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Risk Assessment Studies: Detailed Host Range Testing of Wild-Type Cabbage Moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), Nuclear Polyhedrosis Virus

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The host range of a multiply enveloped nuclear polyhedrosis virus (NPV) (*Baculoviridae*) isolated from the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), was determined by challenging a wide range of insect species with high (10^6 polyhedral inclusion bodies) and low (10^3 polyhedral inclusion bodies) doses of the virus. The identity of the progeny virus was confirmed by dot blotting. Analysis of 50% lethal dose was carried out on selected species, and the progeny virus was identified by using restriction enzyme analysis and Southern blotting. Other than the Lepidoptera, none of the species tested was susceptible to *M. brassicae* NPV. Within the Lepidoptera, *M. brassicae* NPV was infective to members of four families (Noctuidae, Geometridae, Yponomeutidae, and Nymphalidae). Of 66 lepidopterous species tested, *M. brassicae* NPV was cross-infective to 32 of them; however, 91% of the susceptible species were in the Noctuidae. The relevance of host range data in risk assessment studies is discussed.

Naturally occurring baculoviruses have been isolated from a wide range of insects and have been used successfully for the control of many lepidopteran, hymenopteran, and coleopteran pests (11). Recent advances in genetic engineering have meant that baculoviruses are now candidates for genetic manipulation, for example, by the insertion of foreign genes to increase their speed of kill (5). Even with naturally occurring baculoviruses, safety testing and environmental impact assessment have always been required before large-scale release; with the advent of genetically altered viruses, risk assessment studies have assumed even greater importance. The risks attached to the release of an engineered baculovirus can only be assessed against a good background knowledge of the behavior of the wild-type virus (10). One key area in risk assessment is host range testing. It is important to know whether cloning or genetic manipulation alters the virus host range. It is also important to know what effect the virus has on nontarget hosts in order to make better predictions about the impact and fate of the engineered virus after release into the environment.

A certain amount of information is already available on baculovirus host range and how it varies within the baculovirus subgroups (11). In general, nuclear polyhedrosis viruses (NPVs) are thought to have the broadest host range, with the NPV of *Autographa californica* reportedly extending into 11 families of Lepidoptera (24). Whether this is an exception or purely a reflection of the great effort being devoted to this virus will only be shown with wider and more detailed host range testing such as that described in this paper. No beneficial insects have ever been reported to have been harmed by baculoviruses, and no cross-order transmission was thought to occur. However, it has recently been reported that a lepidopterous NPV can infect a member of the Orthoptera (4) and the Isoptera (1), and these findings need to be further explored.

It is also important to consider latent or occult infections in host range testing: although the mechanism is not yet understood, feeding a heterologous virus to an insect may trigger its own latent homologous virus (17, 20, 22). One problem with the earlier cross-infection experiments is that it was not possible to confirm true cross-infection before the use of restriction enzymes to identify the progeny virus. Any host range study must now incorporate these techniques in order to validate the results.

The data reported in this paper form part of a project to assess the risks attached to the possible release of a genetically altered NPV of the cabbage moth, *Mamestra brassicae*. In this project, the behavior of the wild-type virus was assessed to allow comparison of it with cloned and genetically engineered NPVs. This first report presents data on the detailed host range testing of wild-type *M. brassicae* multiply enveloped NPV (*M. brassicae* NPV). Although some host range testing has been carried out on *M. brassicae* NPV (2, 3, 7, 27), no attempt has been made to define a comprehensive host range which includes both beneficial insects and a wide range of Lepidoptera. A stepwise approach was taken to confirm cross-infection. Larval deaths were first screened for occluded virus by smearing, staining, and light microscopy. Those that proved positive were then assessed for the presence of *M. brassicae* NPV by dot blotting. True cross-infection was also confirmed in several instances by restriction enzyme analysis and Southern blotting. A total of 74 species of insects from four orders were tested for susceptibility to *M. brassicae* NPV.

MATERIALS AND METHODS

Origin and production of virus. The *M. brassicae* NPV was originally obtained from A. Gröner (BBA, Darmstadt, Federal Republic of Germany) in 1976 and originated from an epizootic in an insect culture in Darmstadt in 1973. It has since become known as the Oxford isolate and has been both biochemically (6, 19, 25) and biologically (12–14) characterized. In order to bulk up the virus, laboratory-reared third-instar *M. brassicae* larvae were each fed a dose of 10^3 polyhedral inclusion bodies (PIBs) of *M. brassicae* NPV on

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a plug of modified Hoffman tobacco hornworm diet (16). The NPV was purified on a sucrose gradient after macerating larval corpses in 0.1% sodium dodecyl sulfate as described previously by Carruthers et al. (8). Before the virus was frozen, its concentration was determined by a dry counting method (28). The same *M. brassicae* NPV stock was used throughout the study: the virus was stored frozen in 200-μl portions and only thawed once before use in the assays.

Cross-infection of test insects. The eggs of lepidopterous species to be tested were obtained mostly from light-trapped adult females. The eggs were surface sterilized by soaking in a 10% solution of Formalin for 40 min, followed by thorough rinsing in water. A few of the species were obtained from stocks already kept in culture within the Institute of Virology and Environmental Microbiology, Oxford, England. After hatching, the larvae were reared to an appropriate size on artificial diet (where possible) or their preferred food plant. Noctuids and similarly sized lepidopterans were tested in the second instar: using microlepidopterous species of an equivalent size meant that the larvae were either in the second or the third instar. Batches of 20 or 40 larvae, depending on availability, were each exposed to two dose levels: a high dose of 10^6 PIBs per larva and a low dose of 10^3 PIBs per larva. The dose was administered by placing 1 μl of virus suspension on either a small piece of the preferred food plant or a small diet plug in an individual chamber and allowing the larvae to feed for 24 h. Only those larvae which consumed the entire dose were removed onto clean foliage or diet. At this stage, one larva was removed from each dose and frozen to act as a virus-fed control for later probing. Ten virus-free control larvae were kept from each test species and reared under identical conditions. All larvae were maintained at 24°C until death or pupation, when they were frozen for later analysis. Nonlepidopterous species were obtained from various sources and fed the virus as appropriate. For example, young adult bees (*Apis mellifera*) were fed a solution of honey containing the virus dose.

All insects which died before pupation, both treated insects and controls, were smeared onto a microscope slide, stained by use of Giemsa as previously described, (29) and examined under a light microscope for virus replication as indicated by the presence of nuclear groups of PIBs under oil immersion at a magnification of ×1,000.

Dot blotting. Larvae which proved positive for occluded virus by light microscopy were tested further by dot blotting (18) to ascertain the identity of the virus. A single larva was homogenized in 500 μl of deionized water. An equal volume of 0.1 M Na_2CO_3 was added to 200 μl of the macerate, and this mixture was incubated at 37°C for 10 min. A 200-μl volume of lysis buffer (50 mM Tris hydrochloride [pH 8.0], 10 mM EDTA, 5% 2-mercaptoethanol, and 0.4% sodium dodecyl sulfate) was then added plus RNase A to a concentration of 100 μl/ml. This mixture was incubated at 37°C for 30 min, followed by incubation with 200 μg of proteinase K per ml at 37°C for 30 min. After extraction with phenol-chloroform, duplicate 25-μl aliquots of DNA were denatured by heating to 100°C for 3 min and snap-chilled in ice-ethanol. The aliquots were dotted onto nitrocellulose paper (BA85; Schleicher & Schuell, Inc., Keene, N.H.) presoaked in 2× SSC (1× SSC is 150 mM NaCl, 15 mM trisodium citrate). Each filter carried a positive control, consisting of 100 ng of *M. brassicae* NPV DNA and DNA extracted from larvae frozen immediately after feeding with virus, to indicate the degree of hybridization that was a result of the original virus dose. Negative controls consisted of 100 ng of salmon sperm DNA and DNA extracted from control larvae. After being dotted, the filters were air dried and then baked at 80°C for 1 h to bind the DNA.

To minimize the likelihood of hybridization to heterologous baculovirus DNAs, fragments of *M. brassicae* NPV which did not contain the polyhedrin gene were assessed as suitable DNA probes. Radiolabeled cloned *Hin*dIII fragments E and M (25) were hybridized under stringent conditions to dot-blotted DNAs of NPVs from several species of Lepidoptera. Although there was limited hybridization to other DNAs, fragment M appeared to be the most specific (data not shown), and this was used to probe the dot blots for host range testing. The probe was radiolabeled by nick translation as described by Rigby et al. (26). The filters were hybridized for 18 h at 37°C in hybridization buffer consisting of 50% deionized formamide, 3× SSC, 50 mM HEPES (*N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid [pH 7.0]), 0.02% each of bovine serum albumin, Ficoll (Pharmacia Fine Chemicals, Piscataway, N.J.), and polyvinylpyrrolidone (9), plus 100 μg of sonicated denatured salmon sperm DNA per ml. After hybridization, the filters were washed four times for 5 min in 2× SSC at room temperature and then at 65°C for 1 h with two changes. They were then air dried and exposed to X-ray film at −70°C.

Bioassays. Fifty percent lethal doses (LD_{50}s) were calculated for a selection of susceptible species by bioassay. Of the second-instar larvae within a narrow size range (approximately 0.002 ± 0.001 g depending on the species), 50 were individually fed known doses of *M. brassicae* NPV by using the infection procedure described above. Serial dilutions of virus were used, the concentrations of which were determined from the initial infection susceptibility from the two-dose test. Larvae were examined daily, and the corpses were removed, smeared, stained, and diagnosed as previously described. The data obtained were analyzed by using probit analysis (15) to ascertain LD_{50} values.

Restriction enzyme analysis. To confirm cross-infection, DNA was extracted from a single larva infected in the cross-infection tests and bioassays, and restriction enzyme analysis was carried out. Particular emphasis was placed on species closely related to *M. brassicae*, as it was considered more likely that a latent NPV from these hosts would share greater homology with *M. brassicae* NPV than those from more distantly related hosts. Virus was extracted as described above but without a sucrose gradient. The virus pellet was washed and suspended in a small volume of sterile water. A 150-μl volume of the virus suspension was incubated in 0.05 M Na_2CO_3 at 37°C for 30 min. This was followed by incubation in 1% sodium dodecyl sulfate plus proteinase K (200 μg/ml) at 37°C for a further 30 min. DNA was extracted with phenol-chloroform and dialyzed at 4°C against four changes of TE buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA) overnight. A 25-μl volume of virus DNA was digested with the restriction endonuclease *Hin*dIII by using the conditions recommended by the supplier (BRL). Fragments were resolved by electrophoresis on a horizontal 0.6% agarose gel in Tris-borate buffer (89 mM Tris, 109 mM boric acid, 2.5 mM EDTA [pH 8.3]) containing ethidium bromide (0.5 μg/ml), at 40 V for 18 h (21). The gel was visualized and photographed on a UV transilluminator. Gels with faint or indistinct bands were Southern blotted onto a Hybond-N membrane (Amersham Corp., Arlington Heights, Ill.) which was subsequently exposed to UV light for 3 min to bind the DNA. After prehybridization by boiling in 1 mM EDTA for 3 min, membranes were probed with radiolabeled PRO1, a 1,268-base-pair *Sal*I fragment derived from the *Hin*dIII F fragment from the closely related *Panolis*

TABLE 1. Susceptibility of nonlepidopteran species to *M. brassicae* NPV infection[a]

Order, family, and species	Susceptibility to infection as determined by:			
	Insect smear		Dot blot	
	10^{3b}	10^6	10^3	10^6
Hymenoptera				
Apidae				
Apis mellifera	−	−	−	−
Symphyta				
Neodiprion sertifer	+	+	−	−
Nematus ribesii	−	−	−	−
Coleoptera				
Coccinellidae				
Coccinella-7-punctata	−	−	−	−
Carabidae				
Notiophilus biguttatus	−	−	−	−
Agonum dorsale	−	−	−	−
Neuroptera				
Crysopa carnea	−	−	−	−

[a] Results are from analysis of insect smears by using light microscopy and dot blotting with radiolabeled DNA probes.
[b] Dose of *M. brassicae* NPV in PIBs.

flammea NPV, which contained the sequence coding for the polyhedrin gene (23). This fragment had previously been shown to hybridize strongly with DNA fragments from *M. brassicae* NPV and was used to confirm the presence of *M. brassicae* NPV by the position of the fragment containing the polyhedrin gene on the autoradiograph. The membranes were hybridized for 18 h at 37°C in a solution containing 50% formamide, 6× SSC, 50 mM HEPES-NaOH (pH 7.0), 0.01% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone, 0.1% sodium dodecyl sulfate, and 100 μg of sheared denatured salmon sperm DNA per ml. After hybridization, the membranes were washed four times for 5 min each in 2× SSC at room temperature and then at 65°C for 1 h with two changes. They were then air dried and exposed to X-ray film at −70°C.

RESULTS

None of the species outside of the Lepidoptera proved susceptible to *M. brassicae* NPV (Table 1), although many species within the Lepidoptera were positive (Table 2). The analysis of the larval smears provided the preliminary data for deciding whether or not the insects had succumbed to viral infection. Confirmation of true *M. brassicae* NPV cross-infection, rather than initiation of latent or occult infection, was shown by dot blotting plus DNA probing and, for a few species, restriction enzyme analysis and Southern blotting (Table 2). For example, Fig. 1 shows two species, one which was positive from the larval smear (*Xestia xanthographa*) and the other which was negative (*Orthonama obstipata*). Probing the dot blot with the radiolabeled *Hin*dIII M fragment from *M. brassicae* NPV demonstrated that the *X. xanthographa* infection was caused by *M. brassicae* NPV since samples from both the 10^3- and 10^6-PIB doses hybridized with the *M. brassicae* NPV DNA probe. All other controls were as expected. Figure 2 shows a different result: both *Semiothisa clathrata* and *Hyles euphorbiae* were virus positive for one or both doses in the larval smear diagnosis; however, only *S. clathrata* proved positive for the dot blot analysis, indicating that the hawkmoth *H. euphorbiae* was killed by another virus, probably its

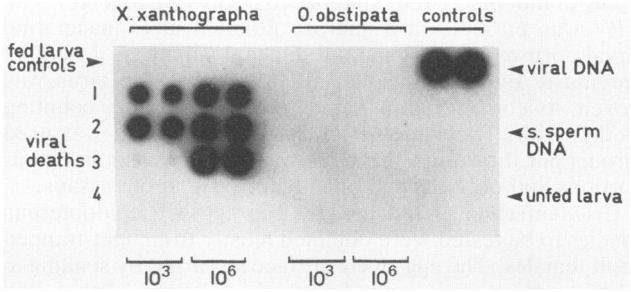

FIG. 1. Autoradiograph of a dot blot of DNA extracted from a single larva of the square spot rustic moth (*X. xanthographa*) and the gem moth (*O. obstipata*) (rows 1 through 4) which were fed *M. brassicae* NPV. The blot was probed with a ^{32}P-labeled *M. brassicae* NPV DNA *Hin*dIII M fragment. The controls were as follows: positive, 100 ng of *M. brassicae* NPV DNA (viral DNA); negative, 100 ng of salmon sperm DNA (s. sperm DNA); fed larva which is DNA extracted from larvae frozen immediately after feeding, one per dose (fed larva controls); and unfed larva. All samples are duplicates. Doses (10^3 and 10^6) shown indicate number of PIBs.

homologous baculovirus. Of those tested, five species (mostly Sphingidae) were positive in the smear diagnosis but negative in the dot-blot analysis.

Restriction enzyme analysis and Southern blotting were carried out, where possible, where *M. brassicae* NPV-positive larvae were obtained from species outside the Noctuidae or where cross-infections were in noctuids closely related to *M. brassicae*. It was not possible to confirm cross-infection in the geometrid *S. clathrata*, as the larvae were very small when they died and did not contain enough virus. Because of the large number of larvae being processed, their variability in both size and degree of infection, and the fact that the DNA degraded very rapidly (even at 4°C), the amount of viral DNA analyzed by using restriction enzymes varied (Fig. 3). In such cases, the gels were Southern blotted and examined by using more sensitive DNA probes. Figure 4 shows the resulting autoradiograph probed with the *P. flammea* NPV PRO1 *Sal*I fragment containing the sequence coding for the polyhedrin gene. Although the DNA fragments on some tracks are weak or obscured, the presence of *M. brassicae* NPV in all of the species tested was confirmed by the position of the polyhedrin gene-containing band on the autoradiograph. It is interesting to note that the *M. brassicae* NPV produced by *Orthosia cruda* (track 2) appears to be a variant of the

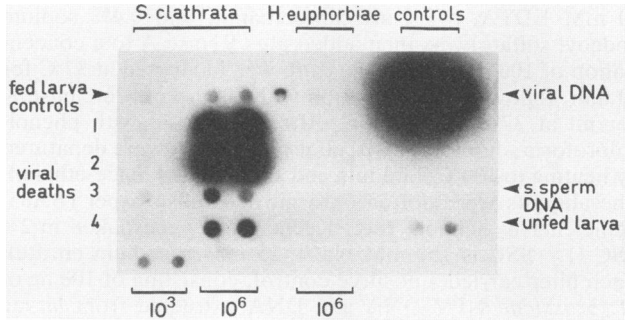

FIG. 2. Autoradiograph which was exposed to the hybridized dot blot of single larval extracts from the latticed heath moth (*S. clathrata*) and the spurge hawk moth (*H. euphorbiae*) fed *M. brassicae* NPV. Labeling and controls are the same as in Fig. 1.

TABLE 2. Susceptibility of lepidopteran species challenged with two doses of *M. brassicae* NPV[a]

Family and species	Susceptibility to infection as determined by:				
	Larval smear		Dot blot		Restriction enzyme analysis
	10^{3}[b]	10^{6}	10^{3}	10^{6}	
United Kingdom residents or immigrants					
Nymphalidae					
Boloriae uphrosyne	−	−	−	−	
B. selene	−	−	−	−	
Aglais urticae	−	+	−	+	+
Cynthia cardui	−	+	−	+	+
Satyridae					
Pararge aegeria	−	−	−	−	
Lycaenidae					
Polyommatus icarus	−	−	−	−	
Pieridae					
Pieris brassicae	−	−	−	−	
Lasiocampidae					
Malacosoma neustria	−	−	−	−	
Philudoria potatoria	−	−	−	−	
Saturniidae					
Pavonia pavonia	−	−	−	−	
Geometridae					
Cyclophora linearia	−	−	−	−	
Idaea aversata	−	−	−	−	
Orthonama obstipata	−	−	−	−	
Chloroclysta truncata	−	−	−	−	
Semiothisa clathrata	−	+	−	+	
Opisthograptis luteolata	−	−	−	−	
Menophra abruptaria	−	−	−	−	
Sphingidae					
Acherontia atropos	−	−	−	−	
Mimas tiliae	+	+	−	−	
Smerinthus ocellata	+	+	−	−	
Hyles euphorbiae	+	+	−	−	
Deilephila elpenor	+	+	−	−	
Notodontidae					
Phalera bucephala	−	−	−	−	
Pheosia tremula	−	−	−	−	
Drymonia ruficornis	−	−	−	−	
Lymantriidae					
Orgyia antiqua	−	−	−	−	
Calliteara pudibunda	−	−	−	−	
Arctidae					
Spilosoma lubricipeda	−	−	−	−	
Tyria jacobaeae	−	−	−	−	
Noctuidae					
Noctuinae					
Agrotis puta puta	+	+	+	+	+
A. segetum[c]	+	+	+	+	
Ochropleura plecta	+	+	+	+	
Noctua pronuba	+	+	+	+	
N. fimbriata	+	+	+	+	
Xestia c-nigrum	+	+	+	+	
X. xanthographa	+	+	+	+	
Anaplectoides prasina	−	−	−	−	
Hadeninae					
Hada nana	+	+	+	+	
Polia nebulosa	−	+	−	+	
Sideridis albicolon	+	+	+	+	
Mamestra brassicae	+	+	+	+	+
Melanchra persicariae	−	+	−	+	
Lacanobia w-latinum	+	+	+	+	+
L. thalassina	+	+	−	+	
L. suasa	+	+	+	+	+
L. oleracea	+	+	−	+	+
Papestra biren	−	+	−	+	+
Ceramica pisi	+	+	+	+	
Hadena bicruris	+	+	+	+	
Panolis flammea	+	+	+	+	+
Orthosia cruda	+	+	+	+	+
O. opima	+	+	+	+	
O. gracilis	+	+	+	+	
Amphipyrinae					
Phlogophora meticulosa	−	+	−	+	
Hyppa rectilinea	−	−	−	−	
Apamea monoglypha	+	+	+	+	
Spodoptera exigua[c]	+	+	+	+	
S. littoralis[c]	−	−	−	−	
Heliothinae					
Heliothis armigera[c]	+	+	+	+	
Chloephorinae					
Pseudoips fagana britannica	−	−	−	−	
Plusiinae					
Trichoplusia ni[c]	+	+	+	+	+
Autographa pulchrina	−	−	−	−	
Yponomeutidae					
Yponomeuta padella	−	−	−	−	
Plutella xylostella		+		+	+
Pyralidae					
Udea olivalis	−	−	−	−	
Non-United Kingdom Lepidoptera					
Nymphalidae					
Vanessa indica	−	+	−	+	+
Noctuidae					
Mythimna separata[c]	+	+	+	+	
Saturniidae					
Antheraea pernyi	−	−	−	−	

[a] Results are from analysis of stained larval smears by using light microscopy, dot blotting plus DNA probing, and restriction enzyme analysis.
[b] Dose of *M. brassicae* NPV in PIBs.
[c] Laboratory-reared species.

original *M. brassicae* NPV inoculum since the 11.67-kilobase-pair fragment is missing and appears to have been replaced by extra fragments farther down the gel. As yet, this variant has not been further examined. However, similar variants have been recovered in previous laboratory and field studies (C. J. Doyle, unpublished data). Track 3 (*Lacanobia w-latinum*) also appears to have another virus present, which is thought to be a cytoplasmic polyhedrosis virus brought in with the wild-caught stock. No species that was positive for the dot blot proved negative after restriction enzyme analysis.

LD_{50} analysis was also carried out on several species where adequate numbers of insects were available (Table 3). Interestingly, several of these species appeared to be more susceptible to *M. brassicae* NPV than *M. brassicae* itself and all were noctuids: *Lacanobia thalassina* and, in one bioassay at least, *Trichoplusia ni* and *Heliothis armigera*. Most of the species tested required in excess of 10^3 PIBs for an LD_{50} value in second-instar larvae and in some cases over 10^4 PIBs.

Overall, 32 out of 66 species of Lepidoptera tested were susceptible to *M. brassicae* NPV, excluding *M. brassicae* itself. Susceptible species covered four families of Lepidoptera: the Noctuidae, the Geometridae, the Yponomeu-

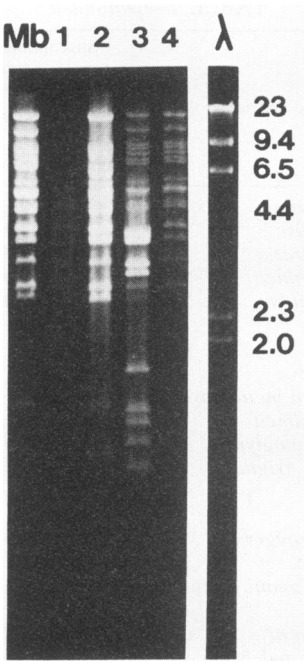

FIG. 3. *Hin*dIII fragments of viral DNA from four species infected with *M. brassicae* NPV and positive for the dot blot, resolved on a 0.6% agarose gel and compared with the profile produced with *M. brassicae* NPV inoculum DNA (lane Mb). Lanes: 1, *P. flammea*; 2, *O. cruda*; 3, *L. w-latinum*; 4, *L. oleracea*; λ, λ *Hin*dIII DNA profile with sizes indicated in kilobase pairs. Sample 3 (*L. w-latinum*) also shows additional bands which are thought to be a cytoplasmic polyhedrosis virus.

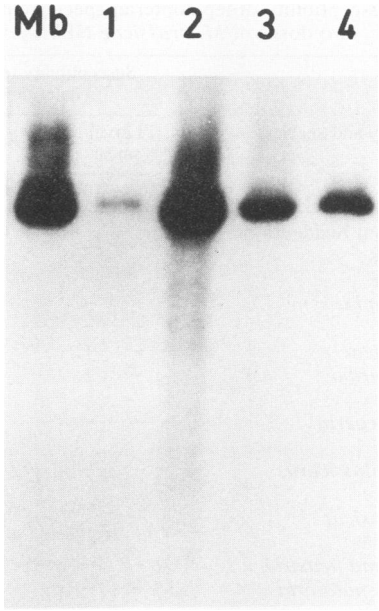

FIG. 4. Autoradiograph of gel shown in Fig. 3 probed with *P. flammea* PRO1, a *Sal*I fragment containing the polyhedrin gene. The *Hin*dIII O band, containing the polyhedrin gene, is lit up in the *M. brassicae* NPV control (Mb) and is at the same position in the other four samples (lanes 1 through 4), confirming cross-infection with *M. brassicae* NPV.

tidae, and the Nymphalidae. However, no insects outside the Noctuidae were susceptible at the lower dose (1,000 PIBs), and most (91%) of the positive cross-infections were found in the noctuids.

DISCUSSION

The data outlined in this paper represent a detailed host range study of a naturally occurring baculovirus. The species tested cover a wide spectrum of families within the Lepidoptera and, additionally, beneficial insects and other species from the Hymenoptera, Coleoptera, and Neuroptera. *M. brassicae* NPV was not infective to any members of the nonlepidopterous orders tested. Within the Lepidoptera, by far the largest number of susceptible hosts came from the Noctuidae, the family of which *M. brassicae* is a member. Exceptions to this were *S. clathrata* (Geometridae), *Plutella xylostella* (Yponomeutidae), and three species from the butterfly family Nymphalidae. Cross-infection in butterflies has not previously been demonstrated, but further details are given by C. J. Doyle and M. L. Hirst (J. Invertebr. Pathol., in press).

This study has shown that the host range of *M. brassicae* NPV is wider than has been previously demonstrated and extends outside the Noctuidae. Several other NPVs have also been reported as extending into two or three lepidopterous families (11), although in many cases host range testing was not extensive and it is likely that the number of susceptible hosts will increase with further work. *A. californica* NPV has been shown to have a wider host range than *M. brassicae* NPV and is currently known to extend into many more lepidopterous families than any other baculovirus. However, the identity of progeny NPV in *A. californica* NPV cross-infectivity studies often has not been adequately established.

Previous studies on *M. brassicae* NPV have only covered a small number of potential hosts within the Lepidoptera. Vlak and Gröner (27) looked at a number of species outside

TABLE 3. LD_{50} values for *M. brassicae* NPV in other lepidopteran species at second instar, calculated from bioassay data[a]

Species (assay)	LD_{50} (PIBs)[b]	95% Confidence limits (PIBs)		Slope
		Upper	Lower	
Noctuidae				
Agrotis segetum[c]	23.4% at 10,000			
Noctua pronuba	3,700	5,213	2,595	1.388
Mamestra brassicae (wild)	819	985	681	2.022
Lacanobia thalassina	480	617	373	2.062
L. oleracea	3,400	4,891	2,332	1.052
Orthosia gracilis	1,300	1,390	659	1.931
O. gothica	4% at 10,000			
Heliothis armigera[c] (1)	48.9% at 100			
Heliothis armigera[c] (2)	97.8% at 100			
Trichoplusia ni[c] (1)	489	598	399	2.506
Trichoplusia ni[c] (2)	635	863	468	1.346
Mythimna separata[c]	34,000	50,251	22,169	1.316
Yponomeutidae				
Plutella xylostella[c]	27,000	60,065	12,492	0.829

[a] No virus deaths occurred in controls.
[b] A percentage is indicated in cases where it was not possible to calculate the LD_{50} from the range of values chosen.
[c] Laboratory-reared species.

the Noctuidae, namely *Cydia* (*Laspeyresia*) *pomenella* (Tortricidae), *Adoxophyes orana* (Tortricidae), *Ostrinia nubilalis* (Pyralidae), *Galleria mellonella* (Pyralidae), *Evergestis forficalis* (Pyralidae), and *Plutella xylostella* (*maculipennis*) (Yponomeutidae), all of which were found to be nonpermissive. *P. xylostella* was found to be susceptible to *M. brassicae* NPV in the current study, although it is not particularly sensitive, with a second-instar LD_{50} of 27,000 PIBs. Similarly sized larvae were used in both bioassays, so this discrepancy was probably a result of an insufficient dose being administered in the bioassay or possibly to differences in the susceptibility of the insect stocks. The hosts recorded as being susceptible to *M. brassicae* NPV are all within the Noctuidae: *Agrotis segetum*, *Spodoptera exigua*, and *T. ni* (7); *Lacanobia* (*Mamestra*) *suasa*, *Noctua pronuba*, and *Autographa* (*Plusia*) *gamma* (27); *Agrotis exclamationis* and *Lacanobia oleracea* (2); and *Euxoa scandens* (3). All of these studies looked at the virulence of *M. brassicae* NPV in these alternative hosts, but the data are difficult to compare because of the different techniques and instars used in the bioassays. However, it is interesting to note that, in agreement with this study, several of the species tested appear to be more sensitive to *M. brassicae* NPV than *M. brassicae* itself, e.g., *A. gamma* (2, 27) and, from this study, *L. thalassina*, *H. armigera*, and *T. ni* (7). *N. pronuba* and *L. oleracea* are also equally or only slightly less sensitive to *M. brassicae* NPV, whereas *A. segetum* is at least 10 times less susceptible to *M. brassicae* NPV than *M. brassicae* (2, 7, 27). There are also several different isolates of *M. brassicae* NPV to consider, although the genotypic difference between the variants is not great (6). Vlak and Gröner (27) compared the host range of two isolates of *M. brassicae* NPV, one from the Netherlands and one from Germany, the latter having the same origins as the virus used in this study. The viruses were distinct but related and showed similar infectivities and host ranges within the noctuids tested (i.e., *M. brassicae*, *L. suasa*, *N. pronuba*, *A. segetum*, and *A. gamma*).

Host range data must form an important part of baculovirus risk assessment studies, particularly in the early stages when the host range data base is still relatively small. It is of obvious importance to know whether cloning or genetic manipulation alters the host range of the virus; therefore, the host range of the parent wild-type virus must be determined. At present, the perceived risks of releasing genetically engineered viruses are, firstly, that they will kill unintended hosts and, secondly, that they will pass on their foreign genetic material to unintended recipients. Nontarget hosts could provide additional pathways for the engineered virus to move through the environment. In order to predict the likelihood of this happening, these questions must be addressed: (i) how can it be decided when enough species have been tested, and (ii) following this, how should the data obtained be interpreted? In this study we have attempted to test as wide a range of species as possible, including representatives of common beneficial insects as well as many species of Lepidoptera that are of ecological interest. Ideally, these cross-infection data could be extrapolated to predict the likelihood of epizootics of *M. brassicae* NPV occurring in susceptible nontarget species after release of the virus into the wild. However, the mechanisms determining the epizootic potential of baculoviruses are complex. The evidence so far collected on host range and infectivity is inadequate for predicting whether or not the widespread use of, for example, *M. brassicae* NPV (wild type or engineered), would be likely to initiate injurious epizootics. It should be noted, however, that in the numerous field trials where naturally occurring baculoviruses have been used, no unintended epizootic in a nontarget host has ever been reported. It should also be borne in mind that although the relatively low concentrations of virus used in spray applications are unlikely to affect all but the most sensitive species, a virus-killed larva constitutes a much more concentrated virus reservoir. More research is needed to consider the probability and impact of such infections in nontarget hosts that would occur in the environment after release of these viruses.

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