

Behavior of a Recombinant Baculovirus in Lepidopteran Hosts with Different Susceptibilities

PEDRO HERNÁNDEZ-CRESPO,[†] STEVEN M. SAIT,[‡] ROSEMARY S. HAILS, AND JENNY S. CORY*

Ecology and Biocontrol Group, National Environmental Research Council Centre for Ecology and Hydrology—Oxford, Oxford OX1 3SR, United Kingdom

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Insect pathogens, such as baculoviruses, that are used as microbial insecticides have been genetically modified to increase their speed of action. Nontarget species will often be exposed to these pathogens, and it is important to know the consequences of infection in hosts across the whole spectrum of susceptibility. Two key parameters, speed of kill and pathogen yield, are compared here for two baculoviruses, a wild-type *Autographa californica* nucleopolyhedrovirus (AcNPV), AcNPV clone C6, and a genetically modified AcNPV which expresses an insect-selective toxin, AcNPV-ST3, for two lepidopteran hosts which differ in susceptibility. The pathogenicity of the two viruses was equal in the less-susceptible host, *Mamestra brassicae*, but the recombinant was more pathogenic than the wild-type virus in the susceptible species, *Trichoplusia ni*. Both viruses took longer to kill the larvae of *M. brassicae* than to kill those of *T. ni*. However, whereas the larvae of *T. ni* were killed more quickly by the recombinant virus, the reverse was found to be true for the larvae of *M. brassicae*. Both viruses produced a greater yield in *M. brassicae*, and the yield of the recombinant was significantly lower than that of the wild type in both species. The virus yield increased linearly with the time taken for the insects to die. However, despite the more rapid speed of kill of the wild-type AcNPV in *M. brassicae*, the yield was significantly lower for the recombinant virus at any given time to death. A lower yield for the recombinant virus could be the result of a reduction in replication rate. This was investigated by comparing determinations of the virus yield per unit of weight of insect cadaver. The response of the two species (to both viruses) was very different: the yield per unit of weight decreased over time for *M. brassicae* but increased for *T. ni*. The implications of these data for risk assessment of wild-type and genetically modified baculoviruses are discussed.

Novel pest control methods based on genetic engineering have increasingly received attention as fears have been raised about their long-term environmental impact (15, 23, 31). Although much of this controversy has been directed at genetically modified plants, other organisms are being altered in the search for more effective pest control agents. Insect pathogens, particularly insect baculoviruses, have been the focus of numerous attempts to improve their efficacy by genetic modification (5, 30). An important area of risk assessment for any modified pathogen must be its host range and the potential effects on nontarget as well as target species (8, 9). The majority of baculoviruses have been isolated from insects and have a limited host range. Some isolates such as the gypsy moth (*Lymantria dispar*) nucleopolyhedrovirus (NPV) (2), appear to infect only a single species, while others, such as the cabbage moth (*Mamestra brassicae*) NPV (11) and the alfalfa looper (*Autographa californica*) NPV (AcNPV), can potentially infect a wider range of species from different lepidopteran families (4, 26). Laboratory assessment of the host range of AcNPV has shown that there is a continuum of susceptibility, ranging from highly susceptible, or permissive, to uninfected (nonpermis-

sive) species, with the majority of species falling into an intermediate category (semipermissive) (4). As host-range testing tends to focus on closely related lepidoptera (which are more likely to be susceptible) or simply represents species available in the field, it is reasonable to assume that most nontarget species which are found to succumb to AcNPV will also fall into this intermediate category of susceptibility when tested in the laboratory. Traditionally, pathologists working with invertebrates have concentrated on selecting and studying virus isolates that are highly pathogenic for target pest species. The behavior of baculoviruses in alternative, less-susceptible (nontarget) hosts has received little attention, with issues relevant to conservation and risk assessment being neglected. Clearly, any species that can be infected could be directly affected by virus release and could also contribute to the dispersal and persistence of the virus in the environment.

Many laboratory studies have already shown that the insertion of heterologous genes, particularly insect-selective toxins, into baculoviruses can result in a more rapidly acting insecticide (14, 19, 27–30). The use of a genetically modified AcNPV which expresses a scorpion toxin (AaHIT) has resulted in a reduction in crop damage that is significant compared to that obtained with the use of the wild-type virus in the field (10, 13). A fundamental requirement for predicting the environmental effects of genetically modified baculoviruses is an understanding of the host-pathogen interaction at the individual level. Clearly, increasing the speed of kill is likely to carry consequences for other biological characteristics of the baculovirus, which may ultimately affect the dynamics of the pathogen.

* Corresponding author. Mailing address: Ecology and Biocontrol Group, NERC Centre for Ecology and Hydrology—Oxford, Oxford OX1 3SR, United Kingdom. Phone: 44(0)1865 281643. Fax: 44(0)1865 281696. E-mail: jsc@ceh.ac.uk.

[†] Present address: CIB-CSIC, Departamento de Biología de Plantas, 28006, Madrid, Spain.

[‡] Present address: School of Biological Sciences, University of Liverpool, Nicholson Building, Liverpool L69 3GS, United Kingdom.

Because larvae infected with AcNPV which expresses AaHIT die more rapidly, less of this virus than of the wild-type virus is produced (21). However, the exact nature of this trade-off has not been determined, nor is it known if there are major differences in the way species with differing susceptibilities respond to recombinant baculoviruses.

The two viruses used in the study were AcNPV clone C6 (AcNPV-C6) and a recombinant based on it, AcNPV-ST3, which encodes an insect-selective scorpion toxin from *Androctonus australis* that increases the speed of kill (28). These viruses were compared in experiments using a model semipermissive, or less-susceptible, host, the cabbage moth, *M. brassicae* (Lepidoptera: Noctuidae), and a highly susceptible, permissive host, *Trichoplusia ni* (Lepidoptera: Noctuidae). Larvae of both species infected with wild-type AcNPV show the typical signs of baculovirus infection leading to lysis. By contrast, larvae infected with the recombinant do not liquefy but exhibit signs of paralysis before death. Laboratory assays were used to estimate several components which will affect the fitness of the virus in the field. These assays allowed the time to death, the yield, and the dose administered to be measured with precision. The aims of the study were to ascertain the answers to the following questions: (i) Does the recombinant virus kill the semipermissive species more rapidly than does the wild-type virus? (ii) How is virus yield influenced by infection in the semipermissive species compared to in the highly susceptible species? (iii) How are these relationships affected by virus dose?

MATERIALS AND METHODS

Insect and virus stocks. Larvae of *M. brassicae* and *T. ni* were obtained from cultures maintained at the Natural Environmental Research Council Centre for Ecology and Hydrology, Oxford, United Kingdom, and reared continuously on artificial diet (20). The multiply embedded nucleopolyhedrovirus wild-type clone AcNPV-C6 and the recombinant AcNPV-ST3 were amplified in a fermenter (SGI Cytotflow Bioreactor) seeded with *Spodoptera frugiperda* Sf9 cells in Sf900II serum-free medium. AcNPV-ST3 is a modified version of clone C6 which expresses a synthetic version of the insect-selective neurotoxin (AaHIT) from the Algerian scorpion, *Androctonus australis* Hector, under the control of a duplicated p10 promoter (28). The insertion of AaHIT does not interrupt any other genes (28). The Sf9 cells were grown to a density of approximately 10^7 viable cells/ml and infected with tissue culture inoculum at 6 PFU/cell (AcNPV-C6) and 4.5 PFU/cell (AcNPV-ST3). Cultures were incubated at 27°C and harvested at 5 days postinfection. Cells and polyhedra were pelleted from the supernatant by centrifugation at $1,350 \times g$ for 1.5 h. The pellet was resuspended in sterile distilled H₂O and stored in aliquots at -20°C. Prior to the bioassay, the pellet was treated with sodium dodecyl sulfate and the polyhedra were purified from cell debris by low-speed centrifugation at $3,250 \times g$ for 5 min and resuspended in sterile water. Suspensions of polyhedra were counted using an Improved Neubauer hemocytometer (B.S.748; Weber, Teddington, England).

Bioassays. Bioassays were carried out on early second-instar larvae of *M. brassicae* and *T. ni* which had been starved overnight. Five concentrations of each virus, to which 4% blue food coloring (Duff's food coloring; Langdale) was added, were administered by droplet feeding. The concentrations used were as follows: for *M. brassicae*, 1×10^8 , 3×10^7 , 1×10^7 , 3×10^6 , and 1×10^6 polyhedra/ml, and for *T. ni*, 2×10^5 , 1×10^5 , 5×10^4 , 2×10^4 , and 1×10^4 polyhedra/ml. Larvae that had ingested virus suspension were transferred individually to 30-ml plastic pots containing artificial diet and maintained at $24 \pm 2^\circ\text{C}$ until pupation or death. Controls were treated with a solution of sterile water and food coloring. Thirty larvae were used per treatment, and the experiment was run twice, with independent dilutions for each block. Larvae were checked after 48 h to eliminate any handling deaths and thereafter every 12 h to record virus mortality, weight, and instar at death. Larvae were recorded as dead when they did not respond to prodding with a toothpick, and the time of death was taken as the point at which the recording was made.

Weight measurements and yield estimation. Since insects infected with the wild-type virus lyse at death, larvae showing severe disease symptoms were transferred to individual microtubes and weighed prior to death. They typically died and liquefied 12 to 24 h later. Larvae infected with the recombinant virus do not lyse, and such larvae were weighed after death. For yield measurement, larvae were individually homogenized with 1 ml of sterile water, the homogenate was sonicated for 2 min, and the virus polyhedra were counted using a hemocytometer. Yield was measured in 15 larvae selected randomly from each of the highest, middle, and lowest concentrations of each virus.

Statistical analysis of data. The data were analyzed using a generalized linear modeling program (GLIM version 3.77, 1985; Royal Statistical Society). Initially, all explanatory variables and their interactions were fitted to the data and the contribution of each term was tested for significance. Nonsignificant terms were removed, leaving the minimal adequate model. Standard model-checking procedures were employed, and polynomial terms were fitted when residual plots suggested nonlinearity. Percentage mortality was modeled using binomial errors, using the scale parameter to adjust deviances if required. The values for times to death had a highly right-skewed distribution, and an inverse transformation was used prior to analysis.

RESULTS

Pathogenicity. As expected, *M. brassicae* was significantly less susceptible to AcNPV-C6 and AcNPV-ST3 than *T. ni* was ($\chi^2 = 93.6$, $df = 1$, $P < 0.001$). Both viruses were equally pathogenic for *M. brassicae* ($\chi^2 = 1.86$, $df = 1$, $P = 0.173$); however, the recombinant was more infective for *T. ni* ($\chi^2 = 17.9$, $df = 1$, $P < 0.001$) (Fig. 1). The 50% lethal concentration (LC₅₀) for both viruses in *M. brassicae* was 2.75×10^6 polyhedra/ml (95% confidence interval, 2.2×10^5 to 3.4×10^6 polyhedra/ml). The LC₅₀s for AcNPV-ST3 and AcNPV-C6 in *T. ni* were 3.6×10^4 polyhedra/ml (95% confidence interval, 3.1×10^4 to 4.2×10^4 polyhedra/ml) and 8.7×10^4 polyhedra/ml (95% confidence interval, 7.4×10^4 to 9.5×10^4 polyhedra/ml), respectively. (No deaths among the controls were attributable to viral infection.) Of the deaths among *M. brassicae* and *T. ni* larvae, 0.5 and 15%, respectively, were attributable to bacterial infection, and these were excluded from the analysis. Gravimetric estimates of drinking volumes have shown that second-instar *M. brassicae* larvae drink on average 0.17 μl and *T. ni* larvae drink 0.094 μl (7, 18) during a 30-min period, which translates to a 50% lethal dose (LD₅₀) of 467 polyhedra per larva in *M. brassicae* and 3 and 8 polyhedra for AcNPV-ST3 and AcNPV-C6, respectively, in *T. ni*.

Speed of kill. Both viruses killed the more susceptible species, *T. ni*, faster than they did *M. brassicae* ($F_{1,598} = 867$, $P < 0.001$). As expected, *T. ni* was killed more rapidly by the recombinant than by the wild-type virus; however, at all concentrations, AcNPV-ST3 took longer to kill *M. brassicae* than AcNPV-C6 did ($F_{1,596} = 169$, $P < 0.001$) (Fig. 2). The speed of kill was not affected by the dose of wild-type virus ($F_{1,596} = 0.396$, $P = 0.5294$), resulting in constant times to death of 167 h for *M. brassicae* and 123 h for *T. ni*. In contrast, larvae of both species treated with recombinant virus died more rapidly as the dose increased, which may indicate some dose-related effect of toxin production ($F_{1,596} = 6.62$, $P = 0.01$). At the concentration of virus closest to the LC₅₀, AcNPV-ST3 takes 20% (33.5 h) longer to kill than does AcNPV-C6 in *M. brassicae*, whereas in *T. ni*, the recombinant reduced the time to death by over 25% (34.5 h) compared to the wild type (Fig. 2).

Relationship between virus yield and time to death. The log virus yield increased linearly with the time to death and decreased linearly with the virus concentration ($F_{1,323} = 10.12$,

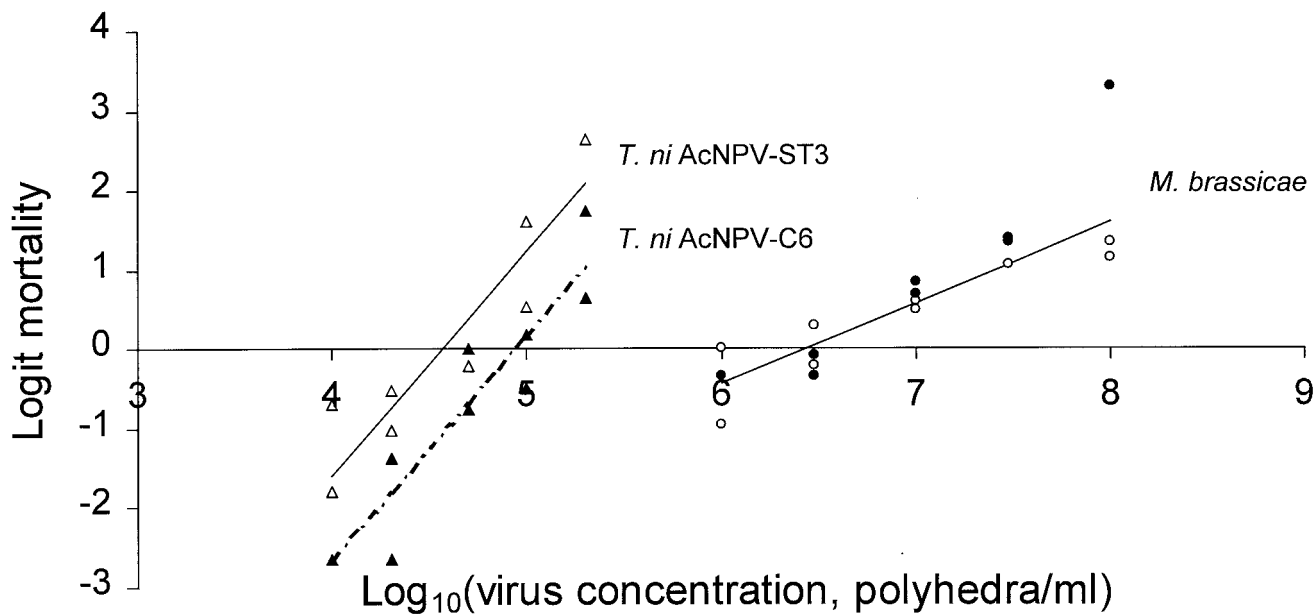


FIG. 1. Dose-response curves for AcNPV-C6 and AcNPV-ST3 in second-instar *M. brassicae* and *T. ni* larvae. The equations for the lines are as follows: for *T. ni*, for AcNPV-C6 (dashed line), $\text{logit}(\text{mortality}) = -14.02 + 2.836 [\log_{10}(\text{concentration})]$, and for AcNPV-ST3 (solid line), $\text{logit}(\text{mortality}) = -12.94 + 2.836 [\log_{10}(\text{concentration})]$; for *M. brassicae* (one solid line for both viruses), $\text{logit}(\text{mortality}) = -7.812 + 1.213 [\log_{10}(\text{concentration})]$ (logit is $\ln[p/(1 - p)]$ and p is proportionate mortality). Δ , *T. ni* AcNPV-ST3; \blacktriangle , *T. ni* AcNPV-C6; \circ , *M. brassicae* AcNPV-ST3; \bullet , *M. brassicae* AcNPV-C6.

$P = 0.0016$). The less-susceptible species, *M. brassicae*, produced a significantly greater virus yield than the more susceptible *T. ni* ($F_{1,323} = 10.49$, $P = 0.0013$). For both species, the yield produced by the recombinant was significantly lower than

that produced by the wild-type virus ($F_{1,323} = 37.6$, $P < 0.001$), despite the fact that the recombinant took longer to kill *M. brassicae* than the wild-type virus did. At the concentration of virus closest to the LC_{50} for the two species, this difference represented a 52% reduction in yield in *M. brassicae* and a 78% reduction in yield in *T. ni* (Fig. 3). The relationship between

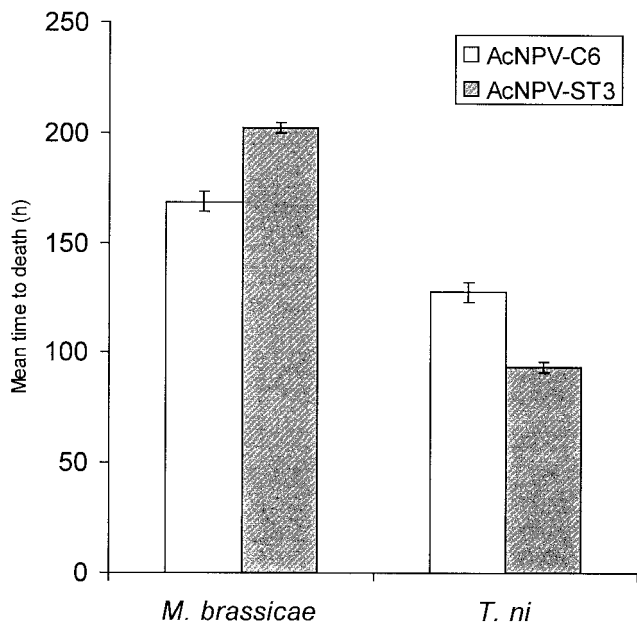


FIG. 2. Time to death (means \pm standard errors [SEs] [error bars]) for second-instar *M. brassicae* and *T. ni* infected with wild-type AcNPV-C6 and AcNPV-ST3. Values have been calculated from approximate LC_{50} s, that is, 3×10^6 and 5×10^4 polyhedra/ml for *M. brassicae* ($n = 29$ and 24 for AcNPV-C6 and AcNPV-ST3, respectively) and *T. ni* ($n = 15$ and 18 for AcNPV-C6 and AcNPV-ST3, respectively), respectively.

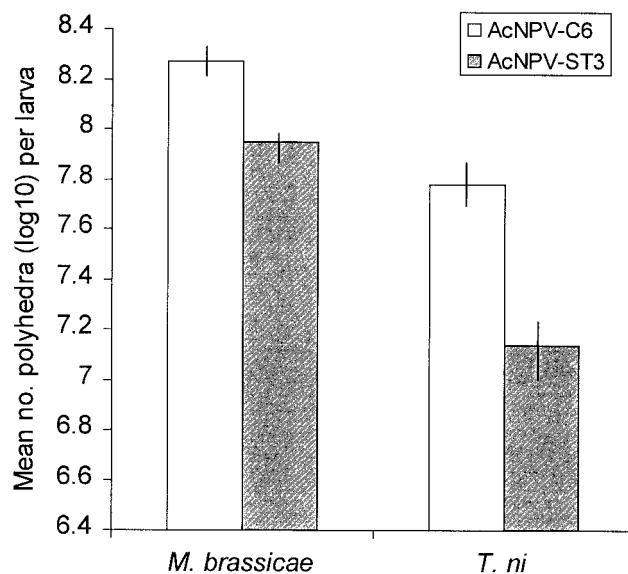


FIG. 3. Virus yield (means \pm SEs [error bars]) for second-instar *M. brassicae* and *T. ni* infected with wild-type AcNPV-C6 and AcNPV-ST3. Values have been calculated from approximate LC_{50} s, that is, 3×10^6 and 5×10^4 polyhedra/ml for *M. brassicae* ($n = 22$ and 24 for AcNPV-C6 and AcNPV-ST3, respectively) and *T. ni* ($n = 6$ and 11 for AcNPV-C6 and AcNPV-ST3, respectively), respectively.

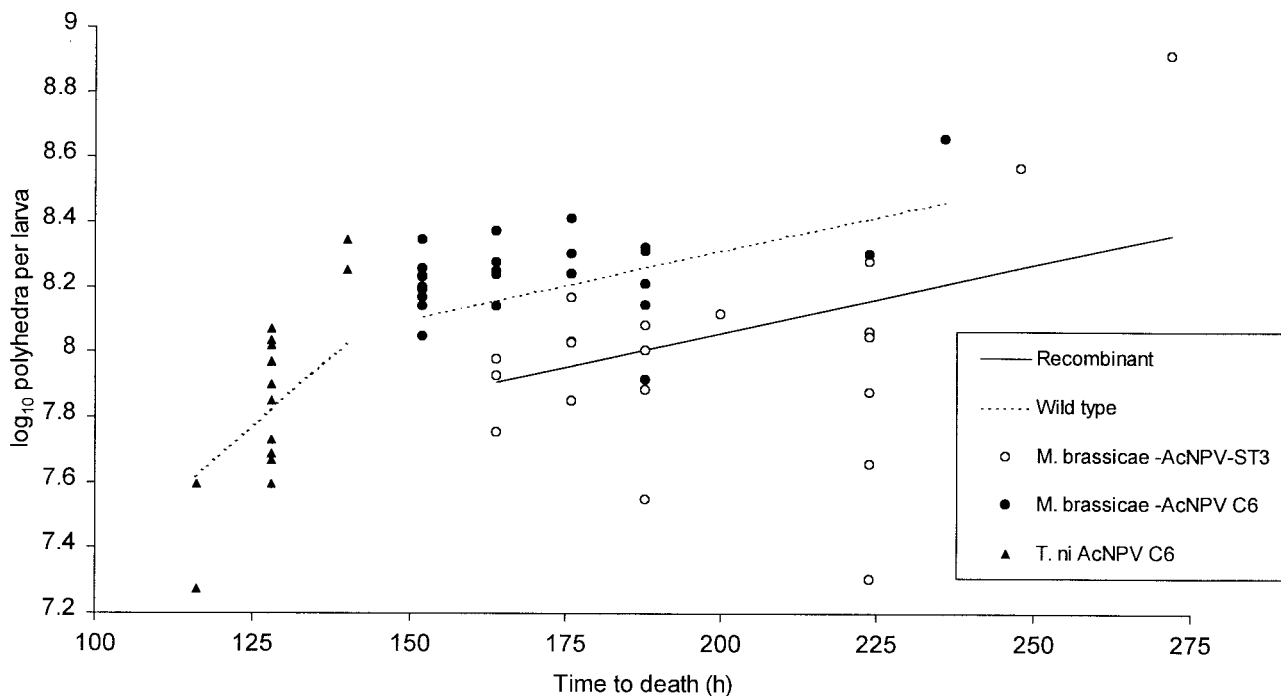


FIG. 4. Relationship between yield per larva and time to death for second-instar *M. brassicae* treated with AcNPV-ST3 and AcNPV-C6 (concentration of each, 10^6 polyhedra/ml) and second-instar *T. ni* treated with AcNPV-C6 (concentration, 2×10^5 polyhedra/ml). (*T. ni* with AcNPV-ST3 has not been included, as the range of times to death at the virus concentration used are not sufficient to demonstrate the relationship.) The relationship for *M. brassicae* with AcNPV-C6 and AcNPV-ST3 can be described by the following equations: yield = $7.702 + 0.004233 \times \text{TTD} - 0.08152 \times \text{LC}$ and $7.9561 + 0.004233 \times \text{TTD} - 0.08152 \times \text{LC}$, respectively. The relationship for *T. ni* with AcNPV-C6 can be described by the following equation: yield = $6.0681 + 0.017083 \times \text{TTD} - 0.08152 \times \text{LC}$. (In the equations given above, yield is stated in \log_{10} units, TTD is time to death, and LC is the \log_{10} virus concentration.)

yield and speed of kill was also different for the two species ($F_{1,318} = 25.96$, $P < 0.001$): the response for *T. ni* has a much steeper slope than that for *M. brassicae*. This is illustrated in Fig. 4, where the highest concentration used against *T. ni* (approaching the level of mortality desired in a field control program, approximately four times the LC_{50}) is contrasted with a similar concentration used against *M. brassicae* (approximately one-third the LC_{50}).

Virus yield per unit of larval weight. A lower yield for the recombinant virus could be the result of a reduction in replication rate. While an estimate of virus yield provides an indication of the relative productivity of the two viruses, the change in the yield of virus per unit of larval weight over time is an indirect measure of the rate of virus replication in the host compared to the rate of host growth. The two species showed a striking difference in their response to AcNPV-C6, with the yield per unit of larval weight declining over time for *M. brassicae* but increasing for *T. ni* (Fig. 5) ($F_{1,325} = 20.5$, $P < 0.001$). There was no difference between the yields of the two viruses in *M. brassicae* ($F_{1,323} = 1.69$, $P = 0.1945$), whereas in *T. ni*, the yield (per unit of larval weight) of the wild type was higher than that of the recombinant at any given time ($F_{1,323} = 5.6$, $P = 0.0185$) (Fig. 5).

DISCUSSION

Studies on the effects of insect microbial control agents on less-susceptible, nontarget species are crucial if we aim to pre-

dict the fate of recombinant, as well as naturally occurring, pathogens in the environment. However, it must be remembered that the risks and benefits associated with the use of wild-type and genetically modified pathogens also need to be compared to the risks associated with other technologies and the costs and benefits of no action. At a fundamental level, understanding how the biological characteristics of genetically modified pathogens contrast with their wild-type counterparts is essential in order to address one of the main questions in assessment of their risk, the impact of recombinant viruses on nontarget species. In this study we have shown that the response of a less-susceptible species to a recombinant baculovirus differs markedly from that of a highly susceptible host in two key parameters that could effect the transmission and persistence of the recombinant virus in the field. In contrast to the response observed for the highly susceptible *T. ni*, the recombinant did not show an enhanced speed of kill when used to infect the less-susceptible species *M. brassicae*. Overall, the yield of virus was greater in *M. brassicae* than in *T. ni*; the recombinant also produced less virus than did the wild-type in both host species. However, this was not for the expected reason, that is, not as a result of the virus having a faster speed of action.

The issue of whether expression of an insect-selective toxin actually increases the mortality produced by the virus is obviously a crucial question for risk assessment. Current trends in genetic engineering are focused on increasing the speed of kill. As a result, most reports on AcNPV expressing AaHIT, one of

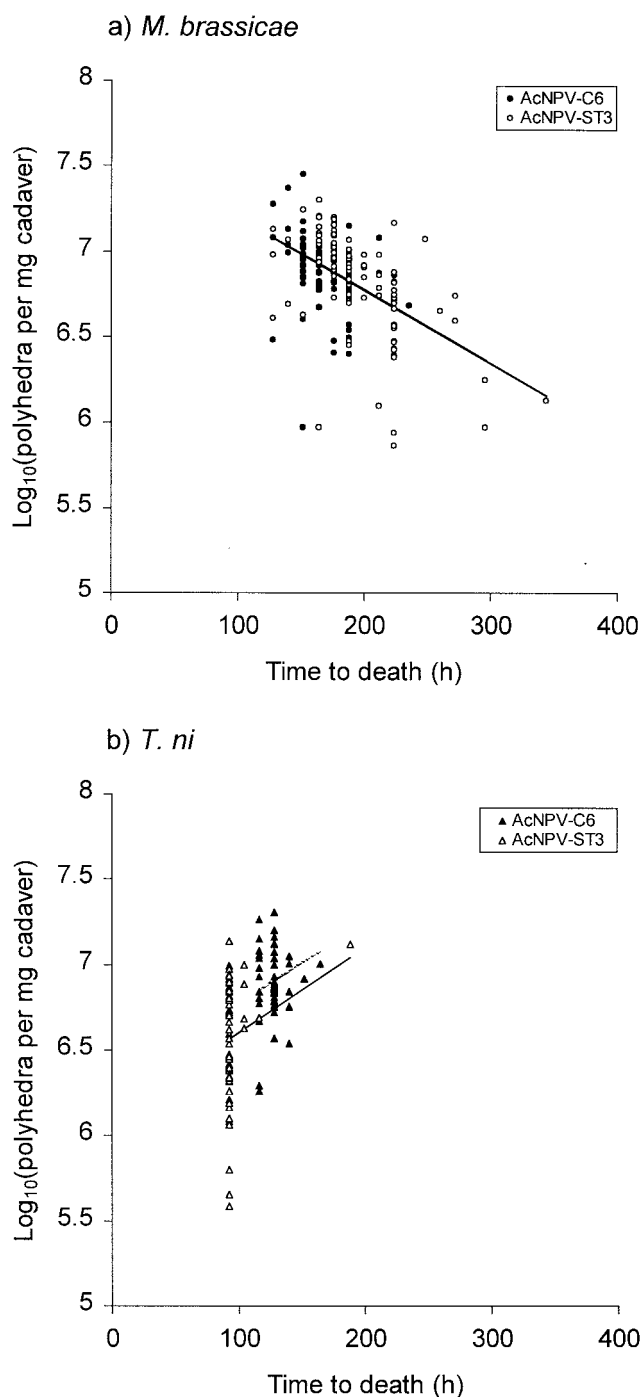


FIG. 5. The relationship between \log_{10} yield per unit of larval weight and time to death for *M. brassicae* and *T. ni* challenged with AcNPV-C6 and AcNPV-ST3. (a) The relationship for *M. brassicae* infected with both AcNPV-C6 and AcNPV-ST3 is as follows: \log_{10} (yield per mg of body weight) = $7.625 - 0.004256 \times \text{TTD}$ ($n = 211$). (b) The relationship for *T. ni* infected with AcNPV-C6 is as follows: \log_{10} (yield per mg of body weight) = $6.269 - 0.00495 \times \text{TTD}$ ($n = 54$) (dashed line). For *T. ni* infected with AcNPV-ST3, the relationship is as follows: \log_{10} (yield per mg of body weight) = $6.11 - 0.00495 \times \text{TTD}$ ($n = 64$) (solid line). (In these three equations, TTD is the time to death.)

the most widely studied recombinant baculoviruses, relate to alterations in the speed of kill and fewer studies have measured the LC_{50} or LD_{50} response. *M. brassicae* did not differ in its response to the two viruses. *T. ni*, however, was more susceptible to the recombinant virus, a result found previously (28). Bioassays using two other sensitive hosts, *Heliothis virescens* (25) and *Pseudoplusia includens* (22), showed no significant differences in LD_{50} between the two viruses. The data for *T. ni* may indicate that this species is particularly sensitive to the toxin or may indicate the existence of a feature peculiar to the recombinant virus. For example, infection with AcNPV-ST3 produces smaller and less complete polyhedra than the wild-type (P. Hernández-Crespo, unpublished data), and it is possible that this could result in the recombinant polyhedra being more readily broken down in the host gut. The fact that the response of *M. brassicae* is the same for both viruses could also indicate that it is not sensitive enough to detect differences between them. The results highlight the importance of testing individual species, rather than extrapolating from one species to another. However, in contrast to the laboratory studies, small-scale field trials with *M. brassicae* and *T. ni* have shown that the mortality induced by AcNPV and AcNPV-ST3 is not significantly different (10, 17). The sensitive nature of highly controlled, detailed laboratory analyses (precise doses administered over a limited amount of time) means that small differences in virus biological activity can be detected. These differences may be negated in the field, however, where conditions can be highly variable and susceptible larvae have the opportunity to experience multiple pathogen hits over a longer time period. While laboratory studies are important for detailing baseline pathological differences between wild-type and recombinant baculoviruses, ultimately, they will always need to be contrasted in the field environment.

An unexpected finding was that the recombinant virus did not kill the larvae of *M. brassicae* faster than the wild-type virus, as it did in *T. ni*. Other studies which have measured speed of action have concentrated on the more susceptible species, and despite the various methodologies used, they all report a significantly more rapid response in larvae treated with the toxin-producing AcNPV recombinant in both the laboratory and the field (10, 30). As the larvae treated with AcNPV-ST3 exhibited paralysis before death, we can confirm that the toxin exerts an effect on *M. brassicae*. Lepidoptera do differ in their sensitivity to this toxin (18), so the slower response could be due to *M. brassicae* being less sensitive than other species to AaHIT. The recombinant virus appears to be producing toxin at critical sites, as the virus-produced toxin is killing at levels well below those needed when injecting pure toxin. However, it is possible that there could be a difference in distribution of the toxin between the two species. Alternatively, a proportion of the toxin could be folding incorrectly in *M. brassicae* (the toxin contains four disulphide bonds which are crucial to activity); this could be monitored using high-pressure liquid chromatography or a combination of bioassay and Western blotting. The reduced response could also be due to a reduced rate of toxin production or an increased rate of toxin breakdown in *M. brassicae*, a feature that could be monitored by bioassay using a host which is highly sensitive to AaHIT, such as *Drosophila melanogaster*. These issues need to be addressed in further studies. As the recombinant actually took

longer to kill the host than the wild-type virus, it might also indicate that some inhibition of virus replication was also taking place. The results highlight the need for the toxin host range to be considered in addition to the host range of the virus as well as for more detailed mechanistic studies on toxin expression. As with infectivity, field trials are needed to examine whether this effect translates into measurable differences in field populations. In a small-scale, short-term field experiment in which second-instar *M. brassicae* were sprayed with the same two viruses, the time-to-death results did not show the same pattern (17). Larvae collected 24 h after spraying did not differ in the time they took to die in the laboratory, whereas of the larvae collected 3 and 5 days postspray, those infected with the recombinant died more rapidly (17). Environmental conditions, such as temperature and prolonged exposure to the pathogen, are likely to alter the relationship between virus and host in the field.

Our results show that a less-susceptible host infected with AcNPV produces a greater yield than a highly susceptible species. They have also demonstrated that production of recombinant virus in both species is always lower than that of the wild-type. This has clear implications for the ecological fitness of the recombinant virus. In agreement with Kunimi et al. (21), in *T. ni* the yield of the recombinant was reduced significantly compared to that of the wild-type virus. The resulting yield of the recombinant was also lower in *M. brassicae*; however, in contrast to what was observed for *T. ni*, this cannot be a result of the virus having less time to replicate. Estimating the virus yield per unit of weight of larva can give some indication as to how much and how rapidly the insect is being converted into virus polyhedra. Interestingly, a very different relationship was found for the two hosts. In *T. ni* the relationship between yield per unit of larval weight and time to death is positive, whereas in *M. brassicae* it is distinctly negative, with larvae that died later yielding less virus per unit of larval weight. In *T. ni*, the permissive species, this implies that viral replication and infection of tissues keeps pace with host growth, whereas the negative relationship in *M. brassicae*, the semipermissive species, may indicate that virus replication is slow relative to larval growth. Additionally, the wild-type virus has a greater yield per unit of larval weight than the recombinant at any point in time (which would exacerbate the yield differences between the two viruses), whereas the response for the two viruses in *M. brassicae* is the same. Measurement of the virus yield per unit of larval weight over time in different hosts might be a means of categorizing species of varying susceptibility.

This study has clearly shown that species that vary in susceptibility can respond very differently to recombinant (and wild-type) baculoviruses. We have demonstrated that there are differences in productivity and the timing of virus release, both of which could have a major impact on virus-host dynamics. The effects of such alterations are difficult to predict. Simple host-pathogen models identify parameters such as productivity, speed of kill, transmission, and persistence as being central to describing the basic reproductive rate of the virus (1, 6, 12, 16). Therefore, a reduction in a key parameter such as yield would result in a reduction in the fitness of a virus and it would be predicted that the wild-type virus would outcompete AcNPV-ST3 in *T. ni* populations. In reality, the agricultural environment will consist of an interaction between the pathogen

and the target as well as nontarget species. With the introduction of less-susceptible, nontarget hosts such as *M. brassicae*, predicting the outcome and associated risks of using recombinant viruses as biopesticides will be more difficult to predict. The yield larva (of either virus) in *M. brassicae* is greater than that in *T. ni*, but this is offset by the lower infectivity of AcNPV for *M. brassicae*. This means that the overall amount of virus produced by *M. brassicae* will be lower and more aggregated than that in a highly susceptible species like *T. ni*, both of which would reduce transmission of the virus. Recent small-scale field studies have shown that the number of virus patches (cadavers) has a greater influence on transmission than the quantity of virus they contain (16a). The later release of virus may also delay rounds of secondary transmission. Thus, in the case of *M. brassicae*, the high levels of recombinant AcNPV needed to infect it suggest that it will not play a major role in the transmission or amplification of the virus in the field. Nevertheless, a baculovirus may still be able to persist in less-susceptible, nontarget populations when they are at sufficiently high density. At low density, they may be at risk from repeated introductions from other reservoir species (3, 24). Our knowledge of the behavior of baculoviruses (and other insect pathogens) in less-susceptible hosts is negligible, and this study demonstrates that this information is not necessarily going to be predictable from the data we have on more-susceptible species. Estimating parameters of the host-pathogen interaction in the laboratory is the first step in the step-by-step process of risk assessment. However, field studies are required to investigate parameters such as transmission and persistence, the knowledge of which is needed to assess the consequences of the wide-scale release of natural or recombinant viruses.

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