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A Survey of Micro-organisms Infecting A Spruce Budworm Populations

by

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Abstract

The occurrence of micro-organisms in developing and declining infestations of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens), was studied over a seven year period. The microsporidium, *Nosema fumiferanae,* was the only organism whose incidence in spruce budworm increased as the populations aged. Other microsporidia, *Thelohania* sp. and *Pleistophora* sp., as well as a nuclear polyhedrosis virus and several fungi, remained at such low levels as to have little or no impact on the populations. The most interesting organism recovered was an entomopoxvirus infecting a natural population of the spruce budworm. This was the first record of such a virus occurring in the eastern spruce budworm.

Résumé

La présence de microorganismes dans des infestations en progrès et en régression de la Tordeuse des bourgeons de l'Épinette (*Choristoneura fumiferana* (Clemens)) a été étudiée au cours d'une période de sept ans. La microsporidie, *Nosema fumiferanae* s'est avérée le seul organisme dont l'incidence chez la Tordeuse ait augmenté avec l'âge des populations. D'autres microsporidies, *Theolania* sp. et *Pleistophora* sp., de même qu'un virus de la polyédrose nucléaire et plusieurs champignons, sont demeurés à un niveau assez faible pour n'exercer que peu ou pas d'influence sur les populations. L'organisme le plus intéressant récupéré a été un virus entomopox infectant une population naturelle de la Tordeuse des bourgeons de l'Épinette. Il s'agissait d'une première observation d'un tel virus apparaissant chez la Tordeuse des bourgeons de l'Épinette.

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Introduction

The Eastern spruce budworm, *Choristoneua fumiferana* (Clemens), is affected by a complex of micro-organisms including viruses, protozoa and fungi. Nuclear polyhedrosis virus (Bergold 1951), cytoplasmic polyhedrosis virus (Bird and Whalen 1954), and granulosis virus (Bergold 1950) are known to occur in the spruce budworm. In addition to these viruses the Eastern spruce budworm is susceptible to an entomopoxvirus isolated from the two year cycle spruce budworm *Choristoneura biennis* Free. which is found in British Columbia (Bird et al. 1971), and to a second entomopoxvirus isolated from the large aspen tortrix, *Choristoneura conflictana* Wlk. (Cunningham et al. 1973).

One species of microsporidia present in the spruce budworm, first reported by Graham (1948), and later described by Thomson (1955) is *Perezia fumiferanae*. It has now been renamed and is considered to be *Nosema fumiferanae* (Wilson 1972). Two other microsporidia, *Thelohania* sp. and *Pleistophora* sp. also occur in the spruce budworm (Wilson 1975). The principal fungi found in populations of the spruce budworm are *Beauveria* sp., *Isaria* sp., and *Entomophthora* sp. Isolated cases of these fungi infecting spruce budworm in the forest have been reported (Burke 1967-1978), and *Entomophthora sphaerosperma* Fresenius has, in some cases caused high levels of mortality in field populations of this insect (Harvey and Burke 1974).

This study was undertaken to see if the incidence of infection by micro-organisms increased in a spruce budworm population as the infestation grew older. Whether or not the number of different organisms affecting the population increased as well as the incidence of infection by individual species of organisms was of interest.

Materials and Methods

Three areas located in Birch township where Burying Creek crosses highway 129, about 165 km north of highway 17; Nimitz Township about 24 km north of Birch township; and Lipsett township about 90 km north of Birch township, in Chapleau district, Ontario, were selected for this study. The infestations of spruce budworm were first recorded in Birch township in 1972, Nimitz township in 1971 and Lipsett township in 1969 (A.H. Rose, personal communication).

The forest stand in the collection areas of Birch and Lipsett townships consisted of white spruce, *Picea glauca* (Moench) Voss, black spruce, *Picea mariana* (Mill.) B.S.P., balsam fir, *Abies balsamea* (L.) Mill., jack pine, *Pinus banksiana* Lamb., trembling aspen, *Populus tremuloides* Michx., white birch, *Betula papyrifera* Marsh and an occasional white pine, *Pinus strobus* L. In Nimitz township the area had been cutover leaving large scattered white spruce, black spruce and balsam fir, particularly along the road sides. The regeneration was, for the most part, 2-4 m high jack pine and trembling aspen.

The larvae diagnosed in 1972 were received from the Forest Insect and Disease Survey, Great Lakes Forest Research Centre, Sault Ste. Marie, Ontario. They were examined shortly after they emerged from hibernaculae. The observed low incidence of infection could be an underestimate as diagnosis of such small insects is not as reliable as with larger insects. Also there was insufficient time for an infection to become apparent if it was present. Wilson (1973, 1977a) demonstrated that the incidence of *N. fumiferanae* increases with the age of the larvae during the summer and suggested that the increase in the levels of microsporidia toward the end of summer may have been due to incipient infection that was not detected in younger larvae. Since we examined only second instar larvae from the study areas in 1972, a low level of infection in the populations was detected regardless of the ages of the infestations.

After 1972 insects were examined in the late 6th instar, pupae or in some cases as adults. In 1973 and 1975 an early collection was made when the larvae were in the 4th and 5th instar, followed by a later collection. The larvae from the early collection were reared on synthetic diet in 28 ml plastic cups (Grisdale 1973) to 6th instar, pupae or adults. There were two advantages in collecting late instars. The first, already mentioned, was that microsporidian infection could be readily diagnosed in older larvae. The second was that the older larvae provided a better opportunity for a deliberate search for entomopoxvirus-infected larvae, especially after encountering the disease in 1973. It had been shown that entomopoxvirus infection prolonged the larval stadium and infected larvae may be found when most healthy larvae had pupated (Cunningham et al. 1973).

Dead insects were diagnosed by crushing them in a small amount of water and examining a drop of the liquid under a dark field microscope at 1.500 x magnification. Smears of fat and gut tissue from living larvae were usually examined by the same method; however on occasions phase contrast optics were used.

Results and Discussion

The results of the collections made from white spruce, balsam fir and black spruce in Birch township are given in Tables

I-III. Table IV shows the results from all host trees combined. The total number of larvae in Table IV does not correspond to the total number of larvae in Tables I-III as the types of foliage from which the larvae were collected in 1975, 1976 and 1977 were not fully recorded. Consequently, some of the results of the diagnosis could not be entered in Tables I-III but are entered in Table IV.

The samples in 1972 and 1973 were collected from a small area at each collecting site. After encountering 10 entomopoxvirus-infected larvae in the population in Birch township in 1973, the samples in subsequent years were taken from a wider area to see if the virus disease was widespread in the spruce budworm population. In 1974 spruce budworm were collected at the site where the virus was first found and at a number of sites for a distance of 11 km to the south. Sixteen entomopoxvirus-infected larvae were found; 12 of these were at the original site and of the remaining 4 larvae, 2 were found 0.8 km and 2 were 2.4 km south of the original site. The following year, 1975, 2 more entomopoxvirus-infected larvae were found; 1 at the original sampling site and the other 5.6 km to the south.

The results of the collections made from white spruce, balsam fir, black spruce and jack pine in Nimitz township are given in Tables V-VIII. The data from 1977 are not entered in these tables as the tree species were not recorded in that year. Table IX shows the combined results from all host trees sampled in Nimitz township and includes the data from 1977. The insects collected from jack pine in 1974 were from a tree that was partially shaded by a large heavily infested white spruce tree. The spruce budworm larvae probably fell from the spruce tree onto the jack pine where they remained and actively fed. As in Birch township, beginning in 1974, the larvae were collected over a 5 km area in a deliberate search for entomopoxvirus-infected larvae. Only 1 entomopoxvirus-infected larva was found.

Tables X and XI show the results for the third collection site in Lipsett township for white spruce and balsam fir, with the combined results shown in Table XII. Other than the microsporidium *N. fumiferanae*, few organisms were found in the population.

The entomopoxvirus which was found over a three year period is of special interest since it is the first entomopoxvirus reported in a natural population of the eastern spruce budworm. For this reason samples of the spruce budworm were collected over a wider area and the results of this study along with a description of the virus inclusion bodies will be reported in detail elsewhere. It would appear that the entomopoxvirus-infected larvae are in "pockets" as 28 of the 29 infected larvae were found within 5.6 km of each other and in fact 23 of the 28 were found within 100 m of each other. Under these conditions infection in the population would not be readily detected. The entomopoxvirus was not found in the population after 1975. This probably could have been expected, because, *C. biennis* entomopoxvirus mass produced in the laboratory using *C. fumiferana* as host and subsequently sprayed on a forest stand infested with *C. fumiferana*, causes infection the year of application, but in following years this infection rapdily disappears. (Howse et al. 1973 and Cunningham et al. 1975). The virus infection at such a low level would not have any significant role in regulating spruce budworm populations. The low level of infection in the areas examined is the percentage of the insects diagnosed and is not related to the insect population as a whole. It is interesting to note that the entomopoxvirus found in *C. fumiferana* is not as infectious as the entomopoxvirus found in *C. biennis*. Four collections of *C. biennis* larvae from the same general area of British Columbia were received in 1972. An examination of 190 larvae revealed that 32 were infected with entomopoxvirus, (unpublished data). Again this infection rate of 16.8% is not related to the insect population as a whole but only to the larvae examined.

The micro-organisms most often encountered were microsporidia with *N. fumiferanae* predominating. The nuclear virus infection and the 3 fungi recorded were at a very low level. Other studies of spruce budworm populations have also indicated a similar picture. Neilson (1963) in a study of a spruce budworm population in New Brunswick reported that microsporidia was the most prevalent microorganism present. He also stated that the occurrence of nuclear and cytoplasmic polyhedrosis viruses in natural populations of the spruce budworm were so rare that it was extremely doubtful that they had a measurable effect on the budworm populations. Bird and Whalen (1949) examined 2980 spruce budworm larvae from numerous areas of northern Ontario, about half by sectioning and half with smear preparations under darkfield illumination. They reported virus infection in 45 insects (1.5%) and fungus in 8 insects (0.3%). However, microsporidian infection was also very low; about 1.5% of the insects examined.

The microsporidian species, *Pleistophora* and *Thelohania* were rarely found in the spruce budworm in this study. This is in agreement with Wilson (1975), who examined another spruce budworm population, and found that infection by *Thelohania* sp. was less than 0.5%, with *Pleistophora* sp. infection somewhat higher. It was also observed, as previously reported by Wilson (1975) that spruce budworm larvae infected with *Thelohania* sp. contained massive numbers of spores and attempts to infect healthy spruce budworm larvae with the spores were unsuccessful.

Table XIII shows the levels of infection by *N. fumiferanae* arranged year by year after the infestations were first reported. When the data are plotted on a graph there is an upward trend in the incidence of infection. This has also been demonstrated by others. A study of the spruce budworm population in Uxbridge Forest, Ontario from 1955 to 1959 by Thompson (1960) showed an increase in microsporidia infection from 36.4% in 1955 to 81.3% in 1959 when the population was greatly reduced. Wilson (1977a) examined the same site from 1973 to 1978 and found that infection increased from 35.9% to 69%.

It is not known if the level of infection by microsporidia in the populations examined in this study would have had an impact on reducing the number of insects in the populations. Although sampling was not done in a manner that would measure an increase or decrease in population, by 1978 there appeared to be a reduction in population at the sampling sites. The Survey Bulletin, Forest Insect and Disease conditions in Ontario, Summer 1978, Great Lakes Forest Research Centre notes that some parts of the Chapleau District were largely free of noticeable defoliation. However, frost damage to balsam fir and white spruce as a result of low temperatures in June was recorded in the Chapleau district and this may have contributed to the reduction in the population. Thomson (1960), with an 81.3% infection level reported a greatly reduced insect population and suggests it was probably due to larval mortality and a reduction in fertility of the adults; both caused by microsporidia. Wilson (1977a) in his study of the same site found 69.0% microsporidian infection at a time when the Survey Bulletin (mentioned above) indicated a decrease in spruce budworm infestations throughout most of Southern Ontario. Shortened adult life and reduced fecundity of spruce budworm by *N. fumiferanae* have been demonstrated by Thomson (1958) and Wilson (1977b). Neilson (1956) following a study of sectioned adults from a field population of spruce budworm that showed 42% microsporidian infection concluded that heavily infected males could probably not mate or produce viable sperm. Also, when a heavily infected female was dissected no development of the ovaries could be detected.

Wilson and Kaupp (1976) field tested *N. fumiferanae* and *P. schubergi* by spraying spores on white spruce and balsam fir trees. They found that the incidence of infection by each microsporidian species was higher in spruce budworm larvae collected from sprayed balsam fir than in those insects collected from treated white spruce trees. It was suggested that the difference in results might be due, in part, to the condition of the buds at spraying time as the balsam fir buds are more flushed allowing larger quantities of spore material to reach the insects. In the work reported here a chi-squared analysis indicates that the incidence of microsporidian infection by *N.*

fumiferanae is significantly higher in insects collected from balsam fir than in those collected from white spruce. Apparently balsam fir does not have the inhibiting effect on microsporidia that it does on certain bacteria. Kishner and Harvey (1960) found that needles of balsam fir contain substances strongly inhibitory to the growth of different *Bacillus* species, while Smirnoff (1963) found that bacterial spores sprayed on balsam fir were inactivated. However, Wilson (1978) has shown that once the microsporidia is established in the insect, diet does not affect the ability of the organism to multiply.

Bacteria were not found during the survey. It is normal to find bacteria in dead material but they are generally considered to be secondary organisms. It was noticed that Bird and Whalen (1949) and Neilson (1963) did not record the presence of bacterial infections in the spruce budworm populations they examined.

This study substantiates results reported in other studies of spruce budworm populations. The only organism that consistently shows an increase as the population grows older is the microsporidium *N. fumiferanae*. Other micro-organisms remain at very low levels. *Entomophthora* fungi infections do occur in some areas as reported by (Harvey and Burke 1974) but there is not a consistent increase in percentage infection as is found with microsporidia. The most interesting organism detected was the entomopoxvirus which will be reported in detail elsewhere.

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Table I

Diagnosis of Spruce Budworm

Collected from white spruce in Birch Township.

Year	1972		1973		1974		1975		1976		1978	
Organism	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	1	1.5	5	25.0	53	34.0	15	30.0	28	43.7	4	16.0
Pleistophora sp.					1	0.6						
Thelohania sp.					1	0.6	1	2.0				
Entomopoxvirus					14	9.0						
Isaria sp.									1	1.6		
Negative	66	98.5	15	75.0	87	55.8	34	68.0	35	54.7	21	84.0
Total Insects	67		20		156		50		64		25	

Table II

Diagnosis of Spruce Budworm

Collected from balsam fir in Birch Township

Year	1973		1974		1975		1978	
Organism	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	18	16.1	208	42.5	6	23.1	48	44.5
Pleistophora sp.					1	3.8	1	0.9
Thelohania sp.							1	0.9
Entomopoxvirus	10	8.9	2	0.4				
Negative	84	75.0	280	57.1	19	73.1	58	53.7
Total Insects	112		490		26		108	

Table III

Diagnosis of Spruce Budworm

Collected from black spruce in Birch Township

Year	1974		1975		1978	
Organism	no.	%	no.	%	no.	%
Nosema fumiferanae	21	31.8	19	31.7	9	31.0
Negative	45	68.2	41	68.3	20	69.0
Total Insects	66		60		29	

Table IV

Diagnosis of Spruce Budworm

Collected from all host trees in Birch Township

Year	1972		1973		1974		1975		1976		1977		1978	
Organism	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	1	1.5	23	17.4	282	39.7	185	39.3	35	36.8	89	53.3	61	38.1
Pleistophora sp.					1	0.1	4	0.85						
Thelohania sp.					1	0.1	8	1.7						
Entomopoxvirus			10	7.6	16	2.2	2	0.4						
Nuclear polyhedrosis							4	0.85						
Entomophthora sp.							46	9.8						
Isaria sp.									1	1.1				
Negative	66	98.5	99	75.0	412	57.9	222	47.1	59	62.1	78	46.7	99	61.9
Total Insects	67		132		712		471		95		167		160	

Table V

Diagnosis of Spruce Budworm

Collected from white spruce in Nimitz Township

Year	1972		1973		1974		1975		1976		1978	
Organism	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	1	2.6	19	38.0	2	4.9	32	30.5	6	17.1	25	26.9
Pleistophora sp.									1	2.9		
Entomophthora sp.							2	1.9				
Negative	37	97.4	31	62.0	39	95.1	71	67.6	28	80.0	68	73.1
Total Insects	38		50		41		105		35		93	

Table VI

Diagnosis of Spruce Budworm

Collected from balsam fir in Nimitz Township

Year	1973		1974		1975		1978	
Organism	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	4	9.3	5	15.6	41	44.6	13	33.3
Entomopoxvirus			1	3.1				
Negative	39	90.7	26	81.3	51	55.4	26	66.7
Total Insects	43		32		92		39	

Table VII

Diagnosis of Spruce Budworm

Collected from black spruce in Nimitz Township

Year	1974		1978	
Organism	no.	%	no.	%
Nosema fumiferanae	4	8.2	4	20.0
Negative	45	91.8	16	80.0
Total Insects	49		20	

Table VIII

Diagnosis of Spruce Budworm

Collected from jack pine in Nimitz Township

Year	1974	
Organism	no.	%
Nosema fumiferanae	3	15.8
Negative	16	84.2
Total Insects	19	

Table IX

Diagnosis of Spruce Budworm

Collected from all host trees in Nimitz Township

	1972		1973		1974		1975		1976		1977		1978	
Organism	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	1	2.6	23	24.7	14	9.9	73	37.1	6	17.1	41	33.3	42	27.6
Pleistophora sp.									1	2.9				
Entomopoxvirus					1	0.7								
Entomopthora sp.							2	1.0						
Negative	37	97.4	70	75.3	126	89.4	122	61.9	28	80.0	82	66.7	110	72.4
Total Insects	38		93		141		197		35		123		152	

Table X

Diagnosis of Spruce Budworm

Collected from white spruce in Lipsett Township

Year	1972		1973		1975		1977		1978	
Organism	no.	%	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	3	7.3	12	23.5	9	15.0	40	28.4	28	27.7
Beauvaria sp.	1	2.4								
Negative	37	90.3	39	76.5	51	85.0	101	71.6	73	72.3
Total Insects	41		51		60		141		101	

Table XI

Diagnosis of Spruce Budworm

Collected from balsam fir in Lipsett Township

Year	1973		1974		1975		1978	
Organism	no.	%	no.	%	no.	%	no.	%
Nosema fumiferanae	7	13.5	6	7.4	9	18.0	12	18.7
Thelohania sp.			1	1.2				
Negative	45	86.5	74	91.4	41	82.0	52	81.3
Total Insects	52		81		50		64	

Table XII

Diagnosis of Spruce Budworm

Collected from all host trees in Lipsett Township

Year	1972		1973		1974		1975		1976		1977		1978	
Organism														
Nosema fumiferanae	3	7.3	19	18.4	6	7.4	18	16.4	15	41.7	40	28.4	40	24.2
Thelohania sp.					1	1.2								
Beauvaria sp.	1	2.4												
Negative	37	90.3	84	81.6	74	91.4	92	83.6	21	58.3	101	71.6	125	75.8
Total Insects	41		103		81		110		36		141		165	

Table XIII

Data on *Nosema fumiferanae* arranged to

show the age of the spruce budworm infestation

Years after infestation began	Birch Twsp. Infestation began 1972			Nimitz Twsp. Infestation began 1971			Lipsett Twsp. Infestation began 1969		
	Total Examined	Total Micro.	% Micro.	Total Examined	Total Micro.	% Micro.	Total Examined	Total Micro.	% Micro.
1	67	1	1.5						
2	132	23	17.4	38	1	2.6			
3	712	282	39.6	93	23	24.7			
4	471	185	39.3	141	14	9.9	41	3	7.3
5	95	35	36.8	197	73	37.1	103	19	18.4
6	167	89	53.3	35	6	17.1	81	6	7.4
7	160	61	38.1	123	41	33.3	110	18	16.4
8				152	42	27.6	36	15	41.7
9							141	40	28.4
10							165	40	24.2