OBSERVATIONS ON THE LEVEL OF INFECTION AND INTENSITY OF *NOSEMA FUMIFERANAE* (MICROSPORIDA) IN TWO DIFFERENT FIELD POPULATIONS OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA*

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ABSTRACT

The levels of infection and intensity (spores/insect) of the microsporidium *Nosema fumiferanae* were investigated in two contrasting field populations of the spruce budworm, *Choristoneura fumiferana*, over a 4 year period. In Gargantua, an area of decreasing budworm infestation, the average yearly infection level of *N. fumiferanae* increased only slightly from 52% in 1982 to 62% in 1985. However the average intensity decreased sharply from 30.7 x 10^6 spores/insect in 1982 to 4.5 x 10^6 in 1985. This spruce budworm population collapsed in 1985. In a population of spruce budworm at Black Sturgeon Lake (area of increasing infestation), the average infection levels for 1983 and 84 were the same at 18%, this increased to 33 and 31% for 1985 and 86 respectively. The average yearly intensity in this population increased for the first 3 years but declined in 1986. The intensity was 0.9 x 10^6, 3.0 x 10^6, 9.4 x 10^6 and 2.3 x 10^6 spores/host for 1983, 84, 85 and 86 respectively. In both host populations the number of spores per mg of tissue fluctuated with sampling date, but in general also increased with host age.

RÉSUMÉ

Les pourcentages d'infection par la microsporidie *Nosema fumiferanae* et l'intensité de l'infection (spores par insecte) ont été étudiés sur le terrain chez deux populations très différentes de la tordeuse des bourgeons de l'épinette *(Choristoneura fumiferana)*, pendant quatre ans. Dans la région de Gargantua où l'infestation par la tordeuse est à la baisse, le pourcentage moyen annuel d'infection par *N. fumiferanae* n'a augmenté que légèrement, passant de 52% en 1982 à 62% en 1985. Toutefois, l'intensité moyenne a diminué nettement, soit de 30,7 x 10^6 spores par insecte en 1982 à 4,5 x 10^6 en 1985. Il y a eu effondrement de cette population de la tordeuse en 1985. Chez une autre population se trouvant dans la région du lac Black Sturgeon (où l'infestation est à la hausse), le pourcentage moyen d'infection est resté le même à 18% en 1983 et 1984, mais il est monté à 33 et 31% en 1985 et 1986 respectivement. L'intensité moyenne annuelle de l'infection dans cette population a augmenté au cours des trois premières années, mais a diminué en 1986. Les chiffres pour les quatre années sont respectivement de 0,9 x 10^6, 3,0 x 10^6, 9,4 x 10^6 et 2,3 x 10^6 spores par hôte. Chez les deux populations hôtes, le nombre de spores par milligramme de tissus fluctuait en fonction de la date d'échantillonnage, mais, en général, il augmentait avec l'âge de l'hôte.

INTRODUCTION

The spruce budworm, *Choristoneura fumiferana* (Clem.), is naturally infected with a wide variety of pathogens including viruses, fungi, bacteria and microsporidia. The most common pathogen of this insect is the microsporidium *Nosema fumiferanae* (Thomson). This pathogen retards larval and pupal development, reduces pupal weight and fecundity, and shortens adult life. All of these effects are more pronounced in females than in males (Thomson 1958; Wilson 1977, 1983, 1985a). Previous field examinations of spruce budworm indicated that the percentage of individuals infected with microsporidia increased with the age of the infestation and that infections tend to increase throughout any one budworm generation (Wilson 1973). Due to the widespread occurrence of this microsporidium any comprehensive model of spruce budworm population dynamics must include a thorough understanding of this pathogen. To this end, a cooperative study was undertaken with GLFC and other FPMI personnel. This study was therefore a small part of a larger study on the population dynamics of the spruce budworm. Study plots were selected by GLFC at Gargantua and Black Sturgeon Lake in Ontario. Gargantua is an area of decreasing budworm infestation (age 10+ years). Sampling started here in 1982, and due to a collapse of the population intensive sampling ended in 1985. Sampling at Black Sturgeon Lake (an area of increasing budworm infestation) started in 1983. This report was prepared to summarize the data up to and including 1986. If this study continues, data will probably only come from the Black Sturgeon Lake area.

MATERIALS AND METHODS

Spruce budworm material for microsporidian analysis was obtained by subsampling budworm picked from foliage samples. These foliage samples were collected before budworm emergence (overwintering) and at least twice weekly throughout the active larval and pupal stages. The insects were frozen upon collection and later divided into two groups. Smears of individuals from one group were examined using phase contrast microscope optics to determine the percent-

age of individuals infected. The other group was ground together, placed in a known volume of water and microsporidian spores were counted using a hemacytometer to determine the number of spores in the sample. Only a portion of the insects were infected with microsporidia and this had to be taken into account before spores per individual (intensity) could be determined. After the first year, the dry weight of the insects was determined before counting so that the intensity of microsporidia relative to dry material could be estimated. Sample size was determined by the availability of insects and is indicated in the tables: the number of insects in the tables represents about one-half of the total. Samples were divided and one group was used to determine percent infection. Insect material was dried in a dry-type incubator at 40-45°C for at least 3 days and then stored at room humidity (40-50% R.H) until spore counts were made.

The insect sample was ground in a known volume of water using a tissue homogenizer. Depending on spore concentration, some suspensions were further diluted before counts were made. A sample of the spore suspension was removed with a Pasteur pipette and placed under the cover glass of the counting chamber. Care was taken to ensure that the spores were evenly distributed in the sample, that the chamber was not overloaded and that no air bubbles were present (for more details see Cantwell 1970). In our study counts were made by two people on each sample and the average of these counts were used in the calculations.

RESULTS

Percent Infection: Based on the total year's collection of insects, the yearly average infection levels of *N. fumiferanae* for spruce budworm in the Gargantua plots for 1982, 83, 84 and 85 were 52, 59, 60, and 62%, respectively (Fig. 1), indicating a slight increase in infection levels of the parasite with the age of the infestation. Tables 1-4 do not show any particular trend in percent infection by the microsporidia within any one season. In fact, there can be considerable fluctuation from one sampling date to the next.

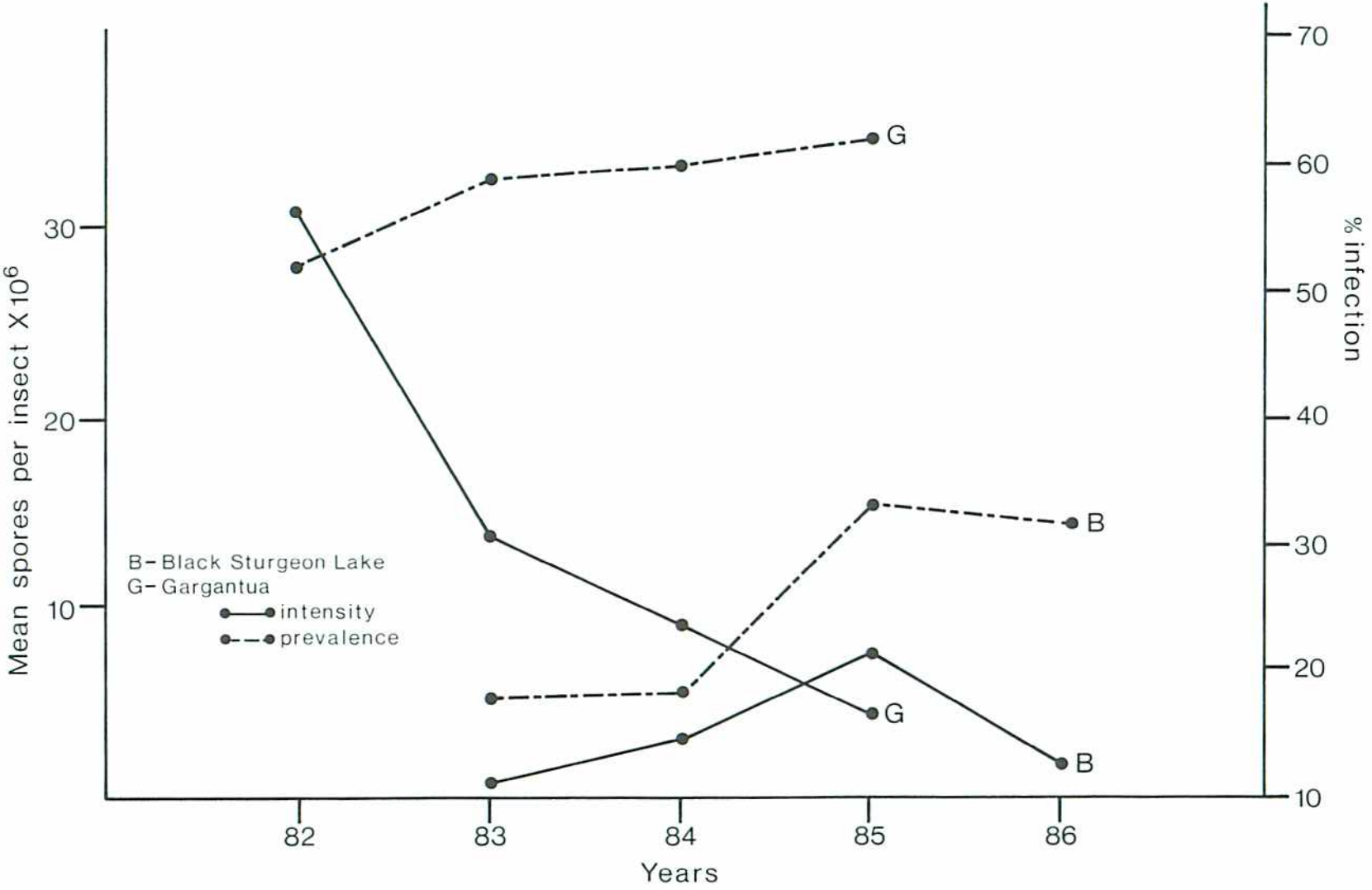

Figure 1. The intensity and prevalence of *Nosema fumiferanae* in spruce budworm collected from Gargantua and Black Sturgeon Lake plots for 1982-1986.

Table 1. The infection levels and intensity of *Nosema fumiferanae* in spruce budworm collected at various times from a plot at Gargantua during 1982

Julian date	Number of insects	Percent infected	Proportion of insects infected	Total spore count	Spores/ sample x 10^6	Spores/ insect x 10^6
145	35	45	16	21	1.1	.07
151	20	38	8	157	7.8	1.0
158	35	67	23	3397	169.8	7.3
162	20	60	12	1322	66.1	5.5
168	40	50	20	4171	208.5	10.4
172	20	60	12	4201	210.0	17.5
175	20	57	11	6695	334.7	30.4
179	20	55	11	9547	477.3	43.4
182	20	67	13	13857	692.8	53.3
188	40	52	21	24105	1205.2	57.4
192	40	60	24	28095	1404.7	58.5
196	40	70	28	28503	1425.1	51.0
198	20	50	10	12647	632.3	63.2

The same general trends were noted for the Black Sturgeon Lake plots, with the average yearly infection levels of *N. fumiferanae* increasing with the age of the host infestation (Fig. 1). Although levels for 1983 and 84 were the same at 18%, they increased to 33 and 31% for 1985 and 86 respectively. As was the case for Gargantua, infection levels fluctuated between sampling dates (Tables 5-8).

Intensity: In the Gargantua plot the average number of *N. fumiferanae* spores per spruce budworm larvae (= intensity) decreased each year, with values of 30.7 x 10^6, 13.9 x 10^6, 9.3 x 10^6 and 4.5 x 10^6 for years 1982, 83, 84 and 85 respectively (Fig. 1). In all cases there was an increase in intensity with an increase in insect age within any one season (Tables 1-4) (Fig. 2). Number of spores per mg of tissue fluctuated with sampling date, but in general also increased with host age (Tables 1-4).

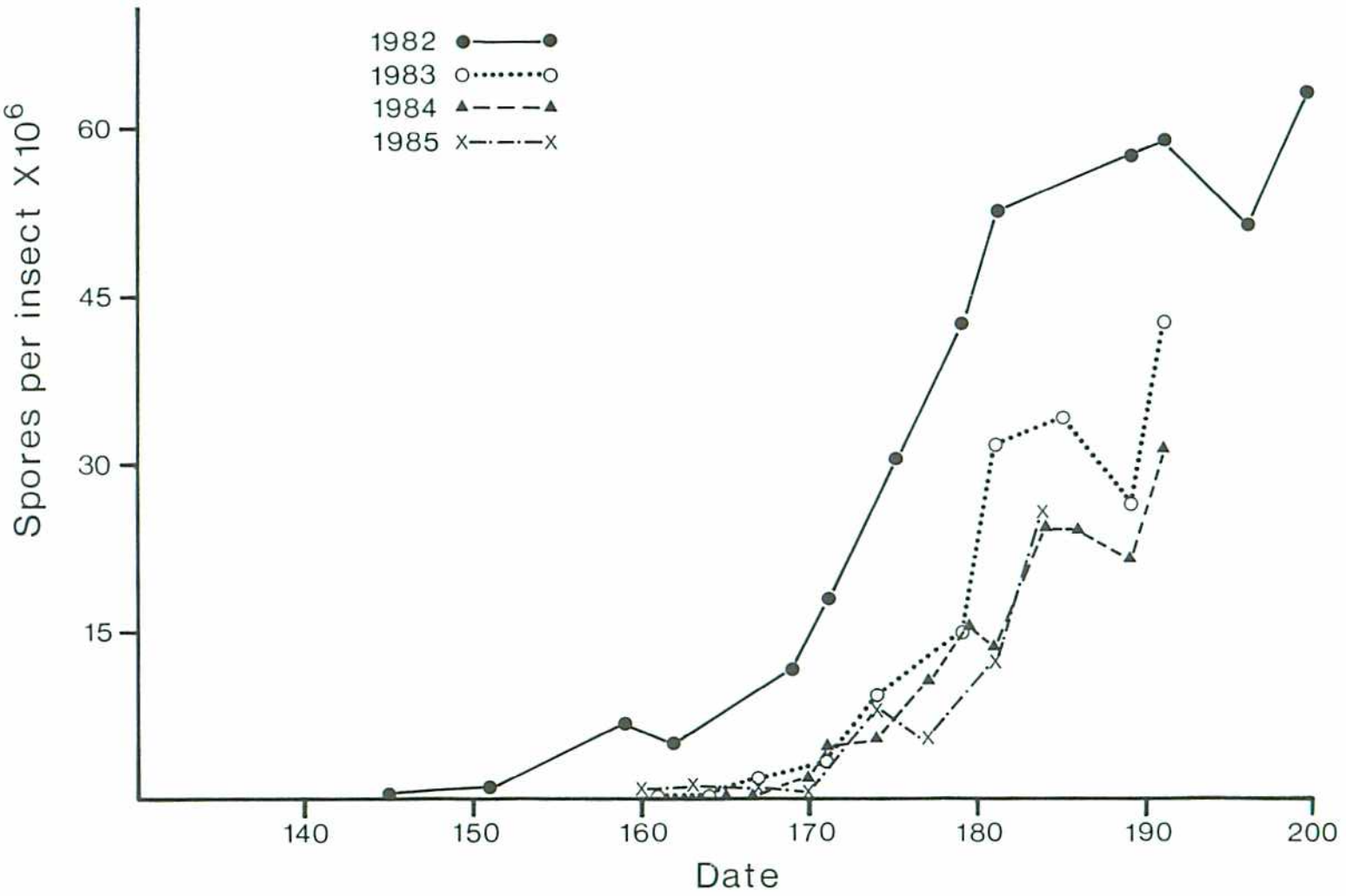

Figure 2. The intensity of *Nosema fumiferanae* in spruce budworm collected from a plot at Gargantua for 1982-1985.

The intensity of *N. fumiferanae* spores per spruce budworm from the Black Sturgeon Lake plot increased for the first 3 years of sampling, but declined in 1986 (Fig. 3). The average intensity was 0.9×10^6, 3.0×10^6, 9.4×10^6 and 2.3×10^6 spores per host for 1983, 84, 85 and 86 respectively (Fig. 1). Spores per mg of tissue showed the same general trend as occurred for the Gargantua spruce budworm population (Tables 5-8). The average number of spores per mg of tissue was 0.2×10^6, 0.4×10^6, 1.2×10^6 and 0.2×10^6 consecutively for the four years of sampling. These values follow the same pattern as spores per insect.

DISCUSSION

Insect pathogens are considered to be a host-density-dependent mortality factor, but if they are widely dispersed in the host environment, they may act as a density-independent factor (Tanada 1976). There is a paucity of quantitative data on the relationship between host-density and microsporidia. There are data available on the levels of infection covering a month, or a year, but long-term studies have not been done. These long-term studies should also relate percent infection with age of the infestation and age of the host, as well as intensity (parasites per host). Investigations into the dynamics of the interaction between a pathogen and its invertebrate host can guide the design of laboratory or field experiments, estimate whether the pathogen is capable of regulating the target population, and, if so, what quantity of it must be introduced to effect a specified level of control (Anderson and May 1981). Franz and Huger (1971) studied an epizootic of *Nosema tortricis* in a population of the green tortrix *(Tortrix viridana)* for a two year period and were able to predict a general collapse of the green tortrix population caused by this microsporidium, enabling the cancellation of planned chemical control measures.

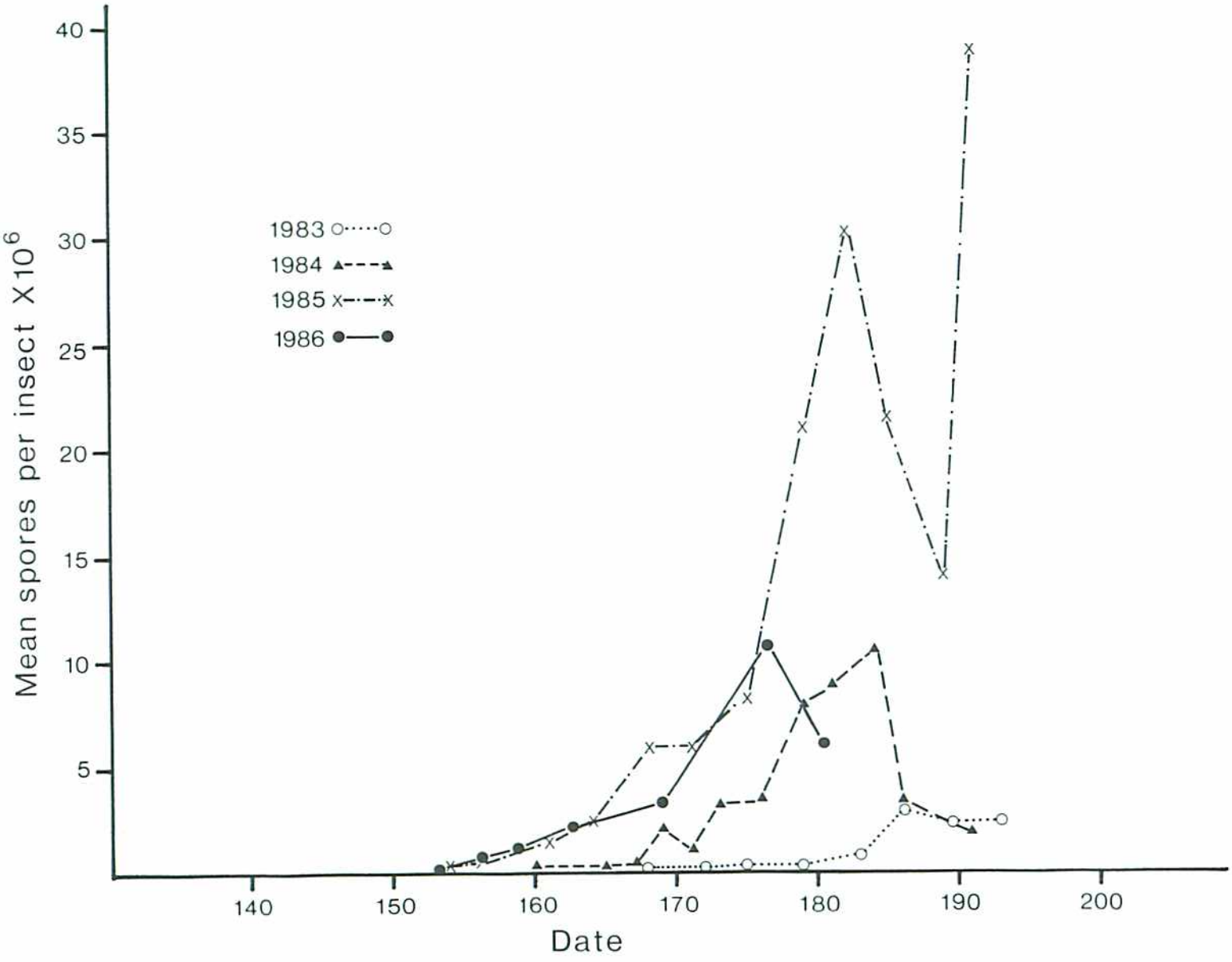

Figure 3. The intensity of *Nosema fumiferanae* in spruce budworm collected from a plot at Black Sturgeon Lake for 1983-1986.

Table 6. The infection levels and intensity of *Nosema fumiferanae* in spruce budworm collected at various times from a plot at Black Sturgeon Lake during 1984

Julian date	Number of insects	Percent infected	Proportion of insects infected	Total dry weight (Mg)	Proportion of dry wt infected	Total spore count	Spores/ sample x 10^6	Spores/mg dry wt x 10^6	Spores/ insect x 10^6
150	87	4	4	13.6	0.5	3	0.2	0.3	0.05
156	142	16	23	58.2	9.3	15	0.7	0.08	0.03
160	141	14	20	146.5	20.5	116	5.8	0.3	0.3
162	215	17	36	251.9	42.8	53	2.7	0.06	0.07
165	100	23	23	144.2	33.1	146	7.3	0.2	0.3
167	100	24	24	239.8	57.5	230	11.5	0.2	0.5
169	100	14	14	229.8	32.1	578	28.9	0.9	2.1
171	110	33	36	1051.3	179.9[a]	503	25.1	0.1	1.3
173	120	20	24	1091.5	116.3[a]	885	44.2	0.4	3.4
177	105	21	22	781.2	74.2[a]	699	34.9	0.5	3.5
179	80	20	16	755.0	58.1[a]	1308	65.4	1.1	8.4
181	36	12	4	364.0	41.4[a]	731	36.5	0.9	9.0
184	27	19	5	298.7	56.7	1052	52.6	0.9	10.5
186	16	14	2	135.1	18.9	153	7.8	0.4	3.9
191	8	16	1	94.2	15.1	43	2.2	0.1	2.2

a = spore counts were performed on a subsample of the dried material.

Table 7. The infection levels and intensity of *Nosema fumiferanae* in spruce budworm collected at various times from a plot at Black Sturgeon Lake during 1985

Julian date	Number of insects	Percent infected	Proportion of insects infected	Total dry weight (Mg)	Proportion of dry wt infected	Total spore count	Spores/ sample x 10^6	Spores/mg dry wt x 10^6	Spores/ insect x 10^6
86	82	27	22	3.6	1.0	23	1.2	1.2	0.05
142	26	31	8	0.5	0.2	3	0.1	0.8	0.01
146	76	25	19	16.2	4.1	40	2.0	0.5	0.1
150	89	23	20	23.5	5.4	61	3.0	0.6	0.1
154	74	41	30	31.3	12.9	237	10.9	0.8	0.4
157	53	65	35	44.7	29.1	426	21.2	0.7	0.6
161	76	33	25	120.5	40.1	593	29.6	0.7	1.2
164	70	30	21	134.4	40.3	1048	52	1.3	2.5
168	49	27	13	156.9	41.6	1560	78	1.9	6.0
171	77	26	20	315.2	83.2	2453	122.6	1.5	6.0
175	110	36	40	929.9	338.5	6629	331.4	1.0	8.3
179	61	34	21	578.8	199.1	8838	439.8	2.2	21.0
182	20	30	6	235.5	70.6	3679	183.9	2.6	30.6
185	25	28	7	421.1	117.9	3046	152.2	1.3	21.7
189	25	46	12	364.6	168.4	3278	163.9	1.0	14.2
192	21	32	7	437.2	139.0	5220	261	1.9	39.0

Table 8. The infection levels and intensity of *Nosema fumiferanae* in spruce budworm collected at various times from a plot at Black Sturgeon Lake during 1986

Julian date	Number of insects	Percent infected	Proportion of insects infected	Total dry weight (Mg)	Proportion of dry wt infected	Total spore count	Spores/ sample x 10^6	Spores/mg dry wt x 10^6	Spores/ insect x 10^6
102	104	32	33	7.4	2.4	17	0.8	0.3	0.03
142	59	20	12	6.6	1.3	4	0.2	0.15	0.02
146	114	30	34	40.9	12.7	70	3.5	0.27	0.1
149	77	24	18	105.5	25.3	100	5.0	0.20	0.3
153	64	33	21	97.5	32.1	225	11.2	0.35	0.5
155	50	27	13	191.6	51.7	270	13.5	0.26	1.0
159	51	53	27	431.8	228.8	870	43.5	0.19	1.6
162	49	28	14	450.8	126.2	550	27.5	0.22	2.0
169	49	36	18	795.4	286.3	1100	55.0	0.19	3.0
176	26	16	4	553.7	86.5	878.7	43.9	0.50	10.9
180	12	42	5	341.9	143.5	670	33.5	0.23	6.7

The study described in this report demonstrates the increased level of *N. fumiferanae* infection as the age of the host infestation increases. This same trend was shown for *N. fumiferanae* in spruce budworm in a previous field study (Wilson 1977), and even earlier by Thomson (1960). Thomson measured levels of *N. fumiferanae* in overwintering populations in the Uxbridge forest of southern Ontario and recorded increases in infection from 36 to 81% over the five year period of 1955 to 1959. This phenomenon has also been recorded for other insects infected with microsporidia. Hurpin (1965) examined populations of *Melolontha melolontha* infected with *Nosema melolonthae* and noted that infection levels rose from 2.5 to 20% in a 3 year period. In a long-term study (over a 16 year period) Hill and Gary (1979) investigated the infection levels and development of *Nosema pyrausta* in field populations of the European corn borer, *Ostrinia nubilalis*. In one county, *N. pyrausta* reached epizootic proportions twice in this 16 year period. The epizootic development (infection levels as high as 100%) followed periods of increasing host density. The same situation probably occurs for *N. fumiferanae* infecting the spruce budworm. However, longer field studies will

be required to determine if definite epizootic peaks occur in this host-pathogen relationship.

There is little information on the relationship between spore intensity and age of the host population or the percent infection by microsporidia. It appears from the spruce budworm study, that a declining host infestation with high levels of infection results in a decrease in the average *N. fumiferanae* spores per infected host. Conversely, an increasing host population with increasing levels of infection results in a higher intensity (parasites/host). The different behavior of the two populations could be due to different starting levels of infection in the study areas. Gargantua had a high level of infection and could only come down, while at Black Sturgeon Lake the opposite was true. The Black Sturgeon Lake population showed an increase in both level of infection and intensity for 1983, 84 and 85, with a slight decrease in both for 1986. This decrease was unexpected and the reason uncertain. It may have been related to two weeks of high temperatures during the first part of the budworm season. Due to the generation time (spore to spore) of *N. fumiferanae* (ca. 1 wk), any increase in the development rate of the host would have a tendency to decrease the number of spores per host.

Issi (1982) reported on similar studies involving the interrelations between the cabbage white butterfly, *Pieris brassicae*, and the microsporidium *Nosema mesnili*. He demonstrated that factors conducive to an increase in the insect population also promote an increase in the pathogen population. The same author found that the highest levels of infection resulted in a reduction of the number of spores developing per unit mass of the host. The interaction of these two factors was due to an increase in the pathogenicity of the microsporidia and a weakening of the physiological condition of the host, resulting in early death, thus shortening the time the microsporidia could multiply. Hill and Gary (1979) reported that the epizootic development of *N. pyrausta* in the corn borer followed periods of increasing host

density. They found that intensity peaked (48.7 x 10^6 spores/larva) at the same time as percent infection, and both then declined followed by a decreasing corn borer population.

In both spruce budworm populations studied the number of *N. fumiferanae* spores per insect peaked toward the end of the yearly budworm cycle. This probably relates to the developmental rate of *N. fumiferanae* and the increase of budworm size. Another phenomenon that occurred in a number of cases was a dip in the intensity at the end of the larval stage followed by a final increase during the larval stadium. The highest intensity of *N. fumiferanae* occurred in spruce budworm collected from Gargantua in 1982 at 63.2 x 10^6 spores per larva. The greatest number of *Nosema locustae* spores present in infected grasshoppers occurred late in the season (Henry 1972). The average number of spores per mg of grasshopper weight also increased with the development of the grasshoppers.

There is virtually no information on the relationship of intensity of *N. fumiferanae* to death of spruce budworm in the field. Wilson (1985b) demonstrated that under laboratory conditions spores per dead larva depend on the initial dose fed to the insects. The spore count ranged from 106.9 x 10^6 to 2.8 x 10^6 spores per dead larvae, when 4th-instar budworm were treated with 5 x 10^5 to 5 x 10^7 *N. fumiferanae* spores. Based on the intensities observed in the present field study it is probable that *N. fumiferanae* kills spruce budworm in the field and could be related to the quantity of *N. fumiferanae* passed from an infected female to her offspring. Any additional spores ingested by larvae during feeding would increase larval mortality.

The data obtained during observations of the interaction of *N. fumiferanae* and its host the spruce budworm reveals a quantitative relationship between the parasite and the host populations. And as suggested by Régnière (1984), vertically transmitted diseases (such as *N. fumiferanae)* even of low virulence may be crucial elements of the population dynamics of many insect pests. Thus any study of the population dynamics of spruce budworm must consider the role of microsporidia.

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