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A COMPREHENSIVE SIMULATION MODEL OF THE EPIDEMIOLOGY OF *Spodoptera exigua* NUCLEAR POLYHEDROSIS VIRUS ON BEET ARMYWORM IN GLASSHOUSE CHRYSANTHEMUMS

Wopke van der Werf[1], Guido H. de Moed[1] & Peter H. Smits[2]

[1]Department of Theoretical Production Ecology,
P.O. Box 430, 6700 AK Wageningen, The Netherlands.

[2]Research Institute for Plant Protection (IPO-DLO),
P.O. Box 9060, 6700 GW Wageningen, The Netherlands.

SUMMARY

We describe a comprehensive simulation model of the epidemiology of *Spodoptera exigua* multiply enveloped polyhedrosis virus (SeMNPV), a pathogen of beet armyworm, *S. exigua*. The model applies to a population of beet armyworm which feeds on a glasshouse crop of chrysanthemums which is sprayed with SeMNPV. The model calculates the likely evolution of the insect-virus-host system by integrating available knowledge of the biology of *S. exigua,* plant growth, spray deposition, virus infectivity and the pathways of virus transmission. The model is used to evaluate various application scenarios of SeMNPV.

INTRODUCTION

Spodoptera exigua is a lepidopterous species of the family Noctuidae. It occurs worldwide in many species of field crops in tropical and subtropical regions and is also found in glasshouse crops in temperate regions like the Netherlands. *S. exigua* has a pest status in glasshouse ornamentals like Gerbera and Chrysanthemum, where feeding injury to the flowers causes large economic losses (Smits, 1987; Smits et al., 1987a). Resistance to insecticides makes chemical control difficult. Therefore, the possibility of control with natural antagonists has been studied. A particularly promising group of potential biological control agents are the nuclear polyhedrosis viruses (NPVs).

NPVs are strictly limited in host range to insects and crustaceans. The infectious unit is the polyhedron which consists of a protein matrix with many virions, the actual virus particles, enclosed in it. Polyhedra are liberated from the dead bodies of infected caterpillars. They contaminate leaves and are taken up orally by other caterpillars. In the gut of the caterpillars, the virions are liberated from the protein matrix, after which they infect the gut cells. The whole body can become infected, and when the caterpillar dies after a few days, up to 30% of the dry weight of the body may consist of polyhedra. Virions are sensitive to inactivation by UV-radiation (Jacques, 1975), but the protein matrix of the polyhedron and the remnants of the dead caterpillar provide some protection (Young & Yearian, 1989).

The life cycle of *S. exigua* is well known, especially from studies on artificial diets at different temperatures (e.g. Fye & McAda, 1972). The number of eggs per female ranges from 200 to 1000, the sex ratio is 1 and a multiplication factor of 50 per generation is easily attained at favourable conditions of temperatures between 20 and 35 °C, high food quality and absence of diseases and predators. The period from egg to egg varies from approximately 20 to 40 days over this temperature range. There are five, sometimes six, larval stages. On chrysanthemums, caterpillars eat approximately 70 cm^2 of leaf surface area during the entire larval period. 75% of the feeding injury is caused by the fifth larval stage, 20% by the fourth instar and 5% by the third (Smits, 1986). The first and second instar eat relatively very little, and their feeding is economically unimportant because they feed on bottom leaves. Eggs are laid in batches of 25 to 50 near the ground (Smits et al., 1986). The caterpillars migrate slowly upwards during their development and disperse from plant to plant (Smits et al., 1987b). Finally, the caterpillars originating from one egg batch occupy about 1 m^2 of crop.

We developed our model for two purposes:

1. to investigate if a biologically realistic model of the epidemiology of SeMNPV on *S. exigua* in chrysanthemums could be built with the data available in the literature and if such a model, that summarizes and integrates our knowledge about the system, would produce reasonable results.
2. second, if the model would produce realistic results, it could be used for generating and testing ideas, e.g. concerning application scenarios for the virus:
   a. dose
   b. moment of application
   c. single or split sprays
   d. scope for improvement of viral efficacy by genetic engineering of persistence or other characters.
   e. usage of SeMNPV for **inoculative** control of beet armyworm in glasshouse ornamentals, versus **inundative** control that had already been proved economically feasible by Smits et al. (1987a). Inoculative control implies that the antagonist is introduced (inoculated) only once, and propagates itself subsequently while keeping the pest below injurious levels. For inundative control, a good immediate killing effect is sufficient. Successful inoculative control requires in addition sufficient survival of virus on the right 'spots' to infect new upsurges of beet armyworm.

An earlier version of the model was described by de Moed et al. (1990). The biological parameters and structure of the present version of the model are largely the same as described in that paper. However, the description of plant growth and the deposition of sprayed virus have been modified (see below). The model version described by de Moed et al. (1990) did not account for periodic harvests of chrysanthemums, which cause removal of virus inoculum. The present model takes this epidemiologically important phenomenon into account.

This paper gives the biologically interested reader an informal introduction to the model and the results that are obtained. A more precise account with mathematical equations will be given elsewhere.

MODEL DESCRIPTION

The model assumes that there is a large (unlimited) number of chrysanthemums in a glasshouse. The plants are grown in beds of 1 m wide with 8 plants across the bed. They are harvested at an age of ca 3 months. Plantings of 10 different ages and stages of development are present simultaneously as beds are harvested and replanted every one or two weeks. Per plant 3 new leaves appear weekly and the average leaf size is 35 cm^2.The leaf canopy grows at a rate of 0.1 LAI-unit (m^2 leaf area per m^2 ground) per day.

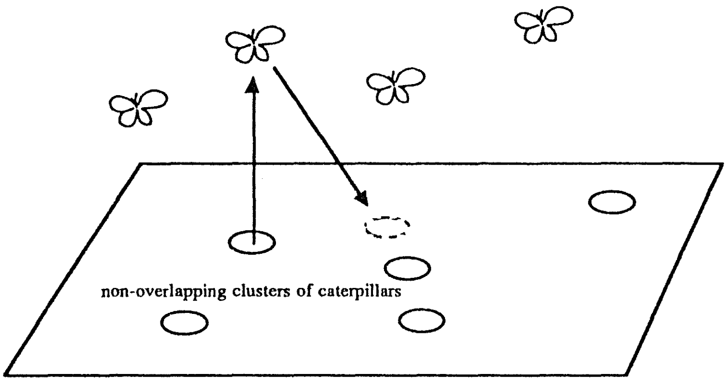

Figure 1: Schematic view of a large glasshouse with few non-overlapping randomly dispersed clusters of beet armyworm. Each cluster results from a single batch of 35 eggs, laid near the ground on one chrysanthemum plant. Caterpillars move randomly between plants, resulting in a ca 1 m^2 patch of infested crop when the caterpillars are in the fifth larval stage.

One single mated female moth flies into the glasshouse at a certain date, day 0, and lays her eggs on chrysanthemum plants of the planting that is in the preferred stage: young plants with 6 leaves. The capacity for egg laying of the female is about 500 eggs. Each egg batch comprises 35 eggs. Thus 14 egg batches are deposited in the course of a few days after the initial immigration. The batches are randomly dispersed. The caterpillar clusters that result from the egg batches will be non-overlapping because the moth is a good flyer and the crop area is large compared to the area occupied by patches of infested crop (about 14 m^2). New adults emerge from the infested patches.

They disperse over the glasshouse and start a new generation. Figure 1 illustrates these events.

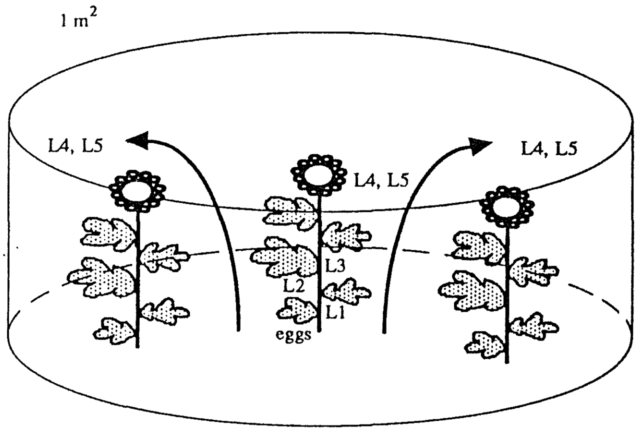

Figure 2: Schematic view of situation within a patch of infested chrysanthemum crop. Eggs are laid at the basis of the central plant. Successive stages crawl slowly upward the stems, with the fifth stage feeding in the upper leaf layers. During larval development, the initially small cluster of caterpillars spreads over more and more plants.

At a finer level of resolution, we observe the events occurring in a single patch. Eggs hatch and the first instar larvae (L1s) start feeding on the lower foliage until they moult into L2s. The age distribution changes gradually from predominantly L1s through L2s, L3s and L4s to the final fifth larval stage. While the age distribution evolves, changes occur in the vertical distribution of the caterpillars in the canopy and in the dispersion pattern in the horizontal plane (Smits et al., 1987b). These events are illustrated in Figure 2.

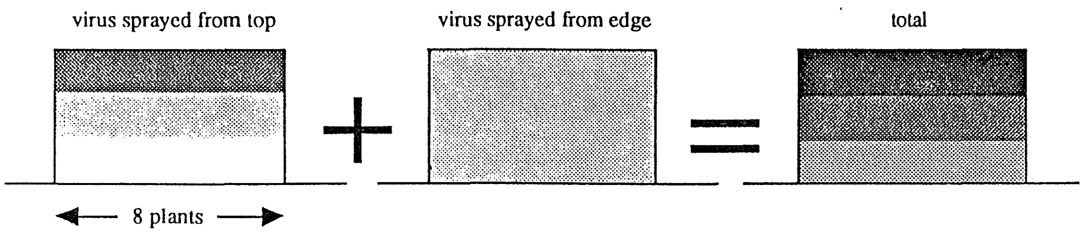

Figure 3: Cross section through chrysanthemum bed showing calculated virus concentration in different canopy layers, resulting from a 50% portion entering the canopy from above and another 50% entering the canopy from the edges. The model calculates with four canopy layers.

At the observation of feeding injury, the grower will apply a virus spray. Sprays are applied by hand. Thereby, the grower sprays not only from the top of the canopy, but also from the edges. Thus a good coverage with polyhedra is obtained on the leaves of edge plants. The model calculates an average virus concentration on the leaves for four heights in the canopy, taking the horizontal and vertical mode of applying virus into account. It is assumed that 50% of the polyhedra applied enter the canopy from above and 50% from the edges. The polyhedra entering from above are intercepted in a random fashion by leaves, resulting in an exponential virus profile. Each LAI unit of leaf area intercepts approximately 40% of the incoming polyhedra. Thus the first layer (from the top) receives a fraction of 0.4 of the amount of polyhedra applied, the second a fraction of 0.6 x 0.4, the third layer a fraction of 0.6 x 0.6 x 0.4, etc. It is assumed that the virus portion entering from the edge is distributed homogeneously in the canopy (Fig. 3).

The caterpillars take up the sprayed polyhedra with ingested leaf material. The rate at which polyhedra are ingested depends upon the feeding rate and the density of the polyhedra on the leaves:

$$\begin{Bmatrix} \text{leaf} \\ \text{consumption} \\ \text{rate} \end{Bmatrix} \times \begin{Bmatrix} \text{polyhedron} \\ \text{density} \\ \text{on leaf} \end{Bmatrix} = \begin{Bmatrix} \text{rate of} \\ \text{polyhedron} \\ \text{consumption} \end{Bmatrix}$$

$$\left(\text{cm}^2 \text{ leaf d}^{-1}\right) \qquad \left(\text{polyhedra cm}^{-2} \text{ leaf}\right) \qquad \left(\text{polyhedra d}^{-1}\right)$$

Due to their greater food intake and the higher polyhedron density in the upper leaf layers, the fourth and fifth instar caterpillars ingest the highest amounts of polyhedra. The relative rate of infection is obtained by multiplying the uptake rate of polyhedra with the infection chance per single polyhedron. This infection chance decreases with stage. For the five instars, these chances are respectively 1/7, 1/4, 1/70, 1/260 and 1/18000 (Smits, 1986; de Moed et al., 1990).

$$\begin{Bmatrix} \text{rate of} \\ \text{polyhedron} \\ \text{consumption} \end{Bmatrix} \times \begin{Bmatrix} \text{infection} \\ \text{chance per} \\ \text{polyhedron} \end{Bmatrix} = \begin{Bmatrix} \text{relative} \\ \text{infection} \\ \text{rate} \end{Bmatrix}$$

$$\left(\text{polyhedra d}^{-1}\right) \qquad \left(\text{fraction infected polyhedron}^{-1}\right) \qquad \left(\text{fraction infected d}^{-1}\right)$$

The portion of the model described in the foregoing is sufficient to calculate the immediate (inundative) effect of a NPV spray. For the simulation of the long term (inoculative) effect of the spray, the transmission pathways must be taken into account. There are three such pathways:

1. horizontal transmission; from caterpillar to caterpillar
2. vertical transmission; from female to eggs
3. vertical transmission; from male to eggs.

Horizontal transmission depends upon the number of encounters between healthy caterpillars and their virus-killed siblings. The model calculates the number of such encounters occurring in a patch, taking the following factors into account:

1. different instars feed at different heights in the canopy (Smits et al., 1987b). Caterpillars of different stages meet each other only on those leaves that belong to the feeding 'territory' of both stages.
2. the number of encounters increases with the number of leaves visited by a caterpillar of a certain instar in a day. The number of leaves visited per day is 1 for L1, L2 and L3, 3 for L4 and 5 for L5.
3. the size of the feeding 'territory' increases with instar (Smits et al., 1987b). L1s are found on 6 plants, including the plant on which the eggs were deposited, L2s on 15 plants, L3s on 30 plants, L4s on 47 plants and L5s on 65 plants.
4. the distribution and movement of healthy and dead caterpillars within their feeding territory is random.

Horizontal transmission is crucial for the 'survival' of the virus within a patch. When the virus is not propagated by horizontal transmission, no infected females can emerge from a patch and no epidemic will start.

Vertical transmission by females occurs when a female caterpillar is infected in the fifth stage (Smits & Vlak, 1988). Half of those caterpillars die while the other half develop into females that carry NPV with them. Approximately 15% of the eggs of these females are infected. The probability that an egg batch will contain only healthy eggs is $(1 - 0.15)^{35} = 0.0034$. Thus only 1 egg batch out of 295 produced by contaminated females will be virus-free. The average number of infected eggs in an egg batch with one or more infected eggs is 5.3.

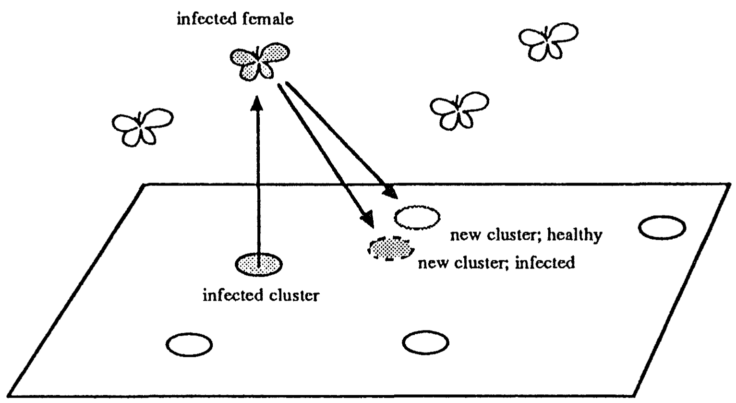

Figure 4: 50% of the infected female L5s develop into infected moths that lay infected eggs at a rate of 15%. The average number of infected eggs per batch laid by such females is 5.3. The proportion of healthy clusters produced by such females is about 0.5%.

Vertical transmission by males occurs when a male caterpillar is infected in the fifth stage. Half of those caterpillars die while the other half develop into males that carry NPV with them. At copulation, the male contaminates the female, upon which the female will lay some infected eggs (Vargas-Osuna & Santiago-Alvarez, 1988). The proportion of infected eggs due to male transmission is 7.5%, half the value taken for female vertical transmission. The probability that an egg batch will contain only healthy eggs is $(1 - 0.075)^{35} = 0.065$. Thus about 1 egg batch out of 15 produced by contaminated females will be virus-free. The average number of infected eggs in an egg batch with one or more infected eggs due to male transmission is 2.8. Females copulate only once. Under the assumption that infected males copulate as often as healthy males, the proportion of females infected at copulation equals the proportion of infected males.

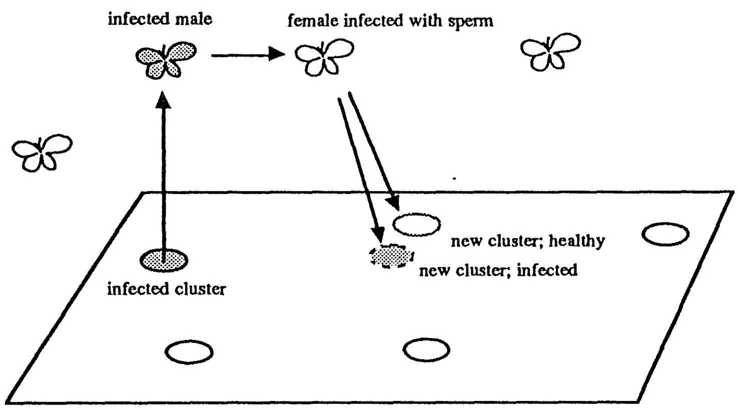

Figure 5: 50% of the infected male L5s develop into male moths that infect females at copulation. The probability of virus transmission at copulation is 1. Females infected in this way lay 7.5% infected eggs. The average number of infected eggs in an infected batch laid by such females is 2.8. The proportion of healthy clusters produced by such females is about 7.2%.

All females, whether or not infected by either pathway, deposit eggs randomly over the glasshouse. Healthy caterpillar clusters are produced by healthy females and by a fraction of the females infected by either vertical transmission mechanism. Infected caterpillar clusters are initiated by infected females. The model distinguishes between three types of patches: healthy patches, patches infected via the male transmission pathway (2.8 infected eggs initially) and patches infected via the female vertical transmission pathway (5.3 infected eggs initially). Sensitivity analyses of the model showed that a finer classification of patches according to the number of infected eggs did not significantly change the results.

Vertical transmission is crucial for the transfer of virus from one caterpillar generation to the next as patch overlap is not accounted for in the model. The simplification of neglecting patch overlap is justified at the low caterpillar densities that are relevant, taking the low damage threshold into account. The model calculates with a time step of about one hour. Patches that are started at adjacent dates are lumped in the analysis, that is: average values are taken for all state variables. Calculations are separated for patches that are initiated at dates that are more than one week apart.

SIMULATION RESULTS

The insect model without virus produces an exponential population growth with distinct generations. As time passes the separation between generations diminishes due to individual variability in development period. Each month one generation is produced. The multiplication factor per generation is ca 100. The relative growth rate of the population is 15% per day (Fig. 6).

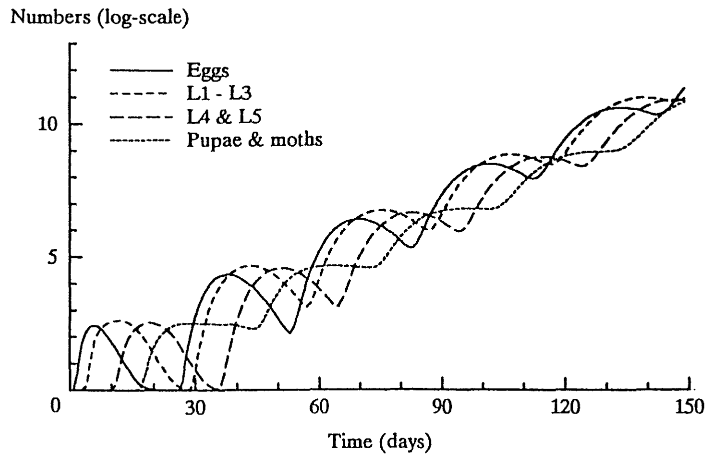

Figure 6: Simulated dynamics of healthy *Spodoptera exigua* population on glasshouse chrysanthemums at 25 °C. Time is reckoned from the day that the crop was infested by one adult female moth.

The optimal timing of the spray was the next thing that we investigated with the model. Best control during the second generation was obtained by a spray somewhat more than 40 days after the initial infestation (data not shown), that is at maximum presence of L2s and L3s. A spray at that time causes an instantaneous reduction in caterpillar numbers of 90 to 95% (Fig. 7). A good effect is obtained with an SeMNPV dose of 10^8 polyhedra/m^2. After one generation, the old pattern of population growth (15% d^{-1}) is however resumed (Fig. 7). Increasing the SeMNPV dose above 10^8 polyhedra/m^2 does not keep the population substantially longer under control. Applying the same dose of 10^8 polyhedra per m^2 in three separate sprays, each of 3 x 10^7 pol./m^2, at intervals of 7 days substantially improves control (Fig. 7).

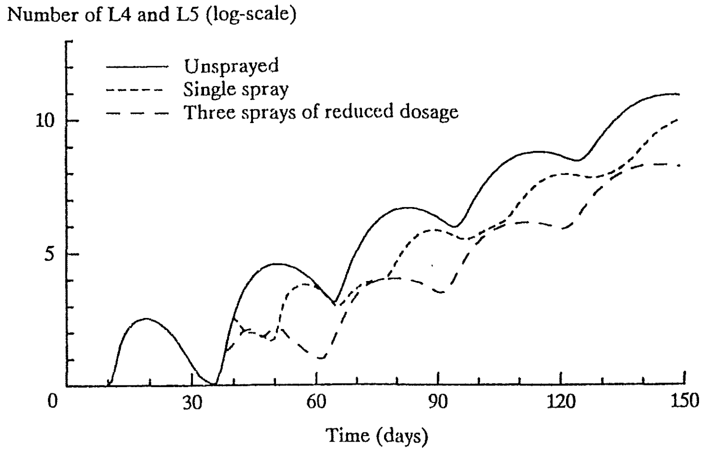

Figure 7: Population development of *Spodoptera exigua* L4 and L5 caterpillars on chrysanthemums in three situations: 1. uncontrolled; 2. single SeMNPV spray of 10^8 pol./m^2 at day 40 after initial infestation of the crop with 1 female moth; 3. three SeMNPV sprays of 3 x 10^7 pol./m^2 at days 38, 45 and 52 after initial infestation.

The effect of the halflife of the virus on the control achieved was also simulated and appeared to be small. Likewise, the effect of introducing a portion of 'stable' virus into the model (which is not inactivated by UV) was very small. The reason of this lack of effect is the spatial structure of the model (and of the system). Stable virus will be present on old leaves of old plants that are not anymore visited by caterpillars due to the rapid growth of the plants and frequent harvests, and is therefore ineffective. Stable virus fractions and slow virus inactivation are of more importance when the crop has not such rapid foliage growth and crop turnover rates as chrysanthemums have.

CONCLUSIONS

1. The model produces results that are logical but not self-evident. For instance the slight importance of the halflife of the virus was not anticipated. Thus model predictions serve as an eye-opener.
2. Experimental validation of model predictions is necessary. Errors may arise from an oversimplified or even incorrect representation of the structure of the system and inaccurate numerical values of the parameters. Although the model is quite comprehensive, still many processes are neglected or aggregated in a single parameter or function.
3. The best immediate control was obtained when the spray was applied at maximum presence of L2s and L3s. These caterpillar stages combine high to moderate susceptibility to the virus with an exposed position in the canopy. L1s are less exposed while L4s and L5s are less

susceptible.

4. Virus persistence in the rapidly growing chrysanthemum crop with its high turnover rate is not important but may be important in less rapidly changing crops.
5. No simulations with a long standing (inoculative) effect of SeMNPV were obtained. The model calculations suggest that the high growth and turnover rate of the chrysanthemum crop, together with the low damage threshold, will obstruct a successful implementation of inoculative control of beet armyworm with NPVs in this crop.
6. A single spray of 10^8 polyhedra/m^2 provides adequate inundative control. Model calculations suggest that three repeated sprays of 3 x 10^7 polyhedra/m^2 provide much better control for the same ingredient costs but higher costs for labour.

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