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Review

Soil as an Environmental Reservoir for Baculoviruses: Persistence, Dispersal and Role in Pest Control

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Abstract: Baculoviruses are DNA viruses that can naturally control insects and form the basis for a range of biological insecticides. These viruses are occluded in a crystalline protein matrix, the occlusion body (OB), which favors their persistence in the environment until consumed by a susceptible insect. This review presents evidence that baculoviruses are perfectly adapted to persist in soil. In support of this assertion, detailed evidence is presented concerning the stability of virus OBs in soil, the mechanisms and processes that affect OB transport to soil, OB persistence and movement through the soil layers, and the transport of the virus back to the host's foodplant for subsequent transmission. The soil reservoir interacts with the host insect, the foodplant and the biotic and abiotic factors in the environment, all of which influence the function of the soil OB reservoir. Agricultural practices that conserve the soil reservoir can result in improved pest control. The soil is also an overlooked source of genetic diversity for the design of baculovirus-based insecticides. Finally, the principal issues that remain to be elucidated are highlighted, particularly with reference to those that affect the soil–baculovirus relationship and the impact of the soil OB reservoir on insect populations.

Keywords: nucleopolyhedrovirus; granulovirus; occlusion body; stability; translocation; Lepidoptera; population

List of Abbreviations and Virus Names Mentioned in this Review

	Genus: *Alphabaculovirus*
AcMNPV	Autographa californica multiple nucleopolyhedrovirus
AgMNPV	Anticarsia gemmatalis multiple nucleopolyhedrovirus
ArviNPV	Arctia virginalis nucleopolyhedrovirus *
CfMNPV	Choristoneura fumiferana multiple nucleopolyhedrovirus
ChinNPV	Chrysodeixis includens nucleopolyhedrovirus (1)
HearNPV	Helicoverpa armigera nucleopolyhedrovirus (2)
HycuNPV	Hyphantria cunea nucleopolyhedrovirus *
HypuNPV	Hyblaea puera nucelopolyhedrovirus *
LaflNPV	Lambdina fiscellaria lugubrosa nucleopolyhedrovirus *
LdMNPV	Lymantria dispar multiple nucleopolyhedrovirus
MbMNPV	Mamestra brassicae multiple nucleopolyhedrovirus
MyseNPV	Mythimna separata nucleopolyhedrovirus
OpSNPV	Orgyia pseudotsugata single nucleopolyhedrovirus
PeriNPV	Pericallia ricini nucleopolyhedrovirus *
SeMNPV	Spodoptera exigua multiple nucleopolyhedrovirus
SfMNPV	Spodoptera frugiperda multiple nucleopolyhedrovirus
TnSNPV	Trichoplusia ni single nucleopolyhedrovirus
WisiNPV	Wiseana signata nucleopolyhedrovirus
	Genus: *Betabaculovirus*
PbGV	Pieris brassicae granulovirus *
PrGV	Pieris rapae granulovirus (3)
SpfrGV	Spodoptera frugiperda granulovirus

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Citation: Williams, T. Soil as an Environmental Reservoir for Baculoviruses: Persistence, Dispersal and Role in Pest Control. *Soil Syst.* **2023**, *7*, 29. https://doi.org/10.3390/soilsystems7010029

Academic Editor: Adriano Sofo

Received: 15 February 2023
Revised: 10 March 2023
Accepted: 20 March 2023
Published: 22 March 2023

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Genus: *Gammabaculovirus*	
GiheNPV	Gilpinia hercyniae nucleopolyhedrovirus
NeseNPV	Neodiprion sertifer nucleopolyhedrovirus

(1) Previously named Pseudoplusia includens single nucleopolyhedrovirus (PsinSNPV). (2) Previously named Heliothis zea single nucleopolyhedrovirus (HzSNPV). (3) Also named Artogeia rapae granulovirus. * not a virus species or tentative species recognized by the ICTV [1].

1. Introduction

The soil habitat harbors an enormous diversity of viruses that are pathogenic to plants, animals and the soil microbiota [2]. Viruses are also extremely abundant in soil, with upper estimates in the range 10^7–10^9 particles/g for agricultural soils and close to 10^{10} particles/g for forest and wetland soils [3]. Despite the complexity of the soil ecosystem and its critical importance to terrestrial biodiversity and human agriculture, the role of viruses in soil ecosystem services remain poorly understood.

Baculoviruses (*Baculoviridae*) are double-stranded DNA viruses with a circular genome of 80–180 Kb [1]. This review focuses on the nucleopolyhedroviruses (genus *Alphabaculovirus*) and granuloviruses (*Betabaculovirus*) that infect Lepidoptera, and to a lesser degree the nucleopolyhedroviruses (*Gammabaculovirus*) that infect sawflies (Hymenoptera). These viruses are important natural enemies of the larval (caterpillar) stages of their hosts and can regulate high-density insect populations by producing epizootics of lethal disease [4,5]. They also have considerable commercial value as natural agents of pest control and form the active ingredient for various biological insecticides [6].

This review will present evidence that baculoviruses are perfectly adapted to persist in soil. It is arguable that the soil is their primary habitat outside the host, as their persistence on the foodplant of the host insect is brief by comparison to that in soil. In support of this assertion, I will briefly describe the physical structure of the transmissible stages of these viruses and then present the evidence related to their stability in soil and the mechanisms and processes that affect virus transport to, persistence within, movement through and exit from the soil environment. I include the leaf litter layer in this analysis given its intimate association with the soil ecosystem. On occasions, baculoviruses are applied directly to the soil to control soil-dwelling pests [7–9], but this is not the focus of this review, which considers how the soil reservoir interacts with the host insect, the insect's foodplant and other biotic and abiotic factors that influence the function of the soil virus reservoir. Finally, I examine how the soil reservoir can be used to improve pest control and highlight some of the many issues that remain to be elucidated concerning the soil–baculovirus relationship and the role of the soil reservoir in virus persistence. Much of the literature dates from several decades ago but has not previously been brought together in a comprehensive, detailed and systematic review.

2. Baculovirus Structure and Infection Cycle

Baculoviruses comprise rod-shaped nucleocapsids that are enveloped singly or in groups to form occlusion-derived virions (ODVs) (Figure 1). The ODVs are occluded individually (granuloviruses) or in groups (nucleopolyhedroviruses) in a crystalline matrix of protein to from the occlusion body (OB). This matrix mainly consists of polyhedrin in nucleopolyhedroviruses or granulin in the granuloviruses. OBs have an exterior layer of polyhedron envelope protein that provides a smooth, sealed outer surface [10]. This results in OBs with a diameter of approximately 0.4–0.6 µm for granuloviruses or 1–3 µm for the nucleopolyhedroviruses.

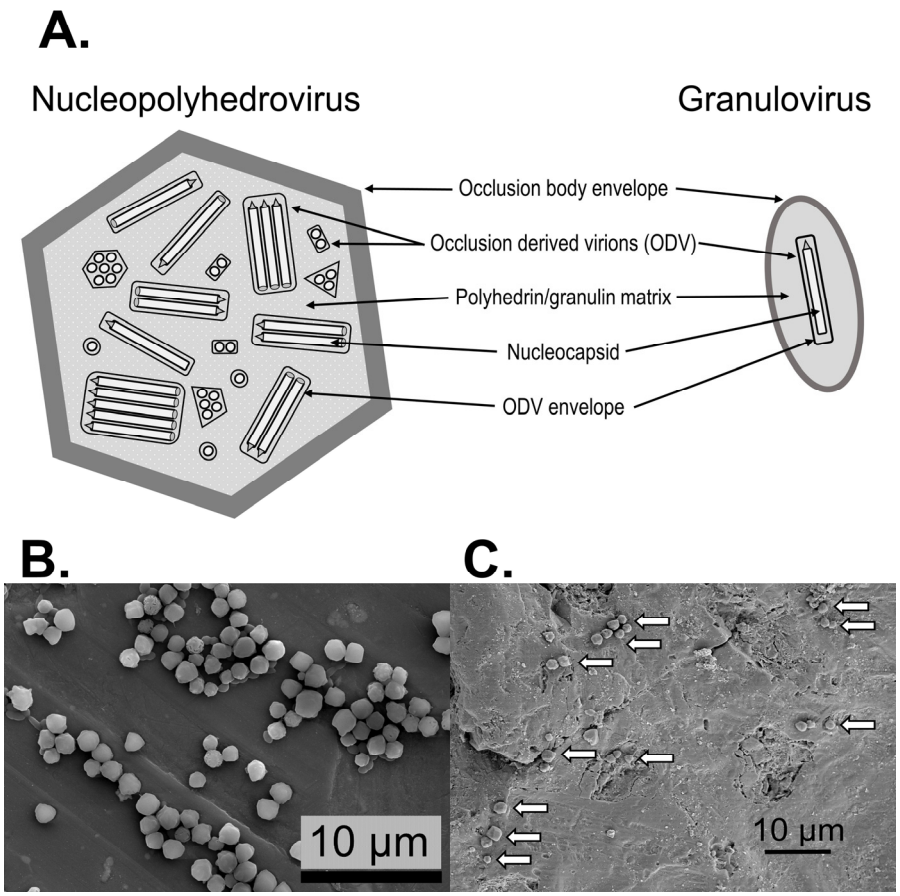

Figure 1. Morphology of baculovirus occlusion bodies (OBs). (**A**) Schematic diagram of the structure of nucleopolyhedrovirus and granulovirus OBs that infect Lepidoptera. The multi-nucleocapsid type of nucleopolyhedrovirus is shown. (**B**) Scanning electron photomicrograph of purified nucleopolyhedrovirus OBs. (**C**) Scanning electron photomicrograph of nucleopolyhedrovirus OBs adhering to a sand particle (indicated by arrowheads). Scale bars indicate 10 µm.

Transmission occurs when a susceptible larva consumes foliage contaminated with OBs. The OBs are pH-sensitive and dissolve in the alkaline midgut of the insect to release ODVs that infect midgut cells. The infection subsequently spreads to most of the cells in the insect. Later in the infection, large numbers of OB progeny are produced. Infected larvae may show increased mobility and climbing activity, a virus-induced behavior [11,12]. Larvae often die on the upper parts of plants and are liquefied by viral enzymes that favor the release of OBs from the cadaver that contaminate foliage for the following cycle of transmission [13,14]. In the case of deltabaculoviruses of sawflies, infection is limited to the intestine and transmission occurs through the release of OB-contaminated feces [15].

In the absence of the OB matrix, non-occluded ODVs are inactivated very rapidly in the environment. For example, a 450-fold reduction in the activity of AcMNPV ODVs was recorded in temperate soil over a 3-day period [16]. Consequently, virus transmission depends critically on the ability of OBs to protect the ODVs, the genomes they carry and the ODV-associated proteins that are essential for primary infection of midgut cells, such as the per os infection factors [17]. These viruses are exposed to solar ultraviolet (UV) radiation [18], elevated temperatures [19] and phylloplane chemistry [20–22] that can limit their persistence on the host's foodplant. The biological and physico-chemical conditions and processes that the viruses face in the soil ecosystem are quite different from those of the foodplant and will be considered in terms of their consequences for virus survival and dispersal.

3. Detection and Quantification of OBs in Soil

Methods for the detection and quantification of OBs in soil can be classified into three main groups: (i) direct counting, (ii) insect bioassay and (iii) molecular methods involving the polymerase chain reaction (PCR).

3.1. Direct Counting

A method of direct counting of MbMNPV OBs extracted from soil was developed in 1980 [23]. For this, soil samples were subjected to a multi-step extraction procedure involving treatment with a desorbent (0.1% sodium dodecyl sulfate, SDS), followed by sonication and several centrifugation steps. The final OB suspension was smeared onto glass slides, dried and stained for microscopic observation and OB counting. The extraction procedure was up to 48–55% efficient, although recovery was more variable (25–38% efficiency) in other samples [24]. The same method had an average efficiency of 26% for the extraction of NeseNPV OBs from pine forest soils [25]. The technique was time-consuming, and the extraction efficiency was lower in dry soil, or in soils with a high clay content [24]. In comparison, SDS-based extraction was 28% efficient for NeseNPV [26] and AgMNPV [27], 15–31% efficient for PbGV depending on soil type [28], and 25% efficient for a recombinant AcMNPV [29]. A simple suspension of soil in water followed by filtration through muslin resulted in 28% recovery of OpSNPV OBs [30].

3.2. Insect Bioassay

Early studies reporting changes in the activity of OBs in soil often used percentages of insect mortality or percentage of original activity remaining (OAR values) as indicators of soil OB populations [31–33]. Mortality values can be misleading as there is not a simple linear relationship between inoculum dose and insect mortality. The OAR metric can also lead to the calculation of erroneous half-life values [34]. Many of the subsequent studies recognized the importance of calibrating the OB concentration–mortality relationship to accurately estimate OB populations in soil.

The insect bioassay can be a highly sensitive method of soil OB quantification and has three advantages over alternative methods. First, it only detects viable OBs, i.e., the fraction of the OB population that is capable of transmission. Second, it produces numerous virus isolates that can be subjected to characterization studies. Third, it is rapid and relatively easy to perform as long as a laboratory colony of the host insect is available. Indeed, simply placing susceptible larvae on the surface of contaminated soil for a brief period followed by rearing on diet can be sufficient to detect soil OBs [35]. Treating leaves or leaf disks with soil slurry and offering these to larvae is also effective for the detection of OBs [36,37].

The simplicity of the bioassay technique is particularly evident if the initial OB extraction steps are omitted and mixtures of dry sieved soil + insect diet are prepared and used to feed insects that are subsequently monitored for lethal virus disease. Such preparations have usually involved 10% soil + 90% diet, although higher proportions of soil have been tested [38]. Differentiating virus deaths from those of other pathogens may require microscopic examination of Giemsa-stained smears of larval tissues. As soil samples can contain several different viruses that are capable of infecting the experimental larvae [39], virus-killed insects can be checked using restriction endonucleases or PCR amplification of specific virus genes to determine the identity of the infecting pathogen [40–42].

The sensitivity of bioassay techniques largely depends on the minimum lethal concentration of OBs required to initiate infection, which varies markedly among host–virus pathosystems (Table 1). For example, *Spodoptera exigua* is highly susceptible to its homologous nucleopolyhedrovirus (SeMNPV), whereas *Spodoptera frugiperda* is notably more resistant to SfMNPV. In this respect, all studies mentioned in Table 1 have used first or second instars which are the most susceptible stages to infection, except for an early study on HycuNPV in Japan [43]. One issue that can arise when using the insect bioassay is that early instars may be less able or less willing to consume large soil particles, such as can

occur in sandy soils, so the use of fine sieves or grinding steps may be necessary before preparing the soil + diet mixtures [44].

Table 1. Studies on the quantification of baculovirus occlusion bodies (OBs) in soil using insect bioassay techniques.

Virus	Insect Instar Tested	Inoculation Method	Lower Threshold of Detection (OB/g Soil)	Reference
AcMNPV	first	Soil extraction + diet surface contamination	30	[29]
AgMNPV	first	Soil extraction + leaf disk contamination	16–318	[44]
HearNPV	first	Soil + diet mixture	26–147 (1)	[38]
HycuNPV	fifth	Soil + diet mixture	1.8×10^7 (2)	[43]
SeMNPV	first	Soil + diet mixture	43 (3)	[45]
SfMNPV	first	Soil suspension on leaf disk	<10–4×10^4 (4)	[46]
SfMNPV	first	Soil + diet mixture	2×10^4	[42]
SfMNPV	second	Soil + diet mixture	~5×10^3	[47]
SfMNPV	second	Soil + diet mixture	1×10^4 (5)	[48]

(1) Threshold sensitivity was 26 OB/g in 25% soil mixture and 147 OB/g in 10% mixture. (2) Lowest concentration tested. (3) Study performed on a volcanic substrate sampled from greenhouses in Spain. (4) Threshold depended on soil type; 0.05% triton X-100 used as a wetting agent. (5) Artificial soil tested in a laboratory.

3.3. PCR Amplification

The unique sensitivity of PCR techniques has been applied to the detection of soil OBs in several ecosystems. Initially, phenol extraction and a magnetic capture-hybridization technique were compared for the extraction of AgMNPV DNA from samples of OBs in soil [49]. Using primers targeted at the *polh* gene, phenol extraction resulted in amplification from as little as 4×10^2 OB/g soil, whereas the magnetic bead technique was much more sensitive (4–40 OB/g). The technique was subsequently validated on soil from soybean plots treated with 10^{11} OB/ha, in which virus DNA was amplified from samples taken over six months [49].

To examine the persistence of recombinant CfMNPV OBs in forest soil, DNA was extracted using sodium pyrophosphate and proteinase K treatment followed by sonication and a Sephadex G75 column. PCR amplification of *p10* and a recombinant gene was achieved from samples of 7.4×10^3–2.1×10^4 OBs in 0.5 g samples of leaf litter [50]. The study was then extended to amplify 10^2–10^3 OB/0.5 g of leaf litter and 10^1–10^3 OB/0.5 g in the underlying layers of acid soil (pH 4.5–4.9). The use of OB desorbents did not improve the sensitivity of the technique [51].

Two studies have tested soil-derived DNA using the quantitative PCR (qPCR) technique. Treatment of leaf litter with phosphate buffer (pH 6.5) and 0.05% Tween 20 followed by filtration and DNA extraction resulted in the amplification of a capsid protein gene from samples down to 9.4×10^4 OB/g of NeseNPV in leaf litter (value calculated from data presented by Krokene et al. [52]). Similarly, qPCR amplification of the *polh* gene was achieved using a DNA extraction kit on soil and sediment samples taken in an area of webworm (*Hyphantria* spp.) nests [53]. PCR-based approaches have also been applied to the detection and quantification of OBs and naked baculoviral DNA in aquatic systems [53–55].

The principal challenge with PCR studies on soil samples is polymerase inhibition by humic substances [56,57]. The presence of inhibitors can be overcome by using Sephadex or spin columns or by dilution of DNA samples, but with a corresponding reduction in the threshold levels of detection [49,55].

Antibody-based methods have been applied to the study of OBs in soil [46] but have now been superseded by PCR-based approaches. The ability to perform metagenomic studies on DNA viruses from the soil is advancing rapidly [58,59], but these techniques have not been applied to the study of baculoviruses to date.

4. Soil as a Virus Reservoir

The soil has been recognized as a virus reservoir since the presence of viable OBs in soil was mentioned in passing by Steinhaus over seven decades ago [60]. The importance of soil OB populations became clear in the 1960s and 1970s, particularly in studies by Jaques [19]. A detailed and quantitative approach was taken by Fuxa in a series of papers in the 1990s and 2000s [61]. It is important to note that molecular methods for the identification of viruses in the environment were not widely used until the 1990s. As a result, for most of the studies prior to this period, the identification of viruses was unconfirmed. Early studies on the isolation of viruses from soil should therefore be viewed with caution, especially with viruses that have overlapping host ranges [39].

As the primary source of OBs is the diseased host insect, the dynamics of soil OB populations involve cycles of inputs from OBs released from virus-killed insects on the foodplant or those that fall to the soil surface and the decay of viable OBs in the soil over time. Therefore, soil OB populations closely track the density of infected insects on the host's foodplant [19,62]. For example, the application of LdMNPV OBs to forest trees resulted in a ~100-fold increase in OBs in soil samples and leaf litter samples taken 22–30 days later, concurrent with an 80–90% increase in the prevalence of lethal polyhedrosis disease in the host population [63]. An average of 56% of the OBs released from MbMNPV-killed larvae of *Mamestra brassicae* were located in the soil directly beneath cabbage plants, although considerable heterogeneity in the distribution of OBs was noted [24]. As a result, fluctuations in soil OB populations are most extreme in the surface layer that receives OBs from virus-killed cadavers, from rain-washed foliage and from contaminated leaves shed from the host's foodplant (Figure 2). Deeper soil layers in contrast harbor more stable populations that can only return to the surface by mechanical disturbance, such as tillage or the action of soil-dwelling animals [61].

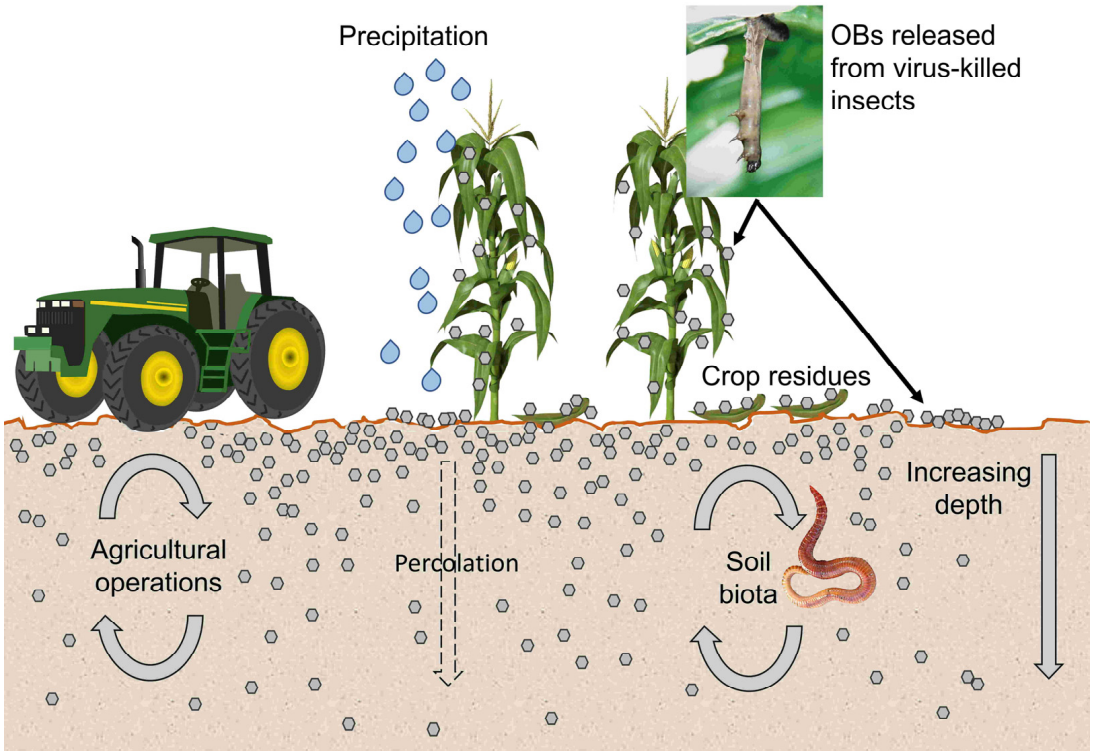

Figure 2. Mechanisms by which virus occlusion bodies (OBs) are transported from diseased insects and contaminated foodplants to the soil and within the soil reservoir. OBs released from host insects enter the soil from virus-killed cadavers, or they contaminate plant foliage and are washed into the soil

by precipitation. Contaminated plant residues also decay on the soil surface and provide an additional source of OBs. The OBs that adhere to soil particles are washed downward by percolation of rainwater (dashed arrow) or are moved through soil layers by agricultural operations such as tillage or by the soil biota (solid curved arrows). The images of plants, earthworm and tractor are available for reproduction under a creative commons license (CC BY; Microsoft Office).

Natural soils that have not been sprayed with OB suspension vary markedly in their OB content depending on fluctuations in the density of the host insect population, the time elapsed since diseased insects were present in the area and agricultural practices that disturb the soil [19,61,64]. Estimates of the density of soil OB populations come from agricultural and forest ecosystems and have been quantified by insect bioassay (Table 2). In southern Mexico, Belize and Guatemala, 18% of maize field soil samples proved positive for SfMNPV OBs [42]. In Canada, 45–68% of crucifer fields were positive for TnSNPV and up to 19% were positive for PrGV [65]. Soil densities of TnSNPV OBs varied annually by over 100-fold in *T. ni*-infested cabbage fields [66], and a stable population of TnSNPV was estimated at 1.9×10^{10}–3.4×10^{10} OB/ha in the top 1 cm of soil at nine years after the host's foodplants were last present [67].

Table 2. Estimates of natural OB populations in untreated soil.

Virus	Crop or Foodplant	OB Density Estimate (OB/g Soil) [(1)]	Reference
CfMNPV	Forest	4.2×10^4 [(2)]	[50]
HearNPV	Sorghum	1.6×10^4	[68]
HearNPV	Sorghum, fenugreek, alfalfa	1×10^3–1.6×10^4	[38]
TnSNPV	Crucifers	5×10^3–6×10^5	[19]
NeseNPV	Pine forest	10^4–10^5 [(3)]	[37,69]
NeseNPV	Pine forest	1.3×10^4–1×10^5 [(4)]	[25]
NeseNPV	Pine forest	9.4×10^4 [(2)]	[52]
SfMNPV	Maize, pastures	2×10^4 [(5)]	[46]
SfMNPV	Maize	10^4–10^5	[42]

[(1)] Estimates based on the results of insect bioassays unless otherwise indicated. [(2)] Values estimated from leaf litter by PCR amplification. [(3)] Values in OB/cm^3 in soil [37] and surface dust [69]. Samples taken 9 years after an application of virus was estimated at 7×10^2 OB/cm^3. [(4)] Values in OB/cm^3 calculated from 39 cm^3 soil core taken from upper 2 cm of soil. [(5)] Average value of 38 fields tested, maximum OB density was ~7.6×10^4 OB/g.

Perhaps the most notable result from these studies is how similar natural OB densities are in forest and agricultural habitats, despite the diversity of ecosystems studied. Most estimates are approximately 10^4 OB/g soil, and all but one fall within the range 10^3–10^5 OB/g soil (Table 2). It is also clear that estimates of the natural densities of granuloviruses in soil are conspicuous in their absence.

The majority of studies on OB quantification have focused on OBs in the top 1–7 cm of soil, although some attempts have been made to estimate the relationship between OB densities and soil depth. Decreasing densities of NeseNPV OBs down to 30 cm depth were estimated by bioassay in pine forest soils in Sweden [37]. Decreasing densities of GiheNPV OBs were detected between 0 and 13 cm depth in pine forest soil in Wales [70]. The density of OpSNPV OBs fell from ~1000 OB/cm^3 at the soil surface (0–2.5 cm depth) to less than 45 OB/cm^3 at 12.5 cm depth [30]. Following applications of TnSNPV OBs to the soil surface in Canada, the distribution of OBs was monitored for between 9 months and 4.4 years at depths of up to 22.5 cm, but the large majority of OBs remained in the upper 2.5 cm of soil, with very little or no virus deeper than 10 cm [71]. Similarly, in Japan, HycuNPV OBs were detected down to 11 cm depth but not at greater depths down to 71 cm [72]. It is clear therefore that the largest fraction of the OB population resides in the topmost few centimeters of soil.

Studies on soil OB persistence have indicated a broad range of decay rates. Using data in these studies on undisturbed soils, the half-life ($t_{1/2}$) of the OBs was calculated using the

formula $t_{1/2}$ = Ln 2/λ, where λ represents the decay rate calculated from the initial and final densities of OBs in soil samples over the period of the study (Table 3). These studies have examined OB inactivation over periods of between 1.5 and 73 months and reported decay rates equivalent to half-life values of between 11 days and 16.8 months (or more in the case of no decay detected in one study [29]) (Table 3).

Table 3. Persistence of virus occlusion bodies (OBs) in agricultural and forest soils.

Virus	Ecosystem	Duration of Study (Months)	Soil pH	Half-Life ($t_{1/2}$) [(1)]	Reference
AcMNPV	Cabbage field	12	-	No decay [(2)]	[29]
ChinNPV	Soybean field	6	-	2 mo [(3)]	[73]
HearNPV	Sorghum plots	5.4	-	2.8 mo [(4)]	[68]
HzSNPV	Cotton plots	11	7.1	2.4–2.9 mo [(5)]	[74]
MbMNPV	Field plots	12	-	2.2 mo	[24]
NeseNPV	Forest	2–3.8	-	13–18 d [(6)]	[26]
NeseNPV	Forest	72		10 mo	[37]
OpSNPV	Forest	12	-	26–39 d [(7)]	[30]
PbGV	Brown soil	12	4.8	1.5 mo	[28]
PbGV	Sandy soil	1.5	7.0	16–54 d	[28]
PbGV	Sandy loam soil	1.5	7.7	11–13 d	[28]
TnSNPV	Field plots	9	4.8–5.2	1.5–2.8 mo [(8)]	[75]
TnSNPV	Field plots	12	6.0–7.2	3.4–5.7 mo [(8)]	[75]
TnSNPV	Field plots	73	-	16.8 mo [(9)]	[66]

[(1)] Half-life values calculated in days (d) or months (mo) from data given in references. The majority of studies sampled the upper 5 cm of soil. [(2)] Recombinant AcMNPV applied. Abundance of OBs in soil did not change between 1990 and 1991. Samples taken from top 15 cm. [(3)] Value estimated from figure for undisturbed period of September 1976—March 1977. PsinSNPV was renamed to ChinNPV by ICTV. [(4)] Study performed during winter months. [(5)] Wild-type virus had a half-life of 2.4 mo at 0–2 cm depth and 2.9 mo at 2–24 cm depth. [(6)] Depending on year of study. Data estimated from figure. Upper 9 cm^3 of soil sampled. [(7)] Top 5 cm layer sampled; data from plots 2, 11 and 12 in 1974. [(8)] Estimated from data in figure. [(9)] Estimated from data in figure.

Plotting the estimated half-lives against the duration of the study, it appears that longer-term studies provide higher estimates of OB half-lives (Figure 3). The fact that there are no published studies of OB persistence of 24–48-month duration highlights the need to validate this relationship through additional long-term studies. This is particularly evident in the case of NeseNPV in forest soil, in which the same virus was estimated to have a half-life of 13–18 days in a study of 2–3.8 months [26] compared to 10 months in a 72-month study [37].

Half-life estimates may have been affected to a degree by methodological differences in the bioassay techniques used and the depth and type of soils sampled in different studies, although pH, where reported, was within the acceptable range for OB persistence (Table 3). However, the much higher estimates generated by long-term studies suggest that there may be different sub-populations of OBs in the soil: a fraction of short-lived OBs that decay at a certain rate during the first few weeks in the soil (giving half-life estimates in days) and a fraction of long-lived OBs that remain stable over periods of many months or years. As the structure of OBs can affect their stability [10], studies on the role of OB size and envelope thickness might provide insights into OB environmental persistence.

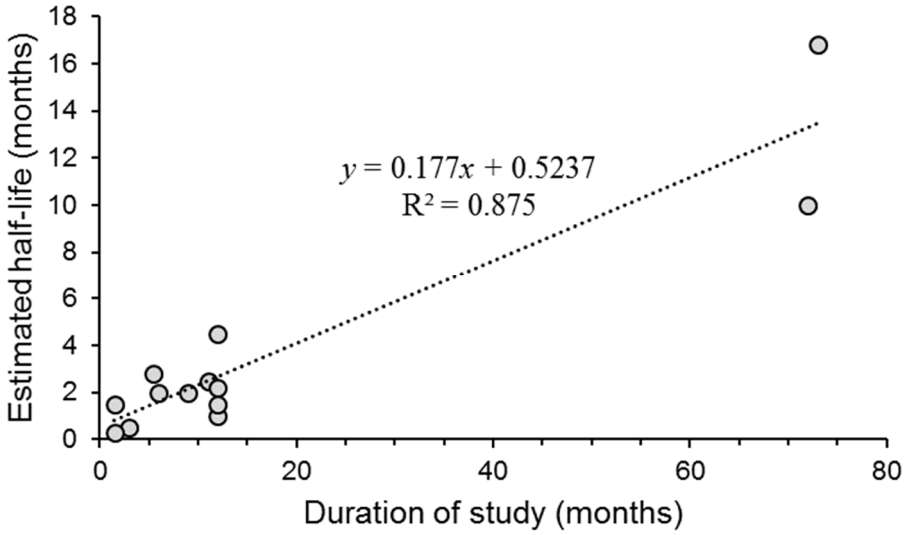

Figure 3. Correlation between estimated half-life of OBs in soil and duration of the study. Equation of the fitted line and R^2 value are given. Data from Table 3 excluding one study [29] in which OB decay was not detected.

5. Transport of OBs to the Soil

As OBs lose activity over time, the persistence of the soil OB reservoir depends on periodic replenishment from virus-killed insects, precipitation run-off from OB-contaminated foliage and senescence of OB-contaminated leaves and crop residues (Figure 2). If the host's foodplant is removed, the OB reservoir will gradually decay, but may still be detectable even after several decades [76].

Precipitation transported LdMNPV OBs released from cadavers on upper branches to the foliage of lower branches and then to the soil, although the soil was not subjected to testing [77]. Similarly, sprinkler irrigation (62.5 L/m^2) transported most of the OBs from AgMNPV-killed and ChinNPV-killed insects from soybean foliage on to the soil [78]. Consistent with this idea, soil densities of NeseNPV OBs were approximately 10-fold higher beneath the canopies of sawfly-infested pine trees compared to the soil collected in gaps between trees [25].

One study estimated an annual input of 1.2×10^{12} OB/m^2 beneath large trees during periods of high densities of *Hyphantria cunea* larvae in Japan, most of which was attributed to contaminated leaves that fell to the ground [43]. The density of ChinNPV OBs in soil increased by ~50% when soybean plants lost their foliage one month after a foliar application of the virus [73]. Similarly, disking of soybean residues into the soil post-harvest resulted in an increase in the soil population of AgMNPV OBs [79]. Following an epizootic of infection, 89% of the OpSNPV OB population was present in the leaf litter beneath infested trees, whereas 10 years later, these OBs appeared to have been incorporated into the soil ecosystem and only 30% of the OB population was present in the litter layer [30]. Similarly, the shedding of contaminated leaves and insect frass (feces) from infected individuals were identified as potential sources of OBs in leaf litter beneath lupin plants that infected larvae of *Arctia virginalis* (Erebidae) the following spring [80].

Larval frass on the soil beneath webworm (*Hyphantria* spp.) nests was found to be positive for nucleopolyhedrovirus by qPCR analysis [53]. Although infected larvae can release biologically significant quantities of a virus in their feces prior to death [81,82], the contribution of OB-contaminated frass to soil OB populations has not been quantified. Small mammals that predate larvae can also release OBs in their feces [83]. Similarly, quantities of up to 5×10^7 OBs can be dispersed over large distances in the feces of birds that feed on virus-infected larvae [84,85], or through virus-contaminated droplets of larval body fluids that land on the soil surface and nearby foliage during bird predation [86].

Birds were estimated to defecate 8.5×10^9 OBs/ha of WisiNPV annually onto pastures in New Zealand [87].

6. OB Dispersal in Soil

OBs can be transported vertically through the soil by the action of percolation or by activities that disturb the soil such as agricultural operations, livestock and soil invertebrates.

6.1. Percolation

Early studies noted that both granulovirus and nucleopolyhedrovirus OBs adhered strongly to soil particles and were not easily removed by treating columns of soil with large volumes of water [31,71]. These studies concluded that movement of OBs by leaching was unlikely to contribute significantly to their downward movement through different soil layers. Soil drying increases the strength of OB binding to soil particles [24,71].

In an experiment involving ^{32}P-labeled OBs of HearNPV, 93% of radioactivity remained in the upper 3 cm of a soil column. No radioactivity at all was detected at depths greater than 10 cm or in the water that passed through the soil column over an 18-day period [88]. The adsorption of HearNPV OBs by red and black soil was estimated by direct counting of OBs that remained in suspension 1 h after being mixed with soil. Between 2×10^5 and 2×10^6 OBs were adsorbed per milligram of soil, and this did not differ markedly between soil types despite differences in their composition and their cation exchange capacity [89]. However, the study was unreplicated, and the methodology may have affected the results as OBs can sediment quickly in water when left undisturbed. The polyhedra of cypoviruses also show a strong affinity for soil and also resist leaching [90]. The term "leaching" is mainly used to describe the movement of solutes in soil [91] and implies that the OBs become detached from soil particles and move freely in the water column during soil drainage.

The percolation of OBs adhering to the smallest soil particles, such as silt and clay particles, is a probable mechanism for the downward displacement of OBs carried by rainwater filtering through the soil. Percolation has not been examined systematically and is often referred to as "leaching" in published studies on baculoviruses. Rainfall during the winter period was implicated in reducing OB densities in the upper 2.5 cm of soil in Louisiana [79]. As OBs have a particularly high affinity for clay and silt [92], their downward movement may differ markedly among soils with different compositions. The contribution of percolation to the downward movement of OBs is also likely to be highly dependent on soil drainage characteristics.

6.2. Agricultural Operations and Livestock

The impact of agricultural operations depends on the distribution of OBs in soil layers. Activities such as harrowing, planting and cultivating did not affect the distribution of AgMNPV OBs in soils of soybean fields, possibly because these operations only disturbed the OB-rich upper layer of soil [79]. Tillage was responsible for removing ChinNPV OBs from the surface layer in soybean plots [93]. Plowing was responsible for burying the OB-rich surface layer and making outbreaks of the pasture pest *Wiseana* spp. more likely in New Zealand [87], but was positively correlated with SfMNPV disease in pasture populations of *S. frugiperda* for reasons that were unclear [94].

OBs in the uppermost layer of soil are also disseminated to other pastures by the movement of farm animals, which affected the prevalence of disease in *S. frugiperda* and *Wiseana* spp. populations [87,94]. The tires of farm machinery are likely to have a similar impact.

6.3. Soil-Dwelling Invertebrates

The earthworm *Eisenia fetida* was capable of transporting SfMNPV OBs from the surface to depths of 22–24 cm within 24 h in laboratory terraria filled with an artificial soil [48]. The gut pH of this earthworm was slightly acidic, so OBs remained viable following passage through the gut. These findings were confirmed in a field study on the

earthworm *Amynthas gracilis* that transported significant quantities of OBs to a depth of 6–8 cm over a 7-day period [47]. Other entomopathogens such as *Beauveria bassiana* and *Bacillus thuringiensis* can also be dispersed by earthworms without loss of activity [95,96].

Adults and larvae of ground beetles (Carabidae) have been implicated in the dispersal of LdMNPV OBs in forests [97]. Adults of the carabid *Harpalus rufipes* that consumed MbMNPV-infected *M. brassicae* larvae released viable OBs onto the soil surface for the following two weeks and also dispersed OBs onto the leaves of cabbages in the field [35].

7. OB Transport from Soil to Plants

Plants that grow in OB-contaminated soil acquire biologically significant quantities of OBs capable of initiating lethal infection in larvae feeding on contaminated plants [27,33,36,39]. The quantity of ChinNPV OBs applied to soil at planting was positively correlated with the prevalence of disease in larvae of *C. includens* on soybean [93]. Similarly, the prevalence of polyhedrosis disease in *Heliothis virescens* larvae (5–50% virus-induced mortality) was directly related to the quantities of OBs applied to soil (1×10^1–1×10^5 OB/g) one month prior to planting cotton in a greenhouse experiment [98]. However, the presence of viable OBs in the soil does not invariably result in disease in the host population on plants [99]. As will become apparent, OB transport to the host plant depends on a complex interaction between soil OB density and depth, soil type, weather conditions, plant height, associated vegetation and the local arthropod fauna.

7.1. Precipitation and Air Currents

The prevalence of infection of *Anticarsia gemmatalis* larvae on soybean increased rapidly after heavy rainfall transported AgMNPV OBs from the soil onto plants [27]. Precipitation was also positively correlated with SfMNPV disease in pastures, presumably due to rain splash transporting OBs onto grasses [94]. In controlled studies, OBs of HearNPV were transported by rain splash more efficiently from sandy soil than clay or silt-rich soils [100]. Amounts of OBs were negatively correlated with the soil-to-plant distance, and ranged from 56 to 226 OBs transported onto cotton plants over distances of 25–35 cm by rain splash in greenhouse experiments [100,101].

Wind currents carry OBs from soil in the form of contaminated dust. Dust was suspected in the contamination of fir trees with significant quantities of OpSNPV OBs [30]. Interestingly, the prevalence of NeseNPV disease in *N. sertifer* larvae on pine trees was correlated with distance from a dusty forest road. Samples of dust from the road surface were shown to contain ~10^4 OB/cm^3, whereas wind-blown dust carried ~75 OB/liter of air [69]. In a greenhouse experiment, OBs were transported from soil to cotton plants for a distance of up to 90 cm by air currents [61]; the quantities of OBs transported were higher from dry soil than wet soil and increased with wind speed [100]. Wind-assisted OB dispersal from clay soil was higher than observed in silt-rich or sandy soils, doubtless because clay particles are markedly smaller than those of sand [100].

Only OBs in the upper 2 cm of soil were transported to plants by simulated rain or wind currents [61]. In general, rain splash transports greater quantities of OBs to plants over shorter distances, whereas air currents carry smaller quantities of OBs but over longer distances. The transport capacity of both factors was quantified on cotton plants at 70–629 OBs for rain splash and 8–94 OB for air currents, from soil densities of 250–12,500 OB/g soil [102]. It is clear that wind and rain interact to transport OBs, and dramatic weather events such as storms may be particularly influential. Ground cover and the presence of plants adjacent to the crop are also likely to influence the transport of OBs from soil to plant [101].

7.2. Plant Height Effects

The vertical distance between the soil surface and the plant foliage influences the probability of contamination from the soil reservoir [103]. SfMNPV OBs in soil did not contaminate maize plants due to their height (distance from the soil surface), whereas

contamination of low-growing grasses was readily achieved in pastures [104]. Similarly, the lower canopy of soybean plants was more contaminated by AgMNPV OBs than the upper canopy by tillage and rainfall [27]. Indeed, rain and wind currents always transported greater numbers of OBs to the lower rather than the upper parts of cotton plants in greenhouse experiments and in the field [98,101], a process that has been quantified on different structures of cotton plants, including leaves, squares, buds and bracts [102].

7.3. Arthropods

Arthropods, particularly insects and spiders, are abundant in agricultural fields. Due to their host-specificity, applications of OB suspensions have little or no effect on arthropod populations [105]. However, a diversity of arthropods have been implicated in the dispersal of OBs in agroecosystems [64]. Predatory and scavenging arthropods are highly active and are likely to transport OBs from the soil surface to plant foliage. Both ants and termites have been mentioned as potential dispersers of OBs, but have not been subjected to empirical studies despite their abundance in agricultural settings [82,106]. In contrast, adults of the moth *Hyblaea puera* (Hyblaeidae) that emerged from pupae in OB-contaminated soil failed to carry HpNPV OBs to the plant in quantities that could infect neonate larvae [82].

8. Factors That Affect Virus Persistence in Soil

Several abiotic and biotic factors can affect the physical integrity and the biological activity of OBs in soil.

8.1. Ultraviolet Radiation

The role of solar radiation (especially the UV portion of the spectrum) on OB persistence on plants has been the subject of numerous studies. These almost invariably agree that OBs exposed to sunlight on plants are inactivated in a few hours [107,108], leading to the development of UV-protective formulations for baculovirus-based insecticides [109]. OBs on the soil surface will be similarly exposed to UV radiation, except where the crop canopy, other vegetation or crevices in the soil provide shade from incident light. Some virus strains are also more resistant to UV exposure than others [110]. However, the rate of UV-inactivation of OBs on the soil surface has not been quantified.

8.2. Temperature

Soil temperatures vary with time and depth, depending on solar radiation, air temperature and numerous interacting factors that influence the heat capacity and thermal conductivity of the soil [111]. These include the moisture content, the soil color (reflectivity) and the presence of vegetation above the soil. Soil temperature also varies seasonally, with slope, latitude and altitude.

Elevated temperatures (>60 °C) can inactivate OBs in a few minutes [112]. However, lower soil temperatures can also inactivate OBs. The soil surface of agricultural fields can reach 35–50 °C, especially in lowland tropical and subtropical regions [34,42,113–115]. Laboratory studies in this temperature range indicate that a significant reduction in OB activity is observed above 35 °C for periods of 8 to 240 h (Table 4). Both wet and dry preparations have been tested. In addition, one study indicated that OB sensitivity to UV radiation increased with increasing temperature in the range 15–45 °C [116]. As the soil is heated for several hours each day during the summer and in tropical regions, the thermal environment near the surface is likely to rapidly inactivate OBs that are not shaded, cooled by evaporation or transported deeper into the soil.

Table 4. Thermal stability of wet and dry laboratory preparations of OBs in the range 35–50 °C likely to be experienced by OBs at the soil surface.

Virus	Temperature (°C)	Duration (h)	Wet or Dry Preparation	Effect on OB Activity	Reference
PeriNPV	35	120	dry	Marked reduction	[117]
SfMNPV	40	8	wet	25% reduction	[118]
PbGV	40	240	wet	Marked reduction	[119]
SfMNPV	45	8	wet	40% reduction	[118]
HearNPV	45	24–48	dry	5-fold to 25-fold reduction	[116]
LaflNPV	45	5–200	wet	Reduced virulence	[113]
PbGV	50	120	wet	Almost eliminated	[119]
MyseNPV	50	96	dry	Almost eliminated	[114]

8.3. Soil pH

At neutral pH, OBs have a negative surface charge [120] and adhere strongly to hydrophobic surfaces [121]. The capacity of soil particles to retain negatively charged anions decreases with increasing pH as the sites of positive surface change decrease [122]. As clay and silt often carry a negative surface charge, their ability to bind OBs is also likely to vary with soil pH.

OBs are sensitive to extremes of pH at both the acid and the alkaline ends of the scale. These extremes do not occur in most soils, but soil pH does affect OB stability over time. By adjusting sterile soil pH using calcium carbonate, TnSNPV OBs at pH 4.8–5.2 had a half-life of 1.5–2.8 months, but at pH 6–7.2, the half-life was 3.4–5.7 months; i.e., OBs were inactivated more rapidly in acid soil compared to neutral soil (values estimated from data presented by Thomas et al. [75]).

Bioassays of greenhouse soil substrates of pH 7.2–8.9 resulted in fewer mixed-genotype infections by SeMNPV in more alkaline soils. Of the genotypes detected, two genotypes appeared to be better adapted to survival in alkaline soil, suggesting that survival in soil may have a genetic basis [45]. In Ontario, Canada, 50% of soil samples from crucifer fields in the pH range 5.1–6.0 were positive for PrGV OBs, but only 4% of samples were positive in soils with more alkaline pH [65]. OBs of GiheNPV were present in an acidic pine forest soil (pH 4) at depths up to 13 cm in Wales [70], and irrigation of a laboratory soil with simulated acid rain (pH 3–4) did not adversely affect the activity of OBs of NeseNPV over one year [123].

8.4. Moisture

Early studies examined the stability of OBs in aqueous suspension at different temperatures and concluded that this family of viruses were highly stable in water, especially at cool or refrigerated temperatures [19,112]. However, only one study has examined the influence of soil moisture on OB stability. In a laboratory experiment, AgMNPV OBs in an agricultural soil lost activity most quickly in moist soil (−0.3 bar matric potential) and most slowly in soil at field capacity (0 bar), whereas the inactivation rate was intermediate in dry soil (−5 bar) over a 28-month period [124].

8.5. Soil Type

Soil is extraordinarily variable in composition and in its physico-chemical characteristics. Soils are characterized by their content of sand (0.05–2.0 mm diameter), silt (0.002–0.05 mm), clay (<0.002 mm) and organic matter [3]. The silt and clay components can comprise many types of minerals with different properties. A recent analysis of soils from maize fields indicated that Leptosol (also known as Lithosol), Luvisol, Andosol, Gleysol and Vertisol soils had a higher-than-average prevalence of SfMNPV OB-positive samples. In contrast, Cambisol soils had a lower-than-average prevalence of OB-positive samples [24]. Luvisols, Andosols, Gleysols and Vertisols are characterized by high clay content, whereas Cambisols are characterized by the absence of a layer of clay, humus, salts

and oxide minerals [125]. These findings suggest that soil type may affect the persistence of OBs and possibly the prevalence of enzootic infection in the host population.

8.6. Soil Microbiota

Despite the enormous diversity of soil microorganisms, only one study has addressed the influence of the soil microbiota on baculovirus OBs. AgMNPV OBs lost activity significantly faster in an agricultural soil compared to soil that had been autoclaved, suggesting that one or more thermolabile agents were involved in OB decay [124]. However, the addition of biocidal agents to eliminate microbial contaminants did not improve the stability of SeMNPV OBs in aqueous suspension [126], so the role of the soil microbiota in OB persistence remains uncertain.

9. The Virus Reservoir as a Resource for Pest Control

By now it should be clear that the soil reservoir is a uniquely valuable resource for year-on-year pest control. The larval stage of the host insect is not present continuously for horizontal transmission, and plant foliage dies during the winter, or during the dry season in the tropics. Consequently, OBs must remain viable in the upper layers of the soil until the host's foodplant and the host become available again in the spring, or the rainy season in the tropics.

Recognizing the importance of pathogens in insect populations, Hochberg [127] adapted a population model developed to investigate forest insect population oscillations [128] by incorporating an environmental reservoir for long-lived infectious stages, such as OBs in the soil. Noting that many insect populations do not experience large cyclic fluctuations in density, Hochberg demonstrated that, in the presence of a pathogen reservoir, host populations could be regulated at low and fairly stable densities. Dampening of cyclic fluctuations was critically dependent on the rate of flow of the pathogen out of the reservoir and into a transmissible habitat, such as soil OBs that are transported on to foliage. The system was most stable at intermediate rates of flow, as particularly high or low flow rates effectively rendered the pathogen's stay in the reservoir excessively transient or excessively protracted, respectively. This model has clear applications in understanding lepidopteran–baculovirus dynamics, especially in systems in which the soil reservoir can be manipulated to improve biological pest control, such as those described in the following examples.

Agricultural practices that conserve the OB soil reservoir can contribute to a reduction in pest densities by increasing the prevalence of enzootic disease in the pest population. These practices include minimizing the use of plowing or other practices that disturb the OB-rich surface layer. Indeed, the stability of the habitat has been identified as one of the major factors affecting the success of biological control involving entomopathogens [103,129]. For example, a no-tillage regime in soybean production allowed soil populations of AgMNPV OBs to contaminate plants and provide the inoculum to trigger epizootics of disease in *A. gemmatalis* infestations for up to two years in Brazil [130]. Similarly, damage to cabbage plants by *Trichoplusia ni* larvae was 43–63% lower in 3–6-year-old plots with an established soil OB reservoir compared to recently plowed plots [66]. High rainfall was associated with natural epizootics of infection in semi-looper populations on soybean in Zimbabwe in which insecticides were not applied, likely as a result of rain-splash dispersal of OBs [131].

In pastures in New Zealand, soil-dwelling larvae of *Wiseana* spp. that are infected by WisiNPV tend to die on the soil surface, which makes OBs available for transmission to conspecifics and for OB dispersal by livestock. Epizootics of infection were estimated to result in the release of as much as 10^{14} OB/ha. A survey of pasture soils revealed that 87% of OB-positive pastures had not been plowed in the past 5 years and had accumulated a large and relatively stable soil OB reservoir [87]. A subsequent study demonstrated that the mortality of *Wiseana* spp. larvae depended on the density of OBs released from infected larvae the previous year in a delayed density-dependent manner in young pastures without

an established virus reservoir. In older pastures, the OB reservoir was large and buffered the annual fluctuations resulting from changes in the density of diseased insects [132].

Another delayed density-dependent effect was observed in populations of *A. virginalis* along the Pacific coast of the United States [133]. The prevalence of lethal disease in larvae was correlated with the prevalence and severity of infection during the previous year. As each generation of larvae feeds on leaf litter at soil level before climbing onto plants, this delayed density-dependent effect appears to have been mediated by the quantity of ArviNPV OBs that survived in the leaf litter from the previous year and the fraction that were inactivated by UV radiation during the previous summer [133].

The use of the insect bioassay to detect OBs in soil samples can generate novel strains of baculoviruses with the potential for pest control. This approach has been adopted for isolates of SfMNPV and SpfrGV in Mexico [42,134–136]. The novel isolates were characterized and shown to have pathogenic and virulence traits that could be applied to the development of biological insecticides [137–140]. Greenhouse soil substrate also proved to be an abundant and valuable source of insecticidal isolates of SeMNPV in Spain [45,141]. Similarly, when soil slurry was applied to potatoes that were fed to larvae of the potato pest *Tecia solanivora* in Costa Rica, 1–47% of larvae died from granulovirus infection. The isolates from these larvae were genotypically diverse and could be a source of novel strains for pest control [41].

Finally, maize farmers in Nicaragua apply mud directly to the whorl, which is the principal feeding site of *S. frugiperda* larvae [142]. It is unclear whether the pest control effect is due to the physical effect of abrasive soil on insect feeding, or a means of inoculating larvae with pathogens from the soil, potentially including SfMNPV OBs. This technique was described as effective when recently tested against this pest in India [143].

10. Future Perspectives and Conclusions

This review has highlighted evidence in support of the concept that the baculovirus OB is primarily adapted to persist in soil. OBs can remain in this environment in a viable state until opportunities for horizontal transmission arise following translocation to the host's foodplant. The use of these viruses as the basis for biological insecticides has focused attention on the relationship between OBs and the factors that affect virus persistence on the crop and the acquisition of lethal infection as pest insects consume OB-treated foliage [6]. Thus, OB persistence in soil has usually been considered as a minor contribution to pest control in the following growing cycles. In some cases, this may be the result of tillage and related operations that disturb the upper layers of OB-rich soil and reduce the translocation of OBs to crop plants.

Soil is a hyperdiverse collection of complex ecosystems comprising solid, liquid and gaseous phases and an enormous abundance of organisms, all of which vary over space and time [3]. This review has highlighted a number of areas that merit examination in order to better understand the function of the soil reservoir.

1. The physico-chemical and biological aspects of OB interactions with each of the principal components of soil (sand, silt, clay and organic matter), alone and in combination, remain largely unknown, as does the effect of the soil microbiome or plant root exudates on OB persistence. This field is set to advance rapidly with advances in metagenomics over the coming decade [144].
2. Related to the previous point, environmental factors that influence OB persistence in soil have not been subjected to systematic examination or have been examined only in early studies before the adoption of modern experimental and statistical methods. Thus, even factors such as soil temperature, incident UV radiation and soil pH have not been the subject of systematic evaluation, and our understanding of these is mostly anecdotal.
3. With the exception of the careful greenhouse studies on OB translocation by Fuxa, the flow of OBs from pest-infested plants to the soil, their fate in the soil and the processes that return them to the host's foodplant are understood qualitatively, but have not

been quantified. Such measurements would be of value in the parameterization of population models to identify the most influential processes driving insect disease dynamics, especially as the importance of environmental pathogen reservoirs has been questioned in the *Lymantria dispar*–LdMNPV pathosystem [145].

4. Some early studies noted a steady decline in OB viability in soil followed by a stable OB population that did not change appreciably for many months or years (see Section 4, Figure 3). This raises the question of whether there are inherent differences in OBs that affect their stability in soil. For example, a fraction of the OBs may differ in the quantity of polyhedrin matrix or the thickness of the polyhedron envelope resulting in structures with different surface area:volume ratios that may affect their susceptibility to proteases or other enzymes produced by the soil microbiota [146]. Addressing this question may provide an additional example of the influence of OB morphology affecting the likelihood of transmission, as observed recently in a laboratory study [147].
5. The impact of agrochemicals on the soil OB reservoir is unknown, although these products can have adverse effects on soil microorganisms of all types [148]. Studies on the interactions of baculoviruses with agrochemicals have mostly focused on the effects of low concentrations of insecticides that can potentiate the insecticidal activity of OBs [149–155]. Alternatively, the compatibility of virus-based insecticides has been evaluated against synthetic insecticides, fungicides and herbicides for application in tank mixes [156–160]. Most studies have reported little or no adverse effects, but where detected, these usually involved alkaline compounds that damage OB integrity [158]. The influence of copper (Cu^{2+}), often applied as a fungicide, can vary depending on concentration [158,161,162]. Common metal ions such as Fe^{2+} and Fe^{3+} can have detrimental effects on OBs [161,163], whereas other metals may have no effect or even potentiate OB activity [163]. As the influence of plant protection products and fertilizers on OBs in soil remains entirely unknown, it would be of considerable interest to compare OB persistence in soils subjected to different fertilization regimes and pest and plant disease management strategies.
6. The intriguing concept that plants can use baculoviruses as bodyguards to reduce herbivory by phytophagous insects [164] is beginning to find empirical support. Plant protection by virus bodyguards can be favored by retaining OB-contaminated foliage from one growing season to the next [80] or by adopting leaf and canopy architecture that reduces the exposure of OBs to UV radiation [164]. Alternatively, the plant could increase the host's susceptibility to infection through the production of volatile compounds that alter the gut microbiome [165], or manipulate the insect's feeding behavior to increase the likelihood of acquiring an infection by adjusting plant defenses [166], or by limiting the availability of new foliage [164]. Whatever the mechanisms involved, examination of the premise that the soil reservoir represents a key source of virus bodyguards that can be recruited for plant protection may provide valuable insights into the mutually beneficial nature of plant–baculovirus interactions.
7. Finally, the soil is a frequently overlooked source of genetic diversity that doubtless has potential applications in the development of virus-based insecticides. Novel isolates can be obtained from soil samples when the pest is present or absent, even years after the host's foodplant was last cultivated. The value of this approach has been demonstrated in *Spodoptera* spp. [42,45,137,138], but it could be applied to virus insecticides targeted at many other pests, as interactions among mixtures of virus genotypes can enhance their insecticidal properties [167].

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: Juan Sebastián Gómez Díaz (INECOL) provided logistical support during the preparation of this review.

Conflicts of Interest: The author declares no conflict of interest.

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