A COMPARISON OF THE EFFECTS OF *NOSEMA FUMIFERANAE* AND A *NOSEMA* SP. (MICROSPORIDA) ON *CHORISTONEURA FUMIFERANA* (CLEM.) AND *CHORISTONEURA PINUS PINUS* FREE.

INFORMATION REPORT FPM-X-77

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1986

Catalogue No: Fo46-16/77E

ISSN: 0704-772X

ISBN: 0-662-15093-7

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Information Services
Forest Pest Management Institute
Canadian Forestry Service
Agriculture Canada
P.O. Box 490
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Cette publication est aussi disponible en français sous le titre *Comparaison des effets de Nosema fumiferanae et d'une autre microsporidie du genre Nosema sur Choristoneura fumiferana (Clem.) et Choristoneura pinus pinus free.*

Wilson, G.G. 1986. A comparison of the effects of *Nosema fumiferanae* and a *Nosema* sp. (microsporida) on *Choristoneura fumiferana* (Clem.) and *Choristoneura pinus pinus* Free. Can. For. Serv., For. Pest Manage. Inst. Inf. Rep. FPM-X-77. 10 p.

ABSTRACT

This report details the effects two microsporidia, *Nosema fumiferanae* and a *Nosema* sp. have when fed to 4th- and 5th-instar larvae of *Choristoneura fumiferana* and *Choristoneura pinus pinus*. Both microsporidia caused increased insect mortality and decreases in pupal weight and adult longevity. In the majority of cases *N. fumiferanae* caused the greatest detrimental effects on both species of pest insects.

RÉSUMÉ

Sont présentés de façon détaillée les effets de deux microsporidies, *Nosema fumiferanae* et *Nosema* sp., lorsqu'elles sont consommées par des larves au 4^{e} et au 5^{e} stade de *Choristoneura fumiferana* et de *C. pinus pinus*. On a observé que les deux microsporidies causaient une augmentation de la mortalité des insectes ainsi qu'une diminution du poids des chrysalides et de la longévité des adultes. Dans la majorité des cas, *N. fumiferanae* a causé les effets les plus dommageables chez les deux espèces d'insectes.

INTRODUCTION

Microsporidian parasites have been isolated from both the spruce budworm, *Choristoneura fumiferana* (Clem.), and the jack pine budworm, *Choristoneura pinus pinus* Free. Both microsporidia were first reported by Thomson (1955, 1959), and are now in the genus *Nosema* sp. The species infecting the spruce budworm is *Nosema fumiferanae* and although the microsporidia occurring in the jack pine budworm is very similar, it has not been assigned to a particular species. Cross-infectivity tests have demonstrated that both forest pest insects are susceptible to either microsporidia (Wilson, unpublished data). Experimental studies (Wilson 1981) have demonstrated that *N. fumiferanae* has definite detrimental effects on its host, the spruce budworm. Because of the serious and dramatic increase in infestations of the jack pine budworm in Ontario, studies were undertaken to compare the effects of these two microsporidian parasites on the spruce and jack pine budworms.

Some difficulty was encountered in rearing the jack pine budworm on the artificial diet, resulting in relatively high mortality in the control insects. Because of this problem and the general nature of the comparisons, detailed statistical analysis was not performed. However mortality values were adjusted using Abbott's formula (Finney 1952).

The Parasites, *Nosema fumiferanae* and *Nosema* sp., from the Jack Pine Budworm

Thomson (1955, 1959) described microsporidia isolated from the spruce and jack pine budworms. The microsporidian parasite of the spruce budworm was originally designated *Perezia fumiferanae*, but is now considered *Nosema fumiferanae*. The microsporidium isolated from the jack pine budworm was similar to *N. fumiferanae* and Thomson suggested that the differences were not great enough to consider it a distinct species. Both microsporidia have spores measuring 2 x 3-5 μ with polar filaments of 60 to 100 μ in length. Most tissues are infected, with the primary site being mid-gut cells.

The Hosts, *Choristoneura fumiferana* and *Choristoneura pinus pinus*

The spruce budworm is the most destructive defoliating forest insect native to North America. In 1985, 12.3 million hectares of Ontario's spruce and fir forests suffered moderate-to-severe defoliation. Infestations of the jack pine budworm in jack pine stands have increased dramatically in Ontario. In 1985 the overall area of moderate-to-severe defoliation peaked at 3.66 million ha. This was the largest infestation ever recorded in Ontario. (Forestry Newsletter, summer 1986, Great Lakes Forestry Centre). The jack pine and spruce budworm are closely related and were considered the same species until 1953 (Martineau, 1984). It is possible that as the respective hosts become isolated that variations will also occur in a once common microsporidian parasite.

MATERIALS AND METHODS

Microsporidian spores used in this study were harvested from their respective hosts, *N. fumiferanae* (Nf) from the spruce budworm and *Nosema* sp. (Cpm) from the jack pine budworm. Both species were reared in the laboratory. Spores were purified and stored at 4°C for up to four weeks before use. Spore suspensions were prepared in water to give concentrations of 10^6, 10^7, and 10^8 spores/mL. These suspensions were applied to the surface (4.7 cm^2) of a synthetic diet (McMorran, 1965) dispensed in plastic cups (Grisdale, 1970) at a rate of 200 µL/cup, resulting in a dose of 2×10^5, 2×10^6 and 2×10^7 spores/cup. Diet for control insects was treated with distilled water. Insects used in all tests were late 4th- or early 5th-instar larvae, 12-14 days out of the hibernacula.

Ten cups were used for each dose; eight larvae were placed in each cup. Larvae were allowed to feed freely on the treated diet for seven days and were then placed on fresh untreated diet. The experiments were repeated twice. Tests were performed under a regime of 16 h light and 8 h dark photo-period, 23±1°C and RH of 60-80%. The cups were placed in an

inverted position and examined every other day to determine larval mortality and to ensure that the diet was in a palatable state. With the onset of pupation the tests were checked daily and the resulting pupae were sexed and weighed within 24 h. Pupae were set up individually in glass vials and emergence and deaths of adults were recorded.

RESULTS

Mortality: As noted in the introduction jack pine budworm did not rear as well as spruce budworm on the artificial diet. Table 1 indicates the control mortality (0 spore dose) for both spruce and jack pine budworm for the different treatments, Nf and Cpm. The total spruce budworm control mortality was 21 out of a total of 238 insects (8.8%). This compares to 46 of 162 insects (28.3%) for jack pine budworm. The effects of the microsporidia have to be weighed against this background.

Mortality in both spruce budworm and jack pine budworm was greatest when larvae were treated with a dose of 2×10^7 spores of either Nf or Cpm. Slightly higher mortality was caused by Nf in both budworm species (Table 1). Total mortality (larval and pupal) for spruce budworm larvae treated with 2×10^7 Nf spores was 59.8%, the same dose of Cpm caused 42.5% mortality. The mortality for treated jack pine budworm larvae was higher than spruce budworm at all dosages for both microsporidia. A spore dose of 2×10^5 caused mortality in the jack pine budworm similar to a dose of 2×10^7 spores for the spruce budworm. A dose of 2×10^7 spores of Nf and Cpm caused total jack pine budworm mortality of 84.9 and 70.8% respectively (Table 1). In both cases Nf caused slightly higher mortality in the two species of insects.

Pupal Weights: Both microsporidia caused a substantial reduction in spruce and jack pine budworm female pupal weights (Fig. 1). The greatest reduction for

Table 1. The effects of various dosages of *Nosema fumiferanae* (Nf) and jack pine budworm microsporidia (Cpm) on mortality of spruce budworm and jack pine budworm when treated as mixed 4th- and 5th-instar larvae

Dose: spores/ diet surface	Percent larval mortality		Percent pupal mortality		Percent total mortality*	
	Nf	Cpm	Nf	Cpm	Nf	Cpm
Host - Spruce budworm						
0	0	5.3	5.2	6.0	5.2 (76)	10.7 (162)
2×10^5	15.2	6.4	16.4	7.9	25.2 (151)	3.2 (154)
2×10^6	19.5	16.4	10.1	16.4	24.3 (157)	19.8 (158)
2×10^7	45.0	34.8	30.7	23.4	59.8 (142)	42.5 (158)
Host - Jack pine budworm						
0	16.4	25.2	9.0	9.6	23.8 (67)	31.5 (95)
2×10^5	39.1	39.8	27.3	33.7	41.8 (156)	41.7 (143)
2×10^6	52.7	63.2	45.7	48.9	66.2 (148)	63.6 (128)
2×10^7	80.5	64.1	41.3	44.2	84.9 (149)	70.8 (121)

* Total mortality was adjusted using Abbott's formula
( ) Parentheses indicate number of individuals in calculations

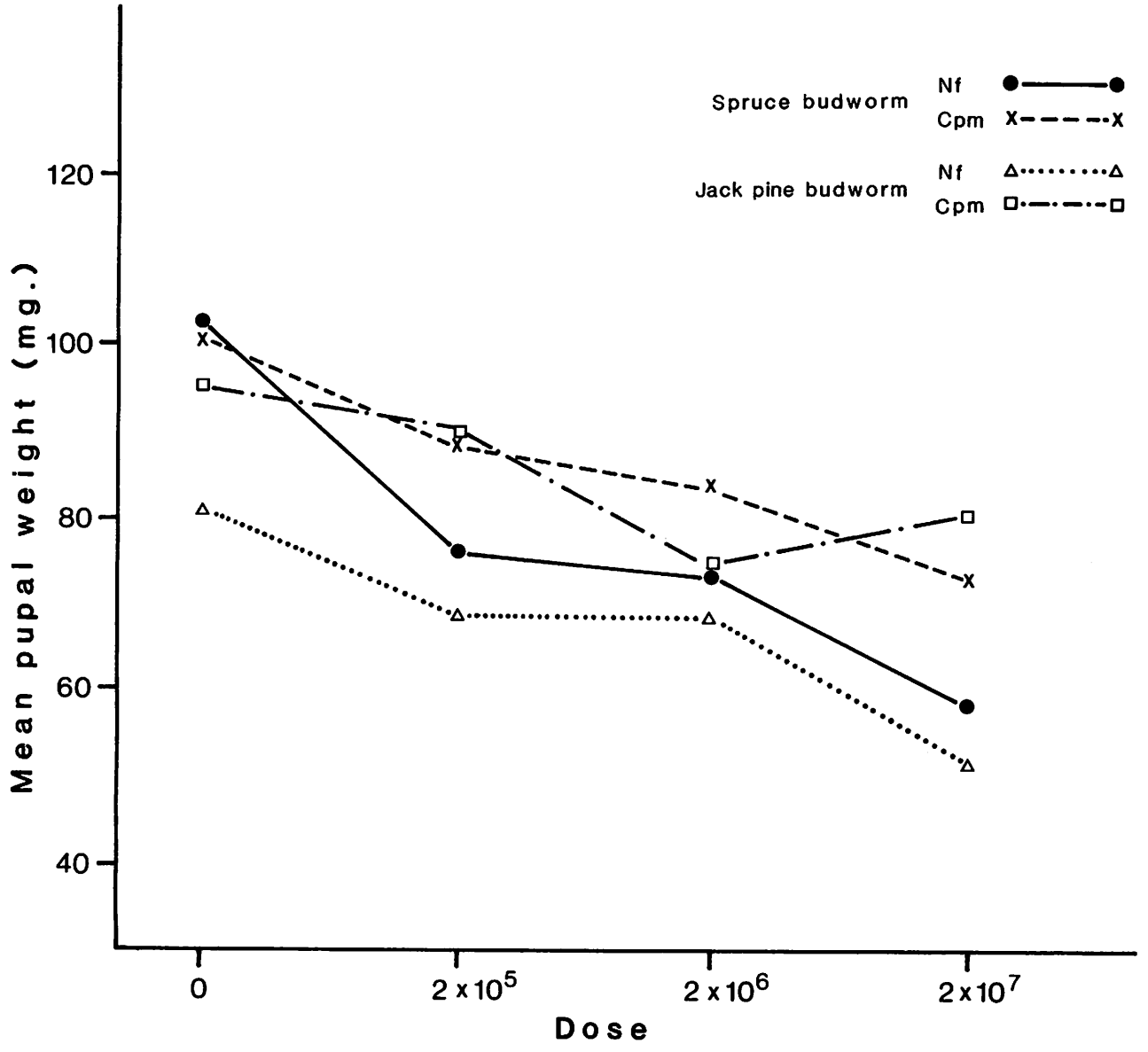

Figure 1. The effects of various dosages of *Nosema fumiferanae* (Nf) and jack pine budworm microsporidia (Cpm) on female pupal weights of spruce budworm and jack pine budworm when treated as mixed 4th- and 5th-instar larvae.

both species of insects was caused by Nf. In general there was only a slight decrease in weights caused by 5 x 10^5 and 5 x 10^6 spores, as compared to controls, with the greatest reduction in pupal weights for those larvae treated with 2 x 10^7 spores. Treatment of jack pine budworm larvae with 2 x 10^7 spores of Nf and Cpm resulted in decreased female pupal weight of 29 and 15 mg, respectively. The reductions were 44 mg (Nf) and 27 mg (Cpm) for spruce budworm treated with the same dose. Similar trends were also noted for male pupal weights when larvae were treated with the microsporidia (Fig. 2). As with the female pupae, Nf infection caused the greatest decline in weight.

Adult longevity: The reduction in adult longevity was similar for each host insect, with even the lowest dose tested, 2 x 10^5 spores, resulting in a decrease in adult life (Table 2). The greatest decrease occurred in adults when larvae were treated with 2 x 10^7 spores/diet surface. The longevity of female spruce budworm treated with 2 x 10^7 Nf and Cpm spores, as compared to the control, was 3.8 and 4.8 days respectively. Similarly, the reduction for female jack pine budworm was 5.7 and 5.9 days. It is apparent that both microsporidia had the greatest effect on the longevity of female jack pine budworm. The decrease in male longevity was similar for both hosts, with Nf and Cpm causing earlier death by 2 to 3 and 1.5 days respectively, when compared to the controls.

DISCUSSION

It is apparent from these tests, that both microsporidia, *N. fumiferanae* and *Nosema* sp., have serious detrimental effects on their hosts. There is an increase in mortality and a decrease in pupal weight and adult longevity. The detrimental effects of *N. fumiferanae* on the spruce budworm have been well documented (Thomson 1958, Wilson 1977, 1983). Wilson (1976) conducted tests to determine the best dose for mass production of Nf spores and reported that a spore concentration of 2 x 10^7 applied to diet surface and fed to 4th-instar spruce budworm

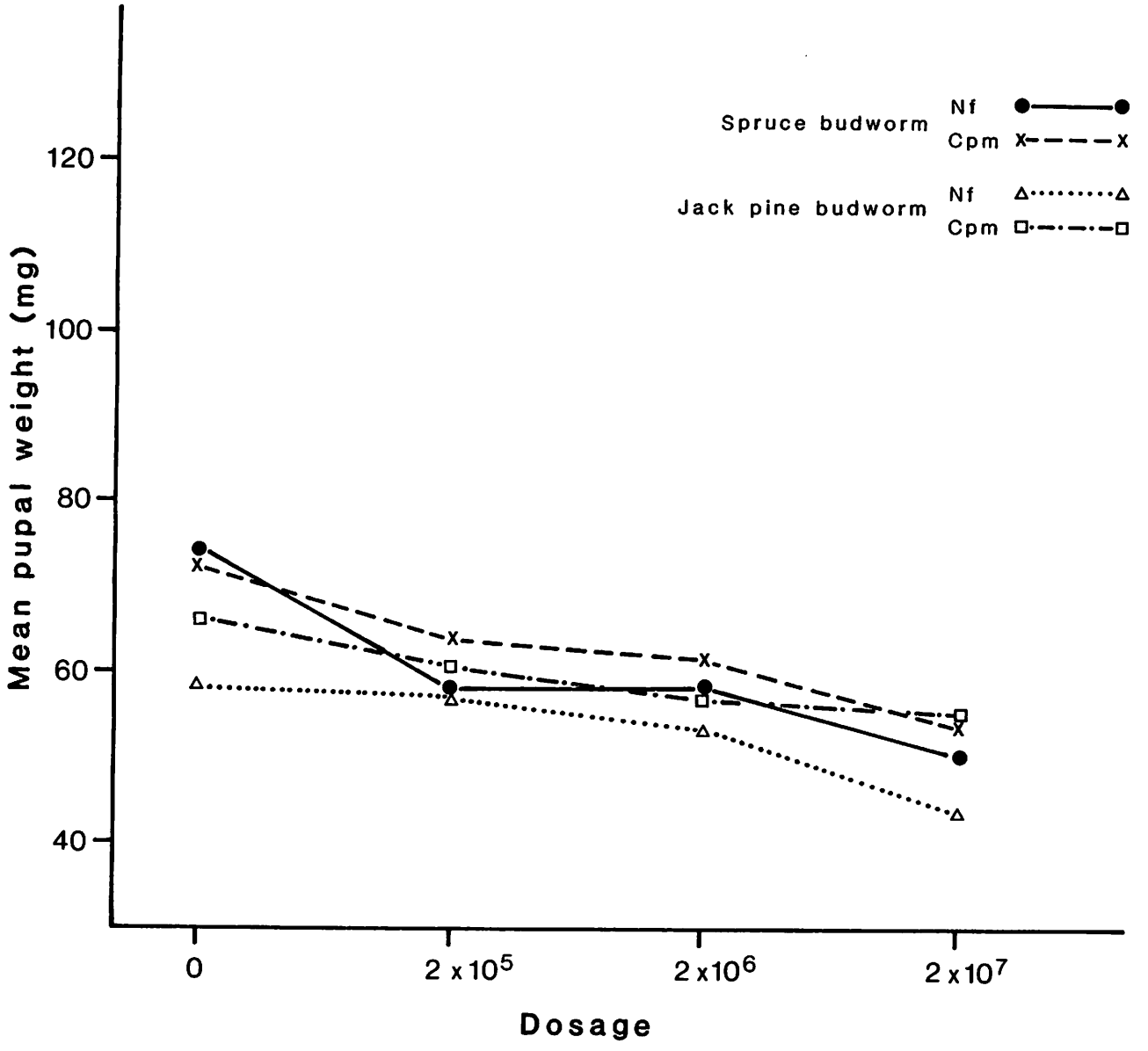

Figure 2. The effects of various dosages of *Nosema fumiferanae* (Nf) and jack pine budworm microsporidia (Cpm) on male pupal weights of spruce budworm and jack pine budworm when treated as mixed 4th and 5th instar larvae.

resulted in 38% larval mortality. Similar results were obtained in the present study, with 2 x 10^7 spores of Nf resulting in 45% spruce budworm larval mortality.

Table 2. The effects of various dosages of *Nosema fumiferanae* (Nf) and jack pine budworm microsporidia (Cpm) on adult longevity of spruce budworm and jack pine budworm when treated as mixed 4th and 5th-instar larvae

Dose: spores/ diet surface	Spruce budworm x days as adults				Jack pine budworm x days as adults			
	Nf		Cpm		Nf		Cpm	
	♂	♀	♂	♀	♂	♀	♂	♀
0	7.9	11.9	8.1	14.1	8.5	10.8	9.2	12.5
2 x 10^5	6.8	9.1	7.4	11.1	7.3	8.6	6.9	10.8
2 x 10^6	6.8	8.8	8.0	10.2	7.1	7.3	6.9	8.6
2 x 10^7	5.4	8.1	6.4	9.3	5.1	5.1	7.6	6.6

Longevity and pupal weights are important considerations in reproductive potential. A reduction in both suggests a depletion of nutritional reserves and a reduced ability to assimilate food efficiently (Nolan and Clovis 1985), resulting in lower reproductive potential. Decreased longevity also shortens the time adults have to mate and lay a full complement of eggs. These effects of microsporidia on pupal weight and adult longevity have been reported for other insects. Gaugler and Brooks (1975) fed 3-day-old larvae of *Heliothis zea* on diet treated with 1.3 x 10^6 spores of *Nosema heliothidis*. Longevity of adults from these larvae was shortened by about 4 days. Similarly, longevity of male and female moths of *Ostrina nubilalis* naturally infected with *Nosema pyrausta*, which exceeded a concentration of 10^6 spores, lived an average of 2.0 and 4.2 fewer days respectively than did moths having no infection (Windels et al. 1976). Thomson (1958) studied a population of spruce budworm

naturally infected with *N. fumiferanae* and reported decreases in longevity of infected adult males and females of 2.4 and 7.1 days respectively, and a reduction in female pupal weights by 23 mg.

This is the first time microsporidia have been tested against the jack pine budworm. This study indicates that results similar to those found with spruce budworm can be expected for jack pine budworm treated with microsporidia. In fact, the data indicate that the effects may be more pronounced in the jack pine budworm. It is also interesting that *N. fumiferanae* seems to cause greater detrimental effects on both the spruce budworm and jack pine budworm than does the microsporidia isolated from the jack pine budworm. However, this does not necessarily indicate a different species of microsporidia. In fact the microsporidia from the jack pine budworm is probably a strain of *N. fumiferanae*. Any comprehensive population dynamics study of the jack pine budworm will have to take into consideration the role of microsporidia in the regulation of this forest pest.

ACKNOWLEDGMENTS

Grateful acknowledgement is extended to Miss E. Young for technical assistance.

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