Annual Research Report To
Western Newfoundland Model Forest

Evaluation of entomopathogenic fungi and viruses for their potential to control the eastern hemlock looper

Shiyou Li

Natural Resources Canada, P.O. Box 960, University Drive, Corner Brook, Newfoundland, Canada

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Introduction

The eastern hemlock looper is one of the most destructive forest defoliators in Newfoundland. Severe defoliation can kill trees in one year. In the past, this insect had caused very serious damage to forests in the island. Localized high populations of the looper are currently occurring in the Northern peninsula, western, central and eastern Newfoundland. This may be the beginning of the next outbreak.

At present, biological insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) is operationally used to control the looper, while tebufenozide (an insect growth regulator) is occasionally applied as well. Heavy use and sole reliance on one product for looper control may face the risk of *Bt* resistance in looper populations because some agriculture pests have developed resistance to *Bt*.

Entomopathogenic fungi and viruses have great potential for control insect pests. At least two fungus species and a NPV virus have been identified from natural populations of the looper in Newfoundland, and they were suspected to cause collapse of outbreak looper populations. However, no solid scientific data are available to support this suspicion.

The study herein is to evaluate potential of entomopathogenic fungi and viruses for looper control. The study is a multi-year project. The ultimate goal of the project is to seek biological control agent(s) for the looper control, through both laboratory and field approaches. The specific objectives for the fiscal year of 2002-2003 were to 1) investigate natural mortality factors of the looper at different population levels and at different larval developmental stages and 2) investigate culture techniques for major fungi.

Progress to Date

Although the project was funded by Western Newfoundland Model Forest in 2002, the actual studies were started in 2001. In the summer of 2001, the looper larvae were individually collected at two different times, each from two different sites of northern Peninsula. The field-collected larvae were individually reared in 1-oz plastic cups with healthy balsam fir foliage under room temperature with natural light. Foliage was changed as needed. Larvae were checked on the regular basis until they either pupated or died. Once a larva died, cause of death was identified.

Larval survivorship was higher with the early collection (1^{st} and 2^{nd} instars) than the late collection (3^{rd} and 4^{th}) (Table 1). A total of 999 larvae were reared from the early collection, and 78.4% of them pupated. A total of 1019 larvae were collected and reared in the late collection, and only 11.4% pupated (Table 1). In both early and late collections, parasitoids were not a major mortality factor. Parasitoids emerged from only 4.6% of dead larvae in the early collection, and 0.6% in the late collection. No virus-caused death larvae were found in the early collection. In the late collection, about 7% of dead larvae were suspected to be killed by viruses. Fungus-caused mortality was much higher in the late collection than in the early collection (31% vs 9.3%). However, unknown mortality factors were much higher than any other factors. These unknown factors included natural mortality, some unidentifiable cause of death with our limited capacity and some handling death.

From the results of 2001, a preliminary conclusion was drawn that entomopathogenic fungi seemed to be a major mortality factor for the hemlock looper, and that fungal infection rate was higher in late instars (3^{rd} and 4^{th}) than early ones (1^{st}

and 2nd) in the field. Thus, field collections of larvae for fungi should be focused late instar stages (3rd and 4th).

In 2002, larvae were only collected at late stages (3rd and 4th instars). The methods of field collections and rearing conditions in the laboratory were the same as those used in 2001. Larvae were collected from eastern, central, western and northern regions in 2002. Three collection sites were chosen for each of these four regions. The site selection criteria within a region were primarily based on accessibility to the site, population level of the looper and distance between two collection sites. For the looper population level, efforts were made to choose a site with as high population as it could be founded within the region. If practical and possible, two collection sites should be as far apart as possible. On each site, about 150 3rd and 4th instars were individually collected and reared to either pupae or death in the laboratory. The results of 2002 were presented in Table 2.

Larval mortality varied significantly from region to region and from one collection site to another within a region (Table 2). Overall larval mortalities were 52.0%, 14.3%, 46.3% and 26.7%, respectively, for eastern, central, western and northern region. Based on collection site, larval mortality was as high as 96.0% (Site 3 in the Western region) and as low as 2.7% (Site 3 in the Central region). These results suggested that the looper mortality factors in the field be localized.

Fungus was a major mortality factor for these field-collected larvae. Overall, 57.3% of larvae died from fungal infection, with the highest of 86.4% in the site 2 of the eastern region and the lowest of 0% in the site 1 of the northern region (Table 2). Viruses and parasitoids were not major mortality factors in 2002 collections.

Fungi that killed larvae were further studied for the purpose of fungal identification and to seek suitable culture medium for future mass production of promising fungi. All fungus-killed larvae were observed daily. Once spores of fungi were observed, photos of infected larva were taken and inoculation of spores onto different culture medium was conducted. Data on fungal identification and culture medium of fungi are still being collected and we will continue these investigations in 2003.

From preliminary analyses, one species of fungi was founded to be predominated over other species. This fungus was tentatively identified as *Entomophthora sphaerosperma*, a different species from *Entomophaga aulicae* that R. Nolan (Memorial University of Newfoundland) was studying for many years in 1980s and early 1990s.

Progresses to date on this multiple-year project are on schedule of the original proposal. No major problems were identified so far and we do not expect major difficulties with the project in the coming fiscal year of 2003 – 2004.

Table 1. Major larval mortality factors of the eastern hemlock looper collected from northern Peninsula of Newfoundland in 2001*

Collection date	Larval stage	Site	Site location	Site description	Larvae collected	% Pupation	% Death	Cause of death			
								Fungus	Virus	Parasit.	Unknow.
July 5	1-2	1	S. Roddickton Rd., 2.5 km E Viking Trail (51^{0}03'N, 56^{0}49'W, 52 m elev)	Semi-mature BF, high looper pop., light defol. on the collection date	504	83.9	16.1	16.0	0.0	9.9	74.1
July 5	1-2	2	N. Roddickton Rd., 2.5 km E Viking Trail (51^{0}03'N, 56^{0}49'W, 52 m elev)	Semi-mature BF, edge of clear cut, low looper pop., light defol. on the collection date	495	72.7	27.3	5.2	0.0	1.5	93.3
	Total				**999**	**78.4**	**21.6**	**9.3**	**0.0**	**4.6**	**86.1**
July 26	3-4	1	S. Roddickton Rd., 2.5 km E Viking Trail (51^{0}03'N, 56^{0}49'W, 52 m elev)	Semi-mature BF, high looper pop., severe defol. on the collection date	509	5.9	94.1	32.4	9.0	0.2	58.4
July 26	3-4	2	N. Roddickton Rd., 2.5 km E Viking Trail (51^{0}03'N, 56^{0}49'W, 52 m elev)	Semi-mature BF, edge of clear cut, low looper pop., severe defol. on the collection date	510	16.9	83.1	29.5	4.7	0.9	64.9
	Total				**1019**	**11.4**	**88.6**	**31.0**	**7.0**	**0.6**	**61.4**

* Stages: 1-2 = 1st & 2nd instars, 3-4 = 3rd & 4th instars; Unknown: including natural and other unknown mortality factors.

Table 2. Major larval mortality factors of the hemlock looper collected from the field, Newfoundland in 2002

Region	Site	Collection date	Site location	Site description	Larvae collected	% Pupation	% Death	Cause of death				
								Fungus	Virus	Parasit.	Natural	Handling
Eastern	1	Jul 25	Hatchet Cove (48^{0}02'N, 53^{0}52'W, 122 m elev)	Semi-mature BF, 30%+ defol. on the collection date, majority L3	149	37.6	62.4	76.4	2.0	0.0	20.5	1.1
	2	Jul 25	TCH, 500 m N. of Hillview joint (48^{0}03'N, 53^{0}56'W, 70 m elev)	Mature BF, majority L3 and L4	150	21.3	78.7	86.4	3.4	0.8	8.6	0.8
	3	Jul 25	TCH, 1.3 km N. of Hodges Cove joint (48^{0}01'N, 53^{0}58'W, 98 m elev)	Semi-mature BF, majority L3 and L4	149	85.2	14.8	54.6	0.0	4.5	40.9	0.0
	Total				**448**	**48.0**	**52.0**	**79.4**	**2.6**	**0.9**	**16.2**	**0.9**
Central	1	Jul 26	Lake Douglas, 3.2 km SW of Lake Douglas Lodge (48^{0}28'N, 56^{0}42'W, 331 m elev)	Semi-mature BF, majority L3 and L4	149	71.8	28.2	2.4	9.5	0.0	88.1	0.0
	2	Jul 26	1 km N. Lake Douglas and 21.4 km S. Harpoon joint (48^{0}32'N, 56^{0}37'W, 322 m elev)	Semi-mature BF and WS, low looper population	150	88.0	12.0	5.6	0.0	16.7	72.1	5.6
	3	Jul 26	Harpoon Camp Rd., 900 m east of joint (48^{0}42'N, 56^{0}35'W, 277 m elev)	Thinned BF, low looper population	149	97.3	2.7	50.0	0.0	0.0	50.0	0.0
	Total				**448**	**85.7**	**14.3**	**6.2**	**6.2**	**4.9**	**81.1**	**1.6**

Region	Site	Collection date	Site location	Site description	Larvae collected	% Pupation	% Death	Cause of death				
								Fungus	Virus	Parasit.	Natural	Handling
Western	1	Jul 30	Big Bonne Bay Pond (49^{0}21'N, 57^{0}32'W, 226 m elev)	Mature thinned BF, majority L4, low looper popu.	149	81.2	18.8	46.4	3.6	7.1	39.3	3.6
	2	Aug 1	Bottomless Pond (49^{0}16'N, 57^{0}36'W, 238 m elev)	Most young BF and WS	150	76.0	24.0	47.2	2.8	2.8	47.2	0.0
	3	Aug 1	Goose Arm (49^{0}11'N, 57^{0}49'W, 66 m elev)	Mature BF, moderate looper population	150	4.0	96.0	81.9	8.3	0.7	9.0	0.0
	Total				**449**	**53.7**	**46.3**	**71.2**	**6.7**	**1.9**	**19.7**	**0.5**
Northern	1	Aug 2	Main Brook Rd., 2.2 km S. Viking Tail (51^{0}21'N, 56^{0}07'W, 23 m elev)	Semi-mature BF, majority L3 and L4	150	86.0	14.0	0.0	0.0	14.3	85.7	0.0
	2	Aug 2	East Castor Pond, 500 m from Roddickton Rd. (51^{0}02'N, 56^{0}37'W, 178 m elev)	Thinned BF, majority L3 and L4, some L2	150	78.0	22.0	30.3	0.0	33.3	36.4	0.0
	3	Aug 2	Main Brook Rd., 1.4 km S. Viking Tail (51^{0}15'N, 56^{0}09'W, 42 m elev)	Semi-mature BF, majority L3 and L4, severe defol.	150	56.0	44.0	16.7	4.5	0.0	78.8	0.0
	Total				**450**	**73.3**	**26.7**	**17.5**	**2.5**	**11.7**	**68.3**	**0.0**
			Overall		**1795**	**65.2**	**34.8**	**57.3**	**4.3**	**3.7**	**34.1**	**0.6**