Application of a Microsporidia, *Nosema fumiferanae*, against Spruce Budworm on Manitoulin Island, 1975.

by

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Abstract

A microsporidian pathogen, Nosema fumiferanae, was tested against the spruce budworm, Choristoneura fumiferana on Manitoulin Island during June 1975. A packsack-type mist sprayer was used to apply suspensions of microsporidian spores on individual spruce trees (Picea glauca). Fourteen trees were sprayed with formulations of varying percentages of microsporidian spores. Eleven trees were sprayed when budworm development was predominantly the fourth instar and three trees were sprayed later when budworm were sixth instar. The formulation applied to one of the latter three trees contained Sandoz adjuvant V.

Spraying of N. fumiferanae in the field increased the level of microsporidia in larvae and adults as compared to unsprayed areas. Early spraying was more effective than late spraying and Sandoz adjuvant V may increase the effectiveness of the pathogen when added to the formulation. Infected adults transmit the pathogen transovarially, therefore most of the resulting offspring can be expected to be infected.

Résumé

En juin 1975, les auteurs testèrent l'effet d'un agent pathogène microsporidien, Nosema fumiferanae, sur la Tordeuse des bourgeons de l'Épinette, Choristoneura fumiferana dans l'île Manitoulin. Un pulvérisateur à dos servait à arroser les Épinettes blanches (Picea glauca) une à une avec une suspension de spores microsporidiennes. On arrosa quatorze arbres avec des formules à teneur variée des dites spores. On arrosa onze arbres lorsque les Tordeuses avaient atteint leur quatrième stade, surtout, et plus tard trois arbres supportant des Tordeuses à leur sixième stade. La suspension qui servit à l'arrosage de l'un de ces derniers contenait aussi de l'adjuvant Sandoz V.

De tels arrosages augmentèrent le niveau de microsporidies dans les larves et les adultes. L'arrosage hâtif fut plus effectif que l'arrosage effectué plus tard, et l'adjuvant Sandoz V peut augmenter l'efficacité de l'agent pathogène. Les adultes infectés transmettent l'agent pathogène "transovairement" et leur progéniture pourra donc en grande partie être infectée.

Introduction

The spruce budworm, Choristoneura fumiferana (Clemens), is the most important forest pest in Eastern Canada. It is the host of a number of pathogens, including the microsporidian, Nosema fumiferanae (Thom.). This protozoan parasite was first reported as occurring in the spruce budworm by Graham, (1948) and later described in more detail by Thomson (1955).

The use of microsporidia in field trials to control pest insects has only recently been attempted. Henry (1971) carried out trials to control grasshoppers using Nosema locustae, and found that the incidence of infection in grasshopper was significantly higher for the treated areas. Hall (1954) attempted to control sodworms, Crambus spp., by using the pathogen Nosema infesta, and concluded that the pathogen would be useful for long-termed control of the insect. McLaughlin et al (1969) were able to provide some control of field populations of the boll weevil, Anthonomis grandis, using the microsporidian Glugea gasti. Although field trials using microsporidia to control pests are few, laboratory evidence indicates that many of these protozoans affect the vigour, longevity and fecundity of the host insect. Our study was initiated to determine if N. fumiferanae

could be successfully introduced into a field population of the spruce budworm and persist sufficiently to control the insect population level in succeeding years.

Materials and Methods

Production of Nosema fumiferanae spores

Propagation of the microsporidia commenced four months prior to field spraying. Second instar larvae (newly emerged from the hibernacula) were placed in one-oz plastic cups 3/4 filled with a synthetic diet (McMorran 1965). The diet surface had been sprayed with 2×10^5 spores/ml of N. fumiferanae in distilled water. Insects were reared on the diet until shortly before pupation, at which time they were removed from the diet so that the microsporidian spores could be harvested in the following way. Infected larvae were homogenized in distilled water and the homogenate passed through two layers of cheesecloth to remove large debris. The filtrate was collected and stored in a refrigerator at 4°C. After the spores had settled (two to three days) most of the supernatant was decanted, thereby reducing the volume of material to be stored. The spores were pooled, mixed, and spore concentration determined, using a hemacytometer.

Experimental plots

The experimental plot was located 1.1 km south of the town of Burpee in Burpee Twp. on Manitoulin Island (fig. 1). The terrain was relatively flat

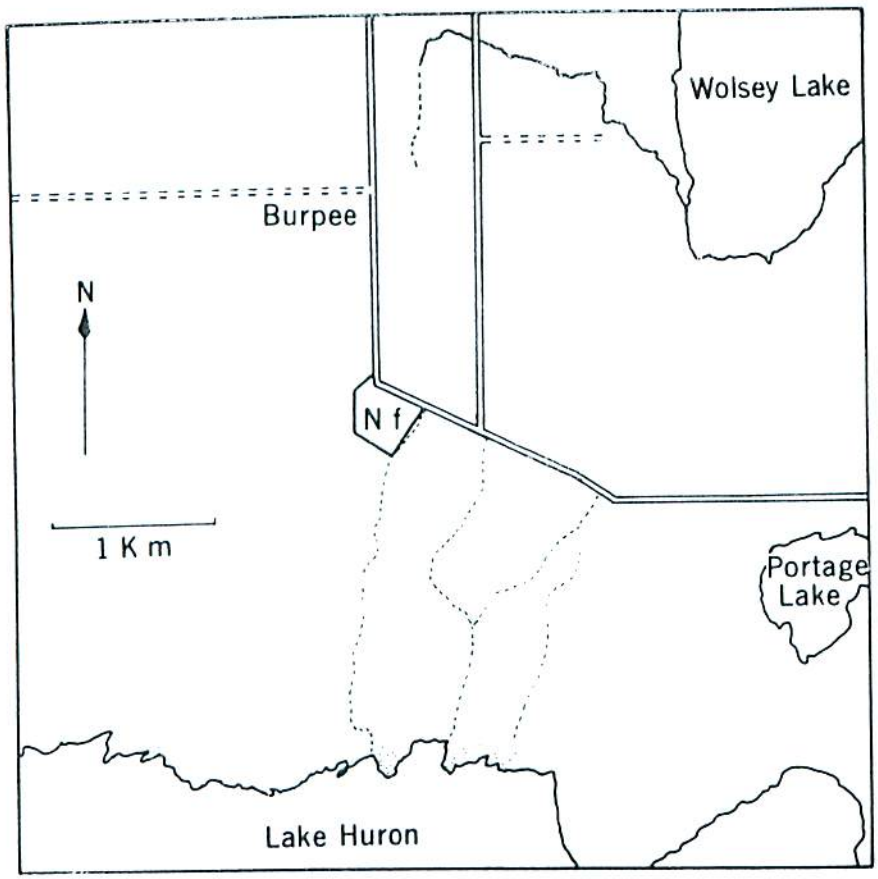

Fig. 1. Plot (nf) sprayed with Nosema fumiferanae spores in Burpee Twp. on Manitoulin Island, Ontario.

covered primarily with open grown white spruce (Picea glauca) stems (fig. 2). Some poplar (Populus tremuloides) and white cedar (Thuja occidentalis) stems were scattered throughout the plot. Fourteen white spruce trees, with a height range of 3.2 to 7.9 m ($\bar{\chi}$ = 5.9 m) were selected for application of N. fumiferanae spores. These trees were labelled M1 through M14. Five check trees were selected in a suitable site in close proximity to the treatment area to insure the same levels of natural microsporidian infection in budworm in both areas.

Formulation and application rate

Sample trees M1 to M12 were sprayed with 1500 ml of a formulation consisting of 12% microsporidian suspension (1×10^9 spores/ml) and 88% distilled water by volume. Approximately 1.8×10^{11} spores were applied per tree.

Sample tree M13 was sprayed at the same rate with a formulation of 12% microsporidian suspension, 44% distilled water and 44% Sandoz adjuvant V[1], a U.V. protectant.

Sample tree M14 was sprayed with 500 ml of a formulation consisting entirely of the microsporidian suspension (approximately 5×10^{11} spores per tree)

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[1] Sandoz-Wander Inc.
Homestead, Florida 33030.

Fig. 2. Area on Manitoulin where Nosema fumiferanae spores were sprayed.

All formulations were prepared in the field immediately prior to spraying.

Spray operation and larval development

The suspension of microsporidia was applied to sample trees with a packsack-type mist sprayer (KWH 26TT Kem. San Ltd.)(fig. 3).

On the evening of June 2, sample trees M1 to M10, and M14 were sprayed under suitable weather conditions. Larval development at the time of application was primarily fourth and fifth instar and is shown in table 1. Trees M11 to M13 were sprayed later (evening of June 13) when larval development was primarily at the sixth instar level (table 1).

Sampling and microscopic examination

All samples consisted of single 46 cm branch tips from the mid-crown of each tree. A pre-spray sample was collected on May 26 from trees in the treatment area, and results from these were used to determine the initial incidence of microsporidia in the budworm population present on the plot. On June 13, eleven days after spraying, the first post-spray sample was collected from trees M1 to M10 and tree M14. Samples were also taken from three check

Fig. 3. Spraying Nosema fumiferanae spores on white spruce using a packsack-type mist sprayer.

trees outside the plot, but close enough to the treated area, to assume that the initial levels of microsporidian infection in spruce budworm was the same in both. A second post-spray sample was collected from trees M1 to M14 and five check trees on June 27.

On July 2, a final sample consisting of live pupae and adults, was collected using a beating mat. Individuals obtained from this sample were used to estimate the level of microsporidia in the adult population of the spray plot, and also the incidence of microsporidia in their progeny.

Adults obtained from the above sample were combined as follows to form 9 groups: Trees 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13, and checks 1 and 2 and 3 and 4. Adults from each group were placed (in mass) in plastic cages (19 x 27 x 10 cm) containing balsam fir foliage and allowed to mate. Adults were examined for N. fumiferanae spores when oviposition was completed. Egg masses on the balsam needles were removed from the cages and allowed to hatch. The offspring (second-instars) were picked at random and examined for microsporidian infection.

All examinations to determine the levels of N.

fumiferanae were performed by examining smear preparation of larvae and adults with an Ortholux microscope using phase contrast optics. The presence or absence of N. fumiferanae spores in the host tissue (fig. 4) was recorded.

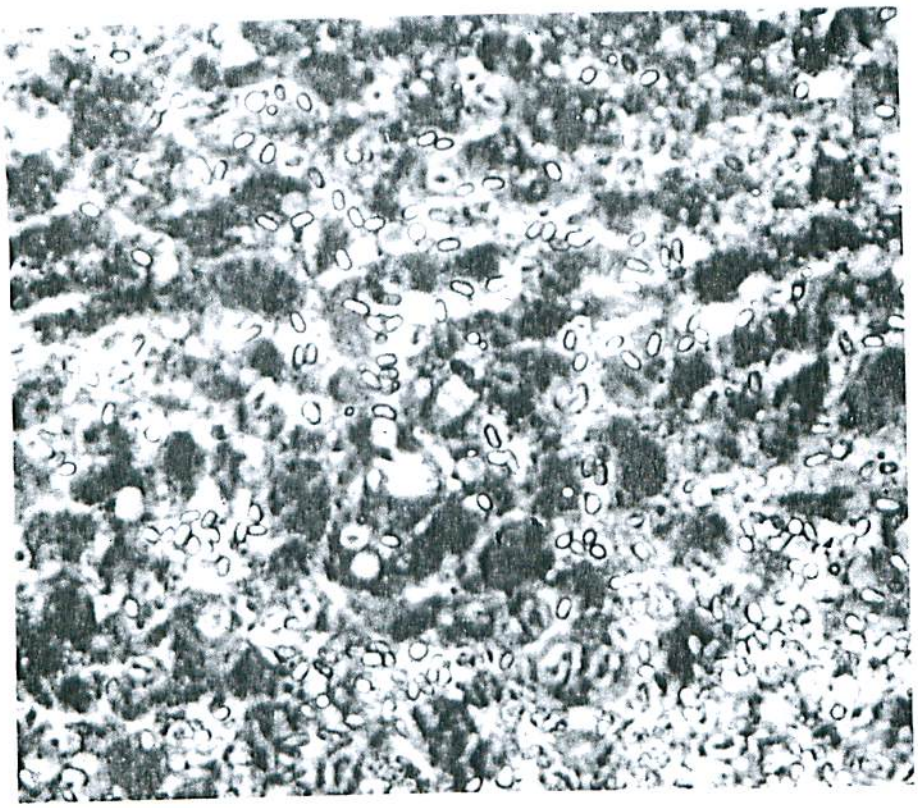

Fig. 4. Spores of Nosema fumiferanae in gut tissue of the spruce budworm. Phase contrast. X750.

Results

The effect on the incidence of N. fumiferanae in spruce budworm larvae following spraying is shown in Table 2. Levels of microsporidia in the larvae were significantly higher in those insects collected from treated trees (M1 to M10 and M14) as compared to untreated trees. This observation is based on the assumption that initial microsporidian infection, in budworm are similar in both treatment and check areas. Due to the close proximity of the two areas it is reasonable to assume the characteristics of the budworm population were the same. Infection in larvae from tree M14, which was sprayed with a 100% microsporidian suspension, was greater than in larvae from trees M1 to M10, however, this difference was not significant. It should be noted that tree M14 received only 500 ml of the formulation as compared to 1500 ml for other trees. Table 2 also shows a slight increase in the levels of N. fumiferanae infection in larvae from both check trees and trees M1 to M10 between sampling dates (June 13-27).

The percent microsporidia in insects from trees M11 to M12, which received the late spray, was not significantly higher than the checks (C1 to C5) (table 3). Infection in larvae sprayed with the formulation containing Sandoz adjuvant V (tree M13),

was significantly higher (at 5% level) than the checks, but not significantly different from larvae on trees M11 and M12 (table 3).

The results in Table 4, indicate that more adults from sprayed trees than the checks were infected. The level of microsporidia was less in those adults collected from trees receiving the later spray. Larvae resulting from the above matings were also examined for microsporidia. It was found that the offspring of adults collected from sprayed trees had higher levels of microsporidia (table 5).

Table 1

Spruce budworm development at time of spraying

Nosema fumiferanae

Spray dates	Number of larvae examined	Percentage Instar III	IV	V	VI	pp*
June 2	104	6	56	35	3	-
June 13	69	-	4	12	78	6

*pp = prepupae

Table 2

Incidence of *Nosema fumiferanae* in spruce budworm larvae collected from spruce trees sprayed June 2, 1975.

Tree numbers[a]	Sample date	Predominate instar(s)	Number insects examined	Incidence of *N. fumiferanae* (%)
In spray area	May 26 (Prespray)	III-IV	613	13.5
M1 to M10	June 13	V-VI	516	45.9**
M14	" 13	V-VI	215	59.6**
Check	" 13	V-VI	52	17.6
M1 to M10	June 27	VI	568	53.0**
M14	" 27	VI	62	54.8**
Check	" 27	VI	174	23.0

a=Trees M1 to M10 sprayed with 1500 ml formulation consisting of 12% *N. fumiferanae* spore suspension (spore concentration 1 x 10^9 spores per ml) Tree M14 sprayed with 500 ml formulation consisting of 100% *N. fumiferanae* spore suspension

**= These values are significantly different from the controls at the 1% level. Statistical analysis was performed using "t" test as applied to percentages (Cox, 1954).

Table 3

Incidence of Nosema fumiferanae in spruce budworm larvae collected from spruce trees sprayed June 13, 1975.

Tree number[a]	Sample date	Predominate instar(s)	Number of insects examined	Incidence of N. fumiferanae(%)
M11 to M12	June 27	VI	135	33.7
M13	June 27	VI	43	41.8*
Check	June 27	VI	174	23.0

a = Trees M11 to M12 same dosage and formulation as trees M1 to M10; see footnote Table 2. Tree M13 same dosage as M11 to M12, however, formulation contained 44% Sandoz adjuvant.

* = This value significantly different from controls at the 5% level; see footnote Table 2.

Table 4

Incidence of *Nosema fumiferanae* in spruce budworm adults collected from sprayed trees.

Tree numbers[a]	Number examined	Incidence of N. fumiferanae(%)
M1 to M10	193	81.3
M11 to M12	100	70.0
M13	49	63.3
Check	148	49

a = For dosage and formulation see footnote Table 2.

Table 5

Incidence of *Nosema fumiferanae* in second instar budworm larvae; offspring of adults collected from sprayed trees

Tree number[a]	Number examined	Incidence of N. fumiferanae(%)
M1 to M10	240	77.1
M11 to M12	50	52.0
M13	50	48.0
Check	150	39.3

a = For dosage and formulation see footnote Table 2.

Discussion

The main objective of this preliminary field trial was to ascertain if the microsporidian pathogen, N. fumiferanae, could be successfully introduced into a population of the spruce budworm. Although no attempts were made to assess different formulations or dosage, the data indicates that higher concentrations of N. fumiferanae spores and the use of Sandoz adjuvant V in the formulation may increase the level of infection in the budworm population.

An analysis of the levels of infection indicate that N. fumiferanae can be successfully used to artificially infect populations of the spruce budworm provided we assume that levels of natural infection was the same in both the check and treated areas. The level of infection of N. fumiferanae in spruce budworm larvae was significantly higher in those sprayed with a formulation consisting of a 12% microsporidian suspension (spore concentration 1×10^9 spores/ml) than in unsprayed larvae.

In general there is an increase in levels of N. fumiferanae as time from spraying increases. However, the percent infection in the checks also increased. This phenomenon has been observed in budworm populations naturally infected with microsporidia (Wilson, 1973). Since viable spores are found in

regurgitated fluids and frass of infected insects, this material may act as an inoculum for any healthy larvae that subsequently come in contact with it. In this way the level of infection can increase with time.

There is some indication that the sunlight protectant we used may increase the levels of infection. It has been demonstrated that 5 to 6 hrs of direct sunlight will inactivate spores of N. fumiferanae (Wilson, 1974a). The results from the late spray may have also been affected by the weather. There was a heavy rain shower approximately 10 hrs after spraying. The extent to which this washed the spores off the foliage or spread the infection is not known.

Levels of infection in adults collected from treated trees was high. However, the sample was apparently taken late because about 70 percent of the pupae had already emerged as adults. This sample therefore consisted of pupae and adults that could be picked from the beating mat. Since late pupae and weakened adults are more likely to be infected with microsporidia the above sample was probably biased in favour of insects infected with the parasite. The level of infection was higher in adults

collected from the sprayed trees than unsprayed trees.

Although it has been demonstrated that N. fumiferanae will cause high mortality in the host, if the initial dosage of spores ingested is high and the host no older than the early third instar (Wilson, 1974b).

It is difficult to infect early instar budworm under field conditions because of their concealment in the unflushed buds of the host tree. However, this study indicates that budworm larvae infected in the fourth or fifth instar, produce infected adults, who readily transmit the pathogen to most of their offspring thus perpetuating the infection in the field.

If protozoans are to be used as biological control agents, effects on the host other than mortality must also be considered. Hall (1954) suggested that Nosema infesta should be considered as a long-term control for sodworms, because it reduced fecundity of the host. Henry (1971) demonstrated that N. locustae reduced densities of some grasshoppers and that others lacked normal reproductive capability because of infection. Thomson (1958) suggested that N. fumiferanae reduces the fecundity of the spruce budworm. This is now

being verified by further tests.

Follow-up studies of the experimental plot are planned to reveal the persistence of the microsporidian parasite on the spruce budworm population and any effects thereof. It is hoped that further field trials with different formulations, spore concentrations, and a sunlight protectant may be useful in establishing microsporidia as a long-term biological control agent.

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