

INCIDENCE OF *LEIDYANA CANADENSIS* (APICOMPLEXA: EUGREGARINIDA) IN
LAMBDINA FISCELLARIA FISCELLARIA LARVAE (LEPIDOPTERA:
GEOMETRIDAE)

C.J. LUCAROTTI¹ and T.L. LECLERC

Atlantic Forestry Centre, Canadian Forest Service, Natural Resources Canada,
P.O. Box 4000, Fredericton, New Brunswick, Canada E3B 5P7

and R.E. CLOPTON

Division of Science and Technology, Peru State College, Peru, Nebraska,
68421 United States

Abstract

The Canadian Entomologist **130**: 583 – 594 (1998)

The gregarine, *Leidyana canadensis* Clopton and Lucarotti was found infecting the guts of larvae in a declining population of the hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée), in Charlotte County, New Brunswick. Total mortality, in rearing, of field-collected larvae and pupae was 75.5% in 1993 and 89.2% in 1994. *Leidyana canadensis* infections were present in 60.1 and 80.5% of these dead insects in the two respective years. The incidence of *L. canadensis* in larvae that died in rearing was greater than 50% for all instars except those collected in the fifth instar, where the incidence was below 30%. Gregarine infections were present in first-instar larvae on the first days of collection for both years and, in 1994, 74% of all first-instar larvae collected were infected with *L. canadensis*. Of 678 larvae collected in 1994, 73 survived to adults and, of these, 44 had harbored *L. canadensis* as larvae. The incidence of other microbes and parasitoids in dead larvae and pupae is also reported.

Lucarotti, C.J., T.L. Leclerc et R.E. Clopton. 1998. Incidence de *Leidyana canadensis* (Apicomplexa : Eugregarinida) chez les larves de *Lambdina fiscellaria fiscellaria* (Lepidoptera : Geometridae). *The Canadian Entomologist* **130** : 583–594.

Résumé

On a constaté que le grégarien *Leidyana canadensis* Clopton et Lucarotti contaminait les intestins de larves d'une population en déclin de l'arpenteuse de la pruche, *Lambdina fiscellaria fiscellaria* (Guenée) dans le comté de Charlotte au Nouveau-Brunswick. La mortalité totale en élevage de larves et de nymphes prélevées sur le terrain a été de 75,5% en 1993 et de 89,2% en 1994. L'infection à *L. canadensis* était présente dans 60,1 et 80,5% de ces insectes morts les deux années respectives. L'incidence de *L. canadensis* dans les larves mortes en élevage était supérieure à 50% pour tous les stades larvaires, sauf pour les larves prélevées au cinquième stade, où l'incidence était inférieure à 30%. Les infections à grégarien étaient présentes dans les larves au premier stade dès premiers prélèvements des deux années et, en 1994, 74% de toutes les larves prélevées au premier stade étaient contaminées par *L. canadensis*. Sur les 678 larves prélevées en 1994, 73 ont survécu jusqu'à l'adulte et, de ce nombre, 44 avaient hébergé *L. canadensis* à l'état larvaire. On signale également une incidence d'autres microbes et parasitoïdes dans des larves et des nymphes mortes.

Introduction

The hemlock looper, *Lambdina fiscellaria fiscellaria* (Guenée) (Lepidoptera: Geometridae), is a pest of balsam fir, *Abies balsamea* (L.) Miller (Pinaceae), in eastern Canada (Mills and Räther 1990), especially Quebec (Jobin and Desautniers 1981) and Newfoundland (Carroll 1956; Otvos et al. 1979). Typical outbreaks of hemlock looper develop quickly, last 2 or 3 years, and end abruptly (Raske et al. 1995). Severe defoliation during this brief period can lead to large-scale mortality of balsam fir (Jobin and Desautniers 1981). In New Brunswick, outbreak populations of hemlock looper were first reported in 1989 (Magasi 1990), and aerial applications of insecticide against this pest were made in 1990 (Hartling et al. 1991). In 1991, there was evidence of severe defoliation in patches of balsam fir in Charlotte County (Magasi 1992), but these hemlock looper populations declined in 1992 (Magasi 1993) through 1993 (Magasi and Hurley 1994), and were all but absent in 1994 (Hurley and Magasi 1995).

A new species of gregarine, *Leidyana canadensis* Clopton and Lucarotti (Apicomplexa: Eugregarinida), was recently described from a hemlock looper population near St. Stephen, Charlotte County. The life-cycle stages of *L. canadensis* were also detailed (Clopton and Lucarotti 1997). Oocysts of *L. canadensis* are ingested by larval hemlock looper. Young trophozoites emerge from the oocysts and attach to the epithelium of the midgut by means of an apical epimerite. Here, the solitary trophozoites enlarge and, after an undetermined amount of time, detach from the epithelium and float freely in the gut lumen as gamonts. Gamonts pair, secrete a cyst wall around themselves, and differentiate to gametes. The gametes pair, forming oocysts which are usually retained within the gametocyst until these are excreted with the frass. Oocysts are extruded, in chains, out of the gametocyst through short spore tubes. These can then be eaten by other hemlock looper larvae. As part of this life-cycle study, the incidence of *L. canadensis* was monitored in the St. Stephen hemlock looper population between 1993 and 1995. The incidence of other microbes and parasitoids infecting hemlock looper larvae and pupae was also recorded. Additional hemlock looper populations in eastern Canada were also sampled to determine the extent of the geographical distribution of *L. canadensis* and its possible impact on these populations.

Materials and Methods

Field Sampling and Laboratory Rearing. Collections of hemlock looper larvae and pupae were made for laboratory rearing from our site off the Mohannes Road (45°16'N, 67°38'W), near St. Stephen, New Brunswick. In 1993, 11 weekly collections were made between 15 June and 17 August; in 1994, 16 collections, at least one a week, were made between 10 June and 15 August. In 1995, larval collections were only made at this site on 28 June and 5 July. On 17 and 30 June 1993 collections for rearing were made from Big Bald Mountain, New Brunswick (47°20'N, 66°40'W). In 1994, additional larvae were collected to serve as field controls from the St. Stephen site on 20, 23, and 28 June and 4, 7, 12, and 14 July. These larvae were killed and examined for disease and (or) parasitoids on the day following collection. All larvae were collected by beating branches of balsam fir trees with a 1-m-long stick, with a 60 × 40 cm tray placed under the branches. A range of size and age classes of trees was sampled each day. Collected larvae and pupae were transported to the laboratory in a covered 7-L plastic tub containing balsam fir foliage collected at the site. All larval instar determinations were made under a dissecting microscope by head capsule measurements (Carroll 1956; Hartling et al. 1991). First, second, third, fourth, and fifth larval instars will be referred to as L₁, L₂, L₃, L₄, and L₅, respectively.

Larvae were individually reared in clear plastic, 30-mL containers at 22°C and 16L:8D and were checked daily. Fresh, cleaned foliage was replenished as required. For use in rearing, current-year and 1-year-old foliage was surface cleansed by rinsing in a 2% solution of commercial bleach for 10 min followed by a further 10-min rinse in 1% bleach and then two 10-min rinses in demineralized water. The numbers reared, as larvae at a given instar or as pupae, were obtained as follows:

$$\begin{aligned} &\text{total number of } L_n \text{ reared} \\ &= L_n \text{ collected} + L_{n-1} \text{ surviving to } L_n \text{ in rearing} \end{aligned} \quad [1]$$

where L_n is larval instar, and $n = 1$ through 5; and

$$\begin{aligned} &\text{total number of pupae reared} \\ &= \text{pupae collected} + L_5 \text{ surviving to pupate in rearing} \end{aligned} \quad [2]$$

Dead larvae and pupae were first examined under a dissecting microscope for the presence of parasitoids. Where the presence of parasitoids was suspected, dead insects were maintained under the rearing conditions until the parasitoids emerged. All dead larvae and pupae were examined for microbial pathogens under a compound microscope using fresh squashes of infected tissues. Because of observations made towards the end of the rearing period in 1993, the accumulated frass in each larval rearing container was examined for the presence of gametocysts with each change of foliage in 1994.

To test whether survival to adult was independent of *L. canadensis* infection, the 1994 data were analyzed in a 2×2 contingency table using Cochran's corrected χ^2 (Zar 1984).

In 1994, hemlock looper nuclear polyhedrosis virus (LafNPV) (Volkman et al. 1995) was detected using molecular probes. Insects that died in rearing and field-control larvae were individually homogenized in 1.5-mL microcentrifuge tubes with a small pestle in approximately an equal volume of sterile distilled water. Three microlitres of homogenate were spotted onto Biotrans+ nylon membranes (ICN, Costa Mesa, California) along with 2 μ L of the recombinant plasmid, pT7/T3 α -18, LafNPV/Sal I, 1.1 kilobases (kb), containing the polyhedrin gene (Levin et al. 1997) which served as a control. Samples on the membranes were denatured at 65°C for 30 min in 1.5 M NaCl, 0.5 M NaOH, neutralized for 1 min at 20°C in 1.5 M NaCl, 1.0 M Tris-HCl, pH 7.0, and soaked once for 10 min at 20°C in 10 times saline – sodium citrate (SSC: 0.15 M NaCl: 0.015 M Na₃C₆H₅O₇·H₂O, pH 7.0). Membranes were air dried and DNA immobilized by ultraviolet crosslinking (125 mJ). Membranes were prehybridized for 5 h at 65°C in Church's buffer (modification 8: Enger-Blum et al. 1993). Hybridization was done overnight at 65°C in Church's buffer (0.1 mL buffer/cm² membrane) plus 0.1 mg/mL salmon sperm DNA and probe consisting of fluoresceinated LafNPV/Sal I, 1.1-kb fragment (RENAISSANCE™, DuPont NEN, Boston, Massachusetts). Hybridized membranes were washed twice for 15 min each in 2 times SSC – 0.1% sodium dodecyl sulfate (SDS), once for 15 min in 0.2 times SSC – 0.1% SDS, and once for 30 min in 0.1 times SSC – 0.1% SDS, all at 65°C. At 20°C, membranes were rinsed in buffer 1 for 1 h, blocked in buffer 2 for 1.5 h, and placed in antifluorescein – horseradish peroxidase conjugate solution (0.1 mL/cm² membrane) for 30 min as per RENAISSANCE kit directions. Membranes were further washed, at 20°C, twice in buffer 1, 10 min each and once for 30 min in 0.1 M Tris-HCl, 0.1 M NaCl, 50 mM MgCl₂, pH 9.5. Membranes were placed in the RENAISSANCE detection reagent and results were recorded on Hyperfilm-ECL (Amersham, Oakville, Ontario).

The geographical range of *L. canadensis* infections in hemlock looper populations in eastern Canada was assessed from additional collections of hemlock looper larvae obtained from Quebec (in 1996, 359 larvae from the Anse-aux-Fraises – Rivière Ste-Mairie region on the southwest coast of Anticosti Island; in 1997, from the Gaspé, 381 from Rivière St-Jean, 385 from Lac Sirois, and 200 from Lac Blanchet), Newfoundland (in 1995, 873 from the Corner Brook region; in 1996, 1000 from the Hawks Bay region), and Nova Scotia (in 1997, 50 each from Maple Ridge, Antigonish County, Boylston Provincial Park, Guysborough County, River Denys Road, and Creignish Hills, Inverness County). Larvae from these collections which were received dead or died in rearing were smeared as described above to determine the cause of death. Larval frass was also monitored for the presence of gregarine gametocysts.

Mode of Transmission of *L. canadensis*. Gregarine gametocysts, collected from the frass of infected individuals, were placed in phosphate-buffered saline until oocyst discharge. Oocysts were collected, rinsed with sterile distilled water, and placed, in a 2- μ L drop of water, onto the tips of new, clean, fir needles. Single contaminated needles were fed to individual laboratory stock hemlock looper L_3 in 2-mL microcentrifuge tubes. After consuming the bait, larvae were individually reared on clean fir foliage as above. Control insects were treated similarly except that fir needle baits received only 2- μ L drops of water. Fourteen adult female hemlock looper that had been collected from the St. Stephen population and had had gregarine cysts in their frass as larvae were placed in a 10-lb (1 lb = 0.453592 kg) plastic bag with five male moths, three of which were known to have harbored gregarines as larvae. The moths were allowed to mate and the females oviposited eggs onto clean cotton gauze placed in the bag. The eggs were overwintered in a Stevenson screen, under the deck of a residential home in New Maryland, New Brunswick. On 3 April 1995, the eggs were brought into the laboratory and counted. Nineteen eggs were prepared for scanning electron microscopy (SEM) by sequential fixation in 2.5% glutaraldehyde – 1% OsO_4 in 0.1 M Na-cacodylate buffer, pH 7.4, followed by two 15-min rinses in buffer, dehydration in a graded ethanol series, critical-point drying, and gold coating as per standard techniques (Cole 1986). These eggs were viewed in a Jeol 6400 SEM at 10 kV. The remaining eggs were hatched in the laboratory and reared on modified corn-soy-milk (CSM) artificial diet (Grisdale 1975). The frass of these larvae was monitored for gametocysts and dead insects were examined microscopically to determine cause of death.

Results

Field Sampling and Laboratory Rearing. In 1993, 1020 hemlock looper larvae and 76 pupae from St. Stephen (Table 1) and 158 larvae from Big Bald Mountain (Table 2) were collected and reared. Only two collections were made from Big Bald Mountain because the plot was sprayed with *Bacillus thuringiensis* Berliner subspecies *kurstaki* (Eubacteria: Bacillaceae) preparations shortly after the last collection date of 30 June. In 1994, 678 hemlock looper larvae were collected for rearing from St. Stephen (Table 1) and 280 for field controls. Collection of all larval stages and pupae was possible in St. Stephen in 1993. In 1994, it became extremely difficult and time consuming to collect larvae after 21 July because of their low numbers. Emphasis was placed on collecting insects for rearing and, as a result, field controls were only collected between 20 June and 14 July. On 30 June only five larvae could be collected because of heavy rain and on 10 August one L_5 and no pupae were found after extensive searching. In 1995, only 13 larvae were recovered on the two collection days, so no further collections were made.

TABLE 1. Mortality in rearing of *Lambdina fuscicollaria fuscicollaria* larvae and pupae collected from St. Stephen, New Brunswick

| Percentage of dead insects infected or parasitized by: ^a | | | | | | | | | | | | | |
|---|------------------|-------------|--------------|-------------------|----------------------------|----------------------------|----------|-------|----------------------|--------------------------|------|------|-------------------|
| | Number collected | Number died | Total reared | Percent mortality | <i>Leidyana canadensis</i> | <i>Entomophaga aulicae</i> | | | | | | | No agent detected |
| | | | | | | LafinPV | Bacteria | Yeast | Diptera ^b | Hymenoptera ^c | | | |
| 1993 | | | | | | | | | | | | | |
| L ₁ | 121 | 14 | 121 | 11.6 | 42.9 | 0 | 0 | 21.4 | 0 | 0 | 0 | 35.7 | |
| L ₂ | 160 | 64 | 267 | 24.0 | 82.8 | 0 | 1.6 | 3.1 | 0 | 0 | 0 | 12.5 | |
| L ₃ | 186 | 133 | 389 | 34.2 | 73.7 | 0 | 3.0 | 0.8 | 0 | 0 | 0.8 | 21.8 | |
| L ₄ | 149 | 213 | 405 | 52.6 | 71.8 | 1.4 | 2.8 | 4.2 | 0 | 1.9 | 0.9 | 16.9 | |
| L ₅ | 404 | 345 | 596 | 57.9 | 44.6 | 2.9 | 9.6 | 3.8 | 1.4 | 10.7 | 2.0 | 24.9 | |
| Pupae | 76 | 58 | 327 | 17.7 | 0 | 0 | 0 | 0 | 1.7 | 34.5 | 13.8 | 50.0 | |
| Adults | 0 | | 269 | | | | | | | | | | |
| Total | 1096 | 827 | | 75.5 | 60.1 | 1.6 | 5.3 | 3.4 | 0.7 | 7.3 | 2.2 | 23.3 | |
| 1994 | | | | | | | | | | | | | |
| L ₁ | 139 | 20 | 139 | 14.4 | 30.0 | 0 | 10.0 | 0 | 0 | 0 | 0 | 60.0 | |
| L ₂ | 121 | 87 | 240 | 36.3 | 86.2 | 3.4 | 4.6 | 0 | 0 | 0 | 0 | 6.9 | |
| L ₃ | 145 | 181 | 298 | 60.7 | 93.9 | 7.7 | 1.7 | 0 | 0 | 0 | 0 | 1.1 | |
| L ₄ | 150 | 145 | 267 | 54.3 | 89.7 | 8.3 | 1.4 | 0 | 0 | 0 | 2.1 | 2.8 | |
| L ₅ | 123 | 145 | 245 | 59.2 | 73.1 | 12.4 | 0.7 | 1.4 | 3.4 | 14.5 | 8.3 | 5.5 | |
| Pupae | 0 | 27 | 100 | 27.0 | 0 | 7.4 | 0 | 0 | 0 | 40.7 | 0 | 51.9 | |
| Adults | 0 | | 73 | | | | | | | | | | |
| Total | 678 | 605 | | 89.2 | 80.5 | 8.1 | 2.0 | 0.3 | 0.8 | 5.3 | 2.5 | 7.6 | |

^aIn 1994, total percentages across individual rows may exceed 100% due to dual infections of larvae by *L. canadensis* and parasitoids or LafinPV.^bTachinidae.^cBraconidae have been reported from larvae and Ichneumonidae from pupae (see Mills and Räther 1990).

TABLE 2. Mortality in rearing of *Lambdina fiscellaria fiscellaria* larvae and pupae collected from Big Bald Mountain, New Brunswick, in 1993

| | Number collected | Number died | Total reared | Percent mortality | Percentage of dead insects infected or parasitized by: | | | | | | No agent detected |
|----------------|------------------|-------------|--------------|-------------------|--|--------|----------|-------|--------------------------|------|-------------------|
| | | | | | <i>Leidyana canadensis</i> | LafNPV | Bacteria | Yeast | Hymenoptera ^a | | |
| L ₁ | 61 | 5 | 61 | 8.2 | 60.0 | 0 | 0 | 0 | 0 | 40.0 | |
| L ₂ | 74 | 11 | 130 | 8.5 | 0 | 0 | 27.3 | 9.1 | 0 | 63.6 | |
| L ₃ | 23 | 21 | 142 | 14.8 | 0 | 4.8 | 19.0 | 14.3 | 0 | 61.9 | |
| L ₄ | 0 | 8 | 121 | 6.6 | 55.6 | 0 | 11.1 | 11.1 | 11.1 | 11.1 | |
| L ₅ | 0 | 35 | 113 | 31.0 | 11.4 | 5.7 | 22.9 | 14.3 | 0 | 45.7 | |
| Pupae | 0 | 6 | 78 | 7.7 | | 0 | 0 | 16.7 | 0 | 83.3 | |
| Adults | 0 | | 72 | | | | | | | | |
| Total | 158 | 86 | | 54.4 | 14.0 | 3.5 | 20.0 | 12.8 | 1.2 | 48.3 | |

^aBraconidae have been reported from larvae and Ichneumonidae from pupae (see Mills and Räther 1990).

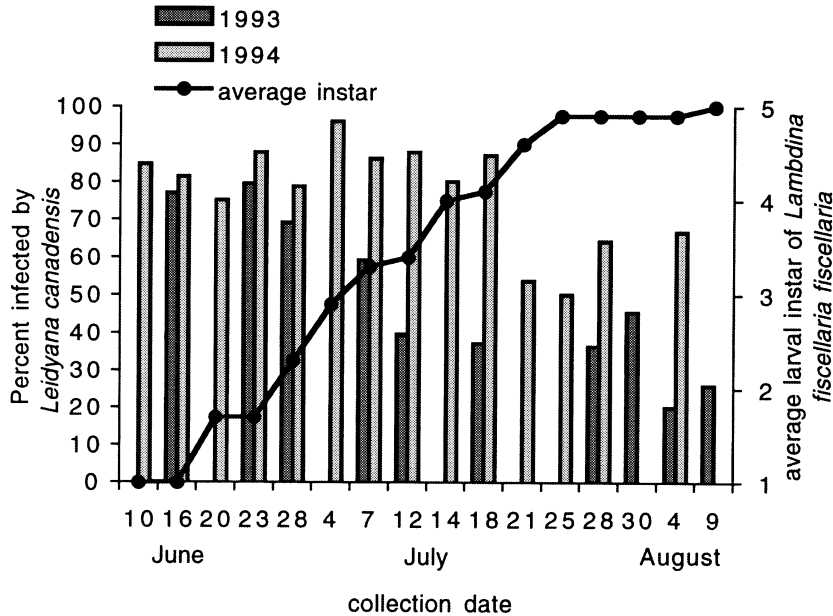


FIG. 1. Incidence of *Leidyana canadensis* infections in *Lambdina fiscellaria fiscellaria* larvae on the date of collection from St. Stephen, New Brunswick, in 1993 and 1994. Percent infected is based on larvae infected by *L. canadensis* which died in rearing as a proportion of the total larval mortality in rearing.

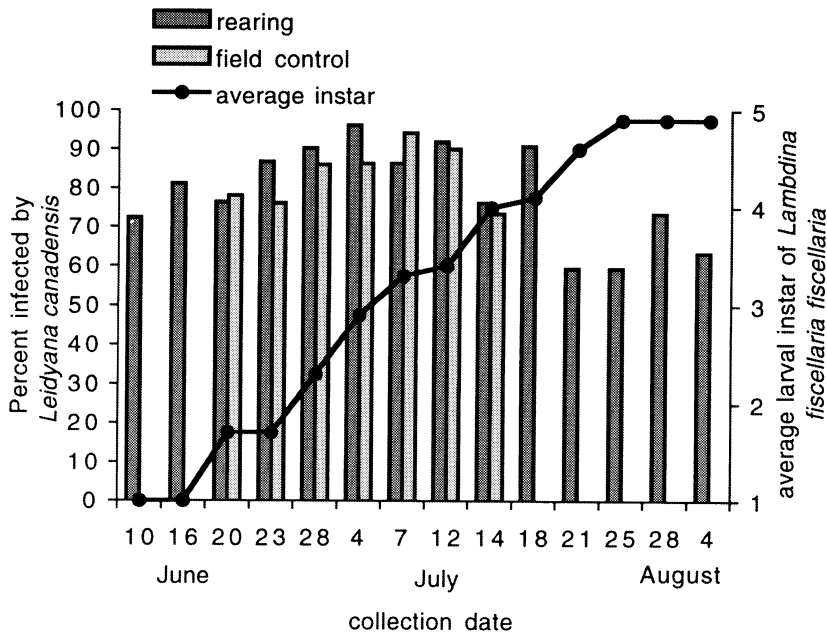


FIG. 2. Incidence of *Leidyana canadensis* infections in all *Lambdina fiscellaria fiscellaria* larvae collected for rearing and field control on the date of collection from St. Stephen, New Brunswick, in 1994.

Mortality in rearing of St. Stephen hemlock looper, at individual larval instars, ranged from 11.6 to 57.9% in 1993 and from 14.4 to 60.7% in 1994. Pupal mortalities were 17.7 and 27.0%, respectively (Table 1). Big Bald Mountain larval mortalities

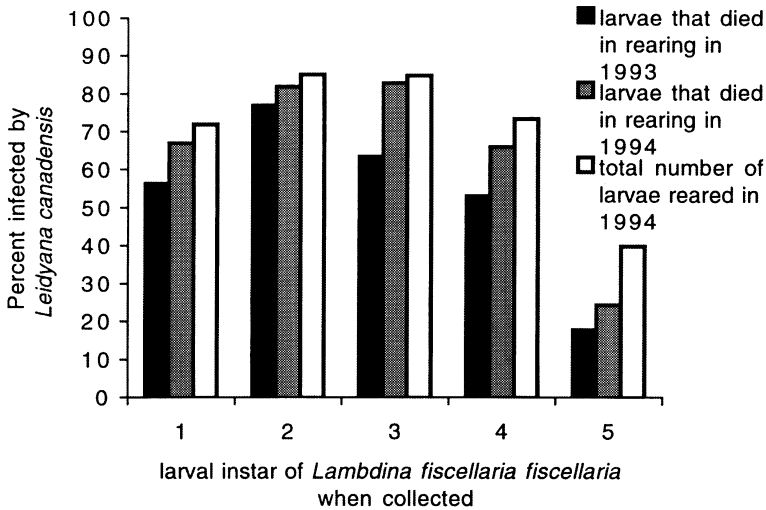


FIG. 3. Incidence of *Leidyana canadensis* infections in *Lambdina fiscellaria fiscellaria* larvae by larval instar at the time of collection from St. Stephen, New Brunswick, in 1993 and 1994.

ranged from 6.6 to 31.0% and pupal mortality was 7.7% (Table 2). Gregarine infections in hemlock looper larvae that died in rearing exceeded 50% in larvae collected from St. Stephen through 7 July 1993, after which *L. canadensis* incidence ranged between 20 and 45% (Fig. 1). In 1994, *L. canadensis* infections in larvae that died in rearing ranged between 50 and 96% (Fig. 1). The total incidence of *L. canadensis* in larvae collected for rearing in 1994 was 72.2% on the first day of collection, increased to 96.1% on 4 July, and remained above 50% for the balance of the collection period (Fig. 2). The incidence of *L. canadensis* in the field controls closely followed the total incidence in the insects collected for rearing (Fig. 2). In the larvae that died in rearing in 1993, *L. canadensis* incidence peaked at 76.9% in L_2 and then declined towards L_5 (Fig. 3). In 1994, the incidence of *L. canadensis* in all larvae brought into rearing and those which died in rearing peaked at over 80% in L_2 and L_3 and then declined in L_4 and L_5 (Fig. 3). The range of gregarine infections for insects that died at a given larval instar was between 42.9 and 82.8% in 1993 and between 30.0 and 93.9% in 1994 (Table 1). Larval mortalities and the incidence of gregarine infections were lower in insects collected from Big Bald Mountain (Table 2).

Gregarine trophozoites were observed in such high densities within the midguts of many of these infected larvae that they could have interfered with the passage of the food bolus. Gamonts and gametocysts were observed in the guts of dead larvae of all instars beginning with L_1 which had died 7 days following collection. However, gametocysts were first observed in the frass of living L_4 and L_5 beginning on 29 July 1994.

Based on microscopic examination, larval mortality attributed to LafinPV in 1993 was 1.4% at L_4 and 2.9% at L_5 (Table 1). The use of molecular probes increased the efficiency of virus detection in 1994, and virus was found in all instars, except L_1 , and pupae (Table 1). Bacteria were not identified, so it is not known whether these were pathogenic or merely saprophytic. The fungus *Aureobasidium pullulans* (de Bary) Arn. (Hyphomycetes) was isolated and identified from larval frass and is suspected to be the "yeast" observed in a number of the dead larvae. Budding cells of *A. pullulans* and free oocysts of *L. canadensis* were observed in the impacted midguts of larvae heavily infected with *L. canadensis*. The incidence of *Entomophaga aulicae* (Reichardt) Humber

(Zygomycetes: Entomophthorales) was low in both years (Table 1). Parasitism by members of the Tachinidae (Diptera) was greatest in L_5 and pupae (Table 1).

The percentage of dead insects with no detectable microbe or parasitoid declined in 1994 because of the use of molecular probes to detect LafiNPV and the examination of larval frass for gregarine gametocysts. Fifty percent of pupae died in 1993 of no apparent cause compared with 51.9% in 1994, but examination of larval frass in 1994 showed that 13 of 27 dead pupae had had gregarine infections as larvae. Similarly, we were able to determine that, of the 73 larvae that went on to pupate and eclose as adults (Table 1), 44 had had gregarine infections as larvae. When the frequency of gregarine infection in 1994 was compared between insects that survived to adults and those that did not in a 2×2 contingency table, Cochran's corrected χ^2 was significant ($\chi^2_c = 19.2$, $P < 0.001$), indicating that gregarine infection was associated with hemlock looper mortality.

In 1995, five of the 13 larvae collected from the field were infected with *L. canadensis*. No evidence of *L. canadensis* infections was found in the hemlock looper larvae collected in Quebec or Newfoundland. Only four (three from River Denys Road and one from the Creignish Hills) of the 200 dead larvae examined from Nova Scotia had *L. canadensis* infections.

Mode of Transmission of *L. canadensis*. Gametocysts were recovered from laboratory stock hemlock looper larvae that were fed oocysts but not from the control insects. Of the 226 eggs that were overwintered from the mating experiment, 19 were taken for SEM and 193 of the remaining eggs hatched between 11 and 19 April 1995. These larvae developed poorly on the CSM diet, and by 19 July 1995 only 37 larvae had survived. These were killed and smeared and six were found to have gregarine infections. No oocysts were observed on the surfaces of the eggs examined in the SEM.

Discussion

The high incidence of *L. canadensis* in the St. Stephen hemlock looper population and its association with larval mortality make it appear that *L. canadensis* was responsible for the population decline. However, gregarines, as a rule, are not considered to be pathogenic to their hosts (Henry 1981), even when infection levels within a population are high. For example, infections of *Comtoides pechumani* Anderson and Magnarelli (Apicomplexa: Eugregarinida) in larvae of *Chrysops fuliginosus* Wiedemann (Diptera: Tabanidae) have been reported to range between 30 and 89% without detrimental effect to their host (Anderson and Magnarelli 1978). Similarly, heavy infections of *Pyxinia frenzeli* Laveran and Mesnil (Apicomplexa: Eugregarinida) in *Attagenus megatoma* (Fabricius) (Coleoptera: Dermestidae) had no effect on larval weight, survival through adult, or length of the life cycle (Dunkel and Boush 1968).

Leidyana canadensis can cause outward symptoms of infection in hemlock looper larvae, such as bloated midsection, sluggishness, and yellowish color, when there are high densities of trophozoites in the larval midgut (see Clopton and Lucarotti 1997, p. 384, Fig. 6). The widespread and chronic infections of *L. canadensis* in this hemlock looper population probably contributed to its decline. Elsewhere, however, gregarines have not been a feature of hemlock looper population declines, as none have ever been reported from *L. fiscellaria fiscellaria* prior to the description of *L. canadensis* (Clopton and Lucarotti 1997) and no gregarines were present in the epidemic hemlock looper populations sampled from Quebec and Newfoundland which have subsequently declined (Dupont 1997; West 1997). *Leidyana canadensis* was reported present in hemlock looper populations in Penobscot, Hancock, and Washington counties in Maine in

1994 and 1995 (Armstrong 1996) but at levels lower than those observed in St. Stephen. These three counties adjoin each other and Washington County is directly across the United States – Canada border from St. Stephen. A “nonpathogenic gregarine protozoan” was found in larvae of *Lambdina athasaria* (Walker) (Lepidoptera: Geometridae) in Connecticut in 1993 (Maier et al. 1994). Here, larval infection rates ranged between 12.6 and 73.0%. Larvae of *L. athasaria* and *L. fiscellaria fiscellaria* may coexist during the summer months in New England (Grehan et al. 1994; Maier and Lemmon 1996), and so it is possible, and perhaps likely, that the gregarine responsible for infections in *L. athasaria* larvae was *L. canadensis*.

The high incidence of *L. canadensis* in L₁ hemlock looper shows that infections are acquired early, possibly during larval emergence from the egg through ingestion of oocysts contaminating the egg surface. Even though oocysts were not observed on eggs examined using SEM, the fact that at least six larvae from the breeding experiment became infected while being fed only artificial diet would implicate the adult female moth in the infection process, possibly by contaminating the egg surface. Larvae might also become infected by contamination of the environment immediately surrounding the oviposition site, either on the bark of the tree bole or on mosses and lichens on the bark (Carroll 1956).

A number of predators and parasites of hemlock looper larvae and pupae have been identified but none has regularly been found responsible for the rapid declines of hemlock looper populations (Raske et al. 1995). In St. Stephen, tachinids were the principal group of parasitoids, accounting for approximately 40% of pupal mortality. Population collapses in Newfoundland have been attributed to *E. aulicae* (Otvos 1973), but larval mortalities, due to this fungus, were low in St. Stephen. *Aureobasidium pullulans*, which was observed in the larval midgut and isolated from the frass, is an endophytic fungus of the needles of balsam fir (Johnson and Whitney 1989) and is not known to be an entomopathogen.

The incidence of LafiNPV was only determined microscopically in 1993, so the apparent increased incidence of this virus in 1994 could be due, at least in part, to the use of DNA probe technology. The use of probes in 1994 showed that larval death due to LafiNPV increased steadily from 0 to 12.4% of larval mortality between L₁ and L₅ and that some pupae were also infected with the virus. The different larval instars of hemlock looper are equally susceptible to LafiNPV (Cunningham 1970a), and being open feeders on fir foliage, larvae should be susceptible to viral epizootics (Lucarotti 1997). However, the rapidity with which hemlock looper populations crest and decline and the apparent lack of longevity of the virus (Cunningham 1970b, 1970c) may not favor natural LafiNPV epizootics.

Populations of hemlock looper in southwest New Brunswick were already in decline in 1992 (Magasi 1993). *Leidyana canadensis* may have been one of the factors contributing to the decline (Royama 1997), however it is not possible to comment on the extent of that contribution because, without bioassays, the level of virulence of *L. canadensis* to its host is not known. Also, because the distribution of hemlock looper larvae in forest stands is patchy and the later instars, in particular, migrate extensively within and between trees (Carroll 1956), methods to estimate hemlock looper densities within a stand have been developed only for eggs (Dobesberger 1989). Thus, we could not extrapolate the data for mortality factors in rearing to determine their potential roles in the hemlock looper population collapse in the field. The high incidence of *L. canadensis* in hemlock looper larvae collected from the St. Stephen population and its increased incidence between 1993 and 1994 demonstrate that it was effectively transmitted within the population and between these two generations.

Acknowledgments

The authors thank A. Carroll, A. Dupont, E. Georgeson, C. Hébert, E. Kettela, M. Leblanc, and R. West for assisting with the additional collections of hemlock looper larvae. Gratitude is also extended to E.S. Eveleigh, D. Ostaff, T. Royama, and J. Sweeney for reviewing the manuscript. J. Sweeney also helped with the statistics. B. Morin and N. MacAfee assisted with the LafiNPV probing. We also thank Janet Proude for bringing the presence of gregarine gametocysts in hemlock looper frass to our attention in 1992. This research was funded by Forest Protection Limited, the Spray Efficacy Research Group, the Canada – New Brunswick COOPERATION Agreement on Forest Development, and the Canadian Forest Service.

References

- Anderson, J.F., and L.A. Magnarelli. 1978. *Cometoides pechumani* sp. n. (Protozoa: Eugregarinida), a gregarine parasite of salt marsh deerflies (Diptera: Tabanidae). *Journal of Invertebrate Pathology* **31**: 324–328.
- Armstrong, C.D. 1996. Natural enemies of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Guen.) (Lepidoptera: Geometridae). M.Sc. thesis, University of Maine, Orono, ME.
- Carroll, W.J. 1956. History of the hemlock looper, *Lambdina fiscellaria fiscellaria* (Guen.) (Lepidoptera: Geometridae) in Newfoundland, and notes on its biology. *The Canadian Entomologist* **88**: 587–599.
- Clopton, R.E., and C.J. Lucarotti. 1997. *Leidyana canadensis* n. sp. (Apicomplexa: Eugregarinida) from larval eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae). *Journal of Eukaryotic Microbiology* **44**: 383–387.
- Cole, G.T. 1986. Preparation of microfungi for scanning electron microscopy. pp. 1–44 in Aldrich, H.C., and W.J. Todd (Eds.), *Ultrastructure Techniques for Microorganisms*. Plenum Press, New York, NY.
- Cunningham, J.C. 1970a. Pathogenicity tests of nuclear polyhedrosis viruses infecting the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae). *The Canadian Entomologist* **102**: 1534–1539.
- . 1970b. Persistence of the nuclear polyhedrosis virus of the eastern hemlock looper on balsam fir foliage. *Canadian Forestry Service Bi-monthly Research Notes* **26**: 24–25.
- . 1970c. The effect of storage on the nuclear polyhedrosis virus of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae). *Journal of Invertebrate Pathology* **16**: 352–356.
- Dobesberger, E.J. 1989. A sequential decision plan for the management of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae) in Newfoundland. *Canadian Journal of Forestry Research* **19**: 911–916.
- Dunkel, F.V., and G.M. Boush. 1968. Biology of the gregarine *Pyxinia frenzeli* in the black carpet beetle, *Attagenus megatoma*. *Journal of Invertebrate Pathology* **11**: 281–288.
- Dupont, A. 1997. Forest protection program against hemlock looper in eastern Québec—1997. Spray Efficacy Research Group 1997 Annual Workshop, Winnipeg, Manitoba, 14–17 October 1997.
- Enger-Blum, G., M. Meier, J. Frank, and G.A. Müller. 1993. Reduction of background problems in nonradioactive Northern and Southern blot analyses enables higher sensitivity than ³²P-based hybridizations. *Analytical Chemistry* **210**: 235–244.
- Grehan, J.R., B.L. Parker, and R.G. Dearborn. 1994. Description of the first and final instar of the hemlock loopers *Lambdina athasaria* (Walker) and *Lambdina fiscellaria* (Guenée) (Lepidoptera: Geometridae). *The Canadian Entomologist* **126**: 1505–1514.
- Grisdale, D.G. 1975. Simplified rearing of the eastern hemlock looper. *Canadian Forestry Service Bi-monthly Research Notes* **31**: 27–28.
- Hartling, L., P.M. MacNutt, and N. Carter. 1991. Hemlock looper in New Brunswick: notes on biology and survey methods. New Brunswick Department of Natural Resources and Energy, Fredericton, New Brunswick.
- Henry, J.E. 1981. Natural and applied control of insects by protozoa. *Annual Review of Entomology* **26**: 49–73.
- Hurley, J.E., and L.P. Magasi. 1995. Forest pest conditions in the Maritimes in 1994. *Canadian Forest Service – Maritimes Region Information Report M-X-194E*.
- Jobin, L.J., and R. Desaulniers. 1981. Results of aerial spraying in 1972 and 1973 to control the eastern hemlock looper (*Lambdina fiscellaria fiscellaria* (Guen.)) on Anticosti Island. *Canadian Forestry Service – Laurentian Information Report LAU-X-49E*.

Johnson, J.A., and N.J. Whitney. 1989. An investigation of needle endophyte colonization patterns with respect to height and compass direction in a single crown of balsam fir (*Abies balsamea*). *Canadian Journal of Botany* **67**: 723–725.

Levin, D.B., A.M. Laitinen, C.J. Lucarotti, B. Morin, and I.S. Otvos. 1997. Characterization of nuclear polyhedrosis viruses from three sub-species of *Lambdina fiscellaria*. *Journal of Invertebrate Pathology* **69**: 125–134.

Lucarotti, C.J. 1997. Baculoviruses as management tools in the control of spruce budworm and other forest pest insects. pp. 7–8 in *Proceedings of the 17th Eastern Spruce Budworm Research Work Conference*, Québec, Quebec, 2–3 April 1997. *Canadian Forest Service – Laurentian Information Report LAU-X-113B*.

Magasi, L.P. 1990. Forest pest conditions in the Maritimes in 1989. *Forestry Canada – Maritimes Region Information Report M-X-177*.

———. 1992. Forest pest conditions in the Maritimes in 1991. *Forestry Canada – Maritimes Region Information Report M-X-181E*.

———. 1993. Forest pest conditions in the Maritimes in 1992. *Forestry Canada – Maritimes Region Information Report M-X-183E*.

Magasi, L.P., and J.E. Hurley. 1994. Forest pest conditions in the Maritimes in 1993. *Canadian Forest Service – Maritimes Region Information Report M-X-188E*.

Maier, C.T., and C.R. Lemmon. 1996. Life cycle of *Lambdina athasaria* (Walker) (Lepidoptera: Geometridae) developing on hemlock in Connecticut. *The Canadian Entomologist* **128**: 995–1003.

Maier, C.T., C.R. Lemmon, R.M. Weseloh, and T.G. Andreadis. 1994. Natural enemies of the spring hemlock looper, *Lambdina athasaria* (Lepidoptera: Geometridae): relative abundance, impact and seasonality. pp. 24–25 in *Proceedings of the Combined Meeting of the Northeastern Forest Pest Council and the Northeastern Forest Insect Work Conference*, Manchester, New Hampshire, 21–23 March 1994.

Mills, N.J., and M. Räther. 1990. Hemlock looper in Canada; biology, pest status and potential for biological control. *Biocontrol News and Information* **11**: 209–221.

Otvos, I.S. 1973. Biological control agents and their role in the population fluctuation of the eastern hemlock looper in Newfoundland. *Canadian Forestry Service – Newfoundland and Labrador Region Information Report N-X-102*.

Otvos, I.S., L.J. Clarke, and D.S. Durling. 1979. A history of recorded eastern hemlock looper outbreaks in Newfoundland. *Canadian Forestry Service – Newfoundland and Labrador Region Information Report N-X-179*.

Raske, A.G., R.J. West, and A. Retnakaran. 1995. Hemlock looper, *Lambdina fiscellaria*. pp. 141–147 in Armstrong, J.A., and W.G.H. Ives (Eds.), *Forest Insect Pests in Canada*. Canadian Forest Service, Natural Resources Canada, Ottawa, Ontario.

Royama, T. 1997. Population dynamics of forest insects: are they governed by single or multiple factors? pp. 37–48 in Watt, A.D., N.E. Stork, and M.D. Hunter (Eds.), *Forests and Insects*. Chapman and Hall, London.

Volkman, L.E., G.W. Blissard, P. Friesen, B.A. Keddie, R. Possee, and D.A. Theilmann. 1995. Baculoviridae. In *Virus Taxonomy: Classification and Nomenclature: Sixth Report of the International Committee on Taxonomy of Viruses*. *Archives of Virology Supplement* **10**: 1118–1121.

West, R.J. 1997. Development of control tactics against the eastern hemlock looper. pp. 18–23 in Hudak, J. (Ed.), *The eastern hemlock looper, Lambdina fiscellaria fiscellaria* (Guen.) (Lepidoptera: Geometridae) in Newfoundland, 1983–1995. *Canadian Forest Service – Newfoundland and Labrador Region Information Report N-X-302*.

Zar, J.H. 1984. *Biostatistical Analysis*. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.

(Date received: 11 February 1998; date accepted: 13 May 1998)