EVALUATING THE RISKS OF ENGINEERED VIRUSES: MODELING PATHOGEN COMPETITION

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Abstract. Recently there has been a great deal of interest in the potential use of genetically engineered baculoviruses as environmentally benign insecticides. Because baculoviruses often have a significant impact on the population dynamics of their hosts, any effort to assess the environmental impact of releasing engineered viruses must confront the question: Will genetically engineered baculoviruses outcompete wild-type strains, thereby altering the natural population dynamics of the host? To begin to answer this question, we develop a mathematical model of competitive interactions between genetically engineered and wild-type baculoviruses. We find that the interactions between these viruses are characterized mostly by dominance of one strain or the other, and that the chance that an engineered strain will outcompete a wild-type strain depends on its particular combination of speed of kill and infectiousness. That is, baculoviruses must kill their host to become infectious, so the faster speed of kill of most recombinant viruses confers a competitive advantage. Most such strains, however, also produce fewer infectious particles and so are less infectious. Our model shows that the extent of this decrease in infectiousness must be rather small for an engineered strain to become dominant. Nevertheless, even engineered strains that are at a substantial competitive disadvantage relative to the wild type may take decades to go extinct. An additional complicating factor is that the outcome of competition depends on the overwinter survival of these viruses, about which little is known even for wild-type viruses. Caution is therefore necessary in predicting the outcome of competitive interactions involving introduced baculoviruses, and further work is needed in understanding pathogen overwinter survival rates.

Key words: baculovirus; biological control; coexistence of virus strains; competition models; competitive exclusion; ecdysteroid UDP-glucosyl transferase (egt); genetic engineering; gypsy moth; insect–baculovirus interaction; nuclear polyhedrosis virus (NPV); recombinant viruses.

INTRODUCTION

Baculoviruses are a diverse group of arthropod pathogens that have long been used as an environmentally benign method of pest control (see Moscardi [1999] for a recent review). Baculoviruses cause severe diseases in many insects (Fuxa and Tanada 1987), and in some cases are believed to play an important role in the population dynamics of their hosts (Myers 1988). These diseases are almost always fatal, and generally must kill their host to be transmitted. The infectious stage, known as an “inclusion body” or “polyhedron,” can survive for long periods in the absence of a host. Consequently, solutions of baculoviral inclusion bodies can be used as microbial insecticides (Podgwaite 1985).

Baculoviruses have an advantage over conventional insecticides in that their high level of host specificity reduces their impact on nontarget organisms; this specificity, however, means that each pest insect requires its own insecticide, which has tended to reduce interest in developing baculoviruses for commercial use. A second problem is that many baculoviruses take from 7 to 14 d to kill their host, which is much longer than conventional insecticides (Possee et al. [1997]). In the time between infection and death, the pest insect can cause much additional damage to a crop or forest. Recently there has been a great deal of interest in the possibility of creating recombinant baculoviruses that have a wider host range, kill more rapidly, or both (Bonning and Hammock [1996] and Possee et al. [1997] review some of this progress). More recently, concerns about nontarget organisms (Possee et al. 1993, Richards et al. 1998) have apparently all but eliminated any efforts to increase the host range of baculoviruses by genetic engineering. Several laboratories, however, have produced (McCutchen et al. 1991, O’Reilly and Miller 1991, Stewart et al. 1991) and tested (Tomalski and Miller 1991, Cory et al. 1994, Kunimi et al. 1996, McCutchen et al. 1996, Ignoffo and Garcia 1997, McCutchen et al. 1997, Treacy et al. 1997, Fuxa et al. 1998, D’Amico et al. 1999) modified baculoviruses that kill more rapidly.
Because the introduction of genetically engineered organisms into the environment is a controversial subject (see Giddings [1998] for a recent review), in this article we use a mathematical model to explore the potential ecological consequences of releasing genetically modified baculoviruses. In general, modification results in reduced transmissibility of the introduced virus relative to the wild type. For example, perhaps the best-known modification is the addition of genes for insect-specific scorpion toxins, which can kill their host as much as 25% more rapidly than wild-type strains (Cory et al. 1994). Scorpion-toxin baculoviruses, however, have reduced transmissibility because unlike the wild type they do not cause the host to lyse, thereby releasing infectious particles, and because they produce as little as one tenth as many infectious particles as does the wild type. Likewise, inactivation of the ecdysteroid glucosyl transferase gene, which normally glycosylates the molting hormone ecdysteroid, produces a virus (known as “egr”) that kills much faster than the wild type, but is less infectious (O’Reilly and Miller 1989, 1991, Slavicek et al. 1999). More generally, on theoretical grounds one would expect that life-history tradeoffs would result in lower transmissibility of more rapidly killing baculoviruses (Anderson and May 1982).

Because of this reduced transmissibility, one might expect that the release of modified baculoviruses would have little ecological impact in naturally occurring insect populations, because modified baculoviruses would be rapidly outcompeted by wild-type strains. Genetically modified baculoviruses might therefore go extinct within a few host generations after release. This is not necessarily the case, however. The delay between infection and death (and thus infectiousness) in baculovirus disease is typically substantial. The faster killing time of the modified strain provides important advantages, both in reaching susceptible larvae earlier in the second and subsequent epidemic generations, and potentially in successfully completing more epidemic generations in one season. Thus, our model is designed to explore the circumstances under which this tradeoff might allow the modified strain to survive, either alone or together with the wild-type strain.

Our work follows most immediately from our previous work with the nuclear polyhedrosis virus (NPV) of gypsy moth, Lymantria dispar (Dwyer and Elkinton 1993, Dwyer et al. 1997, 2001). As is the case with many forest-defoliating insects, the population dynamics of the gypsy moth are strongly affected by the virus (Elkinton and Liebhold 1990). The model that we use assumes that hosts reproduce once a year, like the gypsy moth and other forest-defoliating insects, and it has been extensively tested with data from field experiments and natural epidemics of the NPV of gypsy moth (Dwyer et al. 1997). Nevertheless, the model is general enough to apply to many different insects and baculoviruses.

**Outcomes of competition**

Simple models of competition between two strains typically have three possible outcomes: dominance, where one strain always drives the other to extinction; coexistence, where both strains survive; or mutual exclusion, where one strain goes extinct, but which strain survives depends on the initial density of each strain. A key feature of mutual exclusion is that, once either strain is established it cannot be displaced by the other. This possibility has important implications for the release of genetically modified baculoviruses: if two strains mutually exclude each other, then a strain that seems to be competitively excluded may actually be able to persist if it ever manages to become established. A useful method of evaluating competition models is therefore to evaluate the ability of each competitor to invade when the other competitor has become established at an equilibrium density. In general, we expect to see mutual exclusion when both species fail as invaders, and coexistence when both species succeed as invaders.

In this paper, we assume that the native strain and the introduced strain differ only in speed of kill and in “transmissibility.” In our model, transmissibility is defined in terms of infectious cadavers (see Mosel, below), so this allows for differences both in the number of infectious particles produced per individual, and in the infectiousness of individual particles. Moreover, in previous work we have shown that the transmissibility of baculoviruses can be readily measured in the field, and that the resulting measurements can be used to accurately predict the dynamics of baculovirus–insect interactions at a variety of spatial and temporal scales (Dwyer and Elkinton 1993, Dwyer et al. 1997, 2000, 2001).

As the transmissibility of the introduced strain approaches zero, we expect to find the native strain dominating. As the transmissibility of the introduced strain approaches that of the native strain, we expect to find the introduced strain dominating, because its faster kill time means that it can infect susceptible hosts more quickly in the second and subsequent disease generations. In between, there will normally be some range of transmissibilities where neither strain dominates, but where we find either coexistence or mutual exclusion.

We built this model to investigate competitive outcomes between strains showing tradeoffs between speed of kill and transmissibility, and to test the hypothesis that the tradeoff would lead to mutual exclusion between wild-type and recombinant viruses. We hypothesized that recombinant viruses would do relatively better (compared to wild-type viruses) in conditions where susceptible larvae were depleted rapidly, since their faster kill speed would be important in infecting susceptibles first. We further hypothesized that recombinant viruses would tend to produce faster epidemics, depleting the susceptibles faster. Thus, we ex-
pect recombinant viruses to do relatively better in an environment dominated by recombinant viruses than in an environment dominated by wild-type viruses, and hence we expect mutual exclusion to occur for intermediate values of recombinant transmissibility.

Competitive interactions between virus types may also interact with the complex population dynamics of their hosts. Many insects that are naturally afflicted by baculoviruses have fluctuating population dynamics (Varley et al. 1973, Anderson and May 1981, Turchin 1990). In Dwyer et al. (2001) we showed that under some circumstances we might expect replacement of the native virus by the introduced strain to lead to lower peak densities in gypsy moth outbreaks, but higher mean moth density. Thus, we will also explore competitive interactions under circumstances where we expect pathogen-driven oscillations in the host population.

**Model**

Gypsy moth NPV (nuclear polyhedrosis virus) is transmitted horizontally when the insects, while feeding, accidentally consume foliage contaminated with virus. Larvae that consume a high-enough dose die within about two weeks, and their cadavers further contaminate the foliage. If these virus-infected cadavers are not broken down by the ultraviolet rays in sunlight, they are available for other larvae to consume, completing the process of transmission. Since only larvae can become infected, the seasonal epidemic is terminated when the insects pupate.

Following Dwyer et al. (2000), we use the following epidemic model for this disease:

\[ \frac{dS(t)}{dt} = -[\beta_1 P_1(t) + \beta_2 P_2(t)]m(t)S(t) \]  
\[ \frac{dP_1(t)}{dt} = \beta_1 P_1(t - \tau_1)m(t - \tau_1)S(t - \tau_1) - P_1(t)/L_1 \]  
\[ \frac{dP_2(t)}{dt} = \beta_2 P_2(t - \tau_2)m(t - \tau_2)S(t - \tau_2) - P_2(t)/L_2. \]  

Here \( S(t) \), \( P_1(t) \), and \( P_2(t) \) refer to the density of susceptible larvae and densities of viruses of the two types respectively (in the future we will suppress the \( t \), except when looking at the delay terms); \( \beta_1 \) and \( \beta_2 \) are the transmission terms; \( \tau_1 \) and \( \tau_2 \) are the latency (or “kill”) times; \( m \) is the mean susceptibility of the pool of susceptible larvae; and \( L_1 \) and \( L_2 \) are the mean durations of infectiousness of the infectious particles.

Heterogeneity in larval susceptibility is known to be an important factor in the dynamics of gypsy moth NPV (Dwyer and Elkinton 1993, Dwyer et al. 1997). We include heterogeneity by letting \( m \) decrease through time, as the more susceptible individuals become infected first, following the formula

\[ m(t) = \mu \left( \frac{S(t)}{S(0)} \right)^v \]  

where \( \mu \) is the mean and \( V \) is the squared coefficient of variation of the distribution of susceptibilities (see Dwyer et al. [2000] for details). Note that when \( V = 0 \), \( m \) is constant.

We choose the units for susceptible larvae such that \( \beta_1 = 1 \). This means that the unit density is the density required for an epidemic outbreak of the native strain starting from low density (see Dwyer et al. 2000). We have chosen to count pathogens in the same units as cadavers, thus one unit of infected larvae produces one unit of pathogen. The fact that the two types produce different amounts of virus is reflected instead in the value of \( \beta \), which measures infectiousness per cadaver. Since mean susceptibility \( \mu \) is always multiplied by the transmission coefficients, we are free to set it to 1. A single season is simulated by integrating the within-season model (Eqs. 1–3) from time 0 to a fixed stopping time \( T \), representing pupation and the cessation of new infections. Larvae that are infected at time \( T \) are assumed to die and be converted into infectious particles.

An important omission in this model is that larvae change in size and susceptibility as the season progresses. Specifically, as larval size increases, the rate at which an individual consumes foliage and the number of virus particles it produces if infected both increase (Shapiro and Robertson 1986; G. Dwyer, unpublished data), while the risk of infection given that particles are ingested decreases (Slavicek et al. 1999).

The net effect of these changes is as yet unmeasured in gypsy moth, but is known to be quite complicated in other insect–virus systems (Dwyer 1991). To avoid introducing poorly understand biological details and additional unmeasured parameters into our model, for now we proceed by assuming that the changes that occur in the larvae during the season do not fundamentally alter the population dynamics of the system. The previous success of the epidemic model in predicting epidemics suggests that this assumption may in fact be justified. We recognize, however, that such changes may play a role in competition among virus strains, and in future work we plan to modify our model to allow for this complication.

Our next step is to consider the dynamics of the insect and the virus between epidemics. For the purpose of investigating competition between baculoviruses and their effects on the population dynamics of the insect, we assume that the baculovirus is by far the most important factor in the population dynamics of its host. Little is known about the long-term survival of insect pathogens. In the case of gypsy-moth NPV, it is known (Murray and Elkinton 1989, 1990) that virus produced during an epidemic contaminates the eggs laid at the end of the season, which can in turn initiate disease spread in the following season. We believe that
this is the major process by which virus survives the winter.

To complete our model, we therefore need only consider the reproduction of the host and the between-season transmission of the virus. The between-season behavior of the model is given by

\[
S(y + 1; 0) = \lambda S(y; T) \tag{5}
\]

\[
P_1(y + 1; 0) = \phi_1 Q_1(y) \tag{6}
\]

\[
P_2(y + 1; 0) = \phi_2 Q_2(y). \tag{7}
\]

The indices before the semicolons refer to years; thus the number of hosts at the beginning of year \(y + 1\) is the fecundity \(\lambda\) multiplied by the number of hosts remaining susceptible at the end of the epidemic in year \(y\). \(Q\) gives the total number of individuals infected by each respective strain during the epidemic, so \(Q\) is defined as \(\beta \int P(t) m(t) S(t) dt\).

The “carryover” parameter \(\phi\) provides the link between disease seasons. It is therefore a product of the probability that a virus particle will successfully overwinter, and its relative infectiousness given that it does. Because the probability of overwintering is unknown, but is probably small, whereas the susceptibility of hatching larvae is high, \(\phi\) could conceivably vary over a wide range. As we will see below (Results), it also has important effects on population dynamics.

**Results**

To evaluate the possibility that mutual exclusion might occur, and final results be dependent on initial conditions, we chose biologically plausible values for all of the parameters except for \(\beta_2\), which gives the relative transmission (combined with fecundity) of the introduced virus. We then varied \(\beta_2\) between 0 and 1, to see what sort of behaviors were observed in the model.

As Fig. 1 shows, for values of \(\beta_2\) where neither species dominates we find mutual exclusion, as we hypothesized. In Fig. 1a, we started with a high level of pathogens; this led to a very large proportion of larvae being infected during the first generation of the first epidemic, with no chance for the introduced strain to take advantage of its faster generation time. Using this initial advantage, the native strain will gradually exclude the introduced strain. In Fig. 1b, we started with a low level of pathogens; since the introduced strain can reproduce faster it gains an advantage during the first year’s epidemic and eventually excludes the native strain.

Fig. 2 shows the time course of the within-season epidemic for each of the two species at its own equilibrium. That is, we simulated the model with only one virus type until it reached equilibrium (this simple model reaches a stable equilibrium for these parameters), and then plotted the course of one season’s epidemic at this equilibrium. It can be seen that the introduced strain in fact produces an environment where

the density of susceptibles is higher at first, and later lower on, as hypothesized.

More striking, however, than the fact that we found the pattern that we are looking for is how small the effect is. Mutual exclusion is found only in a small window of parameter space (see Fig. 3); correspondingly, its magnitude is also small: for the parameters we chose, each species can persist at non-negligible densities for hundreds of years on the way to extinction. Fig. 3 also shows estimated data points for the egt strain and for an engineered strain with scorpion toxin (both of which kill faster but are less infectious than the wild-type strain [see Introduction, above]). Although it is very likely that the these strains are dominated by associated wild-type strains, available data cannot absolutely confirm this. Note also that changing the carryover parameter \(\phi\) could make survival either more or less likely.
Host dynamics

An earlier paper Dwyer et al. (2000) hypothesized that outbreaks in gypsy moths might be explained by host–pathogen cycles, and was able to match outbreak data using the model discussed above, by using a large value of $\phi$ and a small value of $V$. In Dwyer et al. (2001), we discussed the possibility that a faster-killing introduced strain might tend to reduce outbreak size. In this section we explore competitive interactions between the two strains.

Fig. 4 shows a time series of our system for plausible values of the parameters chosen to give realistic outbreaking dynamics with the native strain. To our surprise, we find affirmative coexistence between the two strains (each strain can invade the other). This implies that, contrary to our hypothesis, in this parameter range each strain does better in the environment created by the competing strain.

This unexpected result is related to our inclusion of realistic time delays for disease latency, rather than a more mathematically convenient exponential form. With a time delay, the changing ability of the two strains to make use of susceptibles through time becomes more complicated. If the wild-type strain kills in $\sim 2$ wk and the recombinant strain kills in $\sim 1$ wk, then the wild-type strain, with its higher transmissibility, benefits relatively more from the availability of susceptibles during the first week. After the first week, however, the faster speed of kill of the recombinant strain becomes relevant, as secondarily infected larvae die of the recombinant strain, boosting its presence in the population. The relative value of susceptibles in subsequent weeks is more complicated, because it depends on the amount of earlier reproduction of the virus.

Fig. 5 shows the time course of the epidemic at equilibrium, for an intermediate value of the pathogen carryover parameter $\phi$, that shows strong coexistence. The high value of pathogen carryover means that the first disease generation within each epidemic is relatively more important. This reduces the advantage of the recombinant strain, thus requiring a higher value of $b_2$ (infectiousness per cadaver) for it not to be dominated. It also increases the importance of the relative advantage of the wild-type strain during the first disease generation, when more susceptibles are available under the environment created by the recombinant strain than under the environment created by the wild-type strain. The period of time when the environment created by the wild-type strain has more susceptibles is earlier than in Fig. 2, near the time when the recombinant strain has its largest advantage. This means that each strain does relatively better under the environment created by the other, leading to coexistence.
Fig. 4. Host and pathogen density through time for the between-season model. Parameters are $\lambda = 10$, $\phi = 10$, $t_1 = 2$ wk, $t_2 = 1$ wk, $T = 10$ wk, $L_1 = L_2 = 1$ wk, $V = 0.8$, and $\beta_2 = 0.968$. For parameter definitions see Fig. 1.

Fig. 5. Density of susceptible larvae through time in the within-season model, with the starting density normalized to 1, with the hosts in equilibrium with the native strain alone (unmarked curve) and the recombinant strain alone (marked curve). Parameters are as in Fig. 1, except $\phi = 0.5$.

Fig. 6. Regions of dominance, mutual exclusion, and coexistence for the NPV virus strains. In panel (a) the recombinant strain kills 50% faster than the wild-type; in panel (b) the recombinant strain kills 25% faster. Other parameters are as in Fig. 1 (except those on the axes). Note the logarithmic scale on the $x$-axis.

Fig. 7 shows the regions of dominance, mutual exclusion, and coexistence parameters over a wide range of values of $\phi$, the pathogen between-season (over-winter) carryover parameter. It confirms that increasing pathogen carryover sharply reduces the advantage of the recombinant strain, so that for large values of $\phi$ the recombinant strain must have nearly the same transmissibility as the wild-type strain to avoid domination. It also confirms that as $\phi$ increases, the behavior in the intermediate region switches from mutual exclusion to coexistence. As in Fig. 3, however, what is most striking is that the intermediate region is small throughout: for most of this large parameter space, the competition is characterized by complete dominance of one strain or the other.

Although Fig. 6 shows that we can usually expect one strain or the other to dominate in this system, we were also concerned about the slow dynamics seen in our first example. Although an introduced strain may eventually be driven extinct by competitive dynamics, if the process is slow it may allow time for conditions to change, or for spread to a more favorable environment, or for genetic exchange.

In Fig. 7 we investigated the possibility of long-term coexistence in regions where one strain eventually dominates. Using the same parameters as in Fig. 6b (so that the region of true non-dominance is tiny), we started the system in the vicinity of the equilibrium, with equal numbers of both strains, and calculated the parameters for which both species have non-negligible abundance (>1% of total virus) after 100 yr.

**Discussion**

Our model results show that the most likely outcome of competition between genetically engineered and wild-type baculovirus strains is that one strain or the other will go extinct. Specifically, as Fig. 3 shows, engineered strains that kill more rapidly will only go extinct if their transmission is sufficiently less than that
of the wild type. Moreover, as Fig. 6 shows, the extent by which transmission must be reduced to ensure extinction of the engineered strain depends on the overwinter carryover parameter $\phi$. Knowing the degree to which the transmission of the engineered strain is reduced will therefore not always be sufficient to predict the outcome of competition. This is especially true because of how little is known about the overwinter survival of baculoviruses. Clearly, more work is needed on understanding and directly measuring the pathogen carryover parameter $\phi$. We are currently attempting to estimate $\phi$ in the field by quantifying levels of infection in successive years in gypsy moth populations at different densities.

Fig. 3 shows our estimates of the relative transmissibility and speed of kill of an $egr^{-}$ mutant of the gypsy moth nuclear polyhedrosis virus (NPV). Given that the $egr^{-}$ virus is a deletion mutant, one would expect that it would go extinct, and Fig. 3 indeed suggests that it probably will. Available data, however, cannot rule out the possibility that it may persist for hundreds of years in the field (see Fig. 7) or even the unlikely possibility that it may outcompete the wild type.

An additional area of uncertainty arises because of the simplicity of our model. In order to explore specific questions about pathogen competition in a broad way, we necessarily used a simplified model, leaving out a variety of factors. Most of these will probably have little impact on our qualitative conclusions. One important exception, however, is the change that occurs in the system as the season progresses, particularly the changes in size and susceptibility of the larvae. This has the potential to affect our conclusions directly. We hope to extend this model to incorporate larval growth in the future, again using gypsy moth NPV data for guidance.

We had hypothesized that pathogens that kill faster at the expense of lower fecundity would have a tendency towards mutual exclusion in competitive relationships with native strains. Although we demonstrated that the hypothesized mechanism exists, we found that the interacting time scales of discrete pathogen generations make the situation much more complicated than we had supposed. Both mutual exclusion and coexistence were found, although only in a small region of parameter space. Pathogen carryover is critical in determining behavior in the intermediate range where neither strain dominates.

In spite of its simplicity, the current model has nevertheless shown that competitive interactions between native and introduced strains can have important implications for the use of microbial control agents. Above all, the model shows that our current knowledge of insect–baculovirus interactions is probably insufficient to predict the outcome of competition among strains.

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**Literature Cited**


