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DISEASE - PARASITOID RELATIONSHIPS IN NATURAL POPULATIONS OF *LYMANTRIA DISPAR* [LEP. : LYMANTRIIDAE] IN THE NORTHEASTERN UNITED STATES (1)

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Immature *Lymantria dispar* L. were collected from 6 geographically distinct populations over 2 years to determine correlations between parasitoid and disease incidences. Incidence of the nuclear polyhedrosis virus disease (NPV) was found to be positively correlated with incidences of the parasitoids *Apanteles melanoscelus* (RATZEBURG) and *Parasetigena silvestris* (ROBINEAU-DESVOIDY).

The gypsy moth, *Lymantria dispar* LINNAEUS, is a serious defoliator of hardwood trees in the northeastern United States, causing trees to die when heavy defoliation occurs in 2 or more successive years. A broad program of research has been formulated by the United States Department of Agriculture to develop an integrated gypsy moth management system. Part of this program is aimed to develop methods of introducing microbial agents into host populations and to maximize the vectoring of these agents within these populations.

It is generally accepted that parasitoids can function as mechanical vectors of pathogens while feeding on or ovipositing in their respective hosts. WHITE (1943) presented evidence to indicate that *Tiphia popilliavora* (ROHWER) and *Tiphia vernalis* (ROHWER) could enhance the spread of milky disease in the Japanese beetle, *Popillia japonica* (NEWMAN). He found *Tiphia* adults to be carriers of spores of the milky disease bacterium *Bacillus popilliae* DUTKY. TANADA (1955) and BLUNCK (1952, 1954) presented data to indicate that the braconid *Apanteles glomeratus* (LINNAEUS) was capable of transmitting the microsporidian pathogens *Perezia mesnili* PAILLOT and *Nosema polyvora* BLUNCK to the imported cabbageworm, *Pieris rapae* (LINNAEUS). VOUKASSOVITCH (1925) speculated that parasitoids transmitted the fungus *Spicaria farinosa* (FRIES) VUILLEMIN to pupae of *Polychrosis botrana* (SCHIFFERMULLER) during oviposition. BIRD (1961) documented the tree-to-tree dispersal of the nuclear polyhedrosis virus of the European pine sawfly *Neodiprion sertifer* (GEOFFROY) chiefly through the action of parasitoids.

In a series of laboratory experiments, WOLLAM (pers. comm., 1974) demonstrated the mechanical transmission of the nuclear polyhedrosis virus (NPV) of the gypsy moth

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by the braconid *Apanteles melanoscelus* (RATZEBURG). Also, casual field observations in gypsy moth populations have suggested the role of parasitoids as vectors of disease in those populations.

The objectives of our present study were (1) to determine if relationships exist between parasitoids and the incidence of disease (infectious and noninfectious) in several natural gypsy moth populations and (2) to select for further study those parasitoids and pathogens that might be compatible in vectoring studies.

MATERIALS AND METHODS

ESTABLISHMENT OF STUDY AREAS

Five-acre sites supporting gypsy moth populations were established in 6 geographically distinct areas in the northeastern United States (fig. 1). These sites were selected as representing a range of conditions: host density (egg masses/0.4 ha), tree species composition, stand history, and climate (CAMPBELL, 1971). Unfortunately, not all of the 1972 sites were monitored in 1973 (table 1).

TABLE 1

Gypsy moth density and tree species composition of study areas 1972 *and* 1973

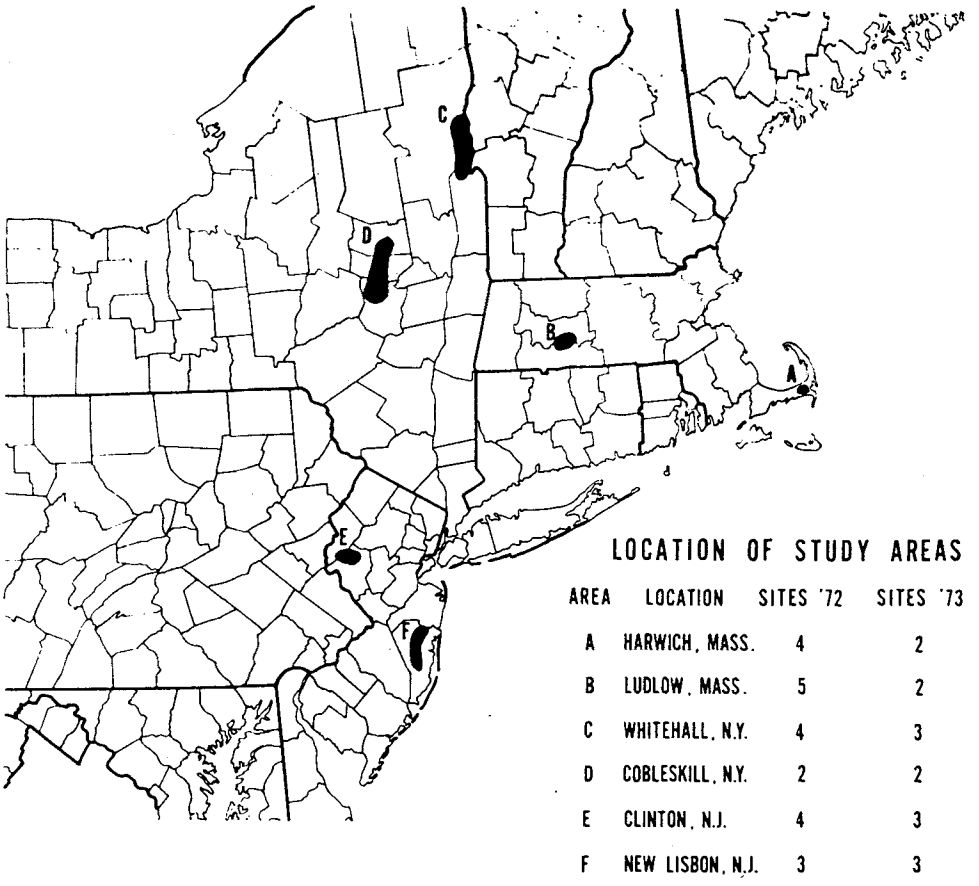
Area	Host density (egg masses per 0.4-ha)		Tree species composition (%)			
	Spring 1972	Spring 1973	Pine	Maple	Oak	Other
(A)	460	311	11	0	84	5
(B)	1899	1804	10	32	31	27
(C)	1491	932	14	10	42	34
(D)	1176	780	11	19	44	26
(E)	972	4362	0	19	23	58
(F)	760	8609	18	0	82	0

Collection of dead larvae and pupae

Five plots, each consisting of a cluster of 10 trees, were established within each site. Each tree in a cluster was sampled by collecting dead and/or moribund larvae from (1) the tree bole to a height of 2 meters above the base and (2) from six .25-m^2 collecting traps positioned under the crown. Each sampling point was visited twice daily from June 1 until adult emergence. Each dead or moribund gypsy moth larva or pupa found was placed individually in a sterile container, refrigerated, and forwarded to our laboratory at Hamden, Connecticut, for examination. Disease conditions were diagnosed according to methods described by CAMPBELL & PODGWAITE (1971) and were classified according to STEINHAUS (1963).

COLLECTION OF PARASITIZED LARVAE AND PUPAE

A 200-x 200-m grid divided into 40-m^2 subunits was established within each site and used to locate (1) sampling points (intersections of 40-m grid lines) and (2) sampling blocks (40-m^2 subunits). Once a week for 8 weeks all larvae and pupae (up to 50) were collected from under burlap bands placed around tree boles at dbh and located at sampling points (1 burlap band/point) or within sampling blocks (2 burlap bands/block) selected randomly within the site. In addition, weekly collections of up to 200 larvae or pupae were taken from numerous locations (understory and overstory foliage, ground litter, and bole) within the sampling blocks. Collections were initiated when gypsy moth larvae were in the 1st stadium, and were continued until at least 75% of the pupae had developed into adults.



LOCATION OF STUDY AREAS

AREA	LOCATION	SITES '72	SITES '73
A	HARWICH, MASS.	4	2
B	LUDLOW, MASS.	5	2
C	WHITEHALL, N.Y.	4	3
D	COBLESKILL, N.Y.	2	2
E	CLINTON, N.J.	4	3
F	NEW LISBON, N.J.	3	3

FIG. 1. Location of study areas, 1972-1973.

Collected larvae were reared in the laboratory at room temperature in 498-g cardboard containers (maximum of 10 to a container) with clear plastic lids and oak foliage as a food source. Larvae and pupae were examined twice weekly, and upon indication of being parasitized were placed individually in Petri dishes. Immature and adult stages of emerging parasitoids were identified from taxonomic keys developed by DOWDEN & REARDON (unpublished, 1968).

RESULTS

A total of 102,443 living gypsy moth larvae and pupae were collected and reared for the recovery of parasitoids during a 2-year period. In 1972, *Apanteles melanoscelus* successfully parasitized 1.5% of the collected hosts, and the tachinids *Blepharipa pratensis* (MEIG.) *Parasetigena silvestris* (R-D.), and *Compsilura concinnata* (MG.) parasitized 5.3%, accounting for 21% and 70%, respectively, of the measured parasitism. In 1973, recoveries of *A. melanoscelus* were down to 10% of the total parasitism, whereas the combined parasitism of the 3 species of tachinids increased to 85% of the total measured parasitism (table 2).

TABLE 2

Parasitoids recovered from living gypsy moth larvae and pupae- 1972 *and* 1973

Area	No. hosts collected		PARASITOID SPECIES (% PARASITISM)									
			Apanteles melanoscelus		*Parasetigena silvestris*		*Compsilura concinnata*		*Blepharipa pratensis*		*Brachymeria intermedia*	
	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973
(A)	11,299	3,711	0.04	0.78	0.75	2.45	0.07	2.86	1.19	7.33	3.22	0.13
(B)	22,064	1,342	1.86	.37	.70	.45	.61	5.00	2.83	.75	.00	.00
(C)	8,610	2,669	6.40	2.36	1.67	2.78	1.20	2.17	3.93	4.60	.02	.00
(D)	4,976	2,358	.72	.64	.32	8.57	.54	9.33	1.49	.89	.00	.00
(E)	14,094	6,182	.85	.76	.75	.18	2.10	2.36	11.34	.42	.00	1.00
(F)	13,408	9,635	.03	.05	.06	.02	.02	.02	1.52	.13	.14	.63
Totals	76,546	25,897	1.47	0.63	0.67	1.49	0.75	2.31	3.88	1.79	0.50	0.49

TABLE 3

Cause of death as a % of dead gypsy moth larvae collected — 1972

Area	No. hosts collected	Infectious disease			Non-infectious disease (a)	Predation (b)	Parasitism	Undetermined (c)
		NPV	Bacteria	Mycoses				
(A)	7,702	4.22	11.00	0.08	43.47	0.26	0.31	40.66
(B)	6,444	21.23	14.25	.12	26.41	.42	3.82	33.75
(C)	3,869	34.81	26.23	.62	22.38	.11	4.64	11.21
(D)	4,094	19.81	11.70	.02	12.53	.68	7.57	47.69
(E)	2,969	14.38	50.45	.20	18.09	1.11	4.72	11.05
(F)	2,782	19.84	11.83	.00	62.47	.14	.65	5.07
Totals	27,855	17.34	18.26	.16	31.25	.42	3.29	29.30

(a) Mortality in which a living microorganism was not involved.
(b) Mortality caused by vertebrate (small mammal) and invertebrate (carabid and pentatomid) predators.
(c) Those insects harboring a "sub-lethal" quantity of an infectious agent or those insects containing two or more agents in combination.

TABLE 4

Cause of death as % of dead gypsy moth larvae collected — 1973

Area	No. hosts collected	Infectious disease			Non-infectious disease (a)	Predation (b)	Parasitism	Undetermined (c)
		NPV	Bacteria	Mycoses				
(A)	6,937	28.01	4.63	0.11	42.97	1.05	1.79	21.44
(B)	1,870	48.51	7.43	.22	31.51	.97	3.26	8.10
(C)	961	29.14	10.41	1.98	24.35	.83	13.32	19.97
(D)	1,798	46.50	12.24	.22	13.85	.78	12.12	14.29
(E)	7,300	48.30	18.58	.55	16.34	.04	1.70	14.49
(F)	9,940	31.54	8.05	.28	50.04	.50	.15	9.44
Totals	28,806	36.89	10.19	.36	35.48	.57	2.33	14.18

(a) Mortality in which a living microorganism was not involved.
(b) Mortality caused by vertebrate (small mammal) and invertebrate (carabid and pentatomid) predators.
(c) Those insects harboring a "sub-lethal" quantity of an infectious agent or those insects containing two or more agents in combination.

TABLE 5

Cause of death by larval stadia, expressed as % total dead larvae collected — 1972 *and* 1973

Cause of death	Stadia											
	1		2		3		4		5		6	
	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973
Predation	0.06	1.19	0.26	1.20	0.25	1.08	0.17	0.96	0.54	0.74	0.55	0.48
Parasitism	.02	.15	10.54	12.91	4.82	5.99	1.20	5.30	2.46	8.50	2.38	9.78
Infectious disease	6.29	7.96	10.23	11.35	22.34	29.33	37.79	42.63	47.36	59.80	51.07	70.09
NPV	.56	1.65	2.70	9.49	5.45	24.17	12.57	31.70	22.35	48.15	30.58	59.91
Bacteria	5.73	6.02	7.53	1.23	16.89	4.63	24.99	10.31	24.84	11.21	20.32	9.58
Mycoses	.00	.29	.00	.63	.00	.53	.23	.62	.17	.44	.17	.60
Noninfectious disease	83.00	81.14	66.46	58.35	61.26	49.05	39.40	31.60	20.10	17.22	12.52	9.40
Undetermined	10.63	9.56	12.51	16.19	11.33	14.55	21.44	19.51	29.54	13.74	33.48	10.25

A total of 56,661 dead and/or moribund gypsy moth larvae were examined during the 2-year period. In 1972 the nuclear polyhedrosis virus (NPV), bacterial infectious, and noninfectious disease (ailments in which a living microorganism was not involved) accounted for 67% of the observed causes of death (table 3). The comparable 1973 figure was 83%, and NPV occurred at a higher level (table 4). The "undetermined" cause-of-death category most often represented either those insects harboring a "sublethal" quantity of an infectious agent or those insects containing two or more agents in combination. In these cases no specific cause of death was determined.

Among all sites, for the collected dead larvae, noninfectious disease was the predominant mortality factor for stadia 1 to 3, whereas the infectious diseases NPV and bacteria predominated in stadia 5 to 6 (table 5).

Correlation matrices were developed for detecting relationships between incidences (%) of parasitism and disease, using the following data sets: (1) 1972 and 1973 dead collections (table 6), (2) 1972 and 1973 living vs dead collections (table 7), and (3) 1972 and 1973 living and dead collections (table 8).

Correlation matrices representing the 1972 and 1973 living and dead host collection data sets were developed for comparing causes of death with specific ecological variables (table 9).

DISCUSSION

About 80% of the determined host mortality was caused by infectious and noninfectious diseases, and these mortality factors were operating at all host-population levels. For the larval stage, the incidence of NPV increased as the larvae developed toward pupation, while the incidence of noninfectious diseases decreased. Pathogenic bacteria and fungi tended to cause a small and relatively constant mortality rate for different stadia.

The greater incidence of noninfectious disease in the earlier stadia was expected, as the earlier stadia are ontogenetically more susceptible to (1) temperature and humidity fluctuations, (2) starvation, and (3) expression of genotypic deficiencies. The later stadia are more likely to be exposed to or to develop an infectious disease, especially NPV, which appears to be present at all host-population levels.

Parasitoids and predators accounted for about 6% of the identifiable host mortality. In general, parasitoids did not appear to be density dependent mortality factors as negative correlations existed with high host densities and percentage of defoliation (table 9). For the larval stage, the incidence of parasitoids and predators was rather constant within a narrow range of mortality.

For the 1972 and 1973 dead, living-vs-dead, and living-and-dead collections, negative correlations existed between noninfectious disease and individual parasitoid species (tables 6, 7, and 8). This was expected, as noninfectious disease tended to be directly related to host density and defoliation, whereas the survival of several species of parasitoids in areas with high host density and/or severe defoliation was adversely affected (table 9).

For the 1972 dead collection, significant positive correlations existed between NPV and the parasitoids *A. melanoscelus* and *P. silvestris* (table 6). These correlations were negative for the 1973 collection, probably due to an average 2-fold increase in host population numbers and subsequent severe defoliation in the majority of the study sites. This combination of high host numbers and severe defoliation probably created an unsuitable habitat for the parasitoids. This significant positive correlation between NPV and the parasitoid *A. melanoscelus* was evident for the 1972 living-vs-dead

TABLE 6

Correlation coefficient (r) values among categories: dead gypsy moth larvae

Disease categories	Parasitism category							
	Apanteles melanoscelus		*Parasetigena silvestris*		*Compsilura concinnata*		*Blepharipa pratensis*	
	1972	1973	1972	1973	1972	1973	1972	1973
Noninfectious	—.20	—.53*	—.39	—.55**	—.40	—.21	—.43*	—.17
Infectious								
NPV	.59**	—.42	.54**	—.43*	.20	—.22	.32	.41
Bacteria	.19	—.35	.05	—.34	.13	—.15	.01	.38
Mycoses	.30	—.10	.31	—.11	—.06	.52*	—.09	—.01

* : Signif. at 5 % level.
** : Signif. at 1 % level.

TABLE 7

Correlation coefficient (r) values: living vs *dead gypsy moth larvae*

Disease categories	Parasitoids from living larvae							
	Apanteles melanoscelus		*Parasetigena silvestris*		*Compsilura concinnata*		*Blepharipa pratensis*	
	1972	1973	1972	1973	1972	1973	1972	1973
Noninfectious	—.36	—.19	—.09	—.44	—.44*	—.59*	—.32	.10
Infectious								
NPV	.69**	—.53*	.06	.27	.01	.15	.01	—.29
Bacteria	.13	.27	.10	.18	.58**	.44	.83**	—.31
Mycoses	.48*	.85**	.72**	—.11	.25	—.03	.15	.20

* : Signif. at 5 % level.
** : Signif. at 1 % level.

TABLE 8

Correlation coefficient (r) values among categories : living and dead collections 1972 and 1973

Disease categories	Parasitism category							
	Apanteles melanoscelus		*Parasetigena silvestris*		*Compsilura concinnata*		*Blepharipa pratensis*	
	1972	1973	1972	1973	1972	1973	1972	1973
Noninfectious	—.40	—.42	—.23	—.69*	—.50*	—.67*	—.44	—.60*
Infectious.....								
NPV	.49*	.15	—.03	.53	—.01	.40	—.16	.38

* : Signif. at. 5 % level.

TABLE 9

Correlation coefficient (r) values: living and dead data sets — 1972 and 1973

Cause of death category	Host density (egg masses/acre)		% defoliation		% pine		% maple		% oak	
	1972	1973	1972	1973	1972	1973	1972	1973	1972	1973
Noninfectious disease	—.10	.66**	.80**	.86**	.26	.17	—.36	—.36	.53*	.58*
Infectious disease										
NPV	.64**	—.46	—.25	—.60*	—.01	—.04	.27	.17	—.32	—.38
Parasitism										
A. melanoscelus	.29	—.42	—.53*	—.46	—.18	—.29	—.04	.47	—.19	—.43
P. silvestris.....	—.15	—.47	—.45	—.50	—.10	.12	—.01	—.04	—.05	—.02
C. concinnata	—.08	—.50	—.64**	—.58*	—.23	—.32	.11	.62*	—.26	—.53
B. pratensis	—.01	—.66**	—.44*	—.51*	—.35	—.08	.16	—.04	—.52*	.04

* : Signif. at 5 % level.
** : Signif. at 1 % level.

and living-and-dead collections. Also, significant positive correlations were evident between bacteria and the parasitoids *C. concinnata* and *B. scutellata.*

The significant positive correlations between the infectious diseases NPV and bacteria and several parasitoid species suggests the possible use of parasitoids to initiate and/or increase disease incidence within gypsy moth populations. As shown in table 5, the incidence of the infectious disease NPV increased with the larval stadia; and an increase in incidence for the small stadia (1 to 3) should result in increased disease incidence for that and subsequent seasons. Thus the parasitoid *A. melanoscelus*, which parasitizes the small (1 to 3) stadia, might be used to vector NPV through several pathways: (1) direct mechanical transmission by insertion of a contaminated ovipositor, (2) oviposition of a contaminated egg within the host, and (3) indirect mechanical transmission by host feeding on foliage contaminated by contact with contaminated parasitoid structures (i.e., tarsi and/or mouthparts).

Also, *A. melanoscelus* possesses several biological characteristics desirable for vectoring. It is distributed over a broad geographical range, completes several generations per year, reproduces by arrhenotoky, is easy to rear in the laboratory in large numbers, and wild stock is readily available for incorporation into laboratory colonies (REARDON *et al.*, 1973).

Future investigations will be directed toward the use of *A. melanoscelus* females to vector NPV in an attempt to initiate and/or propagate the incidence of NPV within field populations of the gypsy moth.

RÉSUMÉ

Relations entre parasitoides et maladies dans les populations naturelles de *Lymantria dispar* [*Lep. : Lymantriidae*] dans la région nord-est des États-Unis.

Les stades juvéniles de *Lymantria dispar L.* provenant de 6 populations géographiquement distinctes ont été recueillis pendant une période de 2 ans dans le but de déterminer des corrélations entre l'incidence des parasitoides et l'incidence de maladies. On a trouvé une corrélation positive entre l'incidence de la polyhedrose nucléaire (NPV) et l'incidence des deux parasitoides *Apanteles melanoscelus* (RATZEBURG) et *Parasetigena silvestris* (ROBINEAU-DESVOIDY).

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