanthemum: measurement and simulation. *In* "Proceedings of the XXV International Horticultural Congress", pp. 386. Brussel, Belgium.

Hogg, D.B., and Gutierrez, A.P. 1980. A model for the flight phenology of the beet armyworm (Lepidoptera: Noctuidae) in central California. *Hilgardia* **48**, 1-23.

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

Hoover, K., Stout, M.J., Alaniz, S.A., Hammock, B.D., and Duffey, S.S. 1998a. Influence of induced plant defenses in cotton and tomato on the efficacy of baculoviruses on noctuid larvae. *J. Chem. Ecol.* **24**, 253-271.

Hoover, K., Yee, J.L., Schultz, C.M., Rocke, D.M., Hammock, B.D., and Duffey, S.S. 1998b. Effects of plant identity and chemical constituents on the efficacy of a baculovirus against *Heliothis virescens*. *J. Chem. Ecol.* **24**, 221-252.

Huber, J. 1998. World survey of virus control of insect pests: Western Europe. *In* "Insect viruses and pest management" (Hunter-Fujita, F.R., Entwistle, P.F., Evans, H.F., and Crook, N.E., Eds.), pp. 201-215. John Wiley and sons, Chichester.

Hughes, P.R., and Wood, H.A. 1981. A synchronous peroral technique for the bioassay of insect viruses. *J. Invertebr. Pathol.* **37**, 154-159.

Hughes, P.R., Wood, H.A., Burand, J.P., and Granados, R.R. 1984. Quantification of the dose-mortality response of *Trichoplusia ni*, *Heliothis zea*, and *Spodoptera frugiperda* to nuclear polyhedrosis viruses: applicability of an exponential model. *J. Invertebr. Pathol.* **43**, 343-350.

Hung, C.F., and Sun, C.N. 1989. Microsomal monooxygenases in diamondback moth larvae resistant to fenvalerate and piperonyl butoxide. *Pesticide Biochem. physiol.* **33**, 168-175.

Hunter-Fujita, F.R., Entwistle, P.F., Evans, H.F., and Crook, N.E. (Eds.) 1998. Insect viruses and pest management. John Wiley and sons, Chichester. 620 pp.

Hunter, D.K., and Hall, I.M. 1968. Cytopathology of a nuclear polyhedrosis virus of the beet armyworm, *Spodoptera exigua*. *J. Invertebr. Pathol.* **12**, 93-97.

Ignoffo, C.M., and Garcia, C. 1994. Antioxidant and oxidative enzyme effects on the inactivation of inclusion bodies the *Heliothis* baculovirus by simulated sunlight-UV. *Biol. Control* **23**, 1025-1029.

Ignoffo, C.M., Garcia, C., and Saathoff, S.G. 1997. Sunlight stability and rain-fastness of formulations of *Baculovirus heliothis*. *Environ. Entomol.* **26**, 1470-1474.

Ignoffo, C.M., Garcia, C., Zuidema, D., and Vlak, J.M. 1995. Relative *in vivo* activity and simulated sunlight-UV stability of inclusion bodies of a wild-type and an engineered polyhedral envelope-negative isolate of the nucleopolyhedrosis virus of *Autographa californica*. *J. Invertebr. Pathol.* **66**, 212-213.

Jacques, R.P. 1972. The inactivation of foliar deposits of viruses of *Trichoplusia ni* (Lepidoptera: Pieridae) and tests on protectant additives. *Can. Entomol.* **104**, 1985-1994.

Jaques, R.P., Laing, J.E., Laing, D.R., and Yu, D.S.K. 1987. Effectiveness and persistence of the granulosis virus of the codling moth *Cydia pomonella* (L.) (Lepidoptera: Olethreutidae) on apple. *Can. Entomol.* **119**, 1063-1067.

References

Jansma, J.E., van Keulen, H., and Zadoks, J.C. 1993. Crop protection in the year 2000: a comparison of current policies towards agrochemical usage in four West European countries. *Crop Protection* **12**, 483-489.

Jones, K.A., Moawad, G., McKinley, D.J., and Grzywacz, D. 1993. The effect of natural sunlight on *Spodoptera littoralis* nuclear polyhedrosis virus. *Biocontr. Sci. Technol.* **3**, 189-197.

Kaupp, W.J. 1981. Studies of the ecology of the nuclear polyhedrosis virus of the European pine sawfly, *Neodiprion sertifer*. PhD Thesis, University of Oxford, UK.

Keating, S.T., Hunter, M.D., and Schultz, J.C. 1990. Leaf phenolic inhibition of gypsy moth nuclear polyhedrosis virus. *J. Chem. Ecol.* **16**, 1445-1457.

Keating, S.T., Yendol, W.G., and Schultz, J.C. 1988. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lyamantriidae) to a bacolovirus and host plant foliage constituents. *Environ. Entomol.* **17**, 952-958.

Kirkpatrick, B.A., Washburn, J.O., and Volkman, L.E. 1998. AcMNPV pathogenesis and developmental resistance in fifth instar *Heliothis virescens*. *J. Invertebr. Pathol.* **72**, 63-72.

Kolodny-Hirsch, D.M., Sitchawat, T., Jansiri, T., Chenrchaivachirakul, A., and Ketunuti, U. 1997. Field evaluation of a commercial formulation of the *Spodoptera exigua* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus for control of beet armyworm on vegetable crops in Thailand. *Biocontr. Sci. Technol.* **7**, 475-488.

Kolodny-Hirsch, D.M., Warkentin, D.L., Alvarado-Rodrigues, B., and Kirkland, R. 1993. *Spodoptera exigua* nuclear polyhedrosis virus as a candidate viral insecticide for the beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **86**, 314-321.

Korth, K.L., and Levings, C.S. 1993. Baculovirus expression of the maize mitochondrial protein URF13 confers insecticidal activity in cell cultures and larvae. *Proc. Natl. Acad. Sci. USA* **90**, 3388-3392.

Kraker, de, J. 1996. The potential of natural enemies to supress rice leaffolder populations. PhD Thesis, Wageningen Agricultural University, The Netherlands, 257 pp.

Lee, S.D., Ahn, S.B., Cho, W.S., and Choi, K.M. 1991a. Effects of temperature on the development of beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). *Res. Rept. RDA (C.P.)* **33**, 58-62.

Lee, S.D., Ahn, S.B., Hong, K.J., and Cho, W.S. 1991b. Effects of three host plants on the development of beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). *Res. Rept. RDA (C.P.)* **33**, 53-57.

Lu, A., Krell, P.J., Vlak, J.M., and Rohrmann, G.F. 1997. Baculovirus DNA replication. *In* "The Baculoviruses" (Miller, L.K., Ed.), pp. 171-191. Plenum Press, New York.

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

Martens, J.W.M., van Oers, M.M., van de Bilt, B.D., Oudshoorn, P., and Vlak, J.M. 1995. Development of a baculovirus vector that facilitates the generation of p10-based recombinants. *J. Virol. Meth.* **52**, 15-19.

Martignoni, M.E., and Iwai, P.J. 1986. A catalogue of viral diseases of insects, mites, and ticks. *USDA forest service*, PNW-**195**. Washington, USA.

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

McLeod, P.J., Young, S.Y., and Yearian, W.C. 1982. Application of a baculovirus of *Pseudoplusia includens* to soybean: efficacy and seasonal persistence. *Environ. Entomol.* **11**, 412-416.

Miller, L.K. (Ed.) 1997. The Baculoviruses. Plenum Press, New York. 447 pp.

MJP-G: Ministerie van Landbouw, Natuurbeheer en Visserij. 1991. Meerjarenplan gewasbescherming; Regeringsbeslissing. Tweede kamer, vergaderjaar 1990-1991, 21677, nr. 3-4. Staatsuitgeverij, Den Haag.

Moed, de, G.H., van der Werf, W., and Smits, P.H. 1990. Modeling the epizootiology of *Spodoptera exigua* nuclear polyhedrosis virus in a spatially distributed population of *Spodoptera exigua* in greenhouse chrysanthemums. *SROP/WPRS Bull.* **8**, 135-141.

Moscardi, F. 1999. Assessment of the application of baculoviruses for control of lepidoptera. *Annu. Rev. Entomol.* **44**, 257-289.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A., and Summers, M.D. (Eds.) 1995. Virus taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses. Springer-Verlag, Wien-New York, pp. 104-113.

Oers, van, M.M., and Vlak, J.M. 1997. The baculovirus 10-kDa protein. *J. Invertebr. Pathol.* **70**, 1-17.

Onstad, D.W., Maddox, J.V., Cox, D.J., and Kornkven, E.A. 1990. Spatial and temporal dynamics of animals and the host-density treshold in epizootiology. *J. Invertebr. Pathol.* **55**, 76-84.

O'Reilly, D.R., and Miller, L.K. 1989. A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. *Science* **245**, 1110-1112.

O'Reilly, D.R., and Miller, L.K. 1991. Improvement of a baculovirus pesticide by deletion of the *egt* gene. *Bio/Technol.* **9**, 1086-1089.

Payne, R.W., Lane, P.W., Digby, P.G.N., Harding, S.A., Leech, P.K., Morgan, G.W., Todd, A.D., Thompson, R., Tunnicliffe Wilson, G., Welham, S.J., and White, R.P. 1993. Genstat 5 release 3 reference manual. Clarendon Press, Oxford, U.K.

Peto, S. 1953. A dose-response equation for the invasion of microorganisms. *Biometrics* **9**, 320-335.

Prikhod'ko, G.G., Robson, M., Warmke, J.W., Cohen, C.J., Smith, M.M., Wang, P., Warren, V., Kaczorowski, G., van der Ploeg, L.H.T., and Miller, L.K. 1996. Properties of three baculovirus-expressing genes that encode insect-selective toxins: μ-AGA-IV, As II, and Sh I. *Biol. Control* **7**, 236-244.

Rabbinge, R., and de Wit, C.T. 1989. *In* "Simulation and systems management in crop protection" (R. Rabbinge, S.A. Ward, and H.H. van Laar, Eds). Simulation Monographs, Pudoc, Wageningen. 420 pp.

Richards, M.G., and Payne, C.C. 1982. Persistence of baculoviruses on leaf surfaces. *In* "Proceedings of 3rd International Colloquium on Invertebrate Pathology", pp. 296-301. Brighton, UK.

Richter, A.R., Fuxa, J.R., and Abdel-Fattah, M. 1987. Effect of host plant on the susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to a nuclear polyhedros virus. *Environ. Entomol.* **16**, 1004-1006.

Roermund, van, H.J.W. 1995. Understanding biological control of greenhouse whitefly with the parasitoid *Encarcia formosa*. PhD Thesis, Wageningen Agricultural University, The Netherlands, 243 pp.

References

Roermund, van, H.J.W., van Lenteren, J.C., and Rabbinge, R. 1997. Biological control of greenhouse whitefly with the parasitoid *Encarsia formosa* on tomato: An individual-based simulation approach. *Biol. Control* **9**, 25-47.

Rossing, W.A.H., Leeuwis, C., and van der Werf, W. 1999. Quality of modelling in fruit research and orchard management: report of a discussion. *In* "Computer modelling in fruit research and orchard management" (P.S. Wagenmakers, W. van der Werf, and Ph. Blaise, Eds). *Acta Horticulturae* **499**, 1213-220.

Russell, R.M., Robertson, J.L., and Savin, N.E. 1977. POLO: A new computer program for probit analysis. *Bull. Entomol. Soc. Am.* **23**, 209-213.

Santiago-Alvarez, C., and Ortiz-Garcia, R. 1992. The influence of host plant on the susceptibility of *Spodoptera littoralis* (Boisd.) (Lep., Noctuidae) larvae to *Spodoptera littoralis* NPV (Baculoviridae, Baculovirus). *J. Appl. Ent.* **114**, 124-130.

Shapiro, M. 1995. Radiation protection and the activity enhancement of viruses. *In* "Biorational Pest Control Agents: Formulation and Delivery" (Hall, F.R. and Barry, J.W., Eds.), pp. 153-164. American Chemical Society, Washington.

Shapiro, M., and Bell, R.A. 1984. Selection of a UV-tolerant strain of the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), nucleopolyhedrosis virus. *Environ. Entomol.* **13**, 1522-1526.

Shapiro, M., and Robertson, J.L. 1992. Enhancement of gypsy moth (Lepidoptera: Lymantriidae) baculovirus activity by optical brighteners. *J. Econ. Entomol.* **84**, 1120-1124.

Slavicek, J.M., Popham, H.J.R., and Riegel, C.I. 1999. Deletion of the *Lymantria dispar* multicapsid nucleopolyhedrovirus ecdysteroid UDP-glucosyltransferase gene enhances viral killing speed in the last instar of the gypsy moth. *Biol. Control* **16**, 91-103.

Smits, P.H. 1987. Nuclear polyhedrosis virus as biological control agent of *Spodoptera exigua*. PhD Thesis, Wageningen Agricultural University, The Netherlands, 127 pp.

Smits, P.H., Rietstra, I.P., and Vlak, J.M. 1988. Influence of application techniques on the control of beet armyworm larvae (Lepidoptera: Noctuidae) with nuclear polyhedrosis virus. *J. Econ. Entomol.* **81**, 470-475.

Smits, P.H., van Velden, M.C., van de Vrie, M., and Vlak, J.M. 1987a. Feeding and dispersion of *Spodoptera exigua* larvae and its relevance for control with a nuclear polyhedrosis virus. *Entomol. Exp. Appl.* **43**, 67-72.

Smits, P.H., van de Vrie, M., and Vlak, J.M. 1986. Oviposition of beet armyworm (Lepidoptera: Noctuidae) in greenhouse crops. *Environ. Entomol.* **15**, 1189-1191.

Smits, P.H., van de Vrie, M., and Vlak, J.M. 1987b. Nuclear polyhedrosis virus for control of *Spodoptera exigua* larvae on glasshouse crops. *Entomol. Exp. Appl.* **43**, 73-80.

Smits, P.H., and Vlak, J.M. 1988a. Biological activity of *Spodoptera exigua* nuclear polyhedrosis virus against *S. exigua* larvae. *J. Invertebr. Pathol.* **51**, 107-114.

Smits, P.H., and Vlak, J.M. 1988b. Selection of nuclear polyhedrosis viruses as biological control agents of *Spodoptera exigua* (Lepidoptera: Noctuidae). *Entomophaga* **33**, 299-308.

Smits, P.H., and Vlak, J.M. 1988c. Quantitative and qualitative aspects in the production of a nuclear polyhedrosis virus in *Spodoptera exigua* larvae. *Ann. Appl. Biol.* **122**, 249-257.

Smits, P.H., and Vlak, J.M. 1994. Registration of the first viral insecticide in the Netherlands: the development of Spod-X, based on *Spodoptera exigua* nuclear polyhedrosis virus. *Med. Fac. Landbouww. Univ. Gent.* **59**, 385-392.

Snedecor, G.W., and Cochran, W.G. 1989. Statistical methods. Iowa State University Press, Ames, Iowa.

Stairs, G.R. 1978. Effects of a wide range of temperature on the development of *Galleria mellonella* and its specific baculovirus. *Environ. Entomol.* **7**, 297-299.

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

Summers, M.D., and Smith, G.E. 1987. A manual of methods for baculovirus vectors and insect cell culture procedures. *Texas Agri. Exp. Stat. Bull.* **1555**.

Teakle, R.E., Jensen, J.M., Giles, J.E. 1986. Age-related susceptibility of *Heliothis punctiger* to a commercial formulation of nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **47**, 82-92.

Tomalski, M.D., Hutchinson, K., Todd, J., and Miller, L.K. 1993. Identification and characterization of tox21A: A mite cDNA encoding a paralytic neurotoxin related to TxP-I. *Toxicon* **31**, 319-326.

Tomalski, M.D., and Miller, L.K. 1991. Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene. *Nature* **352**, 82-85.

Treacy, M.F., and All, J.N. 1996. Imact of insect-specific AaHIT gene insertion on inherent bioactivity of baculovirus against tobacco budworm, *Heliothis virescens*, and cabbage looper, *Trichoplusia ni*. *In* "Proceedings of the Beltwide Cotton Production and Research Conference" (Dugger, P. and Richter, D., Eds.), pp 911-917. Nation Cotton Council, Memphis.

Treacy, M.F., All, J.N., and Ghidiu, G.M. 1997. Effect of ecdysteroid UDP-glucosyltransferase gene deletion on efficacy of a baculovirus against *Heliothis virescens* and *Trichoplusia ni* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **90**, 207-1214.

Tuan, S.J., Tang, L.C., and Hou, R.F. 1989. Factors affecting pathogenicity of NPV preparations to the corn earworm, *Heliothis armigera*. *Entomophaga* **4**, 541-549.

Vail, P.V., Hoffmann, D.F., and Tebbets, J.S. 1996. Effect of a fluorescent brightener on the activity of *Anagrapha falcifera* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus to four noctuid pests. *Biol. Control* **7**, 121-125.

Vasconcelos, S.D. 1996. Alternative routes for the horizontal transmission of a nucleopolyhedrovirus. *J. Invertebr. Pathol.* **68**, 269-274.

Veld, de, A. 2000. De sierteelttoppers van 1999. Vakblad voor de bloemisterij **2**, 30-31.

Vlak, J.M., Klinkenberg, F.A., Zaal, K.J.M., Usmany, M., Klinge-Roode, E.C., Geervliet, J.B.F., Roosien, J., and van lent, J.W.M. 1988. Functional studies on the *p10* gene of *Autographa californica* nuclear polyhedrosis virus using a recombinant expressing a *p10*-β-galactosidase fusion gene. *J. Gen. Virol.* **69**, 765-776.

Webb, R.E., Shapiro, M., Podgewaite, J.D., Ridgway, R.L., Venables, L., White, G.B., Argauer, R.J., Cohen, D.L., Witcosky, J., Kester, K.M., and Thorpe, K.W. 1994. Effect of optical brighteners on the efficacy of gypsy moth (lepidoptera, Lymantriidae) nuclear polyhedrosis virus in forest plots with high or low levels of natural virus. *J. Econ. Entomol.* **87**, 134-143.

Werf, van der, W., Leeuwis, C., and Rossing, W.A.H. 1999. Quality of modelling in fruit research and orchard management: issues for discussion. *In* "Computer modelling in fruit research and orchard

management" (P.S. Wagenmakers, W. van der Werf, and Ph. Blaise, Eds). *Acta Horticulturae* **499**, 151-160.

Werf, van der, W., de Moed, G.H., and Smits, P.H. 1991. A comprehensive simulation model of the epidemiology of *Spodoptera exigua* nuclear polyhedrosis virus on beet armyworm in glasshouse chrysanthemums. *IOBC/WPRS Bull.*, **14**, 108-117.

Wood, H.A., Munkenbeck-Trotter, K., Davis, T.R., and Hughes, P.R. 1993. *Per os* infectivity of preoccluded virions from polyhedrin-minus recombinant baculoviruses. *J. Invertebr. Pathol.* **62**, 64-67.

Xia, J. 1997. Biological control of cotton aphid (*Aphis gossypii* Glover) in cotton (inter) cropping systems in China; a simulation study. PhD Thesis, Wageningen Agricultural University, The Netherlands, 173 pp.

Young, S.Y. 1990. Effect of nuclear polyhedrosis virus infection in *Spodoptera ornithogalli* larvae on post larval stages and dissemination by adults. *J. Invertebr. Pathol.* **55**, 69-75.

Young, S.Y., and Yearian, W.C. 1982. Nuclear polyhedrosis virus infection of *Pseudoplusia includens* (Lep.: Noctuidae) larvae: effect of post larval stages and transmission. *Entomphaga* **27**, 61-66.

Zuidema, D., Klinge-Roode, E.C., van Lent, J.W.M., and J.M. Vlak. 1989. Construction and analysis of an *Autographa californica* nuclear polyhedrosis virus mutant lacking the polyhedral envelope. *Virology* **173**, 98-108.

Summary

This thesis describes the development of a process-based simulation model for the population dynamics of beet armyworm, *Spodoptera exigua*, and baculoviruses in greenhouse chrysanthemum. The model (BACSIM) has been validated for two baculoviruses with clear differences in biological characteristics, *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) and *Spodoptera exigua* MNPV (SeMNPV). The validated model has been used to generate scenario studies, which are used for the evaluation of the potential of baculoviruses as biological control agents.

The first requirement for the construction of BACSIM is quantitative understanding of the processes that determine the chrysanthemum-*S. exigua*-baculovirus system, such as insect behavior and population dynamics, the baculovirus infection cycle and virus transmission routes. These processes have been studied and described in chapters 2 to 5.

S. exigua parameters: The developmental rate and background mortality of populations of *S. exigua* larvae have been quantified on chrysanthemum in a greenhouse situation (chapter 4). In addition, the preference of feeding sites and leaf visit rate of *S. exigua* larvae have been studied on chrysanthemum plants under controlled conditions (chapter 4).

Baculovirus parameters: The infectivity and speed of action of AcMNPV and SeMNPV has been determined for various stages of *S. exigua* larvae that were reared on artificial diet (chapter 2). To study the effect of the host plant on the baculovirus infection cycle the infectivity and speed of action of SeMNPV has also been determined for *S. exigua* larvae reared on chrysanthemum (chapter 4). *S. exigua* larvae reared on chrysanthemum appeared to be more susceptible to SeMNPV than larvae reared on artificial diet. The polyhedron inactivation rate of AcMNPV and SeMNPV on greenhouse chrysanthemum has been quantified in chapter 3 and 7, respectively. Wild-type AcMNPV and SeMNPV have marked differences in infectivity and speed of action, but the relative inactivation rates of AcMNPV and SeMNPV polyhedra were not significantly different. The deletion of the *egt*, *pp34* or *p10* genes from AcMNPV had no or only a marginal impact on the infectivity, speed of action or inactivation rate of this virus (chapter 2). In other virus-host combinations such deletions had a major effect on these parameters. As the AcMNPV recombinants behaved biologically in a similar way as wild-type AcMNPV, wild-type AcMNPV and SeMNPV were chosen as viruses for the model system.

Baculovirus transmission: Horizontal transmission of SeMNPV in *S. exigua* populations with varying densities of primarily SeMNPV-infected larvae has been studied in

chrysanthemum under greenhouse conditions (chapter 5). The distribution of polyhedra released from SeMNPV-killed larvae over chrysanthemum plants has been quantified in detail under controlled conditions (chapter 4). The vertical transmission rate of SeMNPV in *S. exigua* populations has been determined by the quantification of the percentage of first instar larvae originating from egg batches produced by sublethally infected moths that contracted SeMNPV (chapter 5).

The process-based simulation model BACSIM is based on a detailed quantitative description of the behavior and population dynamics of insects, plant growth characteristics, spray deposition, the baculovirus infection cycle and transmission routes (chapter 7). These data were obtained from experiments mentioned above, or were retrieved from literature. The evaluation of the biological control of insect pests with BACSIM offers the possibility to include many treatments and situations, to simulate baculoviruses with extreme biological properties and to include many covariables that may modify the efficacy of baculoviruses in practice, such as crop age and temperature. In addition, this simulation approach allows optimization procedures that can assess optimal virus properties and spraying regimes under varying conditions.

The BACSIM model has been validated with independent data of ten greenhouse trials in which the efficacy of AcMNPV and SeMNPV against synchronized populations of *S. exigua* larvae in chrysanthemum was assessed. Dose- and time-mortality relationships of different virus concentrations and *S. exigua* target stages were determined and crop injury assessed as the total leaf area consumed (chapter 6). The validation of the simulation model BACSIM with the independent data of AcMNPV and SeMNPV applications in greenhouse chrysanthemum was in general satisfying. Mortality levels of AcMNPV and SeMNPV infected larvae were generally predicted within a 25% error margin compared to the observed values. None of the deviations were higher than 40%. All values of simulated foliage consumption, caused by *S. exigua* populations treated with AcMNPV or SeMNPV applications, fell within the 95% confidence intervals of measurements. However, simulated time-mortality relationships gave generally lower survival times than was measured in experiments. A hypothesis that may explain this discrepancy is the functioning of the underside of chrysanthemum leaves as a refuge area where *S. exigua* larvae are exposed much less to polyhedra (chapter 8).

Scenario studies with BACSIM were carried out to evaluate the effectiveness of different spraying regimes, dosages, UV protection agents and speed of action of viruses in different pest situations (chapter 9). The recommended dosages of SeMNPV applications

based on simulations with BACSIM corresponded well with recommended SeMNPV dosages determined for cultivation practice. An early timing of virus applications, soon after egg batch deposition, appeared to be essential for effective control. UV-protection agents may contribute only marginally to effective biological control in the glasshouse. The decay rates of SeMNPV and AcMNPV already ensure a period of exposure to active virus that is long enough for caterpillars to acquire a lethal dose under practical conditions. The effect of genetic improvement towards shortening the survival time of infected larvae depends on the situation in which such a virus is used. When used in a situation with constant immigration of pest insects, a greater reduction of feeding injury is achieved with a faster killing virus than with a wild-type virus. When, however, immigration of pest insects occurs by way of sudden influxes, resulting in synchronous insect populations in the crop, it appears that early timing of virus applications is more critical to reduce crop injury than using a fast-killing virus. In such a situation, good monitoring followed by virus application is indispensable, and may even alleviate the need for a fast killing virus.

Besides the use of BACSIM as a tool to assist in the determination of effective spraying regimes of (genetically modified) baculoviruses or formulations under varying conditions, the model can also be used as a tool to gain insight in the insect-baculovirus-crop system. For example, BACSIM may be used to address fundamental ecological questions or used to evaluate the relative importance of viral characteristics for crop protection purposes. In addition, BACSIM may contribute to the assessment of risks associated with the use of genetically modified baculoviruses by the generation of quantitative information of the population dynamics of recombinant baculoviruses in crops. Finally, the methodology described in this thesis may form a basic concept for process-based modeling of more complex systems, such as the control of bollworm with baculoviruses in cotton.

Samenvatting

In dit proefschrift wordt de ontwikkeling van een simulatiemodel voor de populatiedynamica van insect-baculovirussystemen beschreven. Het model is van toepassing op de floridamot, *Spodoptera exigua*, in de chrysantenteelt in kassen. Het model (BACSIM) is gevalideerd voor twee baculovirussen met duidelijk verschillende biologische eigenschappen, het *Autographa californica* multicapside nucleopolyhedrovirus (AcMNPV) en het *Spodoptera exigua* MNPV. De effectiviteit van baculovirussen als biologisch bestrijdingsmiddel is geëvalueerd aan de hand van scenariostudies.

Een eerste vereiste voor de ontwikkeling van het model is kwantitatieve informatie over de processen die van belang zijn voor het chrysant-*S. exigua*-baculovirussysteem, zoals het gedrag en de populatiedynamica van *S. exigua*, de infectiecyclus van het baculovirus en virustransmissieroutes. Deze processen zijn bestudeerd en beschreven in hoofdstukken de 2 tot en met 5.

S. exigua parameters: de ontwikkelingssnelheid en achtergrondmortaliteit van populaties *S. exigua*-larven is onderzocht in chrysant in de kas (hoofdstuk 4). Daarnaast is het fourageergedrag van *S. exigua*-larven op chrysant bepaald onder geconditioneerde omstandigheden (hoofdstuk 4).

Baculovirus parameters: de infectiositeit en werkingssnelheid van AcMNPV en SeMNPV is bepaald in verschillende larvale stadia van *S. exigua*, waarbij de larven waren opgekweekt op kunstmatig medium (hoofdstuk 2). Om het effect van de waardplant op de baculovirusinfectiecyclus te bepalen, is de infectiositeit en werkingssnelheid van SeMNPV ook bepaald in *S. exigua*-larven, opgekweekt op chrysant (hoofdstuk 4). Het bleek dat larven die opgekweekt waren op chrysant, gevoeliger waren voor SeMNPV dan larven die opgekweekt waren op kunstmatig medium. De bepaling van de inactivatie van AcMNPV en SeMNPV op kaschrysant is beschreven in de hoofdstukken 3 en 7. AcMNPV en SeMNPV hebben duidelijke verschillen in infectiositeit en werkingssnelheid, maar er was geen statistisch significant verschil in relatieve inactivatiesnelheden van polyeders. De deletie van de *egt*, *pp34* of *p10*-genen had geen of nauwelijks effect op de infectiositeit, werkingssnelheid en relatieve inactivatie-snelheid van AcMNPV (hoofdstuk 2). Voor andere virus-insect combinaties hadden dergelijke deleties een groot effect op deze parameters. Uiteindelijk zijn wild-type AcMNPV en SeMNPV gekozen als virussen waarmee kasproeven zijn uitgevoerd om het model te testen.

Baculovirustransmissie: de horizontale transmissie van SeMNPV in *S. exigua*-populaties is bestudeerd in chrysant in een kassituatie, waarbij de dichtheden initieel SeMNPV-geïnfecteerde larven werden gevarieerd (hoofdstuk 5). De verspreiding van polyeders over chrysantenplanten uit door virus gedode larven is nauwkeuriger bepaald onder geconditioneerde omstandigheden (hoofdstuk 4). De verticale transmissie van SeMNPV in populaties van *S. exigua* is bestudeerd aan de hand van het percentage SeMNPV-geïnfecteerd nageslacht van subletaal geïnfecteerde *S. exigua*-motten (hoofdstuk 5).

BACSIM is gebaseerd op een gedetailleerde beschrijving van het gedrag en de populatie-dynamica van *S. exigua*, gewasgroeikarakteristieken van chrysant, virusbespuitingen, de baculovirusinfectiecyclus en transmissieroutes (hoofdstuk 7). Deze gegevens zijn verkregen uit de bovengenoemde proeven en uit literatuur. De evaluatie van de bestrijding van *S. exigua* met baculovirussen met behulp van BACSIM heeft een aantal voordelen. Met deze simulatiebenadering kunnen veel behandelingen en situaties nagebootst worden, kunnen er veel co-variabelen geanalyseerd worden die bepalend zijn voor het verloop van de bestrijding en kunnen optimale viruseigenschappen en bespuitingsregimes onder uiteenlopende omstandigheden bepaald worden.

BACSIM is gevalideerd met onafhankelijke gegevens van 10 kasproeven met AcMNPV en SeMNPV. In deze proeven werd de bestrijding van gesynchroniseerde populaties *S. exigua*-larven met AcMNPV en SeMNPV bepaald (hoofdstuk 6). De voorspelde bestrijding van AcMNPV en SeMNPV met behulp van BACSIM kwam in het algemeen goed overeen met de metingen. De afwijking van gesimuleerde en gemeten mortaliteit als gevolg van AcMNPV- en SeMNPV- bespuitingen was in de meeste gevallen kleiner dan 25% en was nooit groter dan 40%. Gesimuleerde vraat van *S. exigua*-larven na AcMNPV- of SeMNPV-bespuitingen viel altijd binnen 95%-betrouwbaarheidsintervallen van metingen. De gesimuleerde incubatieperiode van AcMNPV en SeMNPV in geïnfecteerde larven was in het algemeen langer dan in de metingen. Een mogelijke verklaring voor dit verschil is dat larven zich verschuilen aan de onderkant van chrysantenbladeren tijdens en na virusbespuitingen, waar zich nagenoeg geen virus bevindt. Larven kunnen dan pas geïnfecteerd worden zodra ze deze schuilplaats verlaten hebben (hoofdstuk 8).

De effectiviteit van verschillende baculovirusdoses, bespuitingsregimes, toediening van UV-absorberende componenten en de werkingssnelheid van het virus is geëvalueerd aan de hand van scenariostudies met BACSIM. De aanbevolen doses voor SeMNPV-bespuitingen, gebaseerd op simulaties, bleken goed overeen te komen met in de praktijk gehanteerde doses. Een goede timing van baculovirusbespuitingen bleek essentieel voor een

effectieve bestrijding van *S. exigua*. Larven moeten bij voorkeur meteen na het uitkomen bestreden worden. Het toevoegen van UV-absorberende componenten aan baculovirusformuleringen zal maar een beperkt effect hebben op de effectiviteit van de bestrijding. Onder kascondities blijft het virus van nature lang genoeg actief om *S. exigua*-larven effectief te kunnen bestrijden. De effectiviteit van genetisch gemodificeerde baculovirussen met een snellere werkingssnelheid hangt af van de situatie waarin het virus toegediend wordt. In geval van continue invlieg van *S. exigua*-motten zal het gebruik van sneller dodend virus tot een beperking van de vraatschade leiden. Als er echter sprake is van invlieg van *S. exigua*-motten in afzonderlijke generaties zal een juiste timing van de bespuiting belangrijker zijn dan de werkingssnelheid van het virus. Een goede monitoring van invliegende motten zal in dit geval het gebruik van virussen met een verhoogde werkingssnelheid overbodig maken.

BACSIM kan behalve voor de bepaling van effectieve bespuitingsregimes voor (genetisch gemodificeerde) baculovirussen ook gebruikt worden om meer inzicht te verkrijgen in het gewas-insect-virussysteem. Zo kan BACSIM licht werpen op fundamentele ecologische vraagstukken en viruseigenschappen, die nodig zijn voor een effectieve bestrijding van plaaginsecten. Daarnaast kan BACSIM gebruikt worden voor de inschatting van de risico's van het gebruik van genetisch gemodificeerde baculovirussen door kwantitatieve informatie te verschaffen over de populatiedynamica van recombinant baculovirus. Tenslotte kan de in dit proefschrift beschreven methodologie een basis concept vormen voor het modelleren van complexere systemen, zoals de bestrijding van *Helicoverpa armigera* met baculovirussen.

Nawoord

Toen ik vijf jaar geleden begon met mijn promotieonderzoek bij de vakgroepen Virologie en Theoretische Productie Ecologie, was ik nagenoeg onbekend met de biologische bestrijding van plaaginsecten met baculovirussen. Als afgestudeerd bosbouwer wist ik alles van het omzagen van bomen en had ik inmiddels ook ervaring met modelering, maar "moleculair biologische begrippen" als PCR, restrictie-enzymanalyse en Eppendorfjes waren in mijn studie aan mij voorbij gegaan. De eerste periode liep ik dan ook regelmatig tegen een muur van moleculair jargon op die het merendeel van mijn moleculair georiënteerde collega's bezigden.

Dat het nu vijf jaar na de start van mijn OIO project toch tot een goed einde is gekomen, is onder andere te danken aan de begeleiding Just Vlak en Wopke van der Werf. Just maakte me bekend met de principes van de virologie, terwijl Wopke me begeleidde met het opstellen van het simulatiemodel. Ook de bijdrage van Rudy Rabbinge, die het project altijd nauwgezet van een afstand in de gaten hield, was weliswaar niet opvallend, maar wel belangrijk. De samenwerking tussen de vakgroepen Virologie en Theoretische Productie Ecologie bleek al snel een duidelijke meerwaarde op te leveren en verdient wat mij betreft dan ook navolging. Ook de input van buitenaf door Peter Smits van Plant Research International en Machiel van Steenis van Brinkman BV bleek zeer nuttig bij de evaluatie van het simulatiemodel. De begeleiding vanuit STW was uitstekend. Tevens wil ik Jacques Withagen bedanken voor zijn hulp met de statistische analyse van onze experimenten. I am further very grateful to Jenny Cory for her continuous interest and her hospitality during a two-month working visit in Oxford in the beginning of the project.

De voltooiing van mijn promotieonderzoek zou verder nooit tot stand zijn gekomen zonder de hulp van de analisten Ineke Snoeijing, Ine Derksen en Nina Joosten. Ineke heeft een belangrijke bijdrage geleverd met het uitvoeren van talloze bioassays in het begin van het onderzoek, terwijl Ine gedurende het gehele project de insectenkweek onderhield. Nina was een geweldige hulp bij de rest van het experimentele onderzoek. Door haar tomeloos enthousiasme, praktische instelling en werklust was het samenwerken met Nina een feest. Gedurende het project werden we dan ook goede vrienden.

Ook de bijdrage van de studenten Serafin Gutiérrez, Nuria Garcia en Tamar Reijnen verdient vermelding. In hun afstudeervakken hebben ze een belangrijke bijdrage geleverd aan het bepalen van parameterwaarden voor het simulatiemodel. Bovendien waren ze regelmatig de klos bij de oogst van kasexperimenten. Hoewel voor de proeven vaak veel en saai werk

verzet moest worden, bleven jullie toch enthousiast. Dit verdient een compliment! De kasproeven waren verder onmogelijk zonder de hulp van vele collega's, die soms in paniek geronseld moesten worden, omdat anders de proef dreigde te mislukken. Allemaal hartelijk bedankt voor jullie hulp!

De prettige sfeer op de vakgroep Virologie is altijd een belangrijke stimulans voor mijn motivatie geweest. Hier hebben zowel de "baculo's" als de "planten" gebroederlijk aan bijgedragen. Natuurlijk mogen mijn diverse kamergenootjes Gorben, Monique, József en Paul niet onvermeld blijven. Zij hebben het piano-gepingel waar ik ze jarenlang mee geteisterd heb goed doorstaan en lijken er geen blijvend letsel aan te hebben overgehouden. Klasse!

Hierbij wil ook mijn familie en vrienden bedanken. Zonder jullie steun zou de afgelopen periode heel wat zwaarder zijn geweest. Door het drinken van Koninckjes met de Henken, voetballen op dinsdag- en woensdagavond en de gitaarlessen van Coen en Eric kon ik de sores op de werkvloer relativeren.

Tenslotte Peet, wil ik jouw bedanken voor alle steun en hulp gedurende de afgelopen vijf jaar. We kunnen terugkijken op een leuke "promotietijd" en zijn nu al begonnen aan de volgende fase in ons leven. Ik hoop dat deze fase net zo leuk wordt als de afgelopen jaren!

Curriculum vitae

Felix-Jan Joost Antoine Bianchi werd geboren op 13 september 1968 in Eindhoven. In 1987 heeft hij het VWO diploma aan het van der Putt-lyceum in Eindhoven behaald waarna hij begon met de studie Bosbouw aan de Landbouwuniversiteit Wageningen. In augustus 1993 studeerde hij met lof af met als afstudeervakken Bosecologie, Bosteelt, Bodemkunde en Plantevoeding en Theoretische Productie Ecologie en een stage bij het Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada.

Van september 1993 tot en met eind 1994 was hij werkzaam als erkend gewetensbezwaarde en vrijwilliger bij het Instituut voor Bos- en Natuuronderzoek te Wageningen. In deze periode ontwikkelde hij een groei- en opbrengstmodel voor opstanden van inlandse eik en douglas. Van februari tot mei 1995 was hij werkzaam als project medewerker bij het Proefstation voor de Fruitteelt in Wilhelminadorp, waar met behulp van een simulatiemodel voor bodemchemie de effectiviteit van calciumfertigatieregimes in fruitopstanden geëvalueerd werd.

Van september 1995 tot december 2000 werkte hij als onderzoeker in opleiding (OIO) bij de vakgroepen Virologie en Theoretische Productie Ecologie aan Wageningen Universiteit. Het onderzoek dat in deze periode is uitgevoerd, staat beschreven in dit proefschrift.

Vanaf december 2000 is hij werkzaam als post-doc bij Alterra in Wageningen waar hij betrokken is bij onderzoek naar functionele biodiversiteit in samenwerking met de leerstoelgroepen Entomologie, Fytopathologie en Gewas- en Onkruidecologie van Wageningen Universiteit.

List of publications

Hendriks, C.M.A., and Bianchi, F.J.J.A. 1995. Root density and root biomass in pure and mixed forest stands of Douglas-fir and Beech. *Netherlands Journal of Agricultural Science* **43**, 321-331.

Bianchi, F.J.J.A. 1998. Simulation of the spread and efficacy of (genetically modified) bacoloviruses in greenhouses. *In*: G.E. de Vries (ed.). *Past, present and future considerations in risk assessment when using GMO's.* Workshop Proceedings of the Commission Risk Assessment Research, 5-6 maart 1998, Noordwijkerhout.

Hu, Z., Luijckx, T., van Dinten, L.C., van Oers, M.M., Hajos, J.P., Bianchi, F.J.J.A., van Lent, J.W.M., Zuidema, D., and Vlak, J.M. 1999. Specificity of polyhedrin in the generation of baculovirus occlusion bodies. *Journal of general Virology* **80**, 1045-1053.

Bianchi, F.J.J.A., Joosten, N.N., Gutierrez, S., Reijnen, T.M., van der Werf, W., and Vlak, J.M. 1999. The polyhedral membrane does not protect polyhedra of AcMNPV against inactivation on greenhouse chrysanthemum. *Biocontrol Science and Technology* **9**, 523-527.

Bianchi, F.J.J.A., Snoeijing, I., van der Werf, W., Mans, R.M.W., Smits, P.H., and Vlak, J.M. 2000. Biological activity of SeMNPV, AcMNPV and three recombinants of AcMNPV against *Spodoptera exigua* larvae. *Journal of Invertebrate Pathology* **75**, 28-35.

Bianchi, F.J.J.A., Joosten, N.N., Vlak, J.M., and van der Werf, W. 2000. Greenhouse evaluation of dose- and time mortality relationships of two nucleopolyhedroviruses for the control of beet armyworm, *Spodoptera exigua*, on chrysanthemum. *Biological Control* **19**, 252-258.

Bianchi, F.J.J.A., van Essen, P.H.A., van der Werf, W., and Smits, P.H. Transmission dynamics of the multicapsid nucleopolyhedrovirus SeMNPV in *Spodoptera exigua* populations in greenhouse chrysanthemum. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society. In press.*

Bianchi, F.J.J.A., and van Steenis, M. Simulatie van de bestrijding van floridamotrupsen met insectenvirus. *Vakblad voor de Bloemisterij. In press.*

Bianchi, F.J.J.A., Joosten, N.N., Vlak, J.M., and van der Werf, W. The influence of greenhouse chrysanthemum on the interaction between the beet armyworm, *Spodoptera exigua*, and the baculovirus SeMNPV: parameter quantification for a process-based simulation model. *Submitted.*

Bianchi, F.J.J.A., Vlak, J.M., Rabbinge, R., and van der Werf, W. Description of a process-based model for the biological control of beet armyworm, *Spodoptera exigua,* with baculoviruses in greenhouses. *Submitted.*

Bianchi, F.J.J.A., Vlak, J.M., and van der Werf, W. Validation of a process-based model for the biological control of beet armyworm, *Spodoptera exigua,* with baculoviruses in greenhouses. *Submitted.*

Bianchi, F.J.J.A., Vlak, J.M., and van der Werf, W. Scenario studies with a process-based model for the biological control of beet armyworm, *Spodoptera exigua,* with baculoviruses in greenhouses. *Submitted.*

Sun, X., Chen, X., Zhang, Z., Wang, H., Bianchi, F.J.J.A., Peng, H., Vlak, J.M., and Hu, Z. Bollworm response to release of genetically modified *Helicoverpa armigera* nucleopolyhedroviruses in cotton. *Submitted.*

Bianchi, F.J.J.A., and van der Werf, W. Description, parameterization and user guide of BACSIM: an epidemiological model of the control of beet armyworm, *Spodoptera exigua,* with baculoviruses. Quantitative approaches in system analysis. CIP-DATA Koninklijke bibliotheek, The Hague. *Submitted.*