MICRO-ORGANISMS INFECTING THE LARGE ASPEN TORTRIX, CHORISTONEURA CONFLICTANA WLK. (LEPIDOPTERA: TORTRICIDAE)

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ABSTRACT

Field-collected specimens of the large aspen tortrix, **Choristoneura conflictana,** are infected by several microorganisms, including granulosis virus, entomopoxvirus, three species of microsporidia, and three species of fungi. There are also mixed infections consisting of two or three microorganisms. Diagnostic results obtained between 1951 and 1978 demonstrate that widely separated geographic populations of this insect are affected by the same complement of microorganisms. Cross infection tests with microsporidia were carried out utilizing both laboratory-reared and field-collected insects. The results are inconclusive concerning the relationship of microsporidia in **C. conflictana** to those occurring in related insect species.

RESUME

Les tordeuses du tremble **(Choristoneura conflictana)** capturées dans la nature sont infectées par divers micro-organismes, y compris le virus de la granulose, le virus de la variole des insectes, trois microsporidies et trois champignons microscopiques. Elles souffrent aussi d'infections mixtes, dues à deux ou trois micro-organismes. Les diagnostics posés entre 1951 et 1978 montrent que leurs populations séparées par de grandes distances souffrent du même ensemble de micro-organismes. Des essais d'infection croisée au moyen de microsporidies ont été tentés chez des insectes élevés en laboratoire ou capturés dans la nature. Les résultats ne sont pas concluants en ce qui concerne la relation entre les microsporidies trouvées chez **C. conflictana** et celles qu'on observe chez des espèces apparentées.

INTRODUCTION

The large aspen tortrix, **Choristoneura conflictana** Wlk., occurs throughout the range of trembling aspen, **Populus tremuloides** Michx., in Canada and the Eastern United States, south to New Jersey and Ohio (Baker 1972). The life history of the insect is described by Prentice (1955), Wickman (1963), and Beckwith (1968). Outbreaks occur over large areas and infestations have been recorded covering nearly 2.6 million hectares in Manitoba, Saskatchewan, and interior Alaska, and about 800,000 hectares in gross land area in Minnesota (Batzer 1972). Outbreaks generally collapse in 2 to 3 years in any particular area. The principle effect of this pest is reduced growth of aspen with little tree mortality (Batzer 1972).

The large aspen tortrix also feeds on balsam poplar, large tooth aspen, paper birch, willow and alder (Baker 1972), but aspen is required for a population increase (Beckwith 1970). It has been suggested that starvation is the primary cause of population decline for this species (Wickman 1963; Beckwith 1968). If complete stripping of aspen occurs prior to the last instar, the population will collapse, as larvae forced to feed on other plants would not receive proper nutrition for larval development and egg production (Beckwith 1970). When food other than aspen was supplied to C. **conflictana** larvae under insectary conditions, the larval period was prolonged (Prentice 1951).

Prentice (1955) reviewed the history of outbreaks and the natural control factors of the insect in Canada from 1912 to 1953 and reported an extensive complement of parasites. Dead and apparently diseased insects were examined but the only disease noted was infection of overwintering larvae by a fungus, **Beauveria bassiana.** Beach (1970) examined 1200 large aspen tortrix pupae collected in three counties in Minnesota and found 26% parasitized and 49% dead of unknown causes. Since flaccid larvae were commonly observed, he speculated that a virus disease was at least partially responsible for the high percentage of dead, unparasitized, pupae.

Descriptions of two organisms affecting C. **conflictana** have been published (Wilson and Burke 1971; Cunningham **et al.** 1973). There is, however, an interesting complex of microorganisms associated with populations of the large aspen tortrix including three species of fungi, three species of protozoa, two definite viruses, and a possible third virus. Because an extensive disease complex probably adds stress to an insect population and may play a role as a regulating factor, it would be advantageous to have all diagnostic results obtained at the Forest Pest Management Institute between 1951 and 1978 brought together into one report.

MATERIALS AND METHODS

This report is not the result of a systematic study of microorganisms affecting the large aspen tortrix, but is rather a summary of results obtained, over a 26 year period, from material submitted for diagnosis or from collections made in an outbreak population when disease organisms were prevalent. 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 1500X magnification. Smears of fat and gut tissue from living larvae were usually examined by the same method. Phase contrast microscopy was also used at times.

As insects became available, and as time permitted, cross-infection tests were conducted with some of the organisms. The inoculum was painted on diet or sprayed on the appropriate foliage while controls were treated in the same manner using distilled water. Efforts to infect the field-collected C. **conflictana** larvae with microorganisms from other insects were confounded by the presence of extensive natural infection in the field population.

The term negative used throughout this report refers to insects in which recognizable pathogenic microorgansms were not observed.

RESULTS AND DISCUSSION

The Insect Disease Survey of the Forest Pest Management Institute was very active between 1950 and 1957. The records from this period however, do not show how many specimens were included in each sample. And, if the sample did contain more than one specimen, the records do not show how many of those were infected, as the aim was to determine occurrence rather than intensity of infection (Cameron 1951). Between 1957 and 1967 the overall amount of material examined was greatly reduced and **C. conflictana** larvae were not examined. This probably resulted from the low population in Ontario where most of the material was collected. Sippell **et al.** (1960), noted that **C. conflictana** infestations had peaked in 1957 and declined in 1958, 1959 and 1960. The species was not mentioned again until 1968 when Sippell **et al.** (1968) reported the first infestation since 1960 in the Port Arthur district. In 1967 the Insect Disease Survey was reactivated, and the amount of material examined increased greatly.

Microorganisms Identified Prior to 1968

The Forest Pest Management Institute's first record of infection in the large aspen tortrix by a microorganism was in 1951 when 6 samples from Manitoba and 24 from Saskatchewan were examined for disease. Five samples were found to be infected with the fungus **Beauveria,** believed to be **globulifera** sp. Two of these were from Manitoba and three from Saskatchewan. About this time Cumming (1953) reported that 90% of overwintering **C. conflictana** larvae in an outbreak near Grenfell, Saskatchewan, were killed by **Beauveria globulifera.** Between 1952 and 1957 a further 8 samples were examined. Four of these were from Saskatchewan; two were negative, one was infected with **Beauveria** sp., and one contained a polyhedral virus. The number of larvae infected with the polyhedral virus was not recorded and the virus was not investigated further. One sample from Ontario in 1957 was infected with the fungus **Entomophthora.** Three samples, composed of 9 larvae, were collected in 1955 from the Fort Vermillion area in Alberta; three of these larvae were negative, one was infected with a granulosis virus, four with microsporidia, and one with both granulosis virus and microsporidia.

Microorganisms Identified from 1968-1978

Ontario

Most of the **C. conflictana** larvae examined after 1967 were collected in Ontario; the results of diagnosis are given in Tables I to III. Table I shows the number and percent samples infected, the number and percent negative and the total samples examined each year. There was an increase in the number of samples examined until 1973, which coincides with the time when the population was heavy in Ontario. In 1972 and 1973 stands of trembling aspen within 13 million hectares showed moderate to severe defoliation by the large aspen tortrix (Sippell **et al.** 1972, 1973). By 1975 there was a general decline in the population in Ontaro (Sippell **et al.** 1975). Table I also shows the number and percent larvae infected and negative, and the total larvae examined for each year. There was a gradual increase in the percent larvae infected as the population outbreaks increased in intensity up to 1974. The number of larvae examined after that was not large enough to show a reliable trend.

Table II shows the number of insects recorded as either infected or negative, and identifies the organisms causing the infection for the years 1968-1977. A total of 1290 insects were examined; of these, 533 were infected with a single organism, 104 with two organisms, and 12 with three organisms. Multiple infections in a single insect have previously been reported by Bird (1959) who investigated the development of polyhedrosis and granulosis viruses causing single and double infections in the eastern spruce budworm, **Choristoneura fumiferana** Clemens.

Table I

Incidence of disease in C. **conflictana** collected in Ontario 1968-1977.

	Samples received					No. of larvae received				
	Infected		Negative			Infected		Negative		
Year	No.	%	No.	%	Total	No.	%	No.	%	Total
1968	0	-	1	100.0	1	0	-	2	100.0	2
1970	4	80.0	1	20.0	5	29	25.4	85	74.6	114
1971	6	66.7	3	33.3	9	31	33.3	62	66.7	93
1972	18	100.0	0	-	18	210	44.8	259	55.2	469
1973	18	94.7	1	5.3	19	299	59.4	204	40.6	503
1974	4	66.7	2	33.3	6	40	72.7	15	27.3	55
1975	3	100.0	0	-	3	25	37.3	42	62.7	67
1976	1	100.0	0	-	1	1	100.0	0	-	1
1977	2	100.0	0	-	2	3	100.0	0	-	3
Total	56	87.5	8	12.5	64	638	48.8	669	51.2	1,307

Table II

Microorganisms found in **C. conflictana** collected in Ontario 1968-1977

	No. Insects Examined		Single infection					Double infection						Triple infection	
	Infected	Negative	Granulosis virus	Entomopoxvirus	Microsporidia sp.	Thelohania microsporidia	Beauveria	Entomophthora	Microsporidia and Granulosis virus	Microsporidia and Entomopoxvirus	Microsporidia and Entomophthora	Granulosis virus and Entomopoxvirus	Microsporidia, Granulosis virus and Entomopoxvirus	Microsporidia, Entomopoxvirus and nuclear polyhedrosis virus
1968	0	2												
1970	29	85			29									
1971	31	62			30		1							
1972	210	259	8	30	161	4	1	1	4	1				
1973	299	204	15	13	172			4	13	54	2	11	9	
1974	40	15	1		31				3	4			1	
1975	42	25	2		27					9		2		2
1976	1	0								1				
1977	3	0			3									
TOTAL	649	641	26	43	453	4	2	5	20	69	2	13	10	2

Table III

Microorganisms found in **C. conflictana** collected in Ontario during 1968-1977 compiled as if all infections were single infections

Year	Total larvae examined	Nuclear polyhedrosis		Granulosis		Entomopox-virus		Microsporidia sp.		**Thelohania** microsporidia		**Beauveria**		**Entomophthora**	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1968	2														
1970	114							29	25.4						
1971	93							30	32.2			1	1.1		
1972	469			12	2.6	31	6.6	166	35.4	4	0.9	1	0.2	1	0.2
1973	486			48	9.9	87	17.9	250	51.4					6	1.2
1974	55			5	9.1	5	9.1	39	70.9						
1975	67	2	3.0	4	6.0	13	19.4	38	56.7						
1976	1					1	100.0	1	100.0						
1977	3							3	100.0						
Total	1,290	2	0.2	69	5.3	137	10.6	556	43.1	4	0.3	2	0.2	7	0.5

Table III shows the total number of insects from Ontario examined, and the number infected by different organisms, compiled as if all cases of infection were single infections. In general, there is an increase in the number of larvae infected by a particular organism as the infestation grows older. This is most noticeable with microsporidia. A similar increase in infection by microsporidia as infestations develop has been shown for the spruce budworm (Thomson 1960: Wilson 1977a: Burke 1980).

Granulosis virus was diagnosed in 69 larvae collected in Ontario. This figure is probably low, as granulosis virus can be detected with a light microscope only when infection is very heavy: light to medium infection would probably be missed. Most granulosis inclusion bodies were quite uniform in shape and size. Only two insects were observed to contain square or cuboidal shaped inclusion bodies in sufficient numbers to be noticeable. One was found in 1973, about 25 miles north of Wawa, Ontario: the second, received in 1978, was found near Fort St. John, B.C. Similar cuboidal inclusion bodies have been reported in spruce budworm (Bird 1959: Stairs 1964).

Nuclear polyhedrosis virus has been reported twice in **C. conflictana.** The first report, as mentioned, was not investigated to describe the organism and the second, obtained in 1975 from two larvae collected near Poplar, Manitoulin Island, may be viewed with suspicion as the sample was collected near a plot that was sprayed with **C. fumiferana** nuclear polyhedrosis virus in 1974 (Cunningham **et al.** 1975).

Western Canada

Only ten samples were received from the four western provinces: these are recorded in Table IV. While the number of insects recorded is low, they were found to be infected with organisms similar to those in the samples from Ontario.

Tables V and VI summarize data from two samples obtained from populations widely separated geographically, and in time. A large sample from Fort St. John, B.C., was received in 1978 (Table V). One or more microorganisms were found in 196 of 320 insects examined. Single infection was observed in 145 insects, double infection in 48 and triple infection in 3. Seven microorganisms were involved in all, and the number of insects and percent infection is shown in Table VI for each microorganism as if all infections were single. Tables V and VI also include results obtained from examination of a large collection made at Bruce Mines, Ontario, in 1973. Results of the Bruce Mines collection are also included in the figures given previously for Ontario.

The microsporidia observed in **C. conflictana** have been tabulated only as microsporidia sp. and **Thelohania** sp. **Thelohania** sp. was identified by spore size and shape compared to **Thelohania** sp. observed in other **Choristoneura** species. **Nosema thomsonii** was found in **C. conflictana** and described by Wilson and Burke (1971). In addition to these, large packets of spores were occasionally observed, which would lead one to believe that the insect was infected by a **Pleistophora** sp. as well. However, it was not possible to differentiate between the microsporidia genera using preparations from crushed insects.

Cross Infection Tests

Nuclear Polyhedrosis

Extensive tests have not been conducted, but in three small trials **C. conflictana** larvae were allowed to feed on diet infected with polyhedra from **C. fumiferana.** Two larvae out of 28 fed the virus became infected.

Table IV

Microorganisms found in **C. conflictana** samples received from the four western provinces[1]

Province	Year	No. of samples			No. of larvae			Microorganisms found		
		Infected	Negative	Total	Infected	Negative	Total	Micro-sporidia	Entomo-poxvirus	Beauvaria
Alberta	1979	1	0	1	1	2	3		1	
British Columbia	1972	0	1	1	0	4	4			
British Columbia	1973	0	2	2	0	20	20			
Manitoba	1968	0	2	2	0	9	9			
Manitoba	1969	2	0	2	10	5	15	7		3
Saskatchewan	1973	1	1	2	1	3	4	1		
Total		4	6	10	12	43	55	8	1	3

[1]The data for British Columbia for 1978 not included in this table.

Table V

Incidence of single and multiple infections in C. **conflictana** from B.C. in 1978 and Ontario in 1973

Type of infection	British Columbia[1]		Ontario[2]	
	Infected Insects	Percent infection	Infected insects	Percent infection
Single infection				
Granulosis virus	58	18.1	1	0.6
Entomopoxvirus	13	4.1	6	3.8
Microsporidia sp.	64	20.0	62	39.0
Thelohania	2	0.6		
Beauveria	1	0.3		
Entomophthora	3	0.9		
Isaria	4	1.2		
Double Infection				
Granulosis virus, Microsporidia sp.	27	8.4	10	6.3
Granulosis virus, Entomopoxvirus	12	3.7	1	0.6
Entomopoxvirus, microsporidia sp.	7	2.2	26	16.4
Thelohania microsporidia sp.	1	0.3		
Thelohania	1	0.3		
Triple infection				
Granulosis virus, Entomopoxvirus, microsporidia sp.	2	0.6	5	3.1
Entomopoxvirus, **Thelohania** **Beauveria**	1	0.3		
Total number of infected insects	196	61.2	111	69.8

[1]The sample contained 320 insects. Microorganisms were found in 196 insects while 124 were negative.
The percent infection is based on the total sample of 320 insects diagnosed.

[2]The sample contained 159 insects. Microorganisms were found in 111 insects while 48 were negative.
The percent infection is based on the total sample of 159 insects diagnosed.

Table VI

Microorganisms found in C. **conflictana** larvae from British Columbia in 1978 and Ontario in 1973 complied as if all infections were single infections.

Organism	Bristish Columbia		Ontario	
	No.	%[1]	No.	%[2]
Granulosis virus	99	30.9	17	10.7
Entomopoxvirus	35	10.9	38	23.9
Microsporidia sp.	101	31.6	103	64.8
Thelohania	5	1.6	-	-
Beauveria	3	0.9	-	-
Entomophthora	3	0.9	-	-
Isaria	4	1.2	-	-

[1]The percent infection is based on the total sample of 320 insects diagnosed.

[2]The percent infection is based on the total sample of 159 insects diagnosed.

Microsporidia

Cross infection tests were undertaken with microsporidia from **C. conflictana.** In one experiment 2nd instar spruce budworm larvae, naturally infected with microsporidia, were placed on diet painted with microsporidia from **C. conflictana.** Controls were treated with distilled water. The microsporidia were identified by spore size. Wilson and Burke (1971) gave the spore size of **Nosema thomsonii** as 1.2 to 1.5 x 2.5 to 3.0 microns. The size of fresh **Pleistophora** sp. spores from **C. fumiferana** is 1.4 x 2.5 microns (Wilson, 1975). Thomson (1955) reported the spore size of **Perezia fumiferanae** (now **Nosema fumiferanae** [Wilson 1972]), as 2.0 x 3.0 to 5.0 microns. The size of the spores in packets in the large aspen tortrix is about the same size as that of **N. thomsonii** and the **Pleistophora** spores in the spruce budworm, and therefore, it is not known if the microsporidia used in the test were **N. thomsonii, Pleistophora** or a mixture of the two organisms. However, there is sufficient difference in spore size to enable identification of **N. fumiferanae** from the others. Therefore, in reporting the results of the experiment the species **N. fumiferanae** is correct but the term **Pleistophora** sp. may refer to **N. thomsonii, Pleistophora** from **C. fumiferana, Pleistophora** from **C. conflictana,** or a mixture of these organisms.

The insects from the experiment were examined 20 days post inoculation and it was found that in the controls 13 insects were infected with **N. fumiferanae** (68%), 4 with mixed **N. fumiferanae** and **Pleistophora** sp. (21%), and 2 were negative (11%). Those fed on treated diet showed 3 with **N. fumiferanae** (13%) and 20 with mixed **N. fumiferanae** and **Pleistophora** sp. (87%). Thus the microsporidia in the **Pleistophora** size range increased from 21 to 87 percent.

Wilson (1975) reported that **Diprion hercyniae** Htg. is susceptible to **Pleistophora** sp. Repeated attempts to infect **D. hercyniae** with microsporidia from **C. conflictana** met with little success (194 negative, 2 with a trace of infection). However when the microsporidia were passed once through **Neodiprion sertifer** Geoff., the result was 29 negative, 9 with a trace of infection and one with medium infection. The results cannot be interpreted to indicate that passage through **N. sertifer** caused a change in the organism as the microsporidia were fresher than the first suspensions used and may have been more viable.

At least one of the microsporidia generally found in **C. conflictana** is infectious to a number of Hymenoptera and Lepidoptera. Most tests were conducted with extra larvae available from samples submitted for examination, so the ideal of having young larvae was not always attained. In order to allow time for the infection to progress to the point at which mature spores were present and could be detected by light microscope, most of the experimental insects were allowed to go through to pupation or even to the adult stage before examination. However, when possible, some were examined fairly soon after being placed on treated food to see how early we could detect infection.

Table VII shows the larvae tested using microsporidian spores from **C. conflictana,** the instar to which the inoculum was fed, the number of days until they were examined, controls used and larvae infected. In some cases very few larvae were available for the test. As a general observation, it was noted, when diagnosing the larch sawfly, **Pristiphora erichsonii** Htg., that infection was light compared to that encountered when the insect was infected in the field with its own microsporidia. Also the infection observed in **Archips negundana** Dyar. was very light. In contrast **Neodiprion lecontei** Fitch became quite heavily infected by the organism. In many cases the percent infection might have been greater had the inoculum been fed to younger larvae, as it has been shown in the case of spruce budworm that it is easier to infect the earlier instars (Wilson 1974).

Table VII

Cross infection tests with microsporidia from **C. conflictana**

	Instar	Days to examination	Control		Inoculated	
			Negative	Positive	Negative	Positive
Hymenoptera						
Neodiprion abietis Harr.	IV	180	-	-	3[a]	3
Neodiprion lecontei Fitch.	I	25/47	3	0	0	21
" " "	II	25/47	5	0	0	16
" " "	II - III	33+	10	0	0	34
Neodiprion p. paradoxicus Ross	-	22	5	0	11	0
Neodiprion p. paradoxicus Ross	-	66	16	0	0	5
Neodiprion sertifer Geoff.	I	7-17	-	-	18	0
	I	18-35	-	-	3	24
" " "	III - IV	-	-	-	3	29
" " "	IV - V	13	2	0	8	0
" " "		95	10	0	2	8
Pikonema alaskensis Roh.	V	15	2	0	1	0
" " "		41	2	0	0	4
Pristiphora erichsonii Htg.	II - IV	29-41	30	0	43	91
Lepidoptera						
Archips negundana Dyar.	-	35	8	0	6	8
Choristoneura occidentalis Free.	III - IV	13	-	-	2	0
	III - IV	20 +	8	0	45	20
Dioryctria reniculelloides M & M	VI	46	11	0	6	18
Malacosoma pluviale (Dyar.)	-	41	5	0	13	2

[a]Figures represent number of larvae; "-" means no untreated controls were kept.

Granulosis virus

A number of attempts were made to infect spruce budworm larvae with large aspen tortrix granulosis virus. In one experiment fresh granulosis virus infected four out of 30 larvae examined (13.3%), while 30 control larvae were uninfected. In another experiment **C. conflictana** granulosis virus that had been stored in water at about 5°C for 18 months was used as the inoculum. Sixty-one larvae were examined and 3 (4.9%) were infected with granulosis virus. The low rate of infection was probably due to the method and duration of storage of the virus. An examination with the electron microscope indicated that many of the capsules were crushed (J. Percy, personal communication). When fresh virus from the latter experiment was fed to spruce budworm larvae, the percent infection increased considerably. Of those larvae fed the granulosis virus 100 pupated and 61 died in the larval and prepupal stage (25 negative, 36 granulosis infected). Six of the 100 pupae were discarded by mistake, while 15 of the remaining 94 pupae failed to emerge as adults (9 negative, 6 granulosis infected). Of the 155 insects considered, 42 (27.1%) were infected with granulosis virus. In the controls, 82 pupated and 2 of these failed to emerge as adults. During the rearing 5 controls died, one as a larva and 4 as prepupae. Virus was not found in the controls. It was observed that of the 61 dead in the virus infected group, 17 died in the larval stage, (4 negative, 13 infected) and 44 died as prepupae (21 negative, 23 infected). Perhaps the larger number of dead prepupae, even when granulosis virus was not detected, was an effect of the virus. It was found in nuclear polyhedrosis infection tests with the Bertha armyworm, **Mamestra configurata** Wlk., that larvae that appeared to be uninfected had difficulty moulting to pupae (Burke, 1974).

Entomopoxvirus

The entomopoxvirus found in the large aspen tortrix is infectious to the spruce budworm (Cunningham **et al.** 1973).

CONCLUSION

It is not known what pressure the microorganisms would place on an outbreak of **C. conflictana.** Microsporidia in spruce budworm shorten adult life and reduce fecundity (Thomson 1958; Wilson 1977b). It is probable that they would have the same effect on **C. conflictana** since the insects are closely related and the organisms are infectious to both species. The percent infection by viruses is low and by fungi even lower so their role in reducing the population would not be very great. Records were not kept of entomogenous parasites, but they were plentiful in the populations examined. While it is considered that starvation ultimately brings about the decline of **C. conflictana** outbreaks, it is possible that the complex of parasites and microorganisms slows the build-up of the insect to epidemic proportions, or accelerates its decline.

It is shown that the same complement of microorganisms may be found in widely separated populations of **C. conflictana** such as those found in Ontario and British Columbia.

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