APPLICATION OF *NOSEMA FUMIFERANAE* AND *PLEISTOPHORA SCHUBERGI* (MICROSPORIDA) AGAINST THE SPRUCE BUDWORM IN ONTARIO, 1976.

by

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Abstract

Two microsporidian pathogens Nosema fumiferanae (Thom.) and Pleistophora schubergi Zwölfer were tested against the spruce budworm, Choristoneura fumiferana (Clem.) in Rose Township, Ontario during the summer of 1976. A packsack-type mist blower was used to apply suspensions of microsporidian spores on individual white spruce (Picea glauca Voss.) and balsam fir (Abies balsamea Mill.) trees. Application of N. fumiferanae to spruce trees when budworm were in the needle-mining stage did not increase the levels of infection. More trees were sprayed when the larvae were predominantly in the fourth and fifth instar. This resulted in levels of infection significantly higher than in check areas. In general, levels of microsporidian infection were higher in insects collected from sprayed balsam fir trees, and infection rates were higher with P. schubergi than N. fumiferanae.

Résumé

On a essayé deux agents pathogènes microsporidiens, Nosema fumiferanae (Thom.) et Pleistophora schubergi Zwölfer contre la Tordeuse des bourgeons de l'Épinette Choristoneura fumiferana (Clem.) dans le township de Rose, Ontario pendant l'été 1976. Un vaporisateur du type sac à dos fut utilisé pour l'application des microsporidies sur des Épinettes blanches (Picea glauca [Moench] Voss.) et des Sapins baumiers (Abies balsamea [L.] Mill.), isolément. L'application de N. fumiferanae sur les Épinettes alors que la Tordeuse était à la phase de mineuse des aiguilles n'a pas augmenté les niveaux d'infection. Un plus grand nombre d'arbres furent arrosés lorsque les larves se trouvaient en plus grand nombre aux quatrième et cinquième stades. Cela eut pour effet de hausser significativement les niveaux d'infection, plus que dans les secteurs témoins. En général, les niveaux d'infection que causent les microsporidies furent plus élevés parmi les insectes recueillis sur les Sapins baumiers et les taux d'infection furent plus élevés avec P. schubergi qu'avec N. fumiferanae.

Introduction

The first attempt at applying a microsporidian Nosema fumiferanae (Thom.) against the spruce budworm, Choristoneura fumiferana (Clem.) was carried out in 1975 on Manitoulin Island (Wilson and Kaupp 1975). Individual white spruce (Picea glauca Voss.) trees were sprayed with suspensions containing spores of N. fumiferanae, using a packsack-type mist blower. The applications were tested when the budworm larvae were predominantly in the IV and V instar. The results indicated that N. fumiferanae could be successfully applied against the spruce budworm.

During the summers of 1973 and 1974 examination of field collected samples of the spruce budworm revealed the presence of a Pleistophora sp. (Wilson, 1975). It has subsequently been determined to be Pleistophora schubergi Zwölfer (Weiser 1961; Weiser personal communication). This microsporidian proved to be highly infectious to the spruce budworm under laboratory conditions.

The present field study was undertaken to confirm some of the results obtained in 1975; to determine if larvae in the needle-mining stage could be infected with N. fumiferanae when applied to the host tree; to compare the results obtained from balsam fir and white spruce; and to determine if P. schubergi could infect the spruce budworm in a field situation.

These protozoans may affect the vigour, longevity and fecundity of the host insect. It is hoped that they can be successfully in-

troduced 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 microsporidian spores

The production of N. fumiferanae spores was the same as that described by Wilson and Kaupp (1975). Propagation of P. schubergi spores commenced about three months prior to field spraying. This microsporidian readily infects the forest tent caterpillar, Malacosoma disstria Hübner, and this insect was used as the host. The use of the M. disstria as the host instead of the spruce budworm was advantageous because of its larger size and it also reduced the possible contamination of P. schubergi preparation with N. fumiferanae as budworm larvae are often naturally infected with N. fumiferanae. Third instar larvae of the forest tent caterpillars were placed in 28 ml plastic cups 3/4 filled with a synthetic diet (Grisdale 1973). The diet surface had been treated with 0.2 ml suspension containing approximately 10^6-10^7 spores/ml of P. schubergi in distilled water. As the larvae increased in size they were removed from the cups and reared in plastic cages measuring 26 x 18 1/2 x 9 1/2 cm. The insects were sacrificed shortly before pupation and P. schubergi spores were harvested and stored using the same procedure as for N. fumiferanae.

Experimental plots

The experimental plots were located 15 km north of Thessalon, Ontario in Rose Township. The major spray area was a white spruce plantation 6.4 ha in area with trees ranging in height from 4.5 to 6.0 m. Twenty-one spruce trees were selected for application of N. fumiferanae spores and were labelled wS1 through wS21. Balsam fir (Abies balsamea Mill.) trees similar in size were selected from the surrounding area to test spores of N. fumiferanae. These were labelled bF1 through bF3.

Open grown white spruce and balsam fir trees labelled wS22 to wS27 and bF4 and bF5 respectively, approximately 1.6 km from the spruce plantation were selected to test the efficacy of P. schubergi spores. These trees were about 7.5 m in height. Check trees were selected in a suitable site in close proximity to the treatment area to insure similar levels of natural microsporidian infection. All trees selected for the experiment had evidence of previous budworm defoliation.

Formulation and application rate

All sample trees were sprayed with 1500 ml of an aqueous formulation containing 25% (v/v) molasses and 30 g/l of IMC 90-001 sunlight protectant[1]. Spores per tree were applied at the following rates; wS1 to wS16 2.5×10^{10} N. fumiferanae spores, wS1 to wS3 received a second application at the same rate, wS17 to wS21 and bF1 to bF3 5×10^{10} N. fumiferanae spores, wS22 to wS24 2.5×10^{10} P. schubergi spores, wS25 to wS27 and bF4 to bF5 5×10^{10} P. schubergi

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1. Sandoz Wander, Inc., Homestead, Florida.

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

Spray operation and larval development

The formulations were applied to sample trees with a packsack-type mist blower (KWH 2677 Kem San Ltd.).

The first spraying took place under calm weather conditions on the evening of May 12. Samples trees wS1 to wS6 were sprayed when the budworm were in the needle mining stage. On the evening of June 3, wS1 to wS3 received a second spray application, while trees wS22 to wS27 and bF1 to bF5 were sprayed for the first time. Sample trees wS7 to wS21 were sprayed during the early morning of June 4. Examination of 71 larvae at this time indicate that the percent in each instar was, III-4, IV-27, V-62 and VI-7%.

Sampling and microscopic examination

The sampling procedure and microscopic examination was similar to that reported by Wilson and Kaupp (1975), with sampling dates indicated in the tables of this report.

Results

The incidence of N. fumiferanae in spruce budworm larvae, following application of the spray on spruce trees when the larvae were in the needle-mining stage is shown in Table 1. There was no significant difference in the levels of N. fumiferanae in those insects collected from treated spruce trees (wS4 to wS6) as compared to untreated trees. The incidence of infection did not increase in samples collected later. Spruce trees wS1 to wS3 were sprayed for a second time on June 3rd, when the other sample

trees were sprayed for the first time. Levels of N. fumiferanae infection in larvae collected from those trees sprayed for the second time, was significantly higher than that for larvae on the check trees (Table 1). However, infection was not greater than that for insects sprayed once in the later instars.

In another experiment comparing dosages, one group of spruce trees was sprayed at a rate of 2.5×10^{10} spores per tree the other at 5×10^{10} spores. Both treatments produced significantly higher levels of infection than in the checks; however there was no significant difference between the two treatments (Table 1). As with other treated areas there was a progressive increase in levels of N. fumiferanae following the spray application.

There was a greater incidence of N. fumiferanae in those larvae collected from sprayed balsam fir as compared to insect collected from sprayed spruce. The levels of infection were higher for those insects on balsam fir (Table 1). This difference is offset in part by a higher natural level of N. fumiferanae in the balsam fir checks.

Spraying of P. schubergi spores resulted in approximately a 60% increase in levels of infection of this microsporidian compared to the checks (Table 2). The effects of using two spore concentrations of P. schubergi were similar to those of N. fumiferanae. Only a slight difference in the levels of infection resulted from the higher dosage, but this was not significant. Again as with N. fumiferanae, higher levels of P. schubergi were encountered in larvae from sprayed balsam fir as compared to white spruce (Table 2). On

both trees infection rates with P. schubergi are higher than those for N. fumiferanae, with levels of P. schubergi as high as 96% being recorded.

Table 1

Incidence of Nosema fumiferanae in spruce budworm larvae collected from white spruce (wS) and balsam fir (bF) trees sprayed with N. fumiferanae in 1976

Tree number and species	Treatment (spores/tree) spray date	Predominate instar when sprayed	Date of sample	Number of larvae examined	Percent incidence of N. fumiferanae
wS4 to wS6	Prespray		May 10	76	35.2
	2.5×10^{10} May 12	II-III	June 21	55	38.1
	-	-	June 28	91	32.9
wS1 to wS3	Prespray	-	May 10	53	22.6
	2.5×10^{10} May 12	II-III	June 2	13	30.7
	2.5×10^{10} June 3	IV-V	June 21	59	45.7*
	-	-	June 28	93	54.8**
wS7 to wS16	Prespray	-	June 2	104	25.0
	2.5×10^{10} June 4	IV-V	June 21	280	46.4*
	-	-	June 28	285	54.7**

Table 1 (cont'd)

Tree number and species	Treatment (spores/tree) spray date	Predominate instar when sprayed	Date of sample	Number of larvae examined	Percent incidence of N. fumiferanae
wS17 to wS21	Prespray	-	June 2	33	24.2
	5×10^{10} June 4	IV-V	June 21	101	44.5*
	-	-	June 28	129	59.7**
bF1 to bF3	Prespray	-	June 2	28	39.2
	5×10^{10} June 3	IV-V	June 21	80	66.2
	-	-	June 28	48	72.9*
wS Check	-	-	May 10	71	25.3
	-	-	June 2	94	22.3
			June 21	64	26.6
			June 28	55	25.4
bF Check	-	-	June 2	36	22.2
	-	-	June 28	67	38.8

Note: statistical analysis was performed using "t" test as applied to percentages.
* = Significantly different from the checks at the 5% level.
** = Significantly different from the checks at the 1% level.

Table 2

Incidence of Pleistophora schubergi in spruce budworm larvae collected from white spruce (wS) and balsam fir (bF) trees sprayed with P. schubergi in 1976

Tree number and species	Treatment (spores/tree) spray date	Predominate instar when sprayed	Date of sample	Number of larvae examined	Percent incidence of P. schubergi
wS22 to wS24	Prespray		June 2	70	0
	2.5×10^{10} June 3	IV-V	June 21	80	61.2**
	-	-	June 28	127	40.1**
wS25 to wS27	Prespray		June 2	28	0
	5×10^{10} June 3	IV-V	June 21	74	64.8**
	-	-	June 28	32	48.6**
bF4 to bF5	Prespray		June 2	35	0
	5×10^{10} June 3	IV-V	June 21	55	96.3**
	-	-	June 28	63	95.2**
wS check			June 2	94	0
			June 21	64	0
			June 28	55	0
bF check			June 2	36	0
			June 28	67	1.4

*, ** see note Table 1.

Discussion

These preliminary field trials confirmed many of the observations made in 1975. Levels of N. fumiferanae in spruce budworm can be increased if this parasite is sprayed on the insect's host tree. However, preliminary results suggest that spraying trees when budworm are in the needle-mining stage is unsuccessful. Although repeated application at this stage may produce better results it is doubtful that the added expense of time and material is justified because the parasite debilitates rather than kills its host. A single application at a later instar will introduce the microsporidia into the population and, being transmitted transovarially, succeeding generations will be infected early in life. As found in the 1975 trials increase in the spore dosage did not produce a significant increase in the levels of infection. The reason for this is unclear, but two fold differences in dosage rates may not be great enough to demonstrate increased infection.

Pleistophora schubergi was tested against the spruce budworm for the first time under field conditions. This microsporidian parasite has a rather wide host range, infecting both hymenopterous and lepidopterous larvae (Weiser 1961; Kaya 1973; Wilson 1975). Kaya (1975) tested this microsporidian against Anisota senatoria (Smith) and Symmerista canicosta Franclemont under field conditions with resulting high incidence of infection. Levels of P. schubergi infection in spruce budworm larvae collected from sprayed balsam fir reached 96%, but was much lower in those insects collected from treated white spruce trees.

This was also true for N. fumiferanae. This may be due in part to the condition of the buds at spraying time. The balsam fir buds are more flushed allowing larger quantities of spore material to reach the insects.

In general spraying with P. schubergi resulted in higher levels of infection in budworm larvae compared to N. fumiferanae. This seems to indicate that P. schubergi may be more infectious to spruce budworm than N. fumiferanae. However, other facts such as environmental and storage conditions may affect the spores of the two species differently. The results obtained to date suggest that N. fumiferanae and P. schubergi show some promise in the biological control of spruce budworm.

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