

DEVELOPMENT OF INSECT VIRUSES AS PESTICIDES: THE CASE OF THE GYPSY MOTH (*LYMANTRIA DISPAR*, L.) IN NORTH AMERICA

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ABSTRACT

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Biological control, one component of integrated pest management, encompasses the use of several types of biological agents to control insect pest populations. Of these biological control agents, the insect viruses appear to offer one logical alternative to the chemical insecticides. One such virus, the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar* (Linnaeus), is discussed in depth in regard to the research leading to its approval as an insecticide against its host.

THE INSECT PEST

The insect, a lepidopteron, was given its common name by English entomologists (Forbush and Fernald, 1896). In 1869 the gypsy moth was imported into the United States from Europe for scientific and commercial purposes. The accidental release of this insect pest in the environment, and its continuous spread in the ensuing years (Fig. 1) has resulted in a serious problem threatening the aesthetic and commercial value of hardwood forests in vast regions of the northeastern United States. Today, the gypsy moth is of major ecological concern in terms of its destructive capacity, and in the measure required for its control.

In 1980 the pest was responsible for the defoliation of over 5 million acres throughout the northeastern United States (Table I). It is estimated that, in saw timber stands, the average immediate value loss after 1 year of heavy defoliation is more than \$75/acre (McCay and White, 1973). Losses can be even higher in residential areas where the presence of trees contributes in large part to real estate values (Payne et al., 1973). Further, during periods of heavy defoliation, managers of recreation areas may lose

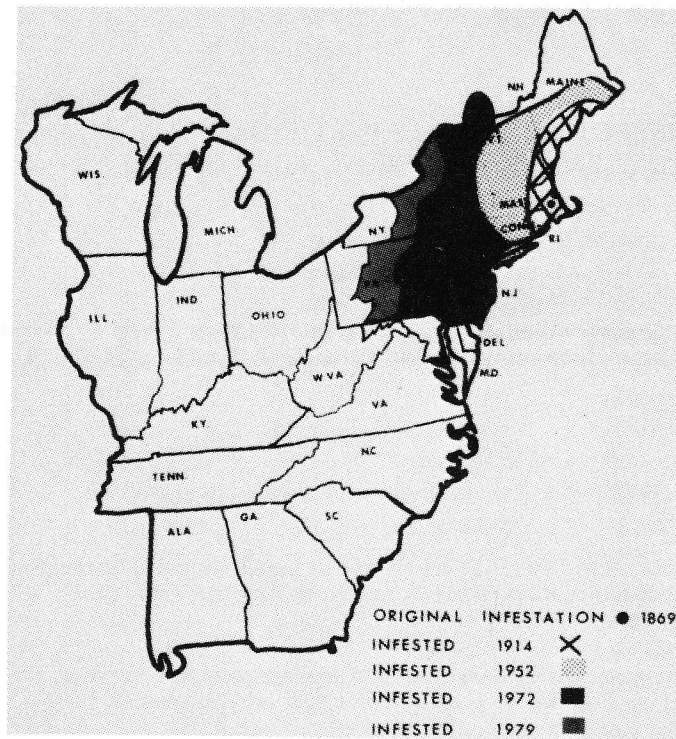


Fig. 1. Spread of the gypsy moth in the United States. The dot shown in the state of Massachusetts (town of Medford) signifies the site of introduction of the insect into the United States in 1868 or 1869. Some 10–12 years later, trees in the immediate area were extensively defoliated by the gypsy moth. By 1914, the insect had spread to New Hampshire, Vermont, Maine, Connecticut and Rhode Island; by 1952, to New York; by 1972, to New Jersey and Pennsylvania; and by 1979, still further west. All the States shown in the map contain forested areas susceptible to gypsy moth infestation. Recently, the insect has established pockets of activity in Michigan and the far-western states of California, Oregon and Washington.

annually up to 240 person-days of use in these facilities (Moeller et al., 1977).

The gypsy moth is a univoltine insect. Overwintering eggs hatch throughout late April and early May coincident with bud-break. Male insects progress through five larval stages (females six) prior to pupation in late July. Adult emergence, mating and egg deposition occur shortly thereafter. Embryonated eggs then pass through a winter diapause and the cycle begins anew. With the development of an artificial diet (Odell and Rollinson, 1966) it has become possible to rear the insect year-round in the laboratory, thus providing a continuous source of insects for virological studies.

TABLE I

Acreage defoliated by the gypsy moth in various States of the United States in 1979 and 1980^a

	1979	1980
New York	162275	2449475
Massachusetts	226260	907075
New Jersey	193700	411975
Connecticut	7486	372213
Maine	23180	221220
New Hampshire	5980	183999
Vermont	15411	75094
Rhode Island	655	43830
Michigan	100	5
Maryland	0	3
Delaware	10	0
Total	643609	5105389

^aSource: NEWS, U.S. Department of Agriculture, 1980.

THE BIOLOGICAL CONTROL AGENT — THE NUCLEOPOLYHEDROSIS VIRUS OF THE GYPSY MOTH

Naturally occurring viruses have been isolated and purified from many insect species (Bergold, 1953). The gypsy moth nucleopolyhedrosis virus (NPV) takes the form of rod-shaped particles (nucleocapsids) embedded within a polyhedral protein matrix, the polyhedral inclusion body (PIB) (Wildy, 1971; Matthews, 1979). After ingestion by larvae, multiplication of the viral particles and their incorporation into the PIB occurs in the nuclei of susceptible cells.

In the laboratory, refrigerated inclusion bodies can be stored for years with little loss of activity (Lewis and Rollinson, 1978). Conversely, free viral particles are highly sensitive to physical and chemical agents and rapidly degrade. Thus, the inclusion bodies are the objects of choice for dissemination into the environment as viral insecticides.

In the field, however, exposed to a variety of meteorological factors, in particular sunlight, the inclusion bodies will, if not protected, rapidly lose their activity. For inclusion bodies of the gypsy moth NPV applied to foliage, such deleterious effect can be complete within one week of application (Podgwaite et al., 1979). However, the inclusion bodies persist for at least a year in litter and in soil. The question of viral persistence is of particular concern to ecologists, who have long objected to the extended activity of chemical insecticides in the environment.

CONTROL OF INSECT PESTS WITH VIRAL INCLUSION BODIES

For effective control of target insects, successive viral applications may be required. In regard to the gypsy moth, NPV has been formulated in an aqueous suspension containing an ultraviolet screen, a feeding stimulant and a sticker-spreader. The formulation is applied from aircraft delivery systems when larvae are in the first and second instars, the stages most susceptible to infection. Also, at this time, leaves of host trees have approached 50–60% expansion and thus provide adequate surface area for deposit. A second application is made 7–10 days later to infect late-hatching larvae and to augment the effects of the first treatment. Larvae older than 4th stage are significantly less susceptible to infection than are younger larvae (Lewis et al., 1979), which makes it important that applications be properly timed.

SPECIFICITY

Many NPVs, including gypsy moth NPV, are highly specific to their respective insect hosts. However, specificity should be proved and never assumed. As a point of interest, the NPV of the alfalfa looper, (*Autographa californica* Speyer) is infectious for a variety of insects, none of which is beneficial to man or to his environment (Vail and Jay, 1973). With regard to the broad but acceptable host spectrum of the alfalfa looper NPV, this feature should be of interest to commercial firms involved in insect virus production, and desiring a greater market demand.

Like other viruses, insect viruses are capable of mutation and possibly of changing their specificity (Reichelderfer, 1975). This possibility should be tested by seeking out and promptly detecting any change in the host range. Moreover, the integrity of the virus should be ascertained by using the most sensitive serological and biochemical tests available (Axelrod, 1975). It should be noted, finally that insect hosts may also change in their sensitivity to NPVs (Maramorosch, 1975).

PRODUCTION OF VIRUS MATERIAL AND SAFETY CONSIDERATIONS FOR THE LABORATORY WORKER

In the laboratory, inclusion bodies of NPVs are prepared in large quantities by in vivo procedures. Gypsy moth NPV is produced by first allowing young larvae to feed on synthetic diet laced with virus and then harvesting the dead. Cadavers are frozen, dehaired, lyophilized and cryogenically milled to produce a mixture of NPV and insect parts. This technical product, which is then used for gypsy moth suppression projects, may contain contaminating bacteria, fungi and possibly other viruses. Thus, it must be carefully monitored to assure the absence of primary mammalian pathogens prior to its acceptance and use (Podgwaite and Bruen, 1978, 1980). The technical virus product may be further purified for laboratory studies by

using density gradient centrifugation techniques (Breillatt et al., 1972).

In vitro production of viral inclusion bodies, while eagerly awaited by investigators, has not as yet matched the high recovery of inclusion bodies produced by in vivo procedures. However, cell, tissue, and organ culture techniques have many advantageous features in insect virus production. Insect host materials such as hairs, setae and other body parts are eliminated. More importantly, far fewer microbial contaminants are present in in vitro cultures than in in vivo technical products.

The question of the safety of the laboratory worker is obviously of prime importance in virus production. No reports of infection or otherwise serious health hazards have been declared for laboratory workers preparing insect NPVs. In the case of NPVs of the gypsy moth and the European pine sawfly (*Neodiprion sertifer* Geoffroy), no circulating antibodies were observed in laboratory workers by serological analysis (Mazzone et al., 1976). Allergic responses have been observed for some laboratory personnel working with gypsy moth larvae, and are due mainly to hairs of the insects becoming airborne and inspired by workers, or by the hairs alighting on the skin of individuals. This hazard can be avoided if laboratory personnel wear protective clothing and masks, and operate in HEPA filtered rooms or hoods.

The essentially reassuring reports on health hazard should not dissuade the laboratory worker from following sound safety precautions. Thus, the contamination of glassware and laboratory surroundings by viral or other microbial agents should be minimized and the disposal of contaminated material and equipment by disinfection and autoclaving should be a standard procedure.

REGISTRATION OF NPVs FOR USE

In the United States, the Environmental Protection Agency (EPA) registers for use the biorational control agents such as the NPVs. Standard protocols for clearing these agents appeared in the 1970's (United States Environmental Protection Agency, 1975), and prescribed procedures are subject to change as more sensitive tests become available. As a rule, the EPA requires not only that NPVs be harmless to users, but also their safety be determined on standard test animals — e.g., dogs, rabbits, mice, rats, guinea pigs, and on animals residing in areas where the virus is to be applied — e.g., birds, aquatic life, and predators.

At present, three NPVs have been registered with the EPA for use against their respective hosts: the NPV of the cotton bollworm (*Heliothis zea* Boddie) in 1975, the NPV of the Douglas fir tussock moth (*Orgyia pseudotsugata* McDunnough) in 1976, and the NPV of the gypsy moth (*L. dispar*) in 1978. Two other NPVs are being readied for registration: the NPV of the European pine sawfly (*N. sertifer*) and the NPV of the alfalfa looper (*A. californica*).

ENVIRONMENTAL AND HEALTH CONSIDERATIONS

To date, no harmful effects of spraying NPVs in the environment have been reported. Thus, for example, the honey bee, other beneficial insects, small mammals, and birds have been examined comprehensively and judged to be unaffected by contact with NPVs (Knox, 1970; Lautenschlager and Podgwaite, 1977; 1979; Lautenschlager et al., 1977, 1978 a and b, 1979, Podgwaite and Galipeau, 1978; Mazzone et al., 1979; Podgwaite et al., 1979). A list of species tested in regard to the gypsy moth NPV is presented in Table II.

TABLE II

Species shown to be unaffected by gypsy moth NPV in susceptibility tests (adapted from Podgwaite, 1980)

Treatment ^a	Test animal
Serology	humans (laboratory workers)
Immunology	mice, guinea pigs
Feeding ^b	rat, dog, white-footed mouse, shorttail shrew, Virginia opossum, red-backed vole, chipmunk, racoon, mallard duck, black-capped chickadee, house sparrow, songbirds, quail, bluegill sunfish, brown trout, water flea, backswimmer, midge, waterboatman, honeybee
Gastric intubation	rat
Inhalation	rat
Carcinogenicity ^c	rat
Dermal toxicity, eye mucosa	rabbit
Intraperitoneal injection	mouse
Tissue culture	fish, snake, raccoon

^aTesting performed by licensed commercial laboratories.

^bIncluded pathology, hematology and urinalysis, where applicable.

^cAs for feeding tests but including tumorigenic analysis.

The possibility that the NPVs may be related serologically to the arthropod borne (arbo) viruses and other viruses which infect man has been investigated. In studies carried out in 1974–1975 at the Yale University Arbovirus Research Laboratories and the U.S. Department of Agriculture's Plum Island Animal Disease Center, all of the known arboviruses were found to bear no serologic relation to the NPVs of the gypsy moth and the European pine sawfly. Other viruses, such as Herpes and lymphocystis chorimeningitis were also found to be serologically unrelated to the insect NPVs cited (Mazzone et al., 1976). In addition, results of studies to determine the effect of the NPVs of gypsy moth and the European pine sawfly in immunodepressed animals, adult mice and guinea pigs, were also negative (Tignor et al., 1976).

Notwithstanding the studies cited above, the release of insect viruses

into the environment should always be planned and executed with strict attention to safety. Since this procedure of pest management is relatively new (Lewis and Etter, 1978), operational applications of insect viruses should be closely monitored to detect promptly the eventual occurrence of undesirable sequels. It is incumbent on those responsible for production, regulation and utilization of these products, to safeguard all concerned against any deleterious effects that might accrue from the use of pathogens as agents of insect pest control.

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