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MINIREVIEW

Vertical Transmission of Nucleopolyhedrovirus in Insects

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A review of the literature on transmission studies of nucleopolyhedroviruses showed that low levels of viral infection were common among studies of pupae of insects but rarer in adults. Virus could be transmitted from parent to progeny and could be found in caterpillars reared from surface-decontaminated eggs. Persistent low levels of infection were observed in many of the studies considered. These could contribute to the persistence of virus in low-density populations, but the dominant source of virus among generations is probably through environmental contamination. © 1999 Academic Press

Key Words: **nuclear polyhedrosis virus; NPV; baculovirus; pupae; adults; transmission; transstadial; horizontal; vertical; persistence; DNA hybridization; PCR.**

INTRODUCTION

The least understood and yet most crucial stage in the dynamics of viral infection is persistence of virus between host generations (Beukema, 1992). Transmission processes determine the spread and the persistence of pathogens and their influence on host population dynamics. In field populations of insects, transmission of pathogens probably consists of a combination of horizontal transmission and vertical transfer (Fine, 1984). In horizontal transfer, pathogens are transmitted among individual hosts within a generation and between generations as environmental contamination, while vertical transmission occurs from parents to offspring (Andreadis, 1987).

Such details of transmission, as whether the pathogen is transferred from host to host, survives in the environment, or is passed through the life stages of the insect, could influence the effectiveness of pathogens in control programs for forest or agricultural pests. The timing, frequency, and patterns of spraying of virus as a bioinsecticide will be influenced by the spread and persistence of virus in the host population.

Research on the transmission of baculoviruses has been conducted for over 30 years. Results of transmission studies of viruses have varied. In this review I examine the studies and assess current knowledge about the transmission of nucleopolyhedroviruses. The focus is on transmission from adult to progeny (via the host) either internally or through environmental contamination. In addition, I discuss some of the issues involved in these studies.

METHODS

To determine if any patterns exist, I have surveyed the literature for studies of vertical transmission which recorded the carryover of virus from one generation to the next and also looked for evidence of a mechanism of transmission by considering studies that investigated virus in developmental stages (transstadial transmission). Results of experiments using laboratory colonies of insects were separated from those using field insects. Due to the volume of literature, I limited the review to studies involving nucleopolyhedroviruses (NPV) and chose studies containing quantified results. I have included a brief description of the experiments for clarity and have summarized results in tables for easy reference. Unless stated otherwise, detection of virus was done by mortality and microscopic examination of samples.

RESULTS

Parent to Progeny Transmission

Experiments discussed in this section involved the viral treatment of larvae from one generation and the subsequent investigation of virus infection in larval progeny.

Laboratory experiments with infected larvae. In this set of studies using laboratory colonies, the insects were infected as larvae, then adults were mated, and mortality of their progeny quantified (Table 1).

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TABLE 1

Survey of the Percentages of Infected Progeny of Experimental Adults Infected with NPV as Larvae and Uninfected Controls

Host	Instar	Control	Exposed to NPV	Reference
Mythimra separata	6th	2.0 (199)	16.5–57.1 (1128)	Neelgund and Mathad, 1978
Pseudoplusia includens	4th	0.5 (750)	0.5–3.4 (750)	Young and Yearian, 1982
	5th	1.2 (750)	1.7–5.2 (750)	
	6th	2.3 (750)	4.3–8.0 (750)	
Spodoptera littoralis	3rd	1–8 (100)	38–48 (100)	Abul-Nasr *et al.*, 1979
	5th	1–8 (100)	44–48 (100)	
Spodoptera exigua	5th	4.2 (60)	10.0–28.0 (60/group)	Smits and Vlak, 1988
Spodoptera frugiperda	3rd	0–0.3	3.6–5.3 (300/group)	Fuxa and Richter, 1991
	5th	0.2–0.8	13.8–23.1	
Spodoptera ornithogalli	4th	0 (250)	0 (250)	Young, 1990
Lymantria dispar	2nd	<2.0 (22)	<2.0 (29)	Murray and Elkinton, 1989
Lymantria dispar	2nd	0 (1440)	4.7–11.5	Shapiro and Robertson, 1987
Trichoplusia ni	1st	0 (157)	0 (947)	Vail and Hall, 1969
Mamestra brassicae	4th, 5th	0 (1021)	0.55 (3528)	Goulson and Cory, 1995

Note. Number of offspring tested in parentheses.

Neelgund and Mathad 1978 fed 6th instar *Mythimra separata* larvae doses of 10^7 and 10^8 polyhedral inclusion bodies (PIBs). Emerging adults were maintained in groups of five males and five females. Adults were mated and some egg masses were decontaminated and some were not. Hatch progeny larvae were checked for infection. Controls had 2% infection ($N = 199$). When progeny from undecontaminated eggs were examined, 52.5–57.1% (498) were infected. Decontaminated eggs gave larvae with 16.6–20.5% (570) infection. The decontamination treatment was 15% formalin for 1 h. Surface decontamination reduced but did not eliminate infection in progeny. High levels of infection in offspring led the authors to conclude that larvae get pathogens through their parents.

Treatment of *Pseudoplusia includens* larvae (Young and Yearian, 1982) at 4th, 5th, and 6th instars inoculated with 4×10^3–1.2×10^6 PIBs/mm^2 diet and mating of survivors gave levels of infection in progeny of between 0.5 and 8%.

There were four experiments using different species of *Spodoptera* and these will now be discussed.

Abul-Nasr *et al.* (1979) fed three concentrations of NPV (1.2×10^6, 1.2×10^7, and 1.2×10^8 PIBs) to 3rd and 5th instar *Spodertera littoralis.* Mortality in the progeny of the infected group was higher (38–48%) than that of the controls (1–8%).

Smits and Vlak (1988) observed that adults developing from *Spodoptera exigua* larvae infected as 5th instars with 1×10^5, 2×10^5, or 1×10^6 PIBs transmitted virus to their progeny. Ten to 28% of second generation neonates developed virus infection but there was no correlation between levels of infection and initial infection doses.

Fuxa and Richter (1991) exposed *Spodoptera fugiperda* 3rd instar larvae to 5 and 5.6×10^4 PIBs and 5th instar larvae to 3 and 3.5×10^7 PIBs. Surviving larvae were reared and mated. A random sample of eggs was collected from each treatment and insects were reared. Total percentage infection of progeny insects ranged from 3.6 to 5.3% when parental insects were exposed as 3rd instars and from 13.8 to 23.1% when 5th instars were exposed. They observed a lower rate (0.35–1.08%; $N = 5000$) of vertical transmission when eggs were sterilized but this treatment did not reduce infection to zero.

Young (1990) found that no progeny larvae of *Spodoptera ornithogalli* in the NPV-treated group died of NPV infection when 4th instars were given 10^6 PIBs/larvae.

Two papers on *Lymantria dispar* gave different results. Shapiro and Robertson (1987) fed 2nd instar larvae with doses of NPV ranging from 5×10^2 to 5×10^4 PIBs/mm^2 diet to obtain larval mortalities from <10–90%. Twenty pairs of surviving adults were mated from each treatment and progeny scored for virus. Virus-induced mortality in progeny ranged from 4.7 to 11.5%.

However, Murray and Elkinton (1989), in combined laboratory and field experiments, found that adults which survived NPV infection as 2nd instars fed a dose of 5×10^3 PIBs did not transmit virus to progeny (infection was <2%). They interpreted the small amount of virus as being due to inadvertent laboratory contamination.

Vail and Hall in 1969 fed *Trichoplusia ni* larvae 1.7×10^3–1.7×10^5 PIBs/larvae as first instars. They detected no diseased progeny.

Goulson and Cory (1995) fed *Mamestra brassicae* 4th and 5th instar larvae 10^3, 10^4, and 10^5 PIBs/larvae. Results indicated that incidence of infection in progeny from adults surviving infection was 0.55% and the authors stated that vertical transmission may occur at low levels.

In summary, for virus-treated insects, progeny were infected in 8 of 10 cases and the range of infection was 0.5 to 57.1%. When control insects are considered, 6 of 10 experiments with control adults had virus present in progeny in the range of 0.2 to 8%. Seven of 10 experi-

ments gave higher infection levels with virus-treated parents compared to control parents.

Field experiments with infected larvae. Of greater interest in terms of naturally occurring virus in field populations are experiments involving detection of virus in larvae from egg masses collected in the field. Experiments on the transmission of virus to progeny in Table 2 involved collection of field egg masses, surface decontamination, and the testing for viral infection of hatched larvae. Parents were exposed to naturally occurring NPV in the field as caterpillars. Researchers used different decontamination techniques and these are stated below.

Doane (1969) decontaminated egg masses with 0.1% sodium hypochlorite for 30 min and found viral infection in 0–0.1% of larvae. Bell *et al.* (1981) decontaminated with 10% formalin for 1 h and had 0–0.7% infection in larvae. Kukan (1996) decontaminated with 2% sodium hypochlorite for 5 min. In these experiments, detection of virus was done by DNA hybridization and infection in larvae was 2–2.5%. Rothman and Myers (unpublished) assessed the number of infected egg masses of *Malacosoma pluviale* after decontamination with 3% sodium hypochlorite and found 9% infection.

In summary, control egg masses produced infected larvae in three of three experiments (2–80%) and decontaminated egg masses produced infected larvae in five of five studies (0.1–9%). In laboratory or field insects in which control and decontaminated samples were compared, decontamination resulted in a decrease of the number of progeny with viral infection.

TABLE 2

Survey of the Percentages of Infected Larvae or Egg Masses with Infected Larvae in Offspring of Parents Exposed to Naturally Occurring Virus in the Field as Caterpillars

Host	Control	Decontaminated	Reference
% Larvae infected			
Lymantria dispar	<10–80 (1000)	0–0.1 (1000)	Doane, 1969
Lymantria dispar	2–28	0–0.7	Bell *et al.*, 1981
Malacosoma pluviale			
1993		2 (61)	Kukan, 1996
1994		2.5 (120)	
% Egg masses infected			
Malacosoma pluviale	22 (36)	9 (35)	Rothman and Myers (unpublished)

Note. Egg masses were either untreated or surface decontaminated.

Sample size in parentheses.

Mechanism of Transmission

The transfer of virus through pupae and adults was examined in two ways. Larvae were infected and then pupae and adults were examined for virus (transstadial transmission experiments) or adults were contaminated and progeny examined for virus.

Transstadial transmission. There were eight studies on transstadial transmission which used insects from laboratory colonies and four which used field insects. Data from these experiments are presented in Table 3. Cases in which no data were available are marked NA.

Virus in pupae and adults; laboratory insects. Stairs (1965) fed 5th instar larvae of *Galleria mellonella* 10^7 PIBs/larvae 8 to 10 days before pupation and examined pupae and adults for virus. While virus was detected in both pupae and adults, no results for control insects were given.

Magnoler (1974) gave 3rd instars *Lymantria dispar* 2.5×10^2–2.5×10^6 PIBs/larvae and the larvae were allowed to pupate. He found no virus in pupae.

Whitlock (1977), using *Heliothis armigera,* fed 6×10^5 PIBs/larvae to 4200 larvae of all ages. He found no NPV in pupae and adults by observation but results for control insects were not given.

Young and Yearian (1982) fed 4th, 5th, and 6th instar *Pseudoplusia includens* larvae with 2×10^3–8.6×10^5 PIBs/mm^2 diet. They examined only pupae for virus and found viral contamination in 0–0.7% of controls and in 1.5–15.2% of virus-treated pupae. Mortality increased with dosage and with instar. Sample sizes were not given.

Evans (1983) infected 5th to 6th instar larvae from a laboratory colony of *Mamestra brassicae* with doses of NPV ranging from 10^3 to 10^8 PIBs/larvae (40 per dose). He found that no adults had viral contamination and that pupae were contaminated with virus when larvae were infected as earlier instars (5–100%). Results for controls were not given.

Shapiro and Robertson (1987) fed 2nd instar *Lymantria dispar* larvae 0.1–10 PIBs/mm^2 diet surface. They expressed their results as the PIB virus yield per insect, not percentage infection. No control insects had virus (accurate to 10^4 PIBs by hemacytometer counting). Virus contamination was found in pupae (6–25%) and adults (5–44%) of both sexes.

Also using *Lymantria dispar,* Murray *et al.* (1991) starved 4th and 5th instar larvae for 48 h and then fed them doses ranging from 7 to 5×10^5 PIBs/larvae. In this experiment, DNA hybridization was used for detection of NPV. They found that some pupae were positive for virus (20%) but no adults were positive.

Fuxa and Richter (1991) gave 3rd to 5th instar *Spodoptera fugiperda* 5×10^4–3.5×10^7 PIBs/larvae. They found vertical transmission of virus to both pupae

TABLE 3

Survey of the Percentages of Pupae and Adults Contaminated with NPV of Insects Experimentally Exposed to NPV as Caterpillars in the Laboratory or Potentially Exposed to Virus under Field Conditions

Host	% Pupae with NPV		% Adults with NPV		Reference
	C	VT	C	VT	
Laboratory colonies					
Galleria mellonella ($N = 50$)	NA	45.0	NA	6.0	Stairs, 1965
Lymantria dispar	0 (146)	0 (1100)	NA	NA	Magnoler, 1974
Heliothis armigera ($N = 4200$)	NA	0	NA	0	Whitlock, 1977
Pseudoplusia includens (*N* not given)	0–0.7	1.5–15.2	NA	NA	Young and Yearian, 1982
Mamestra brassicae (40/dose)	NA	5–100	NA	0	Evans, 1983
Lymantria dispar	0 (100)	6–25 (950)	0 (160)	5–44 (1034)	Shapiro and Robertson, 1987
Lymantria dispar	0 (22)	20 (71)	0 (17)	0 (44)	Murray *et al.*, 1991
Spodoptera frugiperda (300/group)	<0.05	0.8–2	0.2–0.5	8–10	Fuxa and Richter, 1991
Field insects: field collected and reared in the laboratory					
Lymantria dispar (*N* not given)	NA	NA	60	100	Doane, 1967
Malacosoma neustria	0	0	0	0	Magnoler, 1975
	(C total = 108)		(VT total = 633)		
Malacosoma pluviale	6 (50)	3 (90)	0 (40)	0 (65)	Kukan, 1996
Malacosoma disstria	0.5 (199)	NA	0 (143)	NA	Kukan, 1996

Note. C, control insects; VT, virus-inoculated insects; sample size *N* in parentheses.

(controls <0.05% and virus treated 0.8–2%) and adults (controls 0.2–0.5% and virus treated 8–10%).

In summary, six of eight studies of virus-treated larvae produced pupae with virus in amounts of 0.8–100% while two of five experiments involving infection in control pupae had virus present in amounts of 0.05–0.7% of insects. Three of six studies of virus-treated larvae produced virus in adults ranging from 5 to 44%. In three studies of control adults, one study had virus contamination at 0.2–0.5%. Viral contamination in virus-treated insects was greater than in controls in six of eight cases.

Virus in pupae and adults; field insects. These experiments were done using field-collected pupae or larvae reared from field-collected decontaminated egg masses. Larvae were infected and pupae and adults examined for viral contamination.

Doane (1967) decontaminated egg masses of *Lymantria dispar* using 0.1% sodium hypochlorite for 30 min. Three larval instars, 1st, 2nd, and 3rd, were bioassayed on doses ranging from 69 to 0.006 PIBs/mm^2 diet. He found that 100% of viral-treated larvae gave contaminated adults and 60% of untreated insects were also contaminated.

Magnoler (1975) decontaminated field-collected egg masses of *Malacosoma neustria* with 10% formalin for 1 h. He gave 3rd to 4th instar larvae doses of NPV ranging from 3 to 3×10^5 PIBs/larvae. No virus in pupae or adults was detected.

Kukan (1996) reared *Malacosoma pluviale* larvae from surface-decontaminated egg masses from an area whose populations were starting to rise after 4–5 years of decline. Larvae of 5th instar were given 3.6×10^4 and 3.6×10^6 PIBs/larvae and insects allowed to pupate and some left for adult emergence. A PCR assay was used to screen for virus. Primers for the assay had been prepared by sequencing a fragment of DNA from *Malacosoma pluviale* NPV (MpNPV). Testing of the PCR assay indicated that the primers gave a sensitivity of between 1 and 5 fg with pure MpNPV DNA (Kukan, 1996). Virus was detected in pupae (3–6%) but not in adults.

Malacosoma disstria pupae were collected in the field from an area whose population was declining after an outbreak (Kukan, 1996). Surface decontamination of the outside of the pupae was performed and samples were checked for NPV using DNA hybridization. The sensitivity of the DNA dot-blot hybridization assay was between 0.1 and 1 ng pure MpNPV DNA. As with *Malacosoma pluviale*, there were low levels of NPV in pupae (0.5%) and none in adults.

In summary, field experiments were done less frequently than those using laboratory colonies of insects. One of two virus-treated larvae experiments gave results of virus present in the pupae at 3%. Of three studies on transfer to pupae, two control groups of pupae had virus present in amounts of 0.5–6%. One of four experiments with adults from field insects indicated that virus was present in controls and virus-treated samples at 60 and 100%. The results of the study by Doane were very different from the other three studies which had no virus in adults. In these experiments virus-treated insects had higher infection than controls in only one of five cases.

Contaminated adults. In these experiments, adults were contaminated with virus and progeny examined for virus infection (Table 4).

TABLE 4

Survey of the Percentages of Infected Progeny of Insects Contaminated with NPV as Adults and Uninfected Controls

Host	Control	Exposed to NPV	Reference
Colias eurytheme	0 (150)	8–22 (150)	Martignoni and Milstead, 1962
Trichoplusia ni	0–4.0 (20/plant)	16.0–38.0 (20/plant)	Elmore and Howland, 1964
	10–20 plants per treatment		
Lymantria dispar	2.5–5.0 (540–720)	35.0 (340)	Murray and Elkinton, 1990
Heliothis zea	0–7.1 (91)	0.4–94.3 (176)	Hamm and Young, 1974

Note. Number of offspring tested in parentheses.

In a laboratory experiment in 1962, Martignoni and Milstead applied 0.7–0.9 mg of a paste made from NPV (7.87×10^6 PIBs/mg) to the genital armature of 12 adult female *Colias eurytheme*. Egg samples were taken at 2, 4, and 6 days after application and emerged larvae scored for viral mortality. Progeny infection was 0% in controls and ranged from 8 to 22% in viral-treated larvae.

In 1964, Elmore and Howland used laboratory-reared *Trichoplusia ni* in a similar experiment. Twenty pairs of adults were sprayed with a solution of NPV ($31–47 \times 10^9$ PIBs/g) and released into each of 12 cages containing about 88 cabbage plants per cage. Diseased larvae were counted on 5 to 10 plants per cage. Progeny infection was from 0 to 4% in controls and from 16 to 38% with viral treatment.

Murray and Elkinton (1990) allowed laboratory-reared adult *Lymantria dispar* to walk across untreated and virus-treated (drenched with 5×10^8 PIBs/ml) bolts of oak. They found that larvae hatched from egg masses laid on virus-treated bolts had higher mortality from NPV, 35% infected progeny with viral exposure and 2.5–5.0% in controls. They concluded that egg masses can become contaminated with NPV during oviposition on contaminated surfaces.

Ham and Young (1974) fed adult *Heliothis zea* with NPV (10^6–10^8 PIBs per insect). Infection was monitored in progeny in this experiment with laboratory colonies of insects. The authors stated that surface sterilization of the eggs eliminated the transmission. No infection was found in paraffin sections of the adults but polyhedra were visible in the gut lumen and near the tip of the abdomen. Male adults fed PIBs were found to transmit virus to progeny when mated with untreated females. When untreated males were mated first with treated females and then with untreated females, the second females were sufficiently contaminated to transmit virus to some of their progeny. Progeny infection ranged from 0 to 7.1% in controls and from 0.4 to 94.3% with virus treatment.

In summary, three of the studies described relied on environmental contamination of insects and tested whether this virus passed to the progeny. All three studies showed that viral treatment gave progeny with virus (8–38%) and two of three studies had some virus in controls (2.5–5%). In the study in which adults were fed virus, virus was found in progeny (0.4–94.3%) and some in controls (0.06–7.1%). All studies showed an increase in infected larvae with virus treatment over control larvae.

DISCUSSION

Careful consideration of the work previously done in this area is necessary to assess what is known about vertical transmission. Many of the experiments described were done with objectives other than generating data on transmission, but these experiments did quantify data in this area. There are some major problems with the quality of the data available. The studies considered had many differences. This makes it difficult to draw general conclusions. There is some argument for accepting only experiments which had good experimental design, appropriate statistical analysis, use of control insects, and a clear statement that care was taken to rule out contamination. However, the purpose of this review was to assess current knowledge and discuss the issues in doing experiments on vertical transmission. I did not eliminate experiments without control insects since I was looking for presence or absence of virus and even though controls were not included it was interesting to see if virus was detected. Part of the problem in past experiments is the lack of sensitive techniques for detection of virus. I tried to look for patterns in results with the purpose of focusing on what needs to be done in future research.

Parent to Progeny Transmission

Studies summarized in Tables 1 and 4 determined the occurrence of virus in offspring of control insects in comparison to that of parents which were experimentally infected with virus. Overall, in Table 1 investigators found that insects surviving viral treatment as larvae produced detectable virus in progeny. Infection levels in offspring appeared higher if viral treatments were performed at later developmental stages. It is also interesting that the control groups of these experiments had infected progeny although virus levels were much lower. Either laboratory contamination occurs or

the incidence of infection in progeny supports the possibility of vertical transmission in some host species.

In Table 4, for three of the experiments, adults contaminated externally produced infected progeny. Unfortunately, there was only one experiment in which adults were fed PIBs and the conclusion was drawn that virus was transmitted to progeny. One can not draw conclusions on transmission through different life stages until more experiments are done.

In general, if insects were exposed to virus then there was a good chance that their progeny showed some infection and the levels of infection would be higher than in control insects.

Surface Decontamination

One way to avoid environmental contamination is by surface decontamination, which should remove any surface NPV present. This is especially important in studies of field insects in which environmental contamination or transgenerational horizontal transmission is nearly impossible to distinguish from vertical transmission. There were four studies in which field-collected egg masses were surface decontaminated and the progeny examined for incidence of virus (Table 2). The levels of infection were between 0 and 9.0% with decontamination and much higher in controls, which were not decontaminated. So, while decontamination reduced the incidence of virus it did not eliminate it. Whether this virus was the result of incomplete decontamination or due to vertical transmission has not been determined.

Transstadial Transmission

Studies of viral infection in pupae and adults (Table 3), in laboratory-maintained insects, indicate that in three-quarters of the experiments (six of eight studies) of virus contamination in pupae and at least half the studies of virus contamination in adults (three of six studies), virus could be transferred to pupae and to adults. In control insects, two of five studies had virus present.

In field insects generally, virus was rare and more common in pupae than in adults. Considering the results of control samples, in both laboratory colonies and field-collected insects, virus could be detected in pupae and less commonly in adults. Viral treatment of laboratory colonies of insects seemed to result in higher levels of viral contamination than treatment of insects collected recently from field populations. It appeared that virus could be found in some species of pupae. However, virus was rarer in adults, which means either it is not present or it is undetectable by the methods being used.

Almost all of the examples cited identified virus in pupae microscopically. This would require large quantities of virus to be visible and it is uncertain whether these pupae would survive to adults. Studies that stop at the pupal stage rather than rearing through to adults are of questionable value. Contamination of pupae and adults could lead to environmental contamination and progeny infection.

Persistent Low Levels of Infection

Levels of infection in control pupae (laboratory colonies, Table 3) range from 0 to 0.7%. One study with virus in adults had control levels of 0.2–0.5%. In control experiments in Table 4, progeny from insects contaminated as adults had levels of infection in the 0–7.1% range and progeny from insects infected as control larvae (Table 1) also had infection levels in the range 0–8%. Infection in progeny reared from decontaminated egg masses (Table 2) gave detectable virus of 0–9%. Low levels of infection tend to be dismissed as being due to contamination, but the fact that they appear in so many independent studies suggests another explanation. These results suggest that in the species that have been examined a low level of NPV infection persisted which may have resulted by vertical transmission.

How much virus is necessary to cause an infection in an offspring? It may take only one PIB or one virion to cause infection. Viral infection could be maintained at a low level in the population if only a small number of infected individuals exist. Selection for an increased rate of vertical transmission in *Spodoptera frugiperda* (Fuxa and Richter, 1991) suggests a genetic basis for vertical transmission.

Detection Techniques

The majority of the studies reviewed measured infection by mortality and microscopic examination of cadavers for occlusion bodies (PIBs). The lowest level of detection microscopically is about 1×10^6 PIBs (Kaupp and Ebling, 1993). DNA dot-blot hybridization can detect between 0.1 and 1 ng viral DNA, which would be the equivalent of between 20 and 5000 PIBs (Ward *et al.*, 1987; Kaupp and Ebling, 1993; Keating *et al.*, 1989; Kukan and Myers, 1995). Using the PCR assay for virus detection increases the sensitivity to between 1 and 5 fg of pure MpNPV DNA. Therefore, if virus is present at these low levels it should still be detected. These last two techniques can detect virus before polyhedra are formed. However, even the PCR assay did not find NPV in adults of *Malacosoma pluviale*, although it was present in pupae. In contrast to dot-blot methods, it was necessary to extract DNA from samples for use in PCR assay. This may result in the loss of some DNA and viral levels may be below detection. As molecular techniques advance and become more sensi-

tive it will be important to reexamine adults and pupae for viral contamination.

Avoiding Infection or Death

When larvae are infected with virus, some pupae and adults survive. How do the pupae and adults avoid infection and death? Stairs (1965) proposed that the development of disease could be interrupted by metamorphosis. This maturation immunity or hormonal control of virus replication as a result of changes in cell metabolism has been supported by others (Evans, 1983; Whitlock, 1977) but the mechanism is not understood. Recent work (O'Reilly and Miller, 1989) has identified a viral gene which may influence host hormonal levels. Increased knowledge of the interplay of viral genes and host hormones will contribute to our understanding of maturation immunity.

Another method of avoiding infection may be linked to developmental resistance or the increased resistance of larvae to baculovirus infection with age (Stairs, 1965; Whitlock, 1977; Evans, 1983; Engelhard and Volkman, 1995). Resistance may be attributed in part to the sloughing off of infected midgut cells and the ability to clear infection in midgut epithelium by moulting (Engelhard and Volkman, 1995). These processes may decrease the chances of virus becoming established as a systemic infection which would carry through to later life stages. Many insects may avoid infection in this way while others may become infected at sublethal levels and pass the infection to their progeny.

Sublethal Effects

Many of the studies in this review considered and noted sublethal effects (Young and Yearian, 1982; Abul-Nasr *et al.*, 1979; Shapiro and Robertson, 1987; Magnoler, 1974; Murray *et al.*, 1991; Young, 1990). Sublethal viral disease can have debilitating effects on the host population. These include slower development rates, lower pupae and adult weights, and reduced reproductive capacity (Rothman and Myers, 1996). Sublethal infection may play an important role in vertical transmission by contamination of either the egg surface (transovum) or within the eggs (transovarian) (Goulson and Cory, 1995). These effects should not be overlooked.

Latency

Latent infection may also play a role in disease transmission. A common definition for a latent infection is an infection that does not produce visible signs of a disease but may be transmitted to another host (Hale and Margham, 1988). Fuxa *et al.* (1992) state that latency requires the pathogen to be in a noninfective, nonreproductive phase in the host without causing disease and, under stress, the pathogen is transformed to an infective, reproductive phase. Two main mechanisms for latent infections have been suggested. Howard (1986) proposed that the genetic material could integrate into the host cell genome as is found with hepatitis B virus. The other alternative is that the virus may be maintained as independent viral genetic material in the host cell nucleus as with herpes simplex (Mellerik and Fraser, 1987). Cattaneo *et al.* (1988), working with measles virus, suggested that the virus was a persistent infection, that is, one which remains as a low level of infection with the expression of viral genes. Hughes *et al.* (1993) described a latent infection of NPV in *Mamestra brassicae* which was passed from one generation to the next without disease symptoms. Latent virus sequences were not detected by DNA hybridization but were detected by PCR of fat body samples. Latent infection may provide a way for virus to persist in low-density populations. Hughes *et al.* (1997) using the same laboratory colony as in 1993, performed an experiment that provided indirect evidence for a persistent infection in *Mamestra brassicae* which allowed the continuous expression of viral proteins at a low level in fat-body cells. As Fuxa *et al.* (1992) stated it may be advantageous to the NPV to be in a noninfectious state in adults which are involved in dispersal and reproduction.

Implications of Vertical Transmission and Further Research

Much like smallpox, tuberculosis, and diptheria, nucloepolyhedoviruses are transmitted passively. Ewald (1994) describes their transmission by a "sit and wait hypothesis." Susceptible individuals must come in contact with these virulent pathogens and infection success depends on the pathogens' ability to survive in the external environment. Horizontal transmission of virus both within a generation and between generations as environmental contamination can dominate when host population densities are high. However, at low host population densities other means of viral transmission may be important.

Studies on vertical transmission are necessary to understand the ecology and epidemiology of baculoviruses. Vertical transmission does not require a minimum threshold density, so intuitively it could be a significant factor in low-density populations but to date prevalence information is lacking. Vertical transmission enhances virus dispersal, so studies should be done on vertical transmission in migrating and dispersing species. It is important to understand vertical transmission of NPV in a host species to make accurate predictions of the impact of introducing a pathogen into a wild population. Equally important is the impact of vertical transmission on interpreting laboratory research with insects, especially if infections are latent or at persistent low levels. Our understanding of viru-

lence and specificity depends on a clear picture of the cellular infection pathway but it also depends on understanding latency and persistence in host cells. Viral infectivity, host specificity, virus availability, and host density all affect the ecology of NPV. Research is needed to improve the quantitative data base on all these aspects of virus ecology.

CONCLUSIONS

Studies indicate that virus may be maintained and transmitted from larvae to pupae at low levels but is rarely detected in adults. However, virus transmission from parent to progeny can occur and virus sometimes even occurs in caterpillars hatching from surface-decontaminated egg masses. Persistent low levels of infection were observed in many of the studies considered. Sublethal and latent infections may play an important role in persistence of virus. Low levels of infection of insects associated with vertical transmission can allow the pathogen to persist in relatively low host densities and maintain a source of inoculum for epizootics at high host densities.

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