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The Development and Operational Use of a Management System for Control of Douglas-Fir Tussock Moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae), Populations at Pre-Outbreak Levels

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ABSTRACT The Douglas-fir tussock moth is a native defoliator which periodically reaches outbreak proportions in western North America, causing economically important damage to its primary host, the interior Douglas-fir. The research and development of a management system for this defoliator over the past 15 years is reviewed in detail. The various data from field trials supporting this system are presented and components of this management system are briefly mentioned. The operational testing of this management system was successful, and supporting data are presented and discussed. It is recommended that this operationally proven management system be used as a template for the development of similar systems for other defoliators.

THE DEVELOPMENT OF a management system using a virus for the regulation of Douglas-fir tussock moth was the result of cooperative research efforts conducted over a number of years by the Canadian Forest Service (Pacific and Ontario Centres), the B.C. Ministry of Forests, and the USDA Forest Service (Stelzer et al. 1977; Shepherd et al. 1984a, 1984b; Otvos et al. 1987a, 1987b).

The Douglas-fir tussock moth (DFTM), *Orgyia pseudotsugata* (McDunnough), belongs to the family Lymantriidae. It is an important indigenous defoliator in the interior dry-belt forests in British Columbia and the western United States. Although the larvae can feed on the foliage of several trees, the primary host in the interior of B.C. is Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco. The female moth is flightless, and after emerging from her cocoon and mating, lays all her eggs in a single mass on the cocoon from which she emerged. The eggs overwinter and the larvae hatch the following spring after the host trees flush (Wickman and Beckwith 1978, Shepherd et al. 1984). Dispersal of the tussock moth is limited. The small, hairy larvae spin silken threads and are blown to surrounding trees and adjacent stands; this is probably the reason for the patchy pattern of defoliation associated with this insect.

Pages 143-154 in M.L. McManus and A.M. Liebhold, editors. 199 Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

DFTM is a cyclic pest and outbreaks usually occur 7 to 11 years apart in western North America (Harris et al. 1985, Otvos and Shepherd 1991). DFTM populations increase rapidly, usually resulting in severe defoliation and tree mortality (Wickman 1963, 1978). Mature, severely defoliated trees are susceptible to attack by Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins. Outbreaks in an area usually collapse after 2 to 4 years of defoliation (Mason and Luck 1978) due to a naturally occurring nuclear polyhedrosis virus (OpNPV), but by then tree damage is extensive (Dahlsten and Thomas 1969, Shepherd and Otvos 1986). NPVs are so named because they replicate within cell nuclei, and the virus produces an inclusion body which is many-sided, *i.e.* a polyhedral inclusion body.

Two morphotypes of OpNPV have been isolated from DFTM larvae and identified as the cause of epizootics (Hughes and Addison 1970). Unicapsid OpNPVs have rod-shaped virus particles embedded singly in the inclusion matrix. Multicapsid OpNPVs have bundles of five to 15 virus particles occluded in a protein envelope with several bundles per inclusion body. Both viruses belong to a group called Baculoviridae. These viruses are highly species-specific and most of them only infect the host target insect or closely related species of the same genus. Baculoviruses are generally slow acting, and some feeding damage can occur between ingestion of a lethal dose and death of the insect larva. Baculoviruses, like the bacterium, *Bacillus thuringiensis*, must be ingested to cause infection (Cunningham 1982). The inclusion body protein dissolves in the alkaline larval gut juices, releasing the viral particles. The viral particles penetrate the gut cells in susceptible species, after which they usually spread to other organs. In the final stages of infection, more inclusion bodies are produced in infected cells. Following the death of the larva, the cuticle ruptures and the polyhedral inclusion bodies (PIBs) are released into the environment. These may infect more larvae (horizontal transmission) or the next generation of larvae (vertical transmission). Some of these particles end up on other needles, which can then be ingested by additional larvae. Other inclusion bodies are washed off the foliage and end up in the soil. Inclusion bodies in the soil are basically lost from the infection cycle, but are a potential source of inoculum at the next outbreak. Although it is technically feasible to grow these viruses in cell culture, the medium is expensive and the yield is relatively low. It is more economical to produce the virus in host larvae.

The first aerial spray trial in British Columbia using OpNPV against DFTM was conducted jointly by personnel of the Canadian Forest Service, the USDA Forest Service, and the British Columbia Ministry of Forests in the mid-1970s (Stelzer et al. 1977, Shepherd et al. 1984, Cunningham and Shepherd 1984). High populations of DFTM larvae were treated aerially in the second year of the outbreak with laboratory-produced multicapsid OpNPV. The virus was applied as soon as all the larvae had hatched, dispersed, and were actively feeding. Such early application is essential for the second wave of virus infection, causing an epizootic and resulting in a high level of control. The treatments caused high larval mortality, but the treated stands still sustained considerable damage. After these trials, the multicapsid isolate of OpNPV, produced in the DFTM, was registered in the U.S. by the Environmental Protection Agency in 1976 under the name TM BioControl-1®. The same virus, produced in whitemarked tussock moth, *Orgyia leucostigma* (J.E. Smith), received registration in Canada in 1983 under the name Virtuss.® The recommended dosage in polyhedral inclusion bodies on both labels is 2.5×10^{11} PIB/ha (Otvos et al. 1995). Neither Virtuss® nor TM BioControl-1® products were used operationally (only experimentally) in

Canada until 1991, 1992 and 1993, when ca. 200 ha, 450 ha, and 610 ha, respectively, were treated with these products. From 1974 to 1982, the American product TM BioControl-1® was used on eight research plots, with a total area of 638 ha, in a joint project between the Canadian and USDA Forest Service. The Canadian product was used on 10 plots with an area of 150 ha in British Columbia, Canada.

The American product, TM BioControl-1®, was also registered in Canada in 1983 to facilitate its importation and use in British Columbia. The recommended dosage of Virtuss® of 2.5×10^{11} PIB/ha takes 200-300 whitemarked tussock moth, *Orgyia leucostigma*, larvae to produce. *O. leucostigma* is a closely related species that does not have an obligatory diapause, and is much easier to rear and handle than *O. pseudotsugata*. Therefore, in Canada, *O. leucostigma* is the preferred host for producing the virus. Virtuss® is also effective for control of whitemarked tussock moth. It was field tested in Newfoundland (West et al. 1987, 1989) and this species may be added to the label.

There are two main strategies for using viral insecticides. First, there can be a one-shot application, killing only larvae that ingest sufficient amounts of polyhedral inclusion bodies. Secondly, one can initiate an epizootic with horizontal and vertical transmission of the virus, as in the case of the DFTM. The strategy of combining pheromone detection and virus application led to the development of a system for integrated control of DFTM. It was considered desirable to introduce the virus at the beginning of the outbreak to initiate a viral epizootic before it would occur naturally, thus preventing the development of the outbreak and reducing tree damage. Another focus of the study was to determine whether or not it was necessary to spray the entire infested area, or if the virus would spread into untreated areas. In addition, experiments were conducted to determine if the recommended application rate could be reduced and still prevent damage. These points were investigated during the 1981-1984 and 1990-1993 outbreaks.

Materials and Methods

Early treatment of an infested stand is desirable, because the greatest tree mortality results from the first year's defoliation. Application on first and second instar larvae is essential to obtain secondary infection. Introduction of the virus at the beginning of an outbreak requires a reliable monitoring system. In a separate study, concurrent with the virus work, a dependable and sensitive pheromone monitoring system was developed for early warning of outbreaks (Shepherd et al. 1985). Pheromone-baited traps were placed in susceptible stands, which were defined by overlaying previous outbreak, forest type, and biogeoclimatic zone maps. The most susceptible stands tended to be located in the driest part of the range of Douglas-fir, where it mixes with ponderosa pine, *Pinus ponderosa* C. Laws. exo. Laws. Within this forest habitat, 18 permanent monitoring stations were selected and pheromone-baited traps monitored annually to measure male moth density (Shepherd and Otvos 1986). Population trends in pheromone-baited traps were followed from endemic to epidemic levels. The number of successive years of upward trends of male moths caught is used because it is a better indicator of impending outbreaks than the average number of male moths per trap in any one year. Three consecutive years in which the number of male moths caught in the pheromone-baited traps increases and exceeds 25 males/trap indicates that an outbreak is expected to occur within the next 1 or 2 years.

The pheromone-baited trapping program is followed by egg-mass surveys to monitor DFTM populations (Shepherd and Otvos 1986). The pheromone trap system only gives advance warning that an outbreak is imminent and signals that another, more precise, sampling system should be used in the area. Thus, after 2 years of upward trends, an extensive network of additional traps is deployed around the indicator stations to locate the foci of the developing outbreak. An egg-mass survey is then required during the fall or winter to determine the insect density at the centre of the developing infestation and to predict potential damage (Shepherd et al. 1984a).

1981-1984 Outbreak. A developing DFTM outbreak in south-central British Columbia was discovered in 1980 before any defoliation occurred. This led to the experiment conducted in 1981 (Shepherd et al. 1984b), when virus was applied both from the ground and air, at an early phase of the outbreak, to determine whether a viral epizootic can be initiated at low to moderate population levels before it would occur naturally, and to try to prevent or reduce defoliation.

During 1982, one water-based dosage and three dosages in an oil-based formulation containing OpNPV were compared. Four 10-ha plots were treated with the following viral dosages: one plot received the recommended dosage of 2.5×10^{11} PIB/ha in an aqueous formulation containing 25% molasses and 75% water, and a second plot received the same dosage in an emulsifiable oil formulation (25% Dipel 88 blank carrier and 75% water). The other two treatments involved reduced dosages of virus in oil, with Plot 3 receiving about 1/3 and Plot 4 about 1/15 of the recommended dosage (Otvos et al. 1987b). The virus was applied at 9.4L/ha to all four plots. An additional four plots were established as untreated checks. Tree mortality was monitored for 2 years after the treatments in 1982.

1990-1993 Outbreak. Pheromone trap catches followed by egg-mass surveys in 1990 indicated that nine Douglas-fir stands or areas in the Kamloops Forest Region would be defoliated in 1991. Results from the 1982 experiment indicated that a virus dosage of 8.3×10^{10} PIB/ha, which is one-third of the previously recommended dosage, would be adequate, and either tank mix was acceptable. In 1991, 12 infested stands were treated with the "blanket" type approach at the full dose (2.5×10^{11} PIB/ha); four stands with a total area of about 100 ha were treated with ca. 10-year-old stored Virtuss®, four other stands with a total area of about 40 ha were treated with ca. 10-year-old stored TM BioControl-1®, and the remaining four stands with a total area of about 60 ha were treated with a batch of freshly produced Virtuss® to provide a basis for comparison.

In 1992, two new areas were treated with the "blanket" type application, one ca. 240 ha in area east of Kamloops, and one ca. 460 ha in area west of Kamloops. Both areas were treated with the stored Virtuss® at the recommended (full) dosage of 2.5×10^{11} PIB/ha in 9.4L/ha in 25% animal grade molasses, 75% tap water (aerated to get rid of the chlorine) and 6% Orzan LS (I.T.T. Rayonier, Inc., Seattle, WA) added as a sunlight/UV protectant.

The hypothesis that the widely spaced swath treatment approach would provide adequate population reduction and foliage protection was also tested in 1992. This experiment was conducted in the stand used as an untreated check in 1991 where the infestation increased both in size and intensity. The infestation expanded to about 260 ha and the average egg-mass density increased from an average of 2.5 egg masses per tree (based on three lower branches/tree) in the fall of 1990 to 21 per tree in the fall of 1991. Average egg-mass densities over 1.7 per tree (based on three lower branches/tree) indicated severe defoliation

was to be expected in 1992. To test the widely spaced swath treatment, flight lines were set 200 m apart.

Results

1981-1984 Outbreak. In 1981, virus was applied aerially to three DFTM population densities: high, moderate, and low, and an epizootic occurred at all three host densities. Although a natural epizootic also occurred in the check plots containing high and moderate DFTM populations, incidence of viral infection in the treated plots was considerably earlier and higher, indicating the beneficial effects of the virus spray. Treatment effects were excellent even at low population density, and a natural epizootic in the check plot occurred much later. The experiment showed that the virus can be introduced into DFTM populations at an early phase of the outbreak. A viral epizootic can be initiated both by aerial and ground treatment applied at a dosage of 2.5×10^{11} PIB/ha on first and second instar larvae.

Table 1. Population densities and larval reduction of DFTM in the experimental plots treated with Virtuss® at Veasy Lake, Kamloops Forest Region, B.C., 1982

Plot Number	Treatment (PIB/ha)[a]	Tank Mix	Larvae per m² foliage ($\bar{x}$ ± SE) Pre-spray	6 weeks post-spray	Population reduction due to treatment (%)[b]
T1	1.6×10^{10}	Emulsifiable Oil	182.8 ± 12.6	6.7 ± 1.8	64.7
C1	Check		197.5 ± 18.0	20.5 ± 2.9	-
T2	8.3×10^{10}	Emulsifiable Oil	145.8 ± 12.2	2.8 ± 0.7	90.8
C2	Check		136.9 ± 9.4	28.7 ± 2.8	-
T3	2.5×10^{11}	Emulsifiable Oil	302.0 ± 28.7	1.0 ± 0.4	95.0
C3	Check		360.6 ± 34.6	24.1 ± 4.6	-
T4	2.5×10^{11}	Aqueous	41.8 ± 5.3	2.0 ± 0.6	86.6
C4	Check		81.2 ± 16.5	28.9 ± 4.3	-

[a] PIB, polyhedral inclusion bodies

[b] Population reduction was calculated using a modified Abbott's formula (Abbott 1925).

The initial impact of the applications in 1982 was that 10-30% of larvae became infected 2 weeks after the spray. These infected larvae died, liberating polyhedra and increasing the amount of inoculum on the foliage. A secondary wave of virus infection then developed among the surviving larvae (DFTM larvae have a long feeding period) and within 6 to 7 weeks after spraying the population collapsed. Percent infection and the development of the virus epizootic among the larvae in the four treated plots were related to dosage. Population reduction was calculated using a modified Abbott's formula (Abbott 1925), which corrects for natural mortality. Population reduction was 64.7% in the plot receiving the lowest dosage (1.6×10^{10} PIB/ha), 90.8% in the plot receiving the second lowest dose (8.3×10^{10} PIB/ha), and 95.0% in the plot receiving the full dose (2.5×10^{11} PIB/ha) formulated in emulsifiable oil; there was an 86.6% reduction in the plot treated with the water-based formulation at the full dose (Table 1). The lower population reduction in the plot treated with the water-based

formulation is believed to be due to the initial lower population density, about 42 larvae/m^2, compared to a range of 146-302 larvae/ m^2 in the other treated plots. Virus transmission was higher in plots with higher larval densities. In contrast, NPV infection was not detected in the four untreated check plots until 5 weeks after spraying and at a much lower level. Development of the viral disease is temperature dependent and the spread of the disease is influenced by host density.

Six weeks post-spray infection of larvae in the treated plots ranged from 88 to 100%. A high level of naturally occurring viral infection of larvae was found in only one untreated check plot, where it reached 43% at 7 weeks after the spray. Percent infection in the other three check plots was 1.4, 9.1 and 23.0% at this time. All the treatments were considered effective in reducing DFTM larval populations. The higher level of naturally occurring virus in two of the check plots (23% and 43%) was unexpected, because naturally occurring virus is usually not prevalent at the beginning of outbreaks. One additional benefit of the virus application was increased pupal mortality. An average of 11.6% of the pupae emerged as adults from the treated plots, compared to 35.4% emergence in the check plots. Adult emergence from the pupae collected from the four treated plots ranged between 4% and 20%, the latter being in the plot receiving OpNPV in an aqueous formulation (Table 2). The virus application effectively reduced emergence of adults from 45.2% to 86.9%.

Table 2. Reduction of adult emergence of DFTM in experimental plots treated with Virtuss® at Veasy Lake, Kamloops Forest Region, B.C., 1982

Plot Number	Treatment (PIB/ha)[a]	Number of pupae reared[b]	Number of adults emerged	Adult emergence (%)	Emergence Reduction (%)[c]
T1	1.6×10^{10}	107	19	17.8	58.6
C1	Check	219	94	42.9	-
T2	8.3×10^{10}	108	4	3.7	86.9
C2	Check	181	51	28.2	-
T3	2.5×10^{11}	105	10	9.5	71.4
C3	Check	117	39	33.3	-
T4	2.5×10^{11}	52	10	19.5	45.2
C4	Check	265	93	35.0	-
Average	Treatment	372	43	11.6	67.4
	Check	782	277	35.4	-

[a] PIB, polyhedra inclusion bodies.

[b] Collection included some larvae that pupated shortly after collection.

[c] Population reduction was calculated using a modified Abbott's formula (Abbott 1925).

Defoliation attributable to DFTM larvae was impossible to estimate in the test area because western spruce budworm, *Choristoneura occidentalis* Freeman, was also present in all the plots. The *C. occidentalis* population was not affected by the OpNPV application. A

comparison of results of egg-mass surveys conducted in the spring and the fall before and after the spray showed a drastic reduction of egg-mass numbers in all the treated plots. Egg-mass numbers remained the same in one of the check plots, and more than doubled in the other three check plots. The results from these experiments showed that virus can be introduced into a DFTM population at an early phase of the outbreak and a viral epizootic can be initiated by either aerial or ground application on early instar larvae. Levels of virus infection were monitored and all treatments were successful, even at a low population density.

Tree mortality was negligible in the four treated plots: it averaged 0.6% 1 year after treatment and 2.8% 2 years after treatment, compared with 37.8% and 40.7% in the check plots (Table 3) (Otvos et al. 1987a). These results indicate that it is feasible to control DFTM populations with OpNPV (at least at 8.3×10^{10} PIB/ha) at an early stage of an outbreak before significant damage occurs, and this can prevent, or at least minimize, tree mortality. It was concluded that virus treatments in 1982 were successful in preventing tree mortality. The data from ground treatments indicated that OpNPV spread from sprayed to unsprayed areas, and Virtuss® may be applied in widely spaced swaths. This would permit applying the virus aerially in swaths that are about 100 to 200 m apart (instead of the usual "blanket" type approach where the spray swaths are 30 to 40 m apart) and then allowing the spread of the virus into the unsprayed areas. This novel approach would result in considerable cost reduction of spray operations. The results of experiments conducted during the 1981-1984 outbreak provided data for the development of a pest management strategy which was tested operationally in the most recent (1990-1993) outbreak.

Table 3. Population densities and reduction of DFTM larvae in four Virtuss®-treated experimental plots in 1982, the year of application, and cumulative proportion of trees killed 1 and 2 years after treatment at Veasy Lake, Kamloops Forest Region, B.C

Plot Number	Treatments	1982 pre-spray DFTM/m²	% population reduction 1982[a]	% sample trees killed by DFTM 1983	% sample trees killed by DFTM 1984
T1	1.6×10^{10}	182.8	64.7	0	0
T2	8.3×10^{10}	145.8	90.6	2	7
T3	2.5×10^{11}	302.0	95.1	0	4
T4	2.5×10^{11}	41.8	86.6	0	0
Average				0.6	2.8
C1	Control	197.5		53	60
C2	Control	136.9		logged	-
C3	Control	360.6		60	62
C4	Control	81.2		0	0
Average				37.8	40.7

[a] Population reduction was calculated using a modified Abbott's formula (Abbott 1925).

1990-1993 Outbreak. Two of the three treatments used in 1991 gave good population reduction (Table 4). Both the stored and newly produced Virtuss® gave good population reductions at 86% and 82%, respectively (corrected for natural mortality). However, population reduction in the stands treated with the old TM BioControl-1® was only about 8%.

This low and unacceptable population reduction was probably due to the low pre-spray larval population density. When the two untreated check plots with the lowest pre-spray population densities (2.1 and 8.9 larvae/m^2) are excluded from these calculations, the population reduction figures for the two remaining plots (with 27.4 and 23.4 larvae/m^2 in the pre-spray counts) increased to 27.7% (Table 4). The lower population reduction figure is believed to be the result of low population densities prior to the spray and suggests that it was not necessary to treat stands infested with such low densities of DFTM larvae. Egg-mass surveys in the treated stands taken in the fall revealed low counts, indicating a prediction of either no damage or only traces of defoliation in the year following treatment. This was confirmed by both aerial and ground defoliation surveys in 1992.

Table 4. Population reduction for operational application of OpNPV against Douglas-fir tussock moth in Kamloops Forest Region, B.C. 1991

		DFTM per m² at biweekly sampling times					Population reduction due to treatment (Abbott's formula) at biweekly sampling times[a]		
Treatment	Plot	0	1	2	3	4	2	3	4
Old Virtuss®	3	52.34	64.96	22.34	14.31	4.91	42.6	57.6	79.0
	7	163.13	164.88	40.77	3.10	2.57	58.7	96.4	95.7
	7b	39.07	96.70	37.56	30.11	19.12	35.2	40.0	45.0
	9	130.51	121.92	67.43	28.18	2.84	7.7	55.5	93.5
	Avg.	102.85	113.85	41.16	16.67	5.85	39.7	71.8	85.7
New Virtuss®	1	7.92	39.76	14.28	7.47	3.09	40.1	63.8	78.4
	4	51.33	52.32	57.04	11.11	4.50	0	59.1	76.1
	5	86.57	179.70	64.62	15.80	10.36	40.0	83.1	84.0
	6	60.35	102.05	96.31	17.59	6.22	0	66.8	83.1
	Avg.	53.07	90.13	64.69	13.58	5.89	0	71.0	81.8
TM BioControl-1®	8	20.15	27.44	24.71	7.23	4.35	0	49.3	55.9
	10	30.98	23.35	19.04	14.25	8.85	0	0	0
	11	0.64	2.10	2.11	2.11	0.82	0	0	0
	12	7.25	8.85	9.48	7.10	5.49	0	0	0
	Avg.	13.41	13.89	12.45	7.25	4.58	0	0	8.4
Average		61.75	75.16	36.93	12.83	5.44	18.0	67.1	79.9
Control		30.44	33.80	20.26	17.55	12.16			

[a] The calculations for Abbott's formula uses the mean value from collection time 1 week post-spray rather than pre-spray because there was an apparent increase in DFTM populations following the pre-spray sample.

In the widely spaced swath treatment, where the flight lines were 200m apart, Virtuss® was applied at 2.5x10^{11} PIB/ha in 9.4 L/ha on the treated swaths, representing a treatment of approximately 26 ha within the 260-ha plot. The infection was highest directly under the flight lines in the weeks following the treatment, then spread into the untreated areas and gradually increased over time. Population reduction on some sample lines in the area treated with the widely spaced swaths 8 weeks after treatment was over 90% based on larval counts (Table 5). In comparison, population reduction 8 weeks after treatment in 1992 was 94 and 95% in the two DFTM-infested stands given the "blanket" treatment. Population reduction

could not be corrected for natural mortality because all of the infested stands were treated, therefore none could be used as an untreated control. By the time of the last larval collection, 8 weeks after treatment, it was difficult to collect larvae from directly under the flight lines and practically all the larvae collected, irrespective of the location where they were collected from within the plot, were infected with OpNPV, and they either died as larvae in partially formed cocoons, or died as pupae. It should be noted, however, that the results of the widely spaced swath treatment are based on 1 year's data and from one, though large, plot. It is highly desirable to replicate this widely spaced swath treatment in several plots to confirm the results obtained in 1992.

Table 5. Population reduction for experimental application of OpNPV in widely spaced swath treatment of DFTM in the Kamloops Forest Region, 1992

		DFTM per m^2 at biweekly sampling times						Populaton
Treatment	Line	0	1	2	3	4	5	reduction[a]
Sample Trees under flight lines	A	34.55	1.56	15.44	13.58	5.14	1.70	95.1
	B	23.36	18.37	33.87	17.97	2.03	0.43	98.2
	C	50.33	18.76	32.38	48.23	7.27	12.30	75.6
	D	27.30	35.37	24.67	31.63	1.40	0.00	100.0
	Avg.	32.64	20.28	26.77	27.62	3.59	3.06	90.6
Sample trees between flight lines	A	58.71	39.76	56.86	45.29	19.47	2.52	95.7
	B	32.32	52.32	21.49	50.27	1.48	1.07	96.7
	C	120.59	179.70	174.04	75.36	11.12	4.86	96.0
	D	60.71	102.05	47.66	27.72	7.47	0.29	99.5
	Avg.	70.18	90.13	77.03	50.15	8.82	2.15	96.9
All sample trees (both under and between flight lines)	A	44.90	26.07	33.19	27.17	11.28	2.05	95.4
	B	27.61	29.14	27.68	34.12	1.76	0.75	97.3
	C	89.36	60.78	111.08	63.30	9.41	8.17	90.9
	D	42.48	54.52	35.12	29.77	4.16	0.13	99.7
	Avg.	50.63	43.80	51.22	38.73	6.14	2.62	94.8

[a] No untreated check ares were available and population reductions are not corrected for natural mortality.

Egg-mass surveys conducted in the fall, after treatment, showed no new egg masses on sample trees which had an average of about 21 egg masses per tree the fall prior to treatment. In the previous fall, no visible defoliation was forecast for 1992 in any of the treated stands in 1991, and Forest Insect and Disease Survey evaluations in 1993 confirmed this prediction.

In 1993, the B.C. Ministry of Forests treated all stands infested with DFTM, totalling about 440 ha, with either Virtuss® or TM BioControl-1®. Unfortunately, no areas were reserved to repeat the widely spaced swath application. Virtuss® was used on ca. 210 ha of the 440 ha at full dosage and TM BioControl-1® on 230 ha at full dosage (Table 6). Population reduction was about 95% by both of these products on the 440 ha treated at the full dose (2.5×10^{11} PIB/ha). An additional 150 ha was treated with Virtuss® at about 2/3 of the recommended dosage and 25 ha with TM BioControl-1®, also at 2/3 of the recommended dosage. TM BioControl-1® applied at 2/3 of the recommended dose gave an acceptable population reduction of 64%, but in the stand treated with Virtuss® at 2/3 of the full dose,

DFTM population reduction was only 14% (Table 6). No explanation is offered for the low population reduction in these stands.

Table 6. Operational treatment of Douglas-fir tussock moth infested stands in the Kamloops Forest Region, 1993

Treatment	ha	Dosage	% Population Reduction[a]
Virtuss®	100	full	89
	110	full	98
	150	2/3	14
TM BioControl-1®	130	full	90
	100	full	98
	25	2/3	64

[a] No untreated check plots were available and population reduction was not corrected for natural mortality.

Conclusion

The results of the experiments with pheromone traps and OpNPV application over the past number of years indicate that DFTM outbreaks can be prevented by a single application of virus at the early phase of an outbreak. However, foliage protection may be negligible in the year of application, but acceptable in the following years. Tree mortality was prevented when the treatment was applied early enough in the outbreak cycle, and on early instar larvae. A virus application at widely spaced swaths makes it economically more acceptable and gives forest managers a cost-effective and practical alternative to the use of chemical insecticides in controlling DFTM. The operational testing of the management system developed for the DFTM was successful and tree mortality was minimal in stands treated with OpNPV because early infection prevented the development of full-blown outbreaks in treated stands.

It is hoped that the DFTM pest management system will serve as a prototype for the development of pest management systems for other defoliating forest pests. Work has been initiated on the western hemlock looper, *Lambdina fiscellaria lugubrosa* Hulst, and it is hoped that a similar management technique can be developed for this insect.

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Ultra-Low-Volume Aerial Application in Forest Protection

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ABSTRACT The Canadian-Czechoslovak ultra-low-volume (ULV) air-application technology project was conducted from 1991-92. The goals of the project were transfer and modification of Canadian technology and experiences with ULV aerial application of microbial and chemical insecticides for leaf eating insect control into the former Czechoslovakia forest protection program. Two experimental field applications were carried out in spruce and oak forests during 1992. The total sprayed area was 800 ha and experts from both sides participated in these trials. The experimental and practical ULV technology spray program was used for control of *Lymantria dispar* and *L. monacha* on 41,700 ha from 1992 until 1995. A Z-37-Turbo airplane with Micronaire AU 4000 rotary atomizers was used for ULV application of Foray FC, Trebon 30 EC, Trebon 10 F, Dimilin 45 ODC and Dimilin 48 SC.

Materials and Methods

IN 1992, DEMONSTRATIONS were carried out on two experimental plots with a total area of 400 ha in spruce stands of the Forest District Telc and on two experimental plots with a total area of 400 ha in oak stands of the Forest District Lanzhot (Fig. 1). The objective of the experimental applications in spruce stands was to control *Lymantria monacha*, and in the oak stands to control *Lymantria dispar*.

The basic objective of these experimental applications was to confirm the correlation between the deposit of droplets onto spruce needles and oak leaves and the biological effectiveness of the applied preparation.

On 25 April, 1992, in spruce stands and on 2 May, 1992, in oak stands, 2.5 liter/ha (31.75 BIU/ha) of Foray FC, stained with the food colorant Rhodamin, was applied. The Z-37-T airplane was equipped with Micronaire AU 4000 rotary atomizers, a flow meter and application monitor. Important technical data about the course of the applications (time of application, dosing, speed of atomizers) were recorded by a mini-computer in the airplane. Meteorological conditions (wind force, temperature) prior to and during the application were measured with a mini-probe at the height of the airplane during application and at other height levels.

Pages 155-165 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

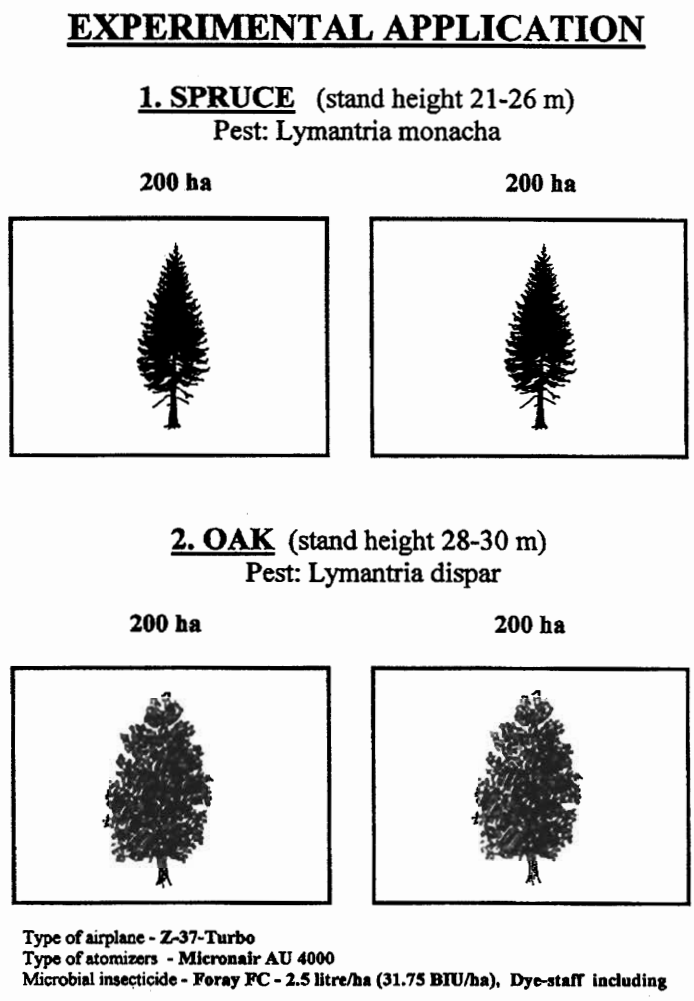

Figure 1. The basic information about experimental plots, tree species, pest, application equipment and volumt of B.t.

Great attention was devoted to studying the penetration of droplets into the stands and evaluating droplet size and the degree of coverage. Partially budded spruce and/or oak transplants were placed on 20 sample trees in each of the spruce and oak stands by means of a simple pulley fixed in the tree tops. Three transplants were placed on each sample tree, i.e. in the upper, middle and lower part of the crown. In addition, an artificial metal twig was fixed to each transplant to intercept the spray droplets (Fig. 2).

Immediately after application, the metal twigs and the needle and leaf samples from the transplants and sample trees were transported to the Department for Forest Protection laboratory of the Mendel University of Agriculture and Forestry in Brno. Droplet size and number of droplets were measured on spruce needles and oak leaves. In total, 7,200 spruce needles were examined. Droplet size in spruce stands was estimated by measuring a set of 600 drops on the artificial twigs, 600 drops on the transplant needles and 180 drops on the needles of mature trees. Droplet size in the oak stands was determined by measuring a set of 300 drops while the number of droplets was measured in 60 places, always on 10 cm^2 of both

the upper and the bottom side of the leaves. Evaluations of the spray composition, in terms of droplet size, volume and estimation of the volume mean diameter (VMD), were performed on a computer according to the respective program.

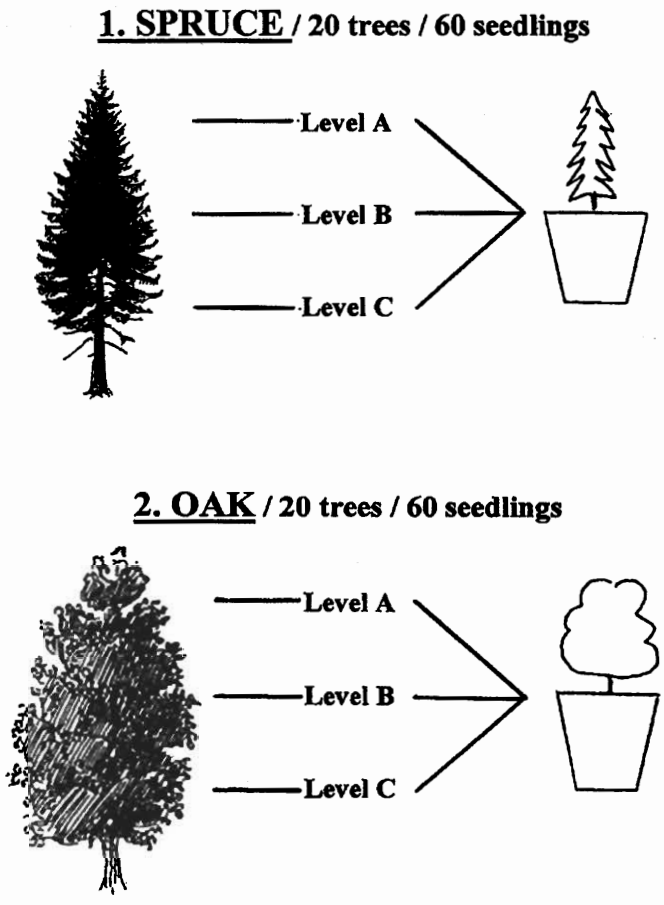

Figure 2. The basic information about tree species, number of control trees and seedlings and placement of seedlings containers during experiments concerning on ascertaining of droplet deposit.

Evaluations of the biological efficiency were based on the mortality of *Orgyia antiqua* caterpillars (2nd instar) and *Lymantria dispar* (3rd and 4th instar) released on the spruce and oak transplants. Following application, 60 spruce and 60 oak transplants in containers were taken down from the tree tops and 10 *Orgyia antiqua*, and/or *Lymantria dispar* caterpillars, covered with monofil, were placed on each transplant. Mortality was evaluated at 5-day intervals for 15 days following treatment under field conditions. A control series was simultaneously established where the caterpillars were released onto untreated transplants.

In 1992 and the following years, pilot and operational aerial ULV applications were carried out based on information obtained from these demonstration applications. In 1992,

pilot applications were made on 100 ha in the LHC Cifáre and on 50 ha in the Forest District Hodonín. Here, the bioinsecticide Foray FC was applied against outbreaks of gypsy moth, *Lymantria dispar.* The following operations in 1993 and 1994 in South Moravia were aimed against the same pest using the same preparation on 5,580 ha, and in 1994-1995 in Slovakia against *Lymantria dispar* and looper moths on 8,420 ha. During the outbreak period of *Lymantria monacha* in the Czech Republic in 1994 and 1995, the ULV technology of application was dominant and was applied on ca 98% of the total area of 28,234 ha. Although Foray FC (i.e. 5 liters/ha) and Trebon 30 EC (and/or Trebon 10 F) were used more frequently, Dimilin 45 ODC (and/or Dimilin 48 SC) in a volume dose of insecticide mixture of 10 liters/ha were used to a lesser extent.

Results

Demonstration applications. Technical data on the ULV demonstration applications are given in Table 1. The table shows that on 25 April, 1992, the flight of the airplane during the treatment of 400 ha of spruce stands, with 94 operational transits, lasted 2 hours and 30 minutes, including departure from and arrival to the advanced airfield 4 km away. The treatment of oak stands of the same area, involving 103 operational transits, lasted 2 hours and 6 minutes.

Table 1. Summary of spray emission parameters recorded by onboard datalogger

Parameters	Conifer sprays	First oak spray	Second oak spray
Start	06:49	08:28	18:30
End	10:18	09:15	19:49
Break	08:21 - 09:22	-	-
Total swaths	94	48	55
Mean flow± s.d. (l/min.)	10,89 ± 2,29	10,93 ± 6,39	16,41 ± 6,43
Mean atomiser speed ± s.d. (r.p.m)			
Outer starboard	9427 ± 699	8795 ± 613	8680 ± 613
Outer port	9708 ± 616	8780 ± 582	8929 ± 613
Inner port	8131 ± 531	7685 ± 525	7718 ± 628

Measurements of the wind force and temperature using a minioprobe (Tables 2 and 3) showed that the wind force at the airplane level at the time of the treatment of spruce stands fluctuated from 2 to 4 m/sec and the temperature ranged from 7 to 11°C. The average rate of flow of the six atomizers at the time of application was 10.89 ± 2.29 litres/min and the average speed of the atomizers ranged from 8,131 ± 531 to 9,708 ± 616 revolutions/min. The wind force at the airplane level at the time of the treatment of oak stands ranged ca from 1.4 to 3.8 m/sec and the temperature ranged between 14 and 17°C. The average rate of flow of the six atomizers at the time of application was 10.93 ± 6.39 to 16.41± 6.43 liters/min and

the average speed of the atomizers ranged between 7,685 ± 525 and 8,929 ± 613 revolutions/min.

Table 2 Minisonde data, No. 1 test site, near Telc (Saturday, April 25, 1992)

Elevation (m)				Wind vel. (m/s)				Wind direction (deg., mag.)				Temperature (°C)			
1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-	6.4	3.4	9.1	12.5
7.9	52.7	12.9	9.6	0.8	2.0	0.7	0.9	150.0	160.0	164.0	163.8	7.0	8.0	10.3	11.7
35.4	79.4	56.3	75.3	4.6	2.1	4.6	2.9	159.4	189.2	176.4	188.3	9.0	10.0	11.9	12.3
69.7	130.1	104.2	157.2	6.2	4.9	5.1	4.0	185.5	198.8	205.5	194.4	10.2	10.4	12.2	13.9
112.6	179.0	166.1	234.7	2.5	2.8	4.1	5.6	257.4	219.8	226.0	200.2	10.0	10.9	12.3	14.5
158.8	226.6	223.7	311.2	0.8	3.2	3.3	9.5	314.1	219.7	216.1	206.6	9.8	11.0	12.1	13.9
189.2	271.7		445.4	0.6	3.6		16.1	281.1	223.4		212.8	9.5	10.9		13.6
211.8	309.8			0.8	3.1			286.2	221.0			9.7	10.9		
226.5	348.9			0.9	2.2			335.3	208.8			9.7	11.0		

Release Times: 1st = 6.10 a.m., 2nd = no data, 3rd = 7.59 a.m., 4th = 9.01 a.m.

Table 3. Minisonde data, No.2 test site, near Zidlochovice (May 2,1992)

Elevation (m)			Wind vel. (m/s)			Wind dir. (deg., mag.)			Temperature (°C)		
1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
0.0	0.0	0.0	-	-	-	-	-	-	14.4	16.0	17.6
53.1	50.0	139.8	3.8	1.4	9.8	149.0	145.1	123.8	14.1	14.8	17.1
79.9	104.8	188.3	6.0	2.8	2.8	148.5	130.8	143.6	14.0	13.4	16.7
151.0	257.9	284.8	8.0	9.2	9.5	151.5	136.9	145.4	13.6	13.2	16.5
196.6	284.2	436.9	8.2	9.4	16.8	149.2	139.0	149.8	13.4	12.8	15.9
218.8	303.1	512.0	4.3	4.9	9.7	147.3	140.5	159.2	12.8	12.4	15.6
250.3	353.4	594.6	2.8	6.9	10.3	147.0	142.0	160.4	12.4	11.8	15.2
263.5			3.0			148.3			12.2		
261.0			2.7			150.0			11.8		

Release Times: 1st = 7.56 a.m., 2nd = 8.34 a.m., 3rd = 9.04 a.m.

Table 4 gives the number of droplets on spruce needles, artificial twigs and oak leaves. The average length of spruce transplant needles was 9.6 mm, and 14.9 mm for mature trees. On the oak leaves, 75% of the droplets were found on the upper side and 25% on the bottom side of the leaves.

Table 4. Number of droplets on the spruce needles (per needle) and oak leaves (per cm^2)

	Tree crown levels			
Tree species	Top	Middle	Bottom	Average
Foliage simulators (needles)	2.58	3.62	2.99	3.06
Spruce- seedling needles	0.20	0.37	0.29	0.29
Spruce- tree needles	0.90	0.36	0.32	0.53
Oak leaves	0.95	0.86	0.63	0.81

Tables 5, 6 and 7 show the results of evaluations of droplet size at the respective heights. Table 8 gives a survey of results of evaluations of the efficiency (mortality) of *Orgyia antiqua* and *Lymantria dispar* caterpillars released on the treated transplants.

Table 5. Magnitude of droplets on the spruce needles and oak leaves (average in μm)

	Tree crown levels		
Tree species	Top	Middle	Bottom
Foliage simulators (needles)	87.1	168.3	93.5
Spruce- seedling needles	95.0	84.8	76.0
Spruce- tree needles	80.9	81.5	76.5
Oak leaves	197.8	229.0	141.5

Table 6. Percentage of droplets by droplet size categories

	Tree crown levels								
	Top (A)			Middle (B)			Bottom (C)		
	Droplet size categories (μm)								
Tree species	0-50	51-150	over 150	0-50	51-150	over 150	0-50	51-150	over 150
Foliage simulators	44.6	54.4	1.0	41.8	53.4	4.8	39.3	59.6	1.1
Spruce- seedling needles	52.9	45.7	1.4	47.8	51.1	1.1	45.3	54.7	0.0
Spruce- tree needles	43.1	56.9	0.0	42.6	57.4	0.0	52.9	47.1	0.0
Oak leaves	21.0	68.5	10.5	24.3	61.6	14.1	33.9	59.3	6.8

Table 7. Percentage of spray volume by droplet size categories

	Tree crown levels								
	Top (A)			Middle (B)			Bottom (C)		
	Droplet size categories (μm)								
Tree species	0-50	51-150	over 150	0-50	51-150	over 150	0-50	51-150	over 150
Foliage simulators	5.6	77.7	16.7	2.3	38.5	59.2	3.8	82.9	13.3
Spruce- seedling needles	4.8	73.4	21.8	6.3	73.9	19.8	6.6	93.4	0.0
Spruce- tree needles	6.9	93.1	0.0	6.4	93.6	0.0	9.9	90.1	0.0
Oak leaves	0.5	30.7	68.8	0.4	22.8	76.8	1.5	53.1	45.4

Table 8. *B.t.* efficacy on spruce and oak seedlings (Czechoslovakia 1992)

Plot	Mean % survival
Control (spruce)	39.33 ± 11.43
Spruce (Level A+B+C)	9.17 ± 7.71
Spruce (Level A)	9.50 ± 8.26
Spruce (Level B)	7.50 ± 9.11
Spruce (Level C)	10.50 ± 12.76
Corrected larval mortality (A+B+C): 76.69 ± 19.61	
Control (oak)	70.00 ± 13.65
Oak (Level A+B+C)	22.63 ± 15.84
Oak (Level A)	22.11 ± 15.84
Oak (Level B)	27.89 ± 17.82
Oak (Level C)	17.89 ± 15.12

Corrected larval mortality (A+B+C): 67.67 ± 11.73

The survey of the above results confirms that the demonstration treatments of spruce and oak stands were carried out under favorable meteorological conditions. The flow rate of the individual atomizers was less than 2 liters per minute, which is one of the prerequisites for producing the desired spectrum of droplets. Due to the operating speed of the airplane and maximal angle of blades, the number of revolutions of the atomizers was on the very limit of the operational maximum (i.e., 9,000 - 10,000 revolutions); the required optimal value would be 12,000 revolutions/min. This influenced the size of the droplets bioinsecticide produced, which was within the range required for this method of application. However, a spectrum of smaller droplets would have been optimal. This was again associated with the coverage of drops, which was indeed very balanced over the whole profile of spruce and oak crowns;

however, the observed values were closer to the limit of the required minimum. In spite of this fact, the biological efficiency against the vapourer moth and the gypsy moth was relatively good.

Small technical defects that occurred during both applications, were accurately identified and explained thanks to the computer data collected during the course of the experiment. These included a defect in the functioning of the flow meter and an error in the control of the application equipment.

The entire program of the project has been accomplished. The possibilities of aerial ULV applications were demonstrated under our conditions and all the participants were provided with information about all the circumstances necessary to apply this technology in practice.

Pilot and operational application. The biological efficiency of ULV applications (4 liters/ha) was compared with classical applications (100 litres of mixture/ha) and its time dynamics were simultaneously studied in detail when applying the Foray FC biopreparation against populations of *Lymantria dispar* (Table 9). No substantial differences were observed when comparing classical and ULV applications. In the first 7, days the efficiency of ULV applications was higher than 95%, sufficiently protecting the leaf area. A similar result was also reached after conventional applications by spraying.

Table 9. Efficacy comparison of LV (100 l/ha) and ULV (4 l/ha) aerial application of FORAY 48B against gypsy math larvae (L2)

Plot (locality)	Spray applicators and spray volume	Efficacy in % (Abbott formula) after 7 days	after 14 days
Bykaret (LV)	Tee-jet (100 l/ha)	93.9	97.3
Mortality on control plot (locality Tajná)		2.6	9.3
Šenkvice (ULV)	Micronair 4000 (4 l/ha)	95.7	97.0
Mortality on control plot (locality Senec)		3.1	5.4

Evaluations of the time dynamics were interesting (Fig. 4) and showed that during the first 5 days after treatment, the efficiency exceeded 90%. The mass mortality of caterpillars occurred between the 3rd and 5th days. Between the 5th and 14th days after application, the efficiency of the Foray FC bioinsecticide did not increase more than 5%. The efficiency of application ULV treatments was found to be optimal, i.e., within 48 hours the population of the pest was infected and within 120 hours mass mortality occurred. This course guarantees that feeding will stop in time to protect the assimilation and reproductive capacity of the host tree.

The results of ULV applications of three different doses of the bioinsecticide Foray FC (Fig. 5) indicated that the doses per hectare of strong concentrations of *B.t.* preparations against *Lymantria dispar* could be reduced. At the same time, they showed that it would be possible to model the per hectare doses of the preparation dependent upon the vitality of the pest and on other conditions to be applied after treatment.

Even after operational applications using Foray FC bioinsecticide against *Lymantria dispar* caterpillars, the effect was altogether quick, particularly against 1st and 2nd instars. In favorable weather conditions, the feeding of the pest stopped within the first two days of the treatment. If the pests were very numerous, or if the applications were carried out later, (i.e., during the 2nd and 3rd instars), and in less favorable weather conditions, part of the caterpillar population survived and the population was not completely destroyed until immediately before pupation.

In 1994 and 1995, the results of aerial ULV treatment against *Lymantria monacha* caterpillars were different; in this case, three types of preparations were used. In the most seriously infested localities, with as much as several thousand caterpillars on one tree, the preparation Trebon 30 EC was used, i.e., 0.2 liter/ha dispersed in 9.8 liter/ha of Petropal oil carrier. The effect of this combination was very quick and effective, with 80-100% mortality. Similarly, the results of Dimilin 45 ODC, applied at a rate of 0.2 liter/ha in the same dose of oil carrier, were somewhat slower, but they were reliable and there was no pest feeding in the treated areas. In 1994, 5 liters/ha of the Foray FC biopreparation were applied to some areas heavily infested with pests to protect drinking water. The weather conditions became worse (cold, rain) immediately after application and the effect of the preparation decreased to 30-50% mortality, so that in places with critical amounts of pests, heavy foci of feeding appeared.

On the contrary, when warm and dry weather prevailed in 1995 after the application of the same preparation in the same dose, the efficiency of the bioinsecticide was on the same level as Trebon (almost 100%).

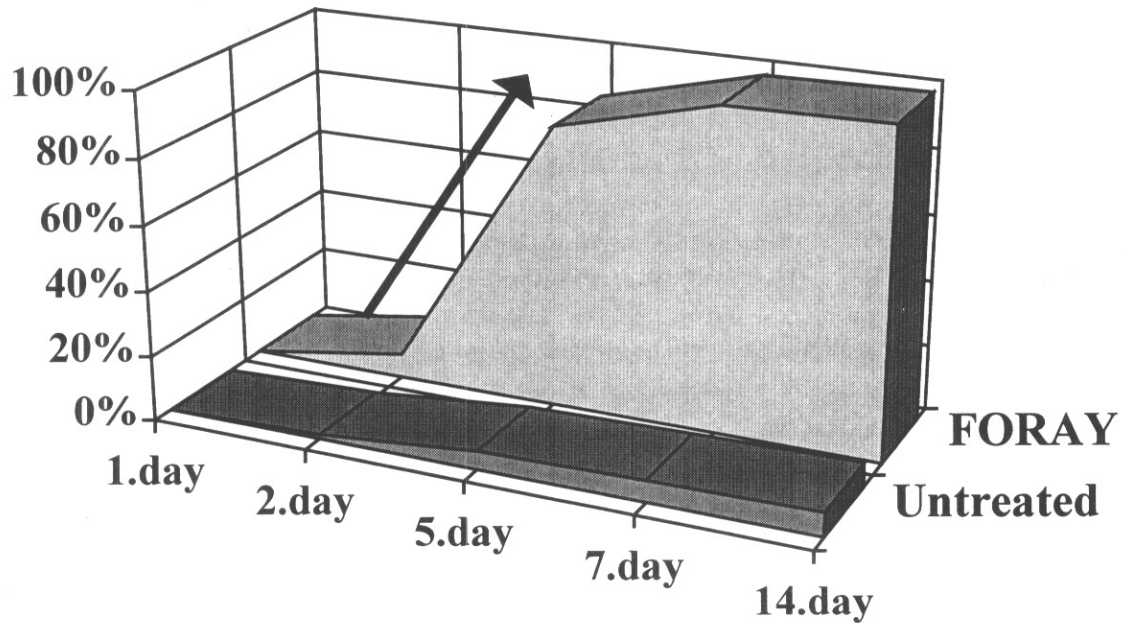

Figure 4. The distribution over time of ULV aerial application efficacy of *Bacillus thuringiensis* (gypsy moth larvae L2).

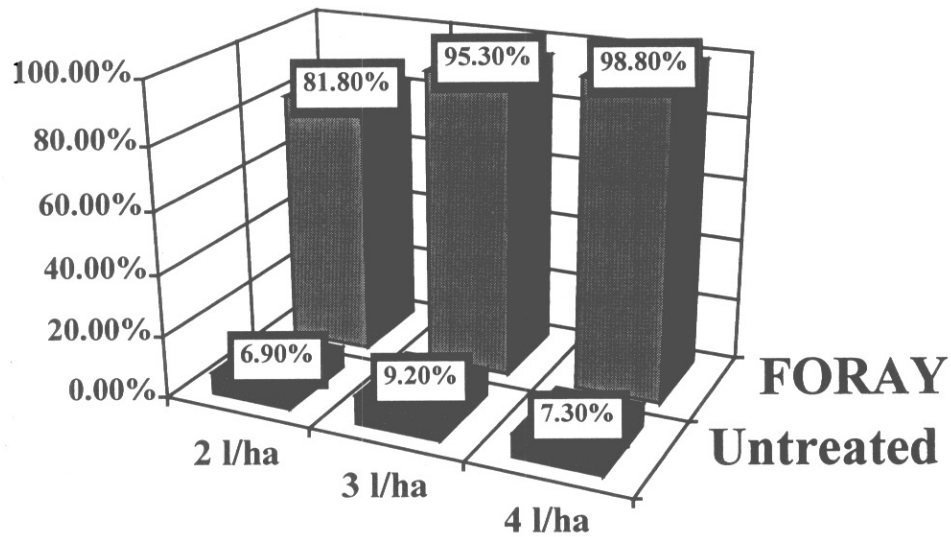

Figure 5. The efficacy of ULV aerial application of *Bacillus thuringiensis* against gypsy moth larvae (L2).

Conclusion

Technology transfer of ULV air-application of microbial and chemical insecticides from Canada into Czech Republic and Slovak Republic was made during 1991-92 based on common research program fulfillment and modifications for Central European conditions to provide an economic method of insect pest control.

The experimental, demonstration and routine ULV applications between 1992-95 have produced the following experience and knowledge about factors limiting the use of ULV technology in the Czech and Slovak Republics. These conditions include:

Suitable type of airplane	Z-37-Turbo
Suitable type of atomizers	Micronair AU 4000
Rotor blade of rotary atomizers	elongated
Optimal hectare dosage	3-5 liters
Fly-speed of aircraft	160 km per our
Optimal flow (1 atomizer)	2 liters per minute
Optimal flow (6 atomizer)	12 liters per minute
Optimal speed of atomizers	10 000 - 12 000 rotations per minute
Swath	20-30 m
Optimal size of droplets (coniferous forests)	15-60 µm
Optimal size of droplets (deciduous forests)	50-150 µm
Optimal number of droplets per	2 per needle
	0.6-1.0 per 1 cm^2 of leaf
Wind speed limit	3 m per sec.
Optimal wind speed	1-2 m per sec.
Wind direction	into treated area

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Atta texana, Texas Leaf-cutting Ant, on Typic Quartzipsamments: Ecological Considerations

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ABSTRACT Pine plantations on Typic Quartzipsamments in East Texas are difficult to establish. Forest management options following clearcutting are limited. An 8-year regeneration study of the growth and survival of loblolly, *Pinus taeda*, L. shortleaf, *P. echinata* Mill., slash, *P. elliotii* Engelm and longleaf pines *P. palustris* Mill. was conducted to determine optimum tree species and treatments for reforestation, and to recommend practical alternative land uses and management strategies for Typic Quartzipsamments. Successful regeneration provides new opportunities for insects and pathogens. Impacts of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), the Deodar weevil, *Pissodes nemorensis*, Germar, Annosus root rot, *Heterobasidion annosum* (Fr: Fr) Bref, fusiform rust, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. fusiforme (hedge and N. Hunt) Burdsall and G. Snow and the Texas leaf-cutting ant, *Atta texana*, (Buckley) will be discussed in the context of droughty site management.

IN THE UPPER Gulf coastal plains of East Texas, sandhills are droughty where alluvial and marine deposits of relatively recent geological origin occur (Burns and Hebb 1972). These droughty sites occur on broad, slightly convex interstream divides at elevations ranging from 100 to 250 meters above sea level and range in depths from 2 to 9 meters. In Nacogdoches and Rusk counties, these sandhills are characterized by Quartzipsamments developed on outcrops of the Carrizo formation, continental stream deposits formed during the Eocene series of the Tertiary system. The Tonkawa soil series is classified as thermic coated Typic Quartzipsamments, and accounts for approximately 5000 ha in Nacogdoches, Rusk, Panola and San Augustine counties (Dolezel 1980). These soils are characterized by low fertility, rapid permeability and extreme acid reaction.

The original vegetation on the sandhills was an association of longleaf pine (*Pinus palustris* Mill.), turkey oak (*Quercus laevis* Walt.) and bluejack oak (*Quercus incana* Bartr.), commonly called scrub oaks, and pineland three-awn (*Aristida stricta* Michx.), commonly known as wiregrass (Hebb 1957). The primary land use on Tonkawa soils today is woodlands (site index averages 55 at 50 years for shortleaf pine, *Pinus echinata* Mill.), although the potential for pine is low due to the droughty and infertile nature of the sand. Watermelons can be grown, but potential is low for any other cultivated crops. Sandhills are resistant to erosion and are considered important ground water recharge areas.

From 1973 to 1975, approximately 1400 ha on Tonkawa were clearcut, followed by extensive site preparation. Removal of all organic matter and surface litter from the site exposed the bare mineral soil to the sun and wind, which greatly decreased the moisture holding capacity of the soil and increased surface temperatures (Kroll et al. 1985). Repeated attempts were made to regenerate the area without success. Intensive management on this sensitive site provided incentive for a regeneration study.

Pages 166-173 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

From 1983 to 1990, a study was conducted (Tracey et al. 1991) on the site to determine the survival and growth of seven species/treatment combinations. These included: 1) untreated loblolly pine, *Pinus taeda* L., 2) Terra-Sorb-treated loblolly pine, 3) kaolin clay slurry-treated loblolly pine, 4) untreated slash pine, *P. elliotii* Engelm, 5) Terra-Sorb-treated slash pine, 6) kaolin clay slurry-treated slash pine, and 7) containerized longleaf pine. The objectives of this study were to determine optimum tree species and treatments for reforestation, and to recommend practical alternative land uses and management strategies for Typic Quartzipsamments.

Containerized longleaf pine yielded the highest survival (> 50%) throughout the study, followed by Terra-Sorb-treated loblolly pine (38%); all other treatments were unacceptable (below 30% by the end of the eighth year). Tracey et al. (1991) recommended: 1) Encourage harvest systems that minimize site exposure and leave residual overstory; underplant pine; avoid clearcutting. 2) Site preparation on previously clearcut sites must be accomplished with minimal site disturbance and topsoil displacement. 3) Reforest droughty sites in East Texas with longleaf pine using container grown seedlings or loblolly pine treated with Terra-Sorb. 4) Manage for non-timber resources, including wildlife, limited recreation and groundwater protection.

Successful regeneration provides new opportunities for insects and pathogens. Artificial monocrop systems in forestry are of recent origin and their effects on the emergence of new pests and diseases are more likely to be the direct result of environmental change (Way 1981). Heavy winter and spring precipitation followed by periods of drought during the summer for the past two years, in combination with soil characteristics have caused undue stress to trees. Minor impacts caused by insects and pathogens on the Tonkawa series include the Nantucket pine tip moth (NPTM), *Rhyacionia frustrana*, (Comstock), the deodar weevil, *Pissodes nemorensis* Germar, annosus root rot *Heterobasidion annosusm* (Fr: Fr) Bref, and fusiform rust, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Hedge and N. Hunt) Burdsall and G. Snow.

Slash pine found scattered throughout the study area grows well on low lying soils with characteristics of Aquic Quartzipsamments. The moisture content of these soils ensured excellent regeneration. Pathogens associated with slash pine, such as Annosus root rot and fusiform rust, are problematic.

The deodar weevil is a minor insect pest on the Tonkawa soil series. The weevil is found throughout the study area in low lying areas. The life cycle of this weevil differs from others in that oviposition occurs in the fall and the larvae feed on terminals during the winter. Adults emerge in the spring and remain inactive during the summer. Adult weevils feed on the inner bark, often girdling a stem or twig. Weevil damage to terminals and the main stem of planted 4-5-year-old loblolly pines was documented in areas of increased surface moisture due to record precipitation throughout the Tonkawa study site.

The Nantucket pine tip moth (NPTM) is widely distributed throughout the eastern and southern United States. NPTM is a larval feeder on the meristematic tissue of young pines, causing significant damage, particularly in areas where forest regeneration practices favor its proliferation (Yates et al. 1981). Larval feeding severs the conductive tissue in the tip, causing it to turn brown and die. Infestations can result in growth loss, excessive branching, multiple terminals and deformed bushy trees and is of primary importance in even-age management of loblolly and shortleaf pines. While NPTM is a major forest insect pest in

pine plantation management, on the Tonkawa study site it is a secondary pest compared to the impacts caused by the Texas leaf-cutting ant, *Atta texana* (Buckley).

Atta texana, confined to Texas and Louisiana, is the northernmost representative of this most specialized genus of Attini, a New World tribe of fungus-growing myrmicine ants. The range of the ant occupies much of the area of Texas and Louisiana lying between 92.5 and 101 degrees of longitude. In Texas, the range extends from near the Oklahoma border to the extreme southern border, with an extension into northeastern Mexico as far south as Vera Cruz.

Atta texana shows a decided preference for nesting in sandy or sandy loam soils, but is also capable of nesting in heavy soils and those of limestone origin (Smith 1963). These nesting areas (mounds) are most often found on the tops and sides of ridges where the water table is deep and nests can reach depths of 25 feet (Moser 1967, 1984). *Atta texana* overturns the soil when excavating tunnels and chambers. In building these tunnels and chambers, materials transported to the surface by ants are mixed with body fluids to form uniform pellets of soil (Weber 1966). The tunnels and chambers that *A. texana* constructs in the soil are numerous and extend deeper than those of vertebrate animals. The nest area is usually marked by crescent-shaped mounds about 15 to 30 cm in height and about 30 cm in diameter. Nests are conspicuous and abundant, reach sizes of 15 to 25 m across, and have a decided impact on the forest landscape.

Atta texana shows a decided preference for grasses, weeds and hardwood leaves. These leaf parts are gathered and used to cultivate their fungus. They prune the vegetation, stimulate new plant growth, break down vegetable material rapidly and in turn enrich the soil (Hölldobler and Wilson 1990). *Atta texana* is a forest pest because it cuts the needles from both natural and planted pine seedlings. The pines usually escape destruction as long as there is other green vegetation, but in the winter pine needles satisfy the ants' need for green plant material (Moser 1967). Spatial distribution of *A. texana* is based on suitable habitat availability. The clearcutting disturbance of the study site quickly became a matrix (the most extensive and most connected landscape element type present, which plays the dominant role in landscape functioning) of ideal ant habitat. Ant densities are normally higher in secondary than in primary vegetation (Haines 1978). Nest dimensions are significantly correlated with distances foraged by various species of leafcutters (Fowler and Robinson 1979). *Atta* foraging patterns are influenced by the availability and locations of preferred plant species in its territory (Waller 1982). Adaptations in their pattern of the nest distribution enable ants to use the food available in the habitat more effectively and to reduce the unfavorable results of competition among societies, which limit their reproduction and numbers (Cherrett 1968).

Objectives

The objectives are to:

1. Determine the overall effects of *Atta texana* on soil texture and organic matter within the mound and adjacent areas;
2. Estimate the landscape area affected by *Atta texana* on different sites within an area on the Tonkawa soil series of thermic coated Typic Quartzipsamments; and
3. Discuss educational activities associated with *A. texana*.

Methods

The study area is located along the FM 1078 road corridor (right of way) and an area of regeneration north of camp Tonkawa, located in northern Nacogdoches and southern Rusk Counties, 10 km west of Garrison, Nacogdoches County, Texas. Distribution of the known nesting areas of *A. texana* was examined on this ecosystem. This study area encompasses many sandy soils and loams that are capable of sustaining *A. texana*. *Atta texana* shows a decided propensity for the Tonkawa soil series of thermic coated Typic Quartzipsamments for their mounds.

Soil samples were collected from 30 *A. texana* mounds found on the Tonkawa soil series. Samples were taken on the surface and at depths of 15 and 50 cm on the *A. texana* mounds (an area currently being impacted by *A. texana*). This procedure was replicated on the inter-mound area (an area once affected by *A. texana*) and from a control area of similar physical characteristics away from the area of influence for a total of nine samples per mound. All soil samples were catalogued, oven dried, and sifted with a 10-gauge soil sieve. Loss on ignition methodology of each soil sample was processed in a muffle furnace at a temperature of 500° C. This determines the percentage of organic matter lost to the nearest 0.01%. Bouyoucos analysis (Bouyoucos 1962) was performed on 100 grams of each soil sample to determine the percent clay, percent silt and percent sand.

Using aerial photographs and ground truthing, all mounds and foraging openings were located in the regeneration study area. All nesting mounds and created forage openings were measured in the four cardinal directions (north, south, east and west). This was done to measure the overall impacts of the nesting and foraging territories on the forest landscapes.

Results and Discussion

Atta texana, by overturning the soil when excavating tunnels and chambers, has a profound effect upon organic matter and texture of the Tonkawa soil series. The tunnels and chambers that *A. texana* constructs in the soil are numerous and extend deeper than those of any vertebrate animals. Materials transported to the surface during tunnel and chamber building by ants are mixed with body fluids to form uniform pellets of soil.

Using Bouyoucos analysis to determine soil texture, it was found that *Atta texana* significantly increases the percent clay. The percent clay in the pellets of nest mound craters was statistically more significant than at the inter-mound surface and the control surface (Table 1) at the $\alpha = .05$ level. In comparing percent clay by depth, the mound surface was statistically more significant than the 15-or 50-cm depths at the $\alpha = .05$ level.

Soil brought to the mound surface by *A. texana* is significantly lower in percent organic matter than the percent organic matter present in the soil at the inter-mound and control surfaces (Table 2). Organic matter for the mound at 15-cm and 50-cm is statistically higher than the same depths in the inter-mound and the control areas at $\alpha = .05$ confidence interval.

Atta texana utilized created openings and disturbances (an event or events that cause a significant change from the normal pattern in an ecological system, Forman and Godron 1986) to create nesting areas and benefit from the use of corridors (a narrow strip of land that

differs from the matrix on either side) in their expansion. *Atta texana* is found along the FM 1087 road corridor and along the edges of stream side corridors. In the regeneration areas, *Atta texana* reacted to the monocultural habitat and dispersed in all directions, causing massive destruction to the loblolly plantation in the area.

Table 1. One-way analysis of variance for mean texture percents of Tonkawa soils (Typic Quartzipsamments) tested by site and depth (means ± standard deviations) using the Bouyoucos method

Site	N	Sand	Silt	Clay
Pellets of Nest Mound Craters	30	90.7 ± 3.7	3.7 ± 1.3	5.6 ± 3.0 a
50 cm Beneath Nest Mound Surface	30	92.0 ± 2.4	4.1 ± 1.8	3.9 ± 1.0 b
Internest Surface	30	91.9 ± 2.5	4.4 ± 1.8	3.6 ± 1.2 b

Currently, there are 52 openings found throughout the study area. The total area of the study is 78 ha, or 78,000 sq. m. Total defoliation attributed to *A. texana* accounts for 16,380 square m or 21.5% of the total landscape area. The immediate nesting areas or mounds account for 1.25% of the total area affected by *A. texana*. Not all disturbance areas contain mounds due to natural mound mortality or chemical treatment with methyl bromide.

Table 2. One-way analysis of variance for mean percent organic matter of Tonkawa (Typic Quartzipsamments) tested by site and depth (means ± standard deviations)

Site	Tonkawa Soils (n = 30 sites)
Pellets of Nest Mound Craters	0.92 ± 0.49 de
15 cm Beneath Nest Mound Craters	1.48 ± 0.68 ab
50 cm Beneath Nest Mound Craters	0.89 ± 0.39 e
Nest Mound Surface	1.45 ± 0.66 b
15 cm Beneath Nest Mound Surface	1.27 ± 0.51 bc
50 cm Beneath Nest Mound Craters	0.71 ± 0.29 e
Internest Surface	1.72 ± 0.61 a
15 cm Beneath Internest Surface	1.14 ± 0.50 cd
50 cm Beneath Internest Surface	0.71 ± 0.28 e

Within columns, means followed by the same letter are not significantly different ($P<0.05$) using Duncan's multiple range test.

Current research includes color infrared photographic coverage at a scale of 1:5000 over a 36 by 36 km area to estimate both numbers of mounds present and percentage of the area defoliated by *A. texana*. The photography includes 60 percent end lap and 30 percent side lap for use as stereo pairs. The relationship of *A. texana* to topography and depth above the water table is being examined to develop a landscape model to ascertain the effects of both terrain and location of the ant mounds and the influence of *A. texana* on the forest landscape. Educational activities for the area include its use in teaching forest entomology,

landscape ecology, environmental science and teacher education in environmental science. Evaluation of the influence of *A. texana* on the landscape includes using the components of structure, function and change to evaluate corridors, patch dynamics and the influence on the forest matrix in long-term evaluation of a droughty landscape. The measurement of change on the forest matrix by *A. texana* gives a graphic example of the influence of social insects on the landscape. Critical thinking skills are honed by evaluating the influence of both soils and openings on the landscape as sculpted by the ants.

Recognition that soil color is indicative of soil texture change and perhaps nutrition raises questions of why the pellets are concentrated in the central nest mound area and what are the influences on the ecology of the landscape? The structure of the ant mound, with its integral tunnels and precise angles of these tunnels to both the surface and to the central nest mound (Moser 1984), add to the overall examination of the impact of *A. texana* on the landscape. Questions arise as to why the central nest mound is large (15 to 25 m across with a depth of 15 to 50 cm of soil deposited on the mound surface). Environmental education and natural history interpretation of the site lead to development of education modules and educational sequences that both intrigue and fascinate all ages and levels of education. As an educator, it is imperative that we stimulate those we are entrusted to teach.

Conclusions

While most consider *Atta texana* an economic pest, in nature they are of fundamental ecological importance. *Atta texana* serves an important ecological function of soil amelioration and increases biodiversity, especially on the very sensitive ecosystem of the Tonkawa study area. Its soil-enriching capabilities outweigh its pest status. *Atta texana* is unique with regard to soil preference, its nesting mounds, foraging areas and spatial distribution.

Plantation forestry, particularly pines on droughty sites, is adversely affected by *Atta* defoliation (Cherrett 1986). They are well-adapted for attacking monocultures (Vilela and Howse 1986) and the most disastrous outbreak of *Atta* can be attributed to the introduction of monoculture systems (Hölldobler and Wilson 1990). Repeated efforts at regeneration and control of *Atta texana* in certain areas of the study area have failed. Low site productivity makes intensive forest silvicultural practices unprofitable. Therefore, our recommendations are that: 1) native vegetation be allowed to grow in the openings created by *Atta texana,* 2) the area be managed for wildlife and limited recreation, 3) *Atta texana* be allowed to continue its biological function of soil improvement and 4) the area be utilized as an important teaching aid for forest pest management and forest entomology laboratories because of the unique nature of the area with regard to the presence of pathogens and insects.

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Pathomorphology of Lepidopteran Chorion during Transgeneration Pathogen Transmission

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ABSTRACT The objectives of this work were (1) to study one of the factors influencing the dynamics of forest phytophagous insects, particularly the transgeneration transmission of entomopathogens, and (2) to study the feasibility of forecasting entomopathogen activity in nature based on their influence on morphological characters of the egg stage. For this purpose we compared the morphological characters of the chorion of intact eggs of forest insects with pathological changes in the chorion of infected insects. Scanning electron microscopy was used to study pathological changes on the surface of the chorion. Practically all changes in the structure of the chorion were of a destructive nature and included closed micropyles or total absence of micropylar canals, closed aeropyle, distortions in the orientation of the rib system, alterations in the polygon structure, and specific "wounds". Data from these studies allowed us to propose a method for estimating the activity of entomopathogens within insect populations by examining the external morphology of eggs. This information would be most important in cultivating insects in the laboratory or when estimating the level of infection caused by entomopathogens in the population dynamics of forest-pest *Lepidoptera.*

FORECASTING PEST POPULATION dynamics is one of the key factors required to determine whether control measures will be necessary to protect agricultural crops. This forecast is based on estimating potential fodder base, weather conditions, natural enemies, and infectious diseases. Each of the above factors is vitally important, and each of them may contribute to the decline of a particular species in the ecosystem.

This is particularly important in the case of entomopathogenic microorganisms. The variety of entomopathogen species and the way they affect host populations vary from almost complete collapse of the population (epizootics), to regulating the abundance of pests for longer durations (i.e., chronic diseases).

The objectives of this study were to determine the factors influencing the dynamics of forest phytophagous insects, particularly the transgenerational transmission of entomopathogens, and to study the possibility of forecasting entomopathogen activity in nature based on their influence on the morphological characters of the chorion in the egg stage. For this purpose, we compared morphological characters and changes in the chorion of eggs of certain forest pests from both healthy and the pathological and infected individuals.

Materials and Methods

Cultivation of Insects. Egg masses of several species of Lepidoptera were collected in nature *(Lymantria dispar, Dendrolimus sibiricus, A. apiformis, P. bucephala, Lymantria monacha, Orgyia antiqua*). Insects from laboratory cultures (*Lymantria salicis, L. dispar, Hyphantria cunea, Bombyx mori*) were cultivated on artificial nutrient media.

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Obtaining Infected Progeny. For all experiments, the later larval stages of the target insects were selected. The larvae were infected by applying entomopathogens to the artificial nutrient media or to the leaf surface. Infected insects were stored in containers or in petri dishes (10 insects per dish) until they pupated.

Activating Latent Viruses and Microsporidioses. We attempted to stress the larvae by altering the air temperature or their feeding, by increasing the density of larvae in their containers and by infecting larvae with pathogens that possessed a long incubation period (e.g., CPV).

Estimating Pathogen Influence. We determined the presence of pathogens and their level of activity by assessing:

- the level of mortality of eggs and larvae.
- the presence of the pathogen in the excrement and meconium of insects.
- the pathological changes in the chorion.

We determined the species of pathogens by using transmission and scanning electron microscopy to observe morphological characters of the microorganisms in the affected tissues of the host.

Electron Microscopy. For studying detailed characteristics of *Lepidoptera* chorion and for identifying pathogens, we used ultrathin sections and negative-stained preparations of purified pathogen microstructures.

We surface sterilized the eggs with Triton-X-100 (Serva) followed by subsequent ultrasonic treatment. The eggs were fixed with 2.5% glutaraldehyde in 0.2 M cacodylate or phosphate buffer (pH 7.2 - 7.4) at 0 to 4°C until eggs settled at the bottom of the vessel (sometimes up to 20 -30 days). We dehydrated the samples by using a series of increasing concentrations of ethanol (from 20% to 100%) followed by 100% acetone. Fixed samples were critical point dried in CO_2 using a Hitachi HCP-2 critical point dryer. Samples of insect eggs with strong chorions (*L. dispar, L. salicis, B. mori*) or dry eggs were not fixed. The samples were partially lyophilized. We used hexamethyldisilazane to prevent shrinking of the samples during air drying (Nation 1983). Dry material was used to prepare fractures of the chorion. Gold, gold and carbon, and platinum shadowing was done in a Jeol JFC-1100 ion sputter. The samples were examined with scanning electron microscopes (Hitachi S 405-A Jeol JSM-50A, S-840, Cambridge Stereo Scan) and in a transmission electron microscope (TEM 200-CX).

Results and Discussion

In classical insect pathology the following characteristics are used to diagnose disease in the egg stage of insects: alteration of color, abnormal size, abnormal shape, inability to hatch, traumas and scars, lack of embryonation, and presence of parasites and infections by microorganisms. The condition of the chorion and its pathological changes were not studied in species of Lepidoptera. The infection of eggs with microorganisms is closely related to the transmission of the pathogens between generations. It appears that the only reason for chorion pathological changes is transmission of entomopathogens from the parent generation to the daughter generation. Such transgenerational transmission of pathogens and symbionts was reported previously by several authors. The transmission of the infection may be either

transspermal (Afzelius et al. 1989, Yefimenko 1989,), or transovarial and transoval (Al-Khalifa 1984, Brooks 1968, Hamm and Young 1974, Kitajima et al. 1985, Nair and Jacob 1985).

Data published previously show that transmission of symbiotic microorganisms in insect females takes place in the early stages of the oocyte formation, in germarium, and that infection by pathogens takes place in vitellarium. In cases of severe infection of germarium, development of oocytes normally does not proceed; therefore, eggs are not laid at all or their quantity decreases significantly.

The influence of entomopathogens on morphological characters of the chorion was studied during transovarial transmission of nuclear polyhedrosis virus and microsporidiosis (*Nosema muscularis* W., *Nosema* sp.) in *H. cunea, Euproctis chrysorrhoea, B. mori, L. dispar, O. antiqua, Mamestra suasa, M. brassicae* and *Agrotis segetum*.

Scanning electron microscopy was used to study pathological changes on the surface of the chorion. Practically all changes were of a destructive nature: closed micropyle or total absence of micropylar canals (Fig. 1), closed aeropyle, distortions in the orientation of the rib system, (Fig. 2), alterations in the polygon structure, and specific "sores." The explanation for these damages may be that the transmission of entomopathogenic viruses (their active form) and protozoa takes place during contact of the follicular epithelium with the oocyte during formation of the chorion. Thus, infection of the cells synthesizing the chorion protein leads to interruption of their functions and, as a result, to formation of distortions on the surface of chorion (Fig. 3).

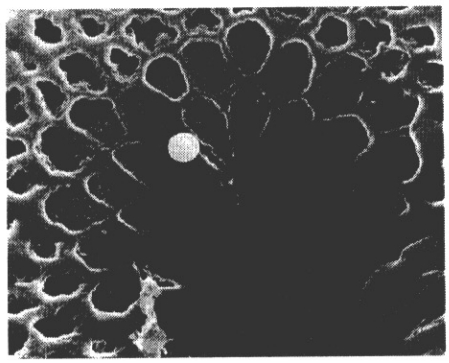

Figure 1. Destroyed micropyle in *Orgyia antiqua* chorion affected by NPV.

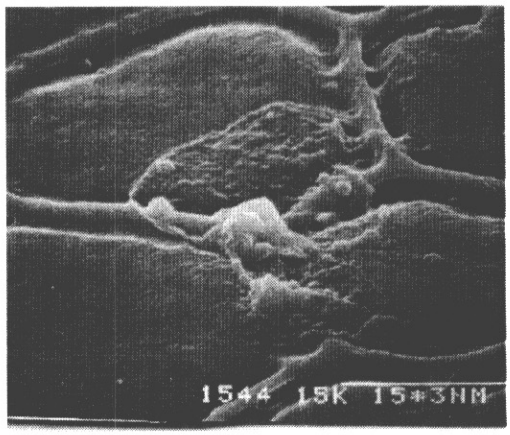

Figure 2. Distortion in the rib system orientation in *Euproctis chrysorrhoea* affected by microsporidia.

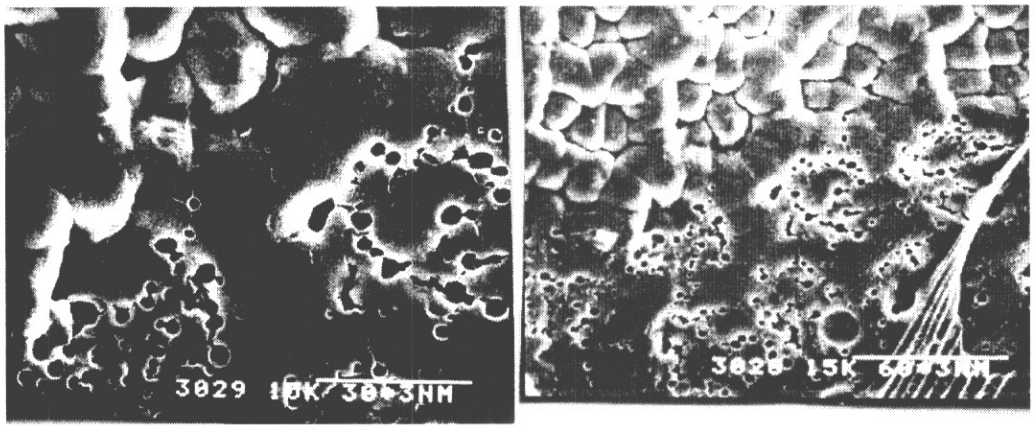

Figure 3. Distortion of chorion of *Bombyx mori* affected by NPV.

Using the scheme of choriogenesis of the silkmoth proposed by Mazur and coworkers (Mazur et al. 1980), we could assume that the most severe changes in practically all chorion structures are associated with heavy infections of the ovariola at III-IV larval stages; the presence of a closed aeropyle may be explained by a comparatively weak influence of the pathogen that leads to formation of "plugs" that did not separate from the top of the aeropyle at the stages Xa – Xd (Fig. 4). To some extent, the above suggestion is confirmed by results obtained during experimental infection of *B. mori* pupae with preparations containing virions of the nuclear polyhedrosis virus.

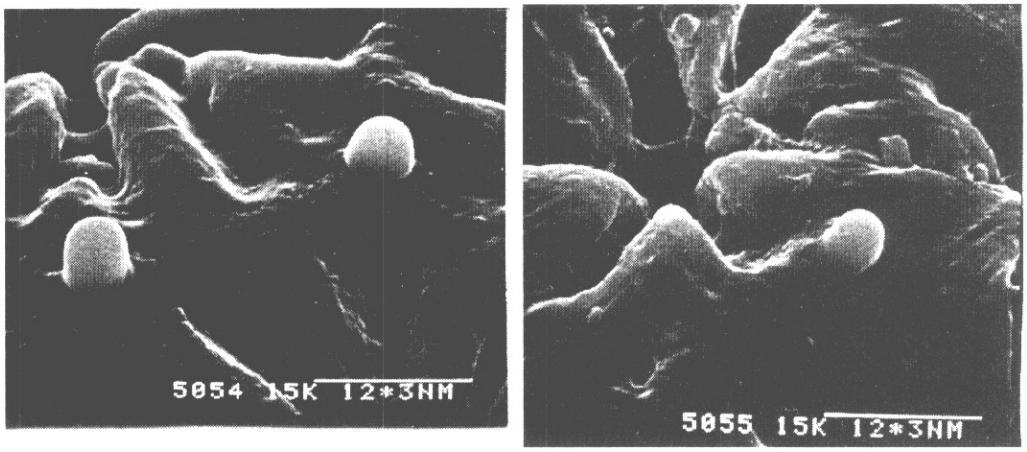

Figure 4. "Plugs" on the aeropyle in *Orgyia antiqua* chorion affected by NPV.

The destructive influence of the pathogen on the follicular epithelium cells and also distortion of vitellogenesis and chorion formation processes were demonstrated (Smith-Johannsen et al. 1986). The pathogen particles were present on the egg surface in 50% of the cases studied, which suggests that there is a coordination between transoval and transovarial pathways of transmission of entomopathogens. The regularity of occurrence that we found may be used to aid in taxonomic descriptions of insects based on the stage of the egg. The data from this experiment allow us to propose a method for identifying the influence of entomopathogens on the egg stage. This is most important in cultivating insects in the laboratory or when estimating the level of infection for use in forecasting the population dynamics of forest-pest Lepidoptera.

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The Effect of Foliage Damage on Transmission of Gypsy Moth Nuclear Polyhedrosis Virus

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ABSTRACT Results of published studies suggest that gypsy moth defoliation may cause elevated tannin levels in trees, which in turn results in reduced larvae mortality caused by the nuclearpolyhedrosis virus (NPV). In a series of field experiments, we tested the hypothesis that gypsy moth defoliation of oaks leads to reduced virus transmission rates. In each of three years, we measured virus transmission rates in gypsy moths feeding on oaks, and tannin levels in oak leaves, with and without experimental defoliation in oak forests with almost no naturally-occurring gypsy moths or virus. In our experiments, we found that there was no effect of gypsy moth defoliation on tannin levels, and consequently virus transmission both in the field and in the lab was unaffected by defoliation. Our results suggest that gypsy moth defoliation does not affect tannin levels early enough in the larval season to have a measurable effect on the interaction between gypsy moth and its nuclear polyhedrosis virus.

NUMEROUS STUDIES HAVE shown that foliage that has been damaged by herbivores changes in ways that can influence herbivore growth or survival (Karban and Myers 1988). One of the more prominent examples of such induced responses is provided by changes in the levels of secondary compounds of oaks following herbivory by the gypsy moth, *Lymantria dispar* (L.). Leaves on red oak (*Quercus rubra*) trees that have experienced significant defoliation contain higher levels of hydrolyzable tannins (Schultz and Baldwin 1982, Rossiter et al. 1988), and these high tannin levels lead to reduced gypsy moth growth rates (Rossiter et al. 1988). More recently, however, this classic story has been complicated by evidence that tannins have an effect on disease transmission by reducing infection rates of the gypsy moth nucleopolyhedrosis virus (NPV), which is consumed with oak foliage (Keating et al. 1988).

Because defoliation can affect oak tannin levels, and oak tannin levels can affect virus transmission, the effect of gypsy moth defoliation on virus transmission has been cited as evidence for a tritrophic interaction among gypsy moths, oaks, and virus (Schultz and Keating 1991). Such an effect would be especially important in gypsy moth population dynamics because the virus has been the most factor causing the collapse of gypsy moth outbreak populations (Elkinton and Liebhold 1990). If defoliation does reduce virus transmission, however, the induced foliage changes in oak could effectively prevent the virus from causing gypsy moth populations to collapse. The possibility of this kind of tritrophic interaction is therefore of great significance for gypsy moth population dynamics. The evidence for this effect is based entirely on either lab data or field observations, therefore the

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objective of our research was to conduct studies to elucidate the effect of gypsy moth defoliation of oaks on the transmission of virus within gypsy moth populations in the field.

Materials and Methods

In our field experiments initited in 1993, we mimicked the natural process virus transmission by placing infected first-instar larvae on oak foliage inside mesh bags along with uninfected third instar larvae (which we refer to as test larvae). After a week, the test larvae were removed and placed in individual cups of artificial diet in the lab. Because the virus requires about 10-14 days to kill larvae in the field, this protocol ensures that only one round of virus transmission occurs. Test larvae that died were autopsied under a light microscope to verify the presence of the virus (Woods and Elkinton 1987). In previous work, we demonstrated that transmission in these experiments is only slightly affected by rainfall (D'Amico and Elkinton 1995), and that the mesh bags have no measurable effect on the concentration of phenolics or hydrolyzable tannins in the confined foliage (Rossiter et al. 1988, Hunter and Schultz 1993).

Study Site. We used mature black oak trees (*Quercus velutina*) in a forest composed of black oak, white oak (*Quercus alba*) and pitch pine (*Pinus rigida*), located on grounds of the Otis Air National Guard Base, Falmouth, MA, USA. In 1994 and 1995, we used 4-6m high red oak saplings in a forest composed primarily of red oak *(Q. rubra*) and red maple (*Acer rubrum*) located in the Cadwell Memorial Forest, Pelham, MA. Neither forest had experienced significant defoliation since gypsy moth outbreaks in the early to mid 1980's. (Otis ANGB - 1986, Cadwell - 1981). In 1993, the trees that we used were part of the canopy, while in 1994 and 1995, the trees that we used were typically in full sunlight along clearings or next to a logging road.

Manipulating Levels of Defoliation. The experiments that we report here consisted of manipulating the extent and timing of defoliation experienced by the trees on which the larvae fed, in order to ascertain how defoliation affects virus transmission. 1993 we used black oaks, for our study while in 1994 and 1995 we used red oak, which is the tree species on which most previous research work has been reported. The timing of our experiments varied slightly from year to year, although all of the experiments took place at that time of the season when virus transmission occurs in natural populations of gypsy moth larvae (see Table 1 for dates). In all of these experiments, we used test larvae that were in the same larval stage as larvae in adjacent natural populations. (1993 - 3rd instars; 1994 and 1995 2nd instars). New trees were used in each year; we were only concerned with quantifying within-season effects.

Because larvae must consume the virus on foliage in order to become infected, defoliation is an intrinsic part of the transmission process. In order to manipulate defoliation levels, we therefore imposed additional defoliation above and beyond what occurred during virus transmission. To accomplish this, in each experiment we established a "damaged" treatment, and an "undamaged" treatment, such that trees in the damaged treatment were exposed to defoliation not just from those larvae that were used to measure virus transmission, but from larvae that were added to effect defoliation (we refer to these as defoliating larvae). The difference in virus transmission rates between the two treatments is a measure of the effects of defoliation on transmission.

The most important differences among experiments were in the extent of defoliation and in the timing of defoliation relative to transmission. In 1993, we infliected defoliation only at the level of the individual branch (about 50% defoliation *within* bags). In 1994 we defoliated both at the level of the individual branch and at the level of the entire tree (30-50% defoliation *inside and outside* the virus-treatment bags). Finally, in 1995 we caused defoliation only at the level of the entire tree (30-50% of tree defoliated *outside* the virus-treatment bags). Hereafter we refer to these experiments as the branch-level, branch- and tree-level, and tree-level experiments, respectively. In the first two experiments, we inflicted defoliation only for the two days before transmission began, but in the third experiment we began defoliation five days before transmission and continued throughout the transmission period. Because defoliation occurred over a longer period in the third year than in the second year, the overall effect was that we increased overall defoliation from experiment to experiment. In other words, as we accumulated negative evidence for any effects of defoliation on transmission, we inflicted increasingly higher levels of defoliation in an attempt to demonstrate a positive effect.

To effect defoliation at the level of individual branches, we added larvae only to the virus transmission bags. Specifically, we added 40 fourth instar larvae to the bags on the damage treatment trees after the initially infected larvae had died. These larvae were removed and discarded after two days, and then test larvae were added to the bags. Because larvae do not avoid virus-contaminated oak foliage (D'Amico unpublished data), the defoliating larvae did not affect the density of virus in bags in the damage treatment. To impose defoliation at the level of the entire tree, we added additional bags that contained only healthy larvae to the trees in the damage treatment (40 fourth instars, no virus, no transmission larvae). To analyze the data from these experiments, we performed one-way ANOVAs on the arc-sine square-root transformed virus mortality.

Laboratory Bioassays. In order to compare better our results to the original published work, which demonstrated the effects of tannins on virus bioassays in the laboratory, we performed a bioassay in conjunction with both the tree-level and branch- and tree-level defoliation experimenst. At the end of the period of transmission in these defoliation experiments, we took samples of some of the unconsumed foliage from the virus-test bags and used them to measure larval infection rates with known doses. First, we surface-sterilized the foliage samples from the two treatments (rinsing thoroughly with distilled water after the bleach treatment), and then added 5 ml of virus (10^6 occlusion bodies per ml) to small (8mm diam) disks from these samples. Each disk was then fed to an uninfected, starved, third-instar larva. To ensure the efficacy of the bleach treatment, we also fed control larvae disks with 5 ml of distilled water instead of bleach. To ensure that all larvae received the same total amount of virus, any larva that did not consume the entire leaf disk after 36 hrs was discarded. The remaining larvae were reared on artificial diet for two weeks to assess levels of infection (Bell et al. 1981). We also assayed foliage from trees from outside of each experimental area which had not experienced defoliation. Finally, in the branch- and tree-level experiments, we looked for an extremely short-term effect of defoliation on oak foliage chemistry by assaying leaves from trees from outside the experiment that we damaged (cut in half with a scissors) 8 h before the assay.

Results

Manipulating Defoliation. No significant differences were observed between virus-caused larval mortality in the damage and no-damage treatments in any field experiment, whether defoliation occurred at the branch level, the tree level, or at both levels. Similarly, there was no effect of larval density on virus mortality in the larval density experiment, and there was no larval density times tree interaction. The results of statistical tests and the mean mortality for each experiment are given in Table 1. In several of these experiments, there was a trend (NS) toward increasing virus mortality with increasing insect density or increasing foliage damage. This is opposite of what we had anticipated since higher densities of insects cause greater levels of defoliation therefore greater induced chemistry effects, which should result in lower virus mortality.

Laboratory Bioassays. In the leaf disk bioassays, there were no significant differences in mean mortality from virus between treatments. Mean mortality and results of statistical tests are again summarized in Table 1.

Table 1. Summary of field experiments and laboratory bioassays and results. Within an experiment, means followed by the same letter are not significantly different (P > 0.05). HT = hydrolyzable tannins, TP = total phenolics

Date	Tree species	Description of leaf treatment	Fraction of larvae dying (SE)		Differences in mortality between treatments detected by ANOVA?		Differences in tannin levels?
			Field test	Leaf assay	Field test	Leaf assay	
June 1993	*Quercus velutina*	previously damaged	0.48 (0.05)		no; $F_{1,36} = 0.21$; $P = 0.65$	-	-
		not previously damaged	0.44 (0.04)				
May 1994	*Q. rubra*	previously damaged	0.92 (0.02)	0.89 (0.03)	no; $F_{1,28} = 3.45$; $P = 0.06$	no; $F_{3,28} = 0.17$, $P = 0.92$	HT; no, $P = 0.09$ TP; no, $P = 0.48$
		not previously damaged	0.84 (0.03)	0.90 (0.03)			
		foliage cut with scissors	-	0.91 (0.01)			
		foliage cut 8 h prior	-	0.93 (0.02)			
May 1995	*Q. rubra*	concurrently damaged	0.60 (0.04)	0.48 (0.03)	no; $F_{1,28} = 0.55$; $P = 0.47$	no; $F_{2,27} = 0.56$, $P = 0.57$	HT; no, $P = 0.27$ TP; no, $P = 0.22$
		not concurrently damaged	0.64 (0.04)	0.52 (0.04)			
		induction control	-	0.47 (0.02)			
July 1994	*Q. rubra*	10 larvae/80 leaves	0.33 (0.08)	-	no treatment effect; $F_{1,29} = 3.78$, $P = 0.06$ no tree effect; $F_{5,29} = 0.22$; $P = 0.95$ no interaction[a]; $F_{5,29} = 2.34$ $P = 0.08$	-	-
		20 larvae/80 leaves	0.32 (0.09)				
		40 larvae/80 leaves	0.35 (0.05)				
		80 larvae/80 leaves	0.46 (0.04)				
		160 larvae/80 leaves	0.46 (0.06)				

[a] Because the interaction effect was not significant, to test for main effects the ANCOVA was redone with a model with no interaction term.

Discussion

Our results suggest that tannin levels are not affected by defoliation at the time when virus transmission occurs in natural gypsy moth populations. Apparently because of this lack of tannin induction, our virus transmission experiments did not show any effects of defoliation on virus-caused mortality, irrespective of the timing or intensity of defoliation,

nor was there any effect of defoliation on virus mortality in our laboratory bioassays. In fact, we were only able to demonstrate a significant effect of defoliation on tannin levels at a site that was defoliated naturally in July. Even in that case, it is not certain whether gypsy moth larvae induced higher tannin levels or simply chose to feed upon hosts with higher levels of constitutive tannin. It is likely also that larval to larvae virus transmission had ceased by the time elevated levels of tannins occurred because larvae were in the process of pupating at the time.

We emphasize that our protocols were designed to eliminate mechanisms that might obscure or counteract induced effects. First, although we are confident that the defoliating larvae did not concentrate virus in the experiments that included branch-level defoliation because larvae do not avoid virus-contaminated foliage, in the tree-wide defoliation experiment and the bioassays any such avoidance effects would be irrelevant. Secondly, although there is uncertainty in the literature as to whether induction effects will occur at the level of the individual branch or the entire tree (Hunter and Schultz 1993, Rossiter et al. 1988), by manipulating defoliation at both levels we allowed for either effect, or both. Finally, because our experiments were performed in synchrony with naturally-occurring gypsy moth populations, in adjacent areas, we believe that they accurately mimicked natural virus transmission processes.

We do not question the finding that tree species affect the susceptibility of gypsy moth larvae to viral infection (Keating and Yendol 1987), or that foliar tannin content may explain differences in virus transmission among tree species. However, our results suggest that the evidence for induction of tannins in oaks is equivocal, especially in the early part of the season (May and June) when most virus transmission occurs in gypsy moth populations. In fact, the results of previous studies have similarly reported low tannin levels in the early part of the gypsy moth season. For example Schultz and Baldwin (1982) and Hunter and Schultz (1995) reported high levels of tannins in July but not in mid-June, when most larval feeding and development occurs.

We realize that our data do not bear on the effects of defoliation between (larval seasons). Although there is a suggestion of such an effect in previous work (Hunter and Schultz 1993), our previous experience with virus transmission in this system (Woods and Elkinton 1987) leads us to suspect that such complex effects do not occur, because the first round of transmission in the gypsy moth larval season occurs on egg masses rather than on foliage.

Our finding that larval density also has no effect on transmission rates is relevant to our earlier work on mathematical models of disease transmission (Dwyer and Elkinton 1993, D'Amico et al. 1996). That is, classical mathematical disease models assume that disease transmission is a linear function of the densities of both host and pathogen. In previous experiments, we demonstrated that this assumption was incorrect, but it was not clear whether the nonlinearity was associated with larval density, virus density, or both. The larval density treatment that we report here strongly suggests that the nonlinearity is not due to host density, because transmission is not affected by larval (i.e. host) density.

In summary, gypsy moths may induce tannin responses in oaks under some conditions, but both our results and the results of others suggest that those conditions do occur at the time of year when most virus transmission takes place. We therefore suspect that

tritrophic interactions among gypsy moths, nuclear polyhedrosis virus, and oak foliage are unlikely.

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Microsporidia Affecting Forest Lepidoptera

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ABSTRACT Forest Lepidoptera are affected by many species of microsporidia which are often important naturally-occurring biological control agents. Although they do not often cause dramatic epizootics, microsporidia interact with their Lepidopteran hosts in a variety of ways. In order to illustrate some of the basic differences that exist among species of microsporidia we discuss the characteristics of three groups of microsporidia: *Nosema*, *Vairimorpha*, and *Endoreticulatus*. Species in the *Nosema* group are moderately pathogenic and are efficiently transmitted, both horizontally and vertically. Vertical transmission occurs usually by transovarial transmission. Species in the *Vairimorpha* group are very pathogenic, are less efficiently transmitted, and are not usually transmitted transovarially. Species in the *Endoreticulatus* group have low pathogenicity, infect only the midgut cells and are transmitted only via the feces. Microsporidia interact both directly and indirectly with most other natural enemies and have an important effect on the population cycles of many species of forest Lepidoptera.

ALTHOUGH MANY SPECIES of microsporidia affect forest Lepidoptera and are often important biological mortality factors, they do not cause dramatic and visible epizootics and frequently go undetected. Microsporidia are important natural enemies of many species of insects but have little if any potential for use as true microbial insecticides (Canning, 1982). Following a brief general introduction to the microsporidia we discuss 1) reports of microsporidia from species of forest Lepidoptera, 2) characteristics of three genera of microsporidia representing different "types" of host/pathogen relationships in forest Lepidoptera, 3) interactions with other biological control agents, and 4) how microsporidia affect forest Lepidoptera.

What are Microsporidia? Microsporidia are unicellular parasitic organisms formerly in the Phylum Protozoa. The Phylum Microspora was erected in 1969 to accommodate this unique group of organisms (Sprague, 1969, 1977). Although the largest number of microsporidian species infect arthropods (especially insects), most animal phyla contain at least a few species that are infected by microsporidia. Microsporidia are small intracellular parasites with an environmentally resistant spore stage characterized internally by the presence of a polar tube and one or more nuclei. Infection occurs when a susceptible insect ingests microsporidian spores. In the gut lumen of a susceptible insect host, spores extrude their hollow polar tube and the sporoplasm, consisting of the cytoplasm, nuclei and other organelles, is injected into the midgut cells of the insect. Extrusion of the spore is caused by specific chemical and environmental conditions in the gut lumen. Injection of the sporoplasm initiates the life cycle of the microsporidium and, depending on the microsporidian species, different tissues may be infected and different types of spores produced. Some species of microsporidia produce chronic infections while others may be very pathogenic and kill the host. In addition to horizontal transmission which occurs by ingestion of spores, many species of microsporidia are transmitted vertically (generation to

Pages 187-197 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

generation) from an infected adult female to her offspring, either on the egg surface or within the egg. The general characteristics of microsporidia are discussed in several excellent reviews (Vavra, 1976a, 1976b).

The extent of microsporidia among species of forest Lepidoptera. Microsporidia are probably far more common among species of forest Lepidoptera than published reports indicate. Because microsporidia do not cause dramatic epizootics such as those caused by fungi and viruses, in which conspicuously large numbers of dead individuals occur, microsporidian infections often go unnoticed and their role in the population dynamics of insects is often not recognized. Nevertheless, microsporidia have been reported as important mortality factors for many species of forest Lepidoptera (Table 1). Based on the number of different microsporidia that have been isolated and/or described from the gypsy moth, *Lymantria dispar* L. (Table 2) we suggest that microsporidia might be much more prevalent in forest Lepidoptera populations than is reported in the literature. A more thorough and comprehensive paper on the systematics of microsporidia isolated from gypsy moth populations in Europe is included in this proceedings (Weiser, 1997). Readers should consult Weiser's paper for more details about the taxonomy of gypsy moth microsporidia. Gypsy moths collected from many areas of Europe have been extensively examined for the presence of insect pathogens. We suggest that many additional species of microsporidia would be recovered from other species of forest Lepidoptera if these species were more closely scrutinized.

Table 1. A partial list of forest insects from which microsporidia have been reported

Common name	Specific name	Reference
Browntail moth	*Euproctis chrysorrhoea*	Puruini and Weiser (1975)
Eastern tent caterpillar	*Malacosoma americanum*	
Fall webworm	*Hyphantria cunea*	Weiser and Veber (1975)
		Nordin and Maddox (1974)
Forest tent caterpillar	*Malacosoma disstria*	Thomson (1959)
Green tortrix	*Tortrix viridana*	Lipa (1976), Franz and Huger (1971)
Larch sawfly	*Pristiphora erichsoni*	Smirnoff (1966), Quednau (1968)
Large aspen tortrix	*Choristoneura conflictana*	Wilson and Burke (1971)
Spruce budworm	*Choristoneura fumiferana*	Thompson (1958)
Uglynest caterpillar	*Archips cerasivoranus*	Wilson and Burke (1978)
Winter moth	*Operophtera brumata*	Canning et al. (1983)

Characteristics of microsporidian genera representing three different types microsporidia/forest Lepidoptera associations. We believe that by separating the microsporidia into the three groups (*Nosema*, *Vairimorpha*, and *Endoreticulatus*), we can better illustrate some of the basic differences that exist among species of microsporidia isolated from forest Lepidoptera and demonstrate how microsporidia may interact with their hosts. Nevertheless we recognize that this separation into three groups undoubtedly represents an oversimplification of the very complex associations that often exist among microsporidia and their hosts. Some described species do not necessarily conform to all of the general features that we ascribe to each group, although they share some characteristics of that group. In addition, we anticipate that some of the species of microsporidia yet to be

described from forest Lepidoptera will not conform to any of the three groups that we have identified.

Table 2. Microsporidia isolated from the gypsy moth, *Lymantria dispar*, and the countries where infected gypsy moths were collected

Microsporidian species	Country	Reference
Nosema lymantriae	Czechoslovakia	Weiser, 1957a
Nosema lymantriae	Yugoslavia	Sidor, 1979
Nosema muscularis	Czechoslovakia	Weiser, 1957
Nosema muscularis	Spain	Romanyk, 1966
Nosema muscularis	USSR, Ukraine	Zelinskaya, 1981
Nosema serbica	Yugoslavia	Weiser, 1964
Nosema serbica	USSR, Ukraine	Zelinskaya, 1981
Thelohania disparis	Not given	Timofejeva, 1956
Thelohania similis	Czechoslovakia	Weiser, 1957a
Vavraia schubergi	Czechoslovakia	Weiser, 1964
Vavraia schubergi	USSR, Ukraine	Zelinskaya, 1981
Nosema sp.	Portugal	Cabral, 1977

Nosema group. A large percentage of the microsporidia reported and/or described from forest Lepidoptera are in the genus *Nosema*. This genus contains more species of microsporidia than any other microsporidian genus, but phylogenetic studies using rRNA sequence data have shown that some *Nosema* species from insect hosts other than Lepidoptera are phylogenetically very distant to the *Nosema* described from Lepidoptera (Baker et al. 1994). These same studies also indicate that *Nosema* species isolated from Lepidoptera are phylogenetically similar.

The genus *Nosema* is characterized by the formation of two spores from each sporont (the basic pre-spore developmental form). These spores are formed singularly and are not enclosed within an envelope. There is only one type of infectious or environmental spore and this spore contains two nuclei. The life cycle is one of the more simple and straightforward among microsporidian genera. Most host tissues are infected (midgut, salivary glands, Malpighian tubules, fat body, gonads, muscles) and these tissues are usually infected sequentially. As indicated in Figure 1, environmental spores are released from infected living hosts in the larval silk and/or frass. Spores may also be released into the environment when an infected host dies and the body disintegrates allowing spores to escape from infected tissues.

Species of *Nosema* are moderately pathogenic; relatively few spores are necessary to initiate an infection, but a large spore dose is typically necessary to produce larval mortality. Figure 1 illustrates how *Nosema*-type microsporidia are transmitted and at what stage mortality occurs. Infected larvae may develop into infected adults and infected females usually transmit microsporidian infections to their progeny. Transmission is both horizontal (from one larva to another) and vertical (generally from an infected female to her offspring via the egg). Transovarially infected larvae (microsporidium infects embryos within the egg) often exhibit a very high mortality rate, much higher than larvae which are infected by ingesting spores. Infectious spores are present in feces and/or silk of live larvae and are also released into the environment when infected larvae die. Infectious spores may overwinter in

the larval habitat (crevices in the bark of trees or in silken mats containing larval or pupal remains, but it is likely that most microsporidia of the *Nosema*-type survive from one season to the next in infected hosts.

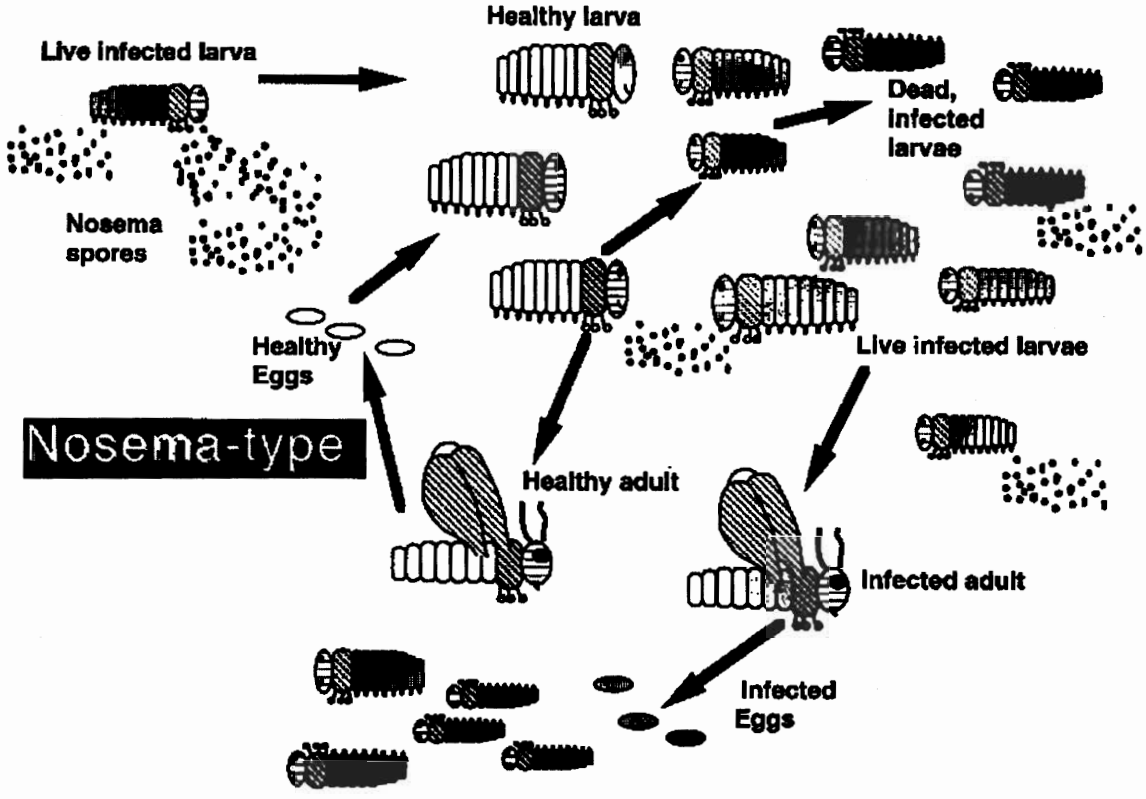

Figure 1. Diagrammatic representation of the interactions between *Nosema*-type microsporidia and their hosts. Healthy larvae become infected by ingesting microsporidian spores which are present in the feces and/or silk of infected individuals. Larvae infected by ingesting spores may die from the infection if they consume many spores at an early larval stage, but many infected larvae can develop into infected adults. Mortality may occur during pupation and emergence as adults. Much of the mortality caused by *Nosema*-type microsporidia occurs in the in the transovarially infected offspring of infected females. Transovarially-infected larvae may be heavily infected and die in early larval stadia.

Sublethal effects have been little studied, but unquestionably have a great effect on populations of many species of forest Lepidoptera (Gaugler and Brooks, 1975; Wilson, 1980). Infected larvae develop more slowly than healthy larvae which increases the time period during which they are vulnerable to additional biotic and abiotic mortality factors; behavior (movement, phototrophic responses, etc.) of both larval and adult hosts may be affected by infection. Effects on mating efficiency, attributed to behavioral differences, pheromone production, sperm production and transfer, may also occur. The total number of eggs laid, as well as the pattern of egg laying, may be affected.

Because *Nosema*-type microsporidia affect all developmental stages of their hosts, they may have an important dampening effect on their host populations; yet, because they do

not cause widespread and extraordinary mortality in any one stage, they often are not recognized as an important component of a pest's natural enemy complex. Unlike many other pathogens, the density dependent effect of microsporidia may occur in the generation following maximum horizontal transmission of the microsporidium; this is because the most significant mortality occurs in the progeny of infected females.

Vairimorpha group. Microsporidian species in the genus *Vairimorpha* are phylogenetically closely related to lepidopteran microsporidia in the genus *Nosema*, but the life cycle and host/pathogen relationships of most *Vairimorpha* species are quite different from that of most *Nosema* species (Baker et al. 1994). All species in the genus *Vairimorpha* produce two types of environmental spores. In addition to the *Nosema*-type life cycle described above, microsporidia in this genus produce an additional type of spore which contains one nucleus and is enclosed in an envelope containing 8 spores. These mononucleated octospores are probably haploid and their role in horizontal transmission is not understood.

Species in the *Vairimorpha* group are usually more pathogenic than species in either of the two other groups. Very few spores are required to initiate infections, and although the length of time required for larval mortality to occur is greatly affected by spore dose and larval age at the time they are infected, most infected individuals die as late instar larvae or prepupae; few infected larvae develop into infected adults (Maddox et al., 1981). Figure 2 illustrates how *Vairimorpha*-type microsporidia are transmitted and at what stages mortality occurs. Spores may or may not be present in silk and/or feces of infected larvae, but even when present in feces and silk, spores are less abundant than those produced in *Nosema* infected individuals. The fat body is usually the primary site of infection, and while other tissues may eventually become infected, the intensity of the infection is usually greatest in the fat body. Thus, *Vairimorpha* spores are not dispersed into the environment throughout the developmental stages of infected hosts, as is the case with *Nosema*-type infections. Rather, environmental spores are released when the host dies. Consequently, horizontal transmission is less effective in this group of microsporidia because healthy larvae are not readily exposed to environmental spores until after infected larvae die and release spores. Vertical transmission may occur, but if it occurs, the frequency of occurrence and the percent of eggs infected is usually relatively low. Thus the phenomenon whereby high rates of mortality are produced in the progeny of infected females is much less important than for the *Nosema*-type infections.

The sublethal effects, so important in the *Nosema*-type infections, have not been well documented for *Vairimorpha* species and although undoubtedly important, are probably less important for *Vairimorpha*-type microsporidia than for the *Nosema*-type microsporidia because the *Vairimorpha* species are more pathogenic.

Even though the *Vairimorpha*-group of microsporidia is more pathogenic than the *Nosema*-group, dramatic epizootics such as those observed for nuclear polyhedrosis viruses (NPV) are seldom reported. Possibly this can be explained by the behavior and gross pathology of virus-infected larvae; shortly after death, larvae literally disintegrate and polyhedra are disseminated to the foliage and other tree structures where they can be transmitted to healthy individuals. *Vairimorpha*-type microsporidia also produce large numbers of infectious propagules but the integument of dead, infected larvae does not rupture and consequently broad dissemination of infective spores does not occur. This is probably one of the major reasons *Vairimorpha* species seldom cause epizootics.

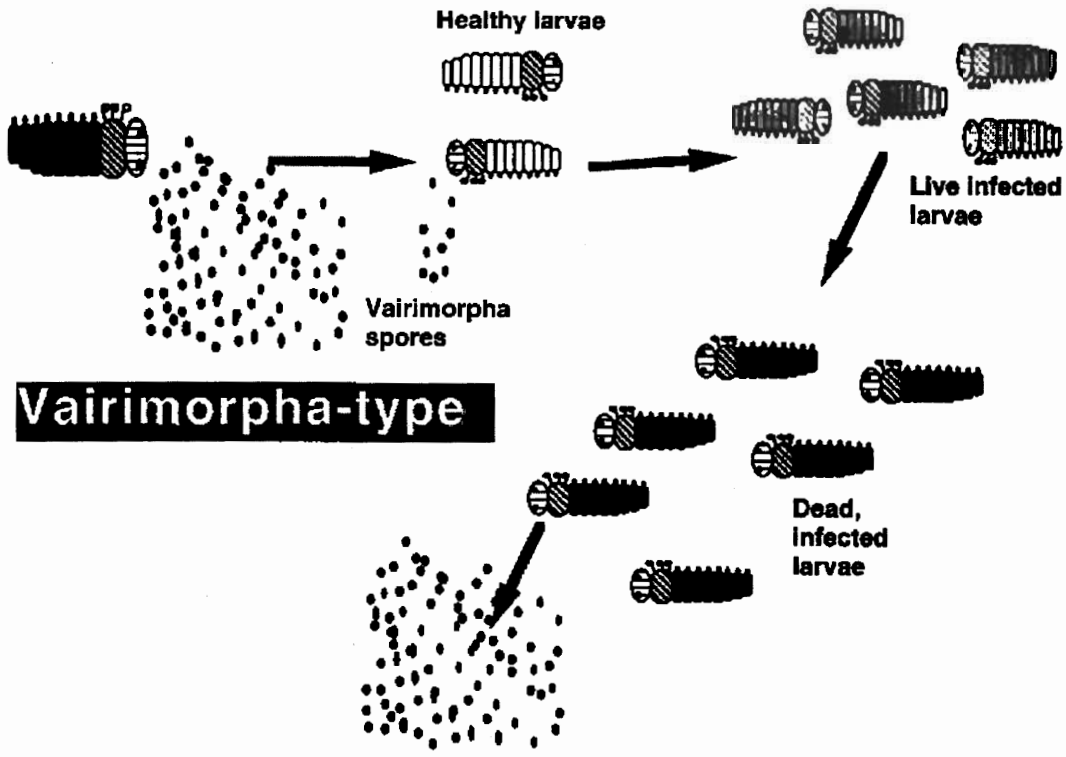

Figure 2. Diagrammatic representation of the interactions between *Vairimorpha*-type microsporidia and their hosts. Healthy larvae become infected by ingesting microsporidian spores which are released when infected larvae die and disintegrate. Spores are seldom present in the feces or silk of infected larvae. Most *Vairimorpha*-type microsporidia are very pathogenic; individuals infected during the larval stage seldom develop into infected adults. These infected individuals usually die as larvae.

Endoreticulatus group. Microsporidia in the *Endoreticulatus* group are very different from microsporidia in the *Nosema* and *Vairimorpha* groups. These differences include their phylogenetic relationships to the other two groups, life cycle, low pathogenicity, and confinement of infection to the midgut epithelial cells.

Species in the *Endoreticulatus* group produce only one type of environmental spore which contains a single nucleus. Spores are enclosed within an envelope which contains 16, 32, or more spores. *Endoreticulatus schubergi*, the species most often encountered in larval populations of forest Lepidoptera, infects only midgut epithelial cells, produces chronic infections, and is not transovarially transmitted. However, it may be transmitted on the egg surface (transovum transmission). This type of vertical transmission usually results in lower percentages of infected larvae than does vertical transmission within the egg (transovarial transmission). Figure 3 illustrates how *Endoreticulatus* -type microsporidia are transmitted and at what stages mortality occurs.

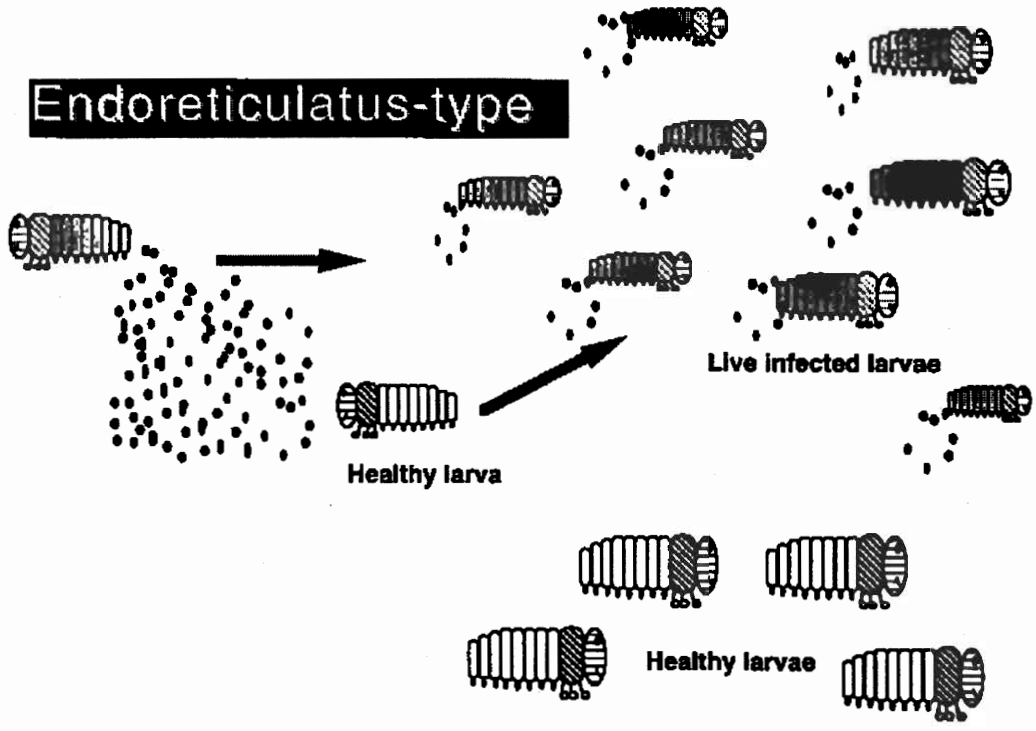

Figure 3. Diagrammatic representation of the interactions between *Endoreticulatus*-type microsporidia and their hosts. Healthy larvae become infected by ingesting microsporidian spores which are present in the feces of infected individuals. The mortality rate is very low in individuals infected with *Endoreticulatus*-type microsporidia. Infected larvae develop more slowly than healthy larvae and produce feces contaminated with spores throughout their larval development.

Endoreticulatus species are frequently recovered from many species of forest Lepidoptera but because of the chronic nature of infection, the impact this group has on insect populations is largely restricted to sublethal effects.

Interactions with other natural enemies. Microsporidia coexist with an array of other natural enemies including parasites, predators and other insect pathogens. Brooks (1993) listed eight different types of interactions that may occur within the host/parasitoid/pathogen system. The following five may apply to microsporidian infections of forest Lepidoptera; premature death of the host, diseased host is not ovipositionally attractive, host is altered nutritionally or physiologically, and direct infection of parasitoids. Parasitoids may also transmit microsporidian infections from diseased hosts to healthy hosts.

If the insect host dies from a microsporidian infection before the parasitoid completes development, the parasitoid usually dies. This has been documented for several species of Lepidoptera infected with microsporidia (Laigo and Paschke, 1968; Cossentine and Lewis, 1988) but not specifically for forest Lepidoptera infected with microsporidia. It is likely that this is also a common phenomenon in populations of forest Lepidoptera.

Insect hosts infected with microsporidia may be unattractive for oviposition by insect parasites for a variety of reasons. For example, sublethal effects of microsporidian infections frequently inhibit development and alter behavioral patterns of the infected host. Because parasitoid females prefer a host of a specific size or a specific larval stadium, any change in the age structure of the host population undoubtedly affects the proportion of the host population attacked by female parasitoids.

Parasitoids may be unable to complete development inside a microsporidian infected insect host because the indigestible microsporidian spores, present in infected host tissues, accumulate in the intestines of immature parasitoids. When this occurs the parasitoid larvae are unable to absorb sufficient nourishment and often die. There are many published examples of this type of interaction (Brooks, 1993; Cossentine and Lewis, 1988; Thomson, 1958).

Parasitoids may be directly infected by the microsporidia of their hosts and in many cases these infections cause a high rate of mortality in the parasitoids (Siegel et al., 1986; Brooks, 1973; Brooks, 1993). Although this has not been documented for any species of forest Lepidoptera, it is likely that this type of interaction occurs.

Parasitoids may also act as vectors of microsporidia. This is probably an important means of dispersing microsporidia both within and between insect populations and has been documented for lepidopteran microsporidia, but not for microsporidia of forest Lepidoptera (Siegel et al., 1986; Brooks, 1993).

Microsporidia also interact with other pathogens. It is often possible to isolate several species of insect pathogens from field populations of insects. Weiser (1987) presented information demonstrating that, over a period of years in a building gypsy moth population, microsporidia appear early in gradation followed by high prevalences of NPV and subsequent collapse of the gypsy moth population. Microsporidia could stimulate the onset of the NPV and/or decrease the rate at which the gypsy moth population increases. It has been suggested that microsporidia increase the transmission efficiency of the NPV, although the specific mechanisms by which this occurs have not been elucidated.

Many of the host/parasitoid/pathogen/microsporidian interactions that have been observed in other insect species undoubtedly occur in forest Lepidoptera. That they have not been observed reflects upon the paucity of research devoted to these topics.

How microsporidia affect forest Lepidoptera. We have discussed at a conceptual level how each of the three groups of microsporidia might affect populations of forest Lepidoptera. Unfortunately, we have relatively few extensive data sets that document the prevalence of microsporidian infections in Forest Lepidoptera over time (seasons, years, etc.) and that relate these infections to the population densities of the host (Maddox, 1987) Relatively long term data sets exist for *Nosema lymantriae* in the gypsy moth, *L. dispar* (see references in Table 1) *Nosema fumiferanae* in the spruce budworm *Choristoneura fumiferana*, (Wilson, 1973), several species of microsporidia in the winter moth, *Operophtera brumata* (Canning and Wigley, 1983), and *Nosema tortricis* in the green tortrix, *Tortrix viridana* (Lipa, 1976; Franz and Huger, 1971). Anderson and May (1980) also presented theoretical evidence that insect pathogens, including microsporidia, are responsible for the population cycles of many forest insects. Nevertheless, our field observations on most species of the microsporidia of forest Lepidoptera consist of one or two collections each year, often unaccompanied with meaningful estimates of the density of host populations. We

must accumulate better long-term data sets to fully evaluate the extent to which microsporidia affect many species of forest Lepidoptera.

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The Effects of Artificial Infections with Microsporidia on Gypsy Moth (*Lymantria dispar*) and Nun Moth (*Lymantria monacha*) - First Results

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ABSTRACT Seven microsporidian isolates from different parts of Europe were fed to laboratory reared larvae of the gypsy moth (*Lymantria dispar*) and nun moth (*Lymantria monacha*). Infections with all isolates were achieved in gypsy moth while in nun moth, six isolates produced detectable infections. The percentage of infected larvae of both species varied significantly, indicating that differences exist in the susceptibility of the larvae to the different isolates. The pathology of infections in German gypsy moth and nun moth does not differ significantly from observations recorded in North American gypsy moths. The isolates have a diverse influence on the larval and pupal mortality. Sublethal infections with the German *Microsporidium* isolate have a significant influence on the development and pupal weight of gypsy moths.

THE GYPSY MOTH (*Lymantria dispar* L.) and nun moth (*Lymantria monacha* L.) are economically and ecologically important defoliators of deciduous and coniferous trees in European forests. Outbreaks of both insects seem to occur periodically (Schwerdtfeger 1981). Pathogens and microparasites like microsporidia are obviously involved in the generation of insect population cycles (Anderson and May 1980). Depending on the nature of the parasite involved, this interaction can also result in maintaining insect populations at a low density level, far beneath the damage threshold (Hochberg 1989). Microsporidia are present in many forest defoliating populations and are suspected to play an important role in the natural regulation of insect populations (Wilson 1981, McManus et al. 1989).

The role of microsporidia in the regulation of European gypsy moth populations is not clearly understood. Zelinskaya (1980) describes microsporidia as an important mortality factor in Russian gypsy moth populations. In addition to this, fertility and larval development are influenced by these parasites. Weiser and Novotny (1987), Novotny (1988) and David et al. (1989) report on attempts to control gypsy moth in the field by applications of microsporidia. To date, no reports on the occurrence of microsporidia in nun moth populations have been published. The objective of this study was to evaluate the potential of microsporidia in the regulation of population density of gypsy moth and nun moth. A cooperative project with the USDA Forest Service and the Illinois Natural History Survey has been established. In this paper, initial results on the susceptibility of German gypsy moth and nun moth larvae to different microsporidian isolates from gypsy moth are presented. In addition, the effects of the infection on individual larvae in the laboratory were also recorded.

Pages 198-205 in M.L. McManus and A.M. Liebhold, editors. 1998. Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. USDA Forest Service General Technical Report NE-247.

Materials And Methods

Seven microsporidian isolates collected from gypsy moth populations in different parts of Europe (Table 1) were obtained from the Illinois Natural History Survey in Champaign/Urbana and refrigerated at 4°C until they were used. The nomenclature of many of the isolates is still preliminary. Gypsy moth larvae were reared in the laboratory on artificial diet (ODell and Rollinson 1966) while nun moth larvae were reared on larch needles.

Table 1: Tissue specificity of the seven different microsporidian isolates from gypsy moth used in the infection experiments[1]

Microsporidian species: (Primary spores)	Infection of gypsy moth	Infection of nun moth
Vairimorpha lymantriae (+)	all tissues, mainly FB	all tissues
German *Microsporidium* isolate (+)	MG, MT, SG (FB)	MG, SG (FB)
Silk gland *Microsporidium* isolate (+)	SG, FB (MG, G)	SG, MG
Romanian *Microsporidium* isolate (-)	MG, FB	MG, (SG)
Endoreticulatus sp. (-)	MG	MG (FB)
Portuguese *Microsporidium* isolate (+)	MG, SG, G	MG, FB (G)
PAV *Microsporidium* isolate (?)	+	-

[1]MG = Midgut; SG = Salivary gland; MT = Malpighian tubules; G = Gonads; FB = Fat body; + = Primary spores were found; - = primary spores not detected

Third instar larvae were infected by feeding spores per os. The spore suspension was fed to individual larvae with an inoculation loop which held approximately 1µl. The concentration of all isolates was adjusted to 10^3 - 10^4 spores/ ml of suspension. For the determination of the pathogenicity of the different isolates, higher doses of spores (maximum $5x10^4$ spores/µl) were fed to gypsy moth larvae. The concentration of spores in the suspension was determined by counting spores in a Petroff-Hauser bacterial counter. A total of 40 to 50 larvae were infected with each isolate. Only larvae that consumed the entire suspension from the loop were used in the experiments.

Infected and healthy (control) larvae were individually reared at 24°C, 40%RH, and 14L:10D photoperiod. Infected and noninfected larvae were inspected every two days and their developmental stage and condition were recorded. Dead larvae, pupae or adults were dissected immediately to determine if they were infected by microsporidia. All larvae in these experiments were killed and autopsied four weeks after they were infected. Wet tissue

mounts were prepared and phase contrast light microscopy was used to confirm the presence of spores and/or vegetative stages. The following tissues were routinely examined: midgut, silk gland, fat body, Malpighian tubules, and nerve tissue.

Results and Discussion

Infectivity. Infections in German gypsy moth larvae were obtained with the following isolates: *Vairimorpha lymantriae,* the German *Microsporidium* isolate, the silk gland *Microsporidium* isolate, the Romanian *Microsporidium* isolate, *Endoreticulatus* sp., and the Portuguese *Microsporidium* isolate. Only very few infections were achieved with the PAV *Microsporidium* isolate (from Slovakia).

The highest infection rates were achieved with the German *Microsporidium* isolate (87%) and the silk gland *Microsporidium* isolate (75%), followed by *V. lymantriae* (70%) (Fig. 1). The German *Microsporidium* isolate was originally isolated from a German strain of gypsy moth in 1993 (Linde and Rappl 1994) which might explain the higher susceptibility of the larvae to this strain.

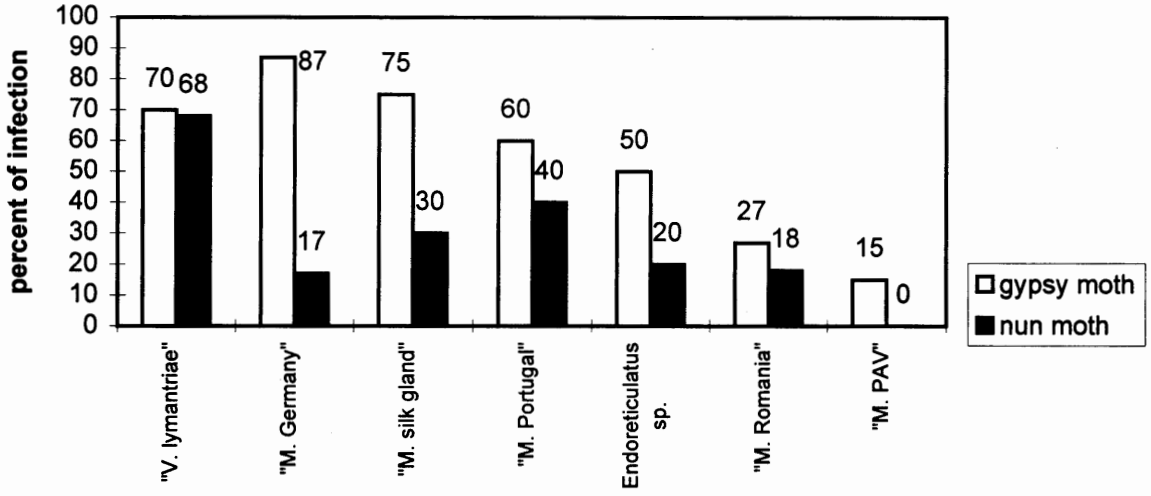

Figure 1. Results of infection experiments with gypsy moth (*Lymantria dispar*) and nun moth (*Lymantria monacha*).

Infections in nun moth larvae were obtained with the following isolates: *V. lymantriae,* the German *Microsporidium* isolate, the silk gland *Microsporidium* isolate, the Romanian *Microsporidium* isolate, *Endoreticulatus* sp., and the Portuguese *Microsporidium* isolate. No infections were achieved with the PAV *Microsporidium* isolate. The negative results with the isolate PAV *Microsporidium* suggest that the spores were not viable. Spores appeared greyish in colour under phase contrast microscopy. The highest infection rates were achieved with *V. lymantriae* (68%) and the Portuguese *Microsporidium* isolate (40%), followed by the silk gland *Microsporidium* isolate (30%) (Fig. 1). The reasons for the variation in susceptibility of the larvae to the different isolates are not yet clear and need further investigation.

The susceptibility of nun moth larvae to gypsy moth microsporidia was not anticipated. No reports on the occurrence of microsporidia in nun moth populations have been published. Gypsy moth and nun moth are taxonomically similar species, but very different in their ecology. Nun moth is more frequent in north central Europe and feeds mainly on coniferous trees, while gypsy moth reaches higher population densities only in southern Europe and mostly feeds on deciduous trees. The susceptibility of an insect to a microsporidian infection depends on the conditions in the midgut, where the spores germinate, and on the suitability of the infected cells for the development of the parasite (Linde 1990). The lower infection rates that we observed in nun moth larvae are probably due to the conditions in the gut (e.g. pH), which are different from gypsy moth as a result of the different food plants and which may inhibit the germination of spores. Nevertheless, the conditions in infected cells obviously support the development of the parasite in both insect species, indicating the close relationship between them.

Pathology. The isolates used in this study differ in their tissue specificity (Table 1). The tissue specificity of the different isolates is almost the same in nun moth, again reflecting the taxonomic similarity of these two lymantriids. *V. lymantriae* was found in all tissues of gypsy moth and nun moth larvae. Usually the fat body was hypertrophied and completely filled with spores. The Portuguese *Microsporidium* isolate was found in the midgut and salivary glands. In a few cases, spores were present in the gonads, suggesting the probability that this isolate might be transovarially transmitted. The silk gland *Microsporidium* isolate was found mostly in the salivary glands and fat body, but also in the midgut. *Endoreticulatus* sp. was found only in the midgut. Infections with the Romanian *Microsporidium* isolate were detected in the midgut and fat body.

These results correspond to those reported by Maddox et al. (1995). *V. lymantriae* and the Portuguese *Microsporidium* isolate infections were also found in the larval gonads, indicating that these isolates are transmitted transovarially as reported by Bauer et al. (1994) and Novotny and Weiser (1993).

So called "primary/early spores" (Sagers et al. 1996, Solter et al. 1993) were produced by most isolates early in the infection cycle. In fact, most infections in the midgut muscle layer were actually "primary spores," which are thought to be responsible for the transmission of the infection from one tissue to another within the insect.

Mortality. The mortality rates of gypsy moth larvae fed with a low spore dose of 10^4 spores per larva resp. (10^3 with *V. lymantriae)* did not differ significantly from the control group; only larvae infected with *V. lymantriae* showed slightly higher mortality in the first two weeks of the experiment.

In a second set of experiments, higher doses of spores ($5x10^4$ spores/µl) were fed to gypsy moth larvae. Two weeks after infection, the percentage mortality was recorded. The results are shown in Figure 2. The highest mortality was caused by *V. lymantriae* and the silk gland *Microsporidium* isolate followed by the Portuguese *Microsporidium* isolate. Much lower mortality was recorded with the German *Microsporidium* isolate (8%) and *Endoreticulatus* sp. The low mortality caused by the German *Microsporidium* isolate probably reflects the long coevolution of this German isolate with the German strain of gypsy moth. In addition to this, the German *Microsporidium* isolate only infects tissues of minor importance for the survival of the larvae (e.g. silk gland).

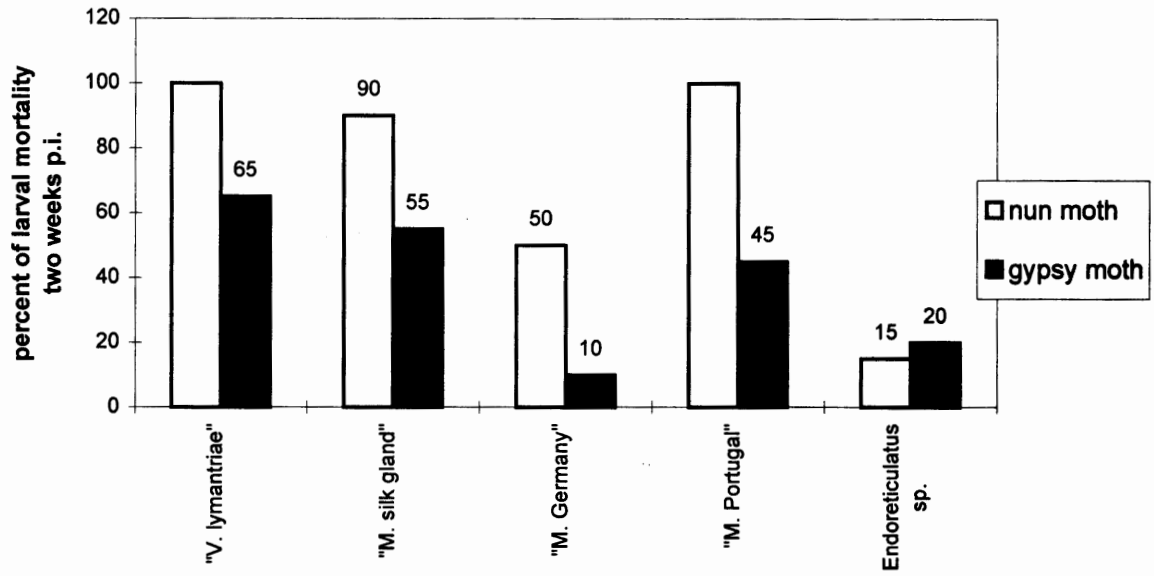

Figure 2. Mortality of gypsy moth and nun moth larvae caused by microsporidia.

The influence of different doses of the German *Microsporidium* isolate on pupal mortality and development of gypsy moth was investigated in detail. The mortality of female pupae significantly increased with the infective dose (Fig. 3). We suggest that the high spore load in the silk gland might interfere with the process of metamorphosis. The silk gland tissue is inactivated, resorbed, and probably incorporated into tissues of the adult insect. This transformation is probably disturbed when the silk gland cells are completely filled with resistant microsporidian spores.

In the nun moth, mortality rates were significantly higher. This is probably due to the fact that nun moth is not the natural host of the microsporidian isolates used in these experiments. The highest mortality rates were found with *V. lymantriae* and the Portuguese *Microsporidium* isolate followed by the silk gland *Microsporidium* isolate. Much lower mortality occurred with the German *Microsporidium* isolate and *Endoreticulatus* sp. (Fig. 2). In this respect, the results correspond to those observed with gypsy moth.

Differences in pathogenicity of microsporidian species is a well documented phenomenon (Brooks 1988). The virulence of the isolates is obviously correlated with the tissue specificity: those species which infect tissues that are important for the survival or development of the larvae (e.g. *V. lymantriae:* fat body) cause the highest mortality rates.

Development. Only few microsporidian species (e.g. *Vairimorpha necatrix*) cause high mortality in their hosts (Brooks 1988). Usually, microsporidia cause sublethal effects which are typical for a chronic disease. In this study, we investigated the influence of infections with *Endoreticulatus* sp. and the German *Microsporidium* isolate on developmental parameters (duration of development, pupal weight) of gypsy moth larvae.

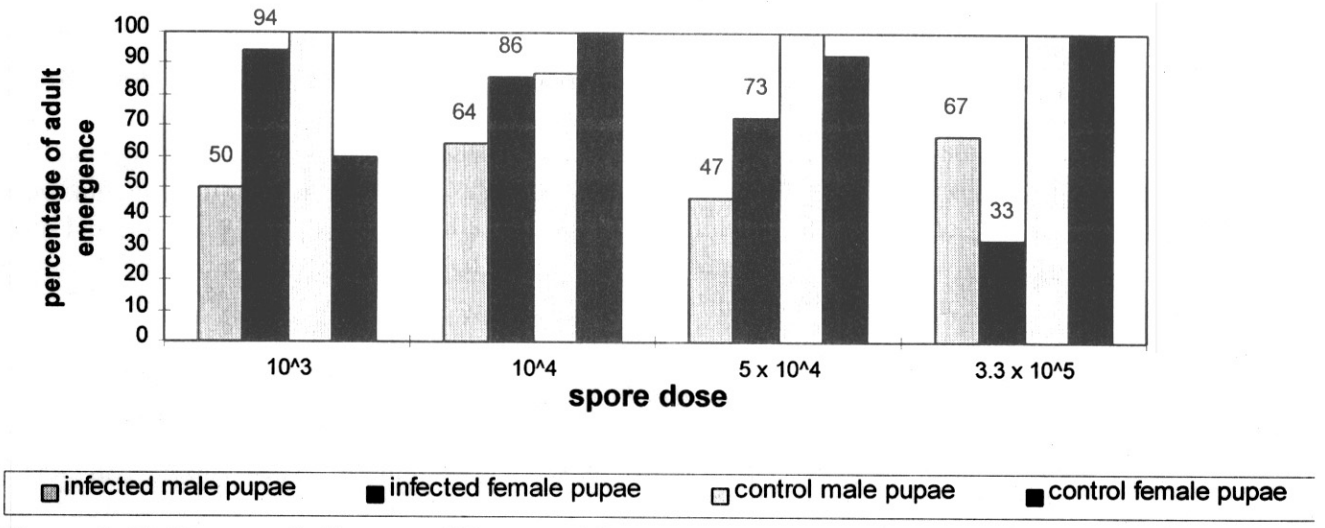

Figure 3. Influence of German *Microsporidium* isolate on the survival of gypsy moth pupae (*Lymantria dispar*).

We observed an influence of the infection on the pupal weight as well as on the development of larvae. The weight of gypsy moth male pupae infected with *Endoreticulatus* sp. was higher compared to healthy pupae (0.38gr resp. 0.35gr.). This is probably due to the prolonged development of the larvae and the heavy spore load in the infected tissues.

Larvae of gypsy moth infected with the German *Microsporidium* isolate pupated significantly later and the duration of the pupation period was prolonged. As a result, the development of infected male and female individuals from egg hatch to adult emergence was significantly longer (Fig. 4). Similar effects of microsporidian infections have been described in other species.

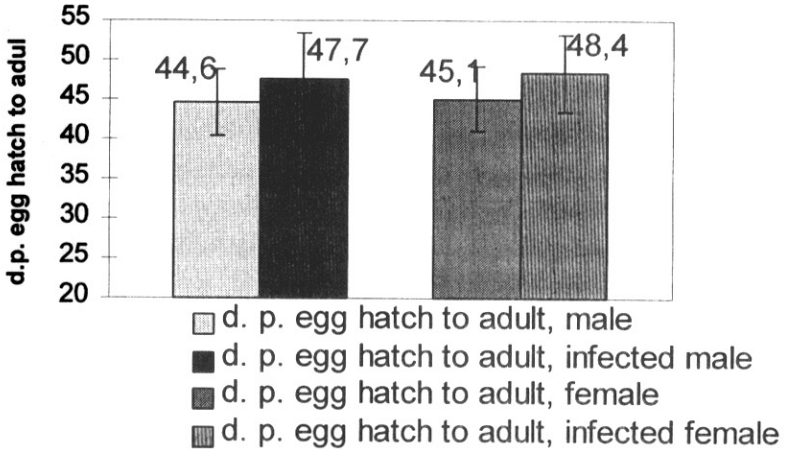

Figure 4. Influence of German *Microsporidium* isolate on the development of *Lymantria dispar*.

The results indicate that microsporidian infections may have an influence on the dynamics of an insect population. Mitchell and Cali (1994) mention the occurrence of a retarded growth and development and lower body weight of lepidopteran larvae that are infected with *V. necatrix*. Bauer and Nordin (1988) observed prolonged development in

spruce budworm infected with *Nosema fumiferanae*. Recently, Habtewold et al. (1995) demonstrated an influence of the infection of grasshoppers infected with *Nosema locustae* on the per capita reproduction and the growth of grasshopper populations.

The prolongation of the development of infected gypsy moth larvae from egg hatch to adult emergence as demonstrated in this study seems negligible, but the experiments were conducted under optimal rearing conditions in the laboratory. In the field, such effects may be enhanced by adverse weather or poor food, leading to a prolonged exposure of larvae to natural antagonists or disadvantageous abiotic conditions.

Conclusions

We were able to successfully infect a German strain of gypsy moth with seven microsporidian isolates collected from gypsy moth populations throughout Europe. In addition to this, nun moth larvae were also infected by most of the same isolates. The effects of the different isolates on the two host insects were varied and mostly sublethal. Our results present further evidence that microsporidian infections can slow down the rate of development of the larvae. Although after four weeks, most of the control larvae had pupated, there were few pupae in the group of larvae infected with *microsporidia*.

The results reported here are preliminary and should be repeated; additional research should concentrate on these long-term effects in order to obtain a realistic view of the potential of microsporidia for use in the long-term regulation of insect population dynamics.

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The Theoretical Basis for Using Baculoviruses to Control Forest Pests

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ABSTRACT The problems associated with using baculoviruses for classical biological control (introduction, colonization and mass release, and the application of microorganisms as an insecticide) are discussed. Based on several years of investigation, differences in the natural occurrence of virus diseases among 23 species of dendrophilous insects have been determined. The *Lepidoptera* and *Hymenoptera* have been divided into 4 groups according to differences in occurrence of viruses observed in those species. The character and intensity of epizootics caused by baculoviruses are associated with a criterion referred to as the "degree of contagion of the virus in the ecosystem." This criterion is formed from two factors that are expressed on a numerical scale (1-5): (1) the virulence and the tissues affected by the virus, and (2) the probability of contact between the feeding larvae and the virus in nature. This probability depends upon the bioecological peculiarities of each insect species. The criterion, "the degree of virus contagion in the ecosystem", is proposed as a method for selecting the best method for the use of the baculoviruses against insect species.

THE THEORETICAL BASIS of the methods for the use of viruses to control harmful insects corresponds to the main objectives of classical biological control, i.e., introduction, colonization, and mass release. The questions of introduction, colonization and the direct use of microorganisms as insecticides were discussed by Hall (1964). There are few known examples of successful introduction of viruses. The importance of sawfly viruses after their introduction from Europe to the USA and Canada was particularly significant (Bird 1953, Bird and Elgee 1957). At first, the effect was very dramatic; however, over time, the interaction between the nuclear polyhedrosis virus (NPV) and its host became more balanced. The NPV now occurs in populations of *Neodiprion sertifer* and *Diprion hercyniae* in North America. In spite of the fact that these viruses are permanent regulators of the population density of these pests in the United States and Canada, preparations of the virus are used occasionally to control these species (Podgwaite et al. 1984, Cunningham et al. 1989).

The introduction of a more virulent NPV strain (than the natural strain) from Japan into populations of *Hyphantria cunea* in Yugoslavia in 1973 caused a decrease of the population density to an insignificant level within one year (Injac 1978).

The possibilities of introducing viruses are not limited to those that are specific to an individual pest species. In 1964, we introduced the granulosis virus (GV) of *Dendrolimus sibiricus* to a Voronezh population of *D. pini*. We treated 12,000 eggs of *D. pini* with GV isolated from the thoracic region of a single butterfly. The treated eggs were placed in paper sacs on 80 pine trees in four plots. Most insects died in the pupal stage (65-80%). Within a year, the population was reduced to an insignificant level and the GV was distributed within a radius of up to 1 km. The introduction of GV into the population of *D. pini,* combined with

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activity of natural entomophages, caused a prolonged suppression (22 years) of the pest population (Orlovskaya et al. 1988).

Regarding colonization, the baculoviruses are periodically introduced only into a small portion of the insect population, after which they reproduce and are transmitted within the ecosystem. As an example of this colonization, we can point to our "ground-focus" method of introducing NPV into the populations of *Lymantria dispar* at the beginning of the gradation phase of the pest. Ten to 20 percent of the egg masses in the artificial focus were treated by NPV before larval emergence (Orlovskaya 1970). This method has been successfully utilized in Russia and some republics of the former USSR for more than 25 years (Orlovskaya 1980).

The third method of utilizing baculoviruses is the application of viral preparations as insecticides. But in this case, the use of this method is limited because of the high price of virus preparations. The spraying of a forest with a Gypchek preparation (NPV) at a rate of 1.25×10^{12} polyhedral inclusion bodies (PIB) per hectare costs $50 (Reardon and Podgwaite 1992). We have developed a virus preparation, VIRIN-ENSH, based on an experimental epizootical NPV strain, that is applied by spraying forests at the rate of 1.5×10^{11} PIB/ hectare. The price of the treatment of 1 hectare is 24,000 rubles ($5). The cost of producing VIRIN-ENSH for seasonal colonization varies from 4,800 to 14,400 rubles ($1 to $3) for every 50 hectares.

Materials and Methods

We made observations on virus diseases of insects in Estonia, Latvia, Central and Southern regions of Russia, Dagestan, Azerbaijan, Western Ukraine, Moldova, Kazakhstan and Kirghizia. Additional investigations were conducted by our colleagues in Byelorussia, Armenia, Ukraine and Siberia. Both living and dead individual larvae were investigated by means of light and electron microscopy. The susceptibility of insects to viruses, their virulence, and specificity were determined by bioassaying host insects and various species that occur in the same biocenosis (Orlovskaya 1968a). The tissue tropism was investigated by analyzing tissues of infected individuals at different periods of development of the disease. The occurrences of latent virus in populations were determined by investigating the effect of various stress factors on the insects. The duration of observations varied among different species, and the size of epizootic foci were defined by special inspections. The artificial epizootics in *L. dispar* populations were created by treating a small portion of egg masses with a virus suspension before larval emergence (Orlovskaya 1970). The active and epizootic NPV strains used for production of the virus preparation VIRIN-ENSH were obtained by adapting introduced viruses to new host insects and by direct selection using symptoms of tissue tropism (Orlovskaya 1975). The virus preparation VIRIN-ENSH was obtained by cultivating the NPV in larvae of *L. dispar* reared on special artificial media and various plant species (Orlovskaya and Shumova 1980).

Results and Discussion

Many years of research on viruses in natural populations of various insects, as well as in laboratory colonies, allowed us to determine some principles for the development of

baculoviruses in individuals and in populations. We have summarized the results of these investigations of virus diseases in 23 species of dendrophilic pest insects.

Table 1. The influence of interaction of baculoviruses with insects upon the occurrence of the viral diseases in nature and upon the methods of their use

The insect species	Virus	The occurrence of viruses in nature	The degree of contagion in the ecosystem: virulence and tissues affected by the viruses*	The degree of contagion in the ecosystem: probability of contacts of larvae with virus**	Methods of use
Lymantria dispar L.	NPV		4	5	
Lymantria monacha L.	NPV		4	5	Seasonal
Dasychira pudibunda L.	NPV	Acute	4	5	colonization
Erannis defoliaria L.	NPV	epizootics	4	5	and as an
Malacosoma neustria L.	NPV		5	4	insecticide
Hyphantria cunea Drury.	GV		5	4	
Neodiprion sertifer Geoff.	NPV		5	5	
Aporia crataegi L.	NPV		4	2	As an
Stilpnotia salicis L.	NPV	Chronic	4	2	insecticide
Selenephera lunigera Esp.	NPV	epizootics	4	3	
Euproctis chrysorrhoea L.	NPV		2	3	The use of
Dendrolimus sibiricus Tschet	GV		3	4	active strains
Diprion pini L.	NPV		3	4	for introduction
Hyponomeuta cognatella Hb.	NPV		3	3	
H. evonymella L.	NPV		4	3	As an
H. malinella Zell.	NPV	enzootics	4	3	insecticide
H. padella Z.	NPV		4	3	
Carpocapsa pomonella L.	GV		4	1	
Cocaecia crataegana Hb.	NPV		4	1	
Panolis flammea Schiff.	NPV	Rare	1	5	The use of
Dendrolimus pini L.	NPV	occurrence	1	2	active strains
Tortrix viridana L.	NPV	in a	1	1	for
Lyda nemoralis Thoms.	NPV	population	1	4	introduction

* The degree of virulence and the tissues affected.
1. The virulence is weak. Separate cells are affected, hypoderma is not.
2. The virulence is moderate. The hypoderma is affected rarely, the other tissues are affected.
3. The virulence is moderate. The various tissues and hypoderma or the gut of sawflies are affected.
4. The virulence is high. The majority of tissues are affected, the hypoderma is affected only before death.
5. The virulence is high. The majority of tissues are affected. The hypoderma or the epithelium of sawfly guts are affected before the muscles.

** The probability of contacts between the feeding larvae and the virus in nature.
1. The contacts are casual.
2. The contacts of the virus dispersed from dead prepupae and pupae with the larvae of the next generation are rare.
3. The contacts with the dead larvae inside the nests are temporary; the infection is limited within the nests.
4. The contacts of the healthy larvae and late instars with the dead larvae on the foliage are moderate.
5. The contacts between the larvae of all instars and the dead and moribund larvae on the foliage are higher.

As a result of the research on the interaction between baculoviruses and their host insects in various insect species, we have developed a table (Table 1) where insects are

grouped according to the peculiarities manifested by their virus diseases in nature. The degree of contagion is expressed by numbers according to the nature of the virus and its probability of contacting the susceptible stage of the host insect in the ecosystem. In the last column, some methods for using the baculoviruses for every insect group and every pair of "virus - host" are proposed.

The reasons for the appearance of virus epizootics in insect populations were determined by many investigators and were correlated mainly with the activation of a latent virus under conditions that were unfavorable for insects (stresses) during periods of mass reproduction of pests (Gershenson 1959, Aruga 1963, etc.). The further development of virus diseases in insects in nature varies among different species; it is connected with the main factor responsible for the epizootic, i.e. the degree of contagion. The latter is provided by pathogen virulence, tissue tropism and bioecological features such as phenology, habitat, mode of life, number of generations and dispersal activity (Orlovskaya 1968b). The investigation of the interaction between baculoviruses and their host insects in individual organisms and in the ecosystem showed that specific NPVs and GVs have different degrees of activity within different species, and that there are differences in the succession of tissues infected in various insect species. This fact has a great influence on the degree of contagion in the ecosystem.

The first group includes mainly free-living insects; their life cycle (or several cycles) is completed within one season. During this period, the highly virulent NPV or GV is accumulated quickly in the population due to intensive secondary infections. Thus, the hypoderma of *Malacosoma neustria* larvae is affected by NPV and that of *Hyphantria cunea* larvae is infected by GV before infection occurs in the muscles. Therefore, the viruses are liberated into the environment through the ruptured cuticle when the larvae are moving on the foliage, which is also where the healthy individuals are feeding. These insect species are designated "5" due to their virus characteristics, but are rated "4" based on the probability of the virus coming in contact with the susceptible larvae; the first instars of these species develop in colonies and contact with the virus occurs more frequently in the middle and later instars. Therefore, these species have a total score of "9".

The hypoderma of the majority of *Lepidoptera* species is affected last, so the viruses are liberated from the organism in the location where the death of individuals occurs; they may be larvae, pronymphs, pupae or even adults in different species. The location of the dead insects is very important for the transmission between the virus and a susceptible stage of the insect. In *Lymantria dispar, L. monacha, Dasychira pudibunda* and *Erannis defoliaria,* viruses are liberated from the dead larvae of all instars onto foliage of deciduous trees or onto the needles of conifers. Therefore, the chances of contact between the virus and healthy larvae in nature is rated a "5" and a "4" according to the activity index due to the fact that the hypoderma is infected last. In *Neodiprion sertifer,* the intestinal NPV is liberated in the feces and therefore continuously contaminates the needles; this provides a high probability for transmitting the infection to healthy larvae. This species has a total score of "10", so it has the highest degree of contagion in the ecosystem. Insects included in the first group have the highest degree of contagion of a baculovirus in the ecosystem. This fact explains the acute course of virus epizootics that occurs in these species in nature. For this group of insects, it is preferable to use the periodic seasonal colonization for introducing baculoviruses into populations. The use of virus preparations as insecticides is also possible.

The second group includes species whose life cycle is interrupted by hibernation in the larval stage. Similarly, in some species, contact of the virus with the susceptible insect stage are limited. As a result these insects (larvae of e.g. *Aporia crataegi*) very seldom die in nature. The dead pronymphs and pupae are situated mainly on branches of the tree. In 2-3 weeks, a new generation of larvae emerges from egg masses in the peripheral part of the crown and begin feeding there. These healthy individuals consume a very small dose of the virus which was dispersed passively by wind. In addition, because the second instars are preparing to hibernate, their rate of development is slow, which does not promote the rapid development of the virus within the organism. In spring, the overwintering larvae develop at low temperatures, which also doesn't promote the intensive reproduction of virus in the organism. Consequently, most mortality occurs in the pronymph and pupal stages. We have observed such a situation for many years in the western Ukraine during the period of gradation of *A. crataegi.* The infection accumulated in the population slowly, so the epizootic was chronic for 11 years (Orlovskaya 1968b). Similar epizootics have been observed in populations of *Stilpnotia salicis* and *Selenephera lunigera* (Gorochovnikov et al. 1968). In these species, there is a small possibility of contact between the virus and the feeding larvae in nature; however, the virulence of the virus is high. Such a small possibility of contact with healthy individuals (rating = "2"), even with highly virulent NPV, results in the development of a chronic viral epizootic in nature. We recommend that these baculoviruses be used only as insecticides against these species.

Some insects of this group are affected by the less virulent baculoviruses in populations (a rating of "2-3"), but there is a great potential possibility for contacts of the healthy larvae with the virus in populations of *D. sibiricus* (Lukyanchikov 1962) and *Diprion pini.* For this group of insects, the search for active strains and their introduction are necessary. In the case of *D. sibiricus,* the introduction of the cytoplasmic polyhedrosis virus (CPV) that infects the intestines is worth investigating.

The third group includes those insects whose virus diseases develop in nature locally, in the form of an enzootic, due to the isolated habitat of the larval stages. In the majority of these species, healthy larvae contact the virus only in colonies; however, the virulence of their baculovirus is very high. The dead individuals remain isolated in abandoned colonies, or in rolled leaves. The colonies of larvae that remain alive settle in new sites, escaping the external infection. In *Carpocapsa pomonella* and *Cocaecia crataegana,* contact of the larvae with the virus is very rare because the larvae develop within apples or in separately rolled leaves. These species can be controlled by using baculoviruses as an insecticide.

The fourth group includes insects that are different in their biological features, so that epizootics in nature are either extremely rare or nonexistent. The NPV isolated from all these species has a weak virulence. By artificially infecting these host species, we showed that their baculoviruses affected only separate cells and tissues. For these insect species, it will be necessary to search for active strains that can be introduced. As mentioned previously, the introduction of the Asian strain of GV, isolated from *D. sibiricus,* into European populations of *D. pini* proved to be very effective (Orlovskaya et al. 1988).

The advantage of the method of introducing very active strains over other methods is the relatively prolonged control of the pest populations (10 to 20 years); therefore, costs are low both for the virus material and for the treatment of populations.

Conclusion

The criterion "the degree of virus contagion in the ecosystem" is offered to select the most promising method for using the baculoviruses for the control of pest species. This criterion can be determined by the use of two indices, in which a scale of "1 to 5" is used for both indices. The indices of virulence and the tissues affected by the virus are included in the first scale. The second scale considers the probability of contact between the feeding larvae and the virus in nature, and takes into account the bioecological peculiarities of each insect species.

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