Sterile Insect Technique as a Tool for Increasing the Efficacy of Gypsy Moth Biocontrol

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Abstract

Characteristics such as the duration of the larval stage and attraction of larvae to oviposition by an endoparasitic wasp were evaluated between groups of irradiated and non irradiated gypsy moth larvae. Untreated larvae required a shorter time to reach the adult stage (both male and female). The mortality of larvae was highest at the highest irradiation dose. Pupae that developed from larvae or eggs irradiated at 110 Gy possessed a high degree of morphological abnormalities (93.18%) whereas in the control group, only 3% were affected. Larval mortality caused by *G. liparidis* reached 17% in the control group and 6 % in the irradiated treatment. The natural population density of gypsy moth at the study plot was low during the experiment and corresponded to a period of latency. Of 750 larvae collected at all sites in 2001, 21.3% were killed by parasitoids and 54.4%. by pathogens and unknown causes. There was a significant difference in the mortality of gypsy moth larvae between selected treatments under field conditions. The parasitic fly *Compsilura concinnata* was the most important parasitoid species present in the sites.

Key words: gypsy moth, *Glyptapanteles liparidis*, gamma radiation, larvae developmental time, SIT, parasitoids.

Introduction

The use of the sterile insect technique for suppressing pest populations was proposed initially by Knipling (1955). Since that time the effectiveness of using the sterile insect technique (SIT) has been demonstrated by various models and has been applied operationally and successfully against many insect species. This technique is based on overflooding the feral population with a large numbers of sterile individuals (mostly males). When there is a high over flooding ratio of sterile: feral individuals in the field, and the sterile individuals are fully competitive, the probability of mating of females by fertile males declines. The sterile insect technique is well suited for population management of the gypsy moth (*Lymantria dispar* L.) because it has one generation per year, and though males may mate several times, females mate only once and produce an egg mass which may contain from 200-1600 eggs (Reardon and Mastro 1993).

Mastro and Schwalbe (1988) summarized various methods and strategies for using SIT against the gypsy moth. Three different application techniques have been used: (1) deployment of totally sterile male pupae; (2) deployment of male pupae treated with a substerilizing dose of radiation; and (3) broadcasting F1 sterile eggs. Our objective was to evaluate a fourth method – the introduction of larvae irradiated as eggs into the field to evaluate the effect of irradiation on larval behavior and to determine if the introduction of larvae irradiated as eggs during the latency period would enhance the activity of parasitoids and predators and thus facilitate biological control of the gypsy moth. Reardon et al. (1987), reported that the release of F1 gypsy moth eggs in Maryland significantly increased parasitism by *Compsilura concinnata*. It is suggested that SIT alone or in combination with low doses of pesticides, could be used as an integrated approach to manage gypsy moth populations.

Material and Methods

For all laboratory experiments we used a laboratory strain (NJ) of the gypsy moth supplied by the USDA – APHIS Methods Development Center in Otis, MA (U.S.A.). In the field tests, field

collected gypsy moth egg were received through the cooperation of the Forest Research Institute Bucharest, and Forest District Oradea, Romania.

Development of Irradiated and Non-irradiated Larvae

Gypsy moth eggs were irradiated at 10, 20, 30, 40, 50, 60, 80, or 110 Gy respectively a few days before hatching and larvae were tested for differences in their development by comparison with non-irradiated controls (Gamacel 300 of the Entomology Unit of the IAEA´s Seibersdorf Laboratory in Seibersdorf, Austria was used).

Larvae were reared initially (5 per a cup) on an artificial diet (Bell et al. 1981); from the third instar, larvae were reared separately and were checked daily. The duration of each larval stage was determined by the time of molting. Dead larvae were removed from rearing cups and recorded. Pupae that developed successfully were stored in boxes (60 cm x 60 cm x 100 cm high). The condition and health of the pupae was determined two days after pupation at which time they were weighed. The number of adults that emerged successfully was recorded.

Attraction of Sterile Gypsy Moth Larvae to Oviposition by the Endoparasitic Wasp *Glyptapanteles liparidis* L.

Gypsy moth eggs were irradiated at 50 Gy using a source of cobalt radiation located at the Roosevelt Hospital in the city of Banska Bystrica. The larvae were reared on artificial diet until they reached the fourth larval stage.

We used laboratory strains of the endoparasitic wasp *Glyptapanteles liparidis* L., which was provided by the Department of Entomology, Phytopathology and Forest Protection in the University BOKU of Vienna (Austria).

To investigate the preference of *G. liparidis*, four replicates of 25 fourth instar larvae from irradiated eggs and 25 non-irradiated fourth instars were placed in a rearing box along with five males and five females of *G. liparidis.* The non-irradiated larvae were marked with dots of yellow paint; all larvae were exposed for 24 hours. In order to create a more natural condition, a cage (50 x 50 x 75 cm, covered with monofil) containing oak twigs and arborvitae branches was placed on a table in the lab. The windows in the lab were darkened and artificial light was provided on all four sides. After the period of exposure to the wasps, the larvae were removed and placed individually in 30 ml plastic cup. Larvae were reared on artificial diet until parasitoids emerged, pupation occurred, or larvae died.

The Natural Enemy Complex of *L. dispar* on Experimental Plots

Field collected gypsy moth eggs were subjected to 25 Gy or 50 Gy respectively in early May, a few days before hatching occurred. Three study plots were established in three young (10-15 years) oak stands in the forest of Parovske Haje located near Nitra in southeast Slovakia; *Quercus cerris* and *Q. petraea* were the main tree species. The distance of the study plots from each other was about 100 m. Gypsy moth population density at the experimental sites was estimated by counting the number of egg masses per tree in one plot located about 100 m from the study plots following the method of Turcek (1956). Because of the low density of natural gypsy moth populations on the experimental plots, we supplemented the populations so that an adequate number of larvae were available for subsequent sampling. Therefore 600-1500 g of field collected, non irradiated, *L. dispar* eggs were placed in the first site in the Spring and at the same time, an equal number of irradiated *L. dispar* eggs were released in the second plot (25 Gy) and in the third plot (50 Gy). To determine levels of parasitism, *L. dispar* larvae were collected from experimental plots at different stages of larval development. We collected approximately 100 each of first and second instars (L1+L2), third and fourth instars (L3+L4), and fifth and sixth instars (L5+L6) respectively. Early instars were collected from twigs of the lower crown, stems of trees, understory vegetation, and litter. We used burlap bands to collect late instar larvae.

Table 1.—Mean development (mean ± SE) of L1-L5 gypsy moth larvae exposed as eggs to cobalt irradiation and reared on artificial diet under laboratory condition.

Treatment	Instar of larvae					SUM
	L1[a]	L2[b]	L3[c]	L4[d]	L5[e]	
10 Gy eggs	10.70 ± 1.31de	5.39 ± 1.13ab	5.38 ± 1.00d	4.58 ± 0.77e	8.42 ± 2.10b	34.49
20 Gy eggs	10.68 ± 1.18de	4.56 ± 1.00d	5.56 ± 0.58cd	5.36 ± 0.81c	6.44 ± 2.08b	32.60
30 Gy eggs	10.89 ± 1.09cd	5.24 ± 1.51abc	6.18 ± 1.46ab	4.89 ± 0.89dd	8.36 ± 2.21b	35.58
40 Gy eggs	11.08 ± 0.98c	5.28 ± 1.55abc	5.85 ± 1.14bc	5.22 ± 0.71c	8.36 ± 2.17b	35.80
50 Gy eggs	10.55 ± 0.58e	5.32 ± 0.60abc	4.82 ± 0.65e	6.75 ± 1.31a	9.43 ± 3.12a	36.87
60 Gy eggs	11.50 ± 0.56b	5.52 ± 1.26ab	5.93 ± 1.01bc	5.85 ± 1.25b	8.87 ± 3.16a	37.68
80 Gy eggs	11.42 ± 0.57b	5.10 ± 0.41bc	6.54 ± 1.24a	6.16 ± 1.00b	9.55 ± 4.05a	38.77
110 Gy eggs	12.24 ± 0.91a	5.56 ± 0.57a	6.42 ± 0.70a	5.52 ± 0.81c	9.59 ± 4.26a	39.33
Control 2002	9.45 ± 0.84f	4.95 ± 1.06cd	5.69 ± 0.93cd	4.43 ± 0.74e	8.27 ± 2.48b	32.80

Means followed by different letters (within a columns) are significantly different at P ≤0.05 by Tukey HSD. [a] *F = 49.08; df = 8. 723; P ≤ 0.01;* [b] *F = 5.95; df = 8. 716; P ≤ 0.01;* [c] *F = 14.53; df = 8. 697; P ≤ 0.01;* [d] *F = 42.85; df = 8. 679; P ≤ 0.01;* [e] *F = 3.32; df = 8. 668; P = 0.010.*

Field collected larvae were reared in groups of 20 or 30 larvae in glass cages on oak foliage under room conditions (23 -25°C). Larvae were checked daily and dead larvae as well as larvae from which parasitoids had emerged were removed. Adult parasitoids that emerged were identified and Tachinid larvae that emerged were identified as pupae (Zubrik 1998). Giemsa stained smears were prepared from dead larvae from which no parasitoid had emerged and viewed under light microscopy to determine the presence of pathogens.

Data Analyses

Analyses of variance (ANOVA) was performed on larval duration and pupal weight to determine the effects of irradiation dose (unequal number of replicates, Tukey HSD test) using SAS. The same method was used in case of *G. liparidis* weight of cocoons and weight of male and female adults. We used analyses of variance (ANOVA, equal number of replicates, LSD test) for evaluation the impact of irradiation dose on the hatching ability of eggs (SAS). Significant differences were tested at $P<0.05$.

Results and Discussion

Development of Irradiated and Non-irradiated Larvae

The duration of development of gypsy moth larvae subjected to doses of radiation (10-110 Gy) is provided in Table 1. The duration of development of all larval instars subjected to irradiation differed significantly from the controls. The control group developed 20 % faster than larvae exposed to the 110 Gy dose. Similar data were obtained by Mastro and Schwalbe (1988), who found that the developmental time of F_1 larvae from irradiated eggs varied from ca 30 days (20 Gy) to 34 days (100 Gy).

Differences in the condition of larvae and pupae were also recognized. Table 2 shows that the mortality of larvae was highest when the higher irradiation doses were used. In the control group, the mortality reached 5 % as compared to 20 % in the 60 Gy treatment. Pupae that developed from irradiated eggs showed a high degree of morphological abnormalities (93.18% at 110 Gy) as compared to 3% in the control group. The thoracic segments of the pupae were undeveloped and the antennae were also abnormal. These abnormalities affected the emergence of adults, which was very

Table 2.—Mortality of larvae, condition, and weight of pupae from irradiated and control groups of *L. dispar* fed on artificial diet in laboratory conditions (N= total No. of larvae per treatment).

Treatment	N	Larval mortality		No. of pupae	Deformed pupae		Unhatched pupae		Weight of pupae (g)	
		No	%		No.	%	No	%	Male[a]	Female[b]
10 Gy eggs	100	4	4.0	96	8	8.33	18	18.75	0.5739 ± 0.045a	1.6622 ± 0.163a
20 Gy eggs	100	7	7.0	93	21	22.58	26	27.95	0.4708 ± 0.088b	1.4133 ± 0.218bc
30 Gy eggs	100	12	12.0	88	20	22.72	28	31.81	0.4738 ± 0.072b	1.3571 ± 0.147c
40 Gy eggs	100	16	16.0	84	22	26.19	38	45.23	0.4631 ± 0.031b	1.2400 ± 0.110d
50 Gy eggs	50	6	12	44	8	18,18	12	27.27	0.5726 ± 0.102a	1.5149 ± 0.164b
60 Gy eggs	100	20	20.0	80	29	36.25	53	66.25	0.4605 ± 0.054b	1.2190 ± 0.181d
80 Gy eggs	50	4	8	46	24	52.17	36	78.26	0.4407 ± 0.066b	1.0568 ± 0.277e
110 Gy eggs	50	6	12	44	41	93.18	39	88.63	0.3631 ± 0.038c	0.8984 ± 0.113f
Control 2002	100	5	5.0	95	3	3.15	7	7.36	0.5574 ± 0.030a	1.8625 ± 0.940b

Means followed by different letters (within a columns) are significantly different at P ≤ 0.05 by Tukey HSD. [a] *F = 45.83; df = 8. 419; P ≤ 0.01;* [b] *F = 43.09; df = 8. 232; P ≤ 0.01*

low in all treated groups. In the control group these symptoms were not recognized. Somatic abnormalities are relatively common in the production of F1 larvae (Reardon et al. 1987).

We also evaluated how irradiation dose influenced the hatching of eggs. In the control group, the number of unhatched eggs was 9.66% whereas the number of unhatched eggs varied from 28.89-71.93% at doses over 60 Gy. This is quite important if irradiated eggs or larvae are destined for use in field augmentation (Table 3).

Attraction of Sterile Gypsy Moth Larvae for Oviposition by the Endoparasitic Wasp *Glyptapanteles liparidis* L.

Larval mortality caused by *G. liparidis* was 17% (17 of 100) in the control group as compared to 6% (6 of 100) in the irradiated groups. There was recognized differences between the total number of larvae killed by pathogens in the irradiated group as compared to the control group (48% vs. 10%).

Table 3.—Impact of irradiation dose on the hatching ability of eggs (N= number of egg masses).

Treatments	N	Average no. of eggs X ±S.D.	No. of unhatched eggs X ±S.D.	% of unhatched eggs X ±S.D.
10 Gy eggs	10	363.9 ± 87.2	41.6 ± 9.9	11.43 ± 0.52a
20 Gy eggs	10	416.6 ± 118.0	51.8 ± 15.5	12.39 ± 0.42ab
30 Gy eggs	10	436. 3 ± 88.7	61.6 ± 12.1	14.14 ± 0.56bc
40 Gy eggs	10	396.6 ± 91.1	61.8 ± 15.1	15.53 ± 0.62c
50 Gy eggs	-	- - -		
60 Gy eggs	10	411.8 ± 134.5	116.0 ± 35.6	29.18 ± 2.58d
80 Gy eggs	10	520.8 ± 159.5	249.5 ± 71.1	48.47 ± 4.36e
110 Gy eggs	10	452.1 ± 105.3	325.2 ± 66.8	72.31 ± 11.41f
Control	10	412.8 ± 110.7	39.9 ± 13.2	9.58 ± 1.47a
Statistical differences	-	-	-	SD

Means followed by different letters (within a columns) are significantly different at P ≤ 0.05 by LSD. SD= significantly different at P ≤ 0.05 by LSD. *F = 839.94; df = 7. 72; P ≤ 0.01*

Table 4.—Mortality induced by parasitoids, pathogens and total mortality in each treatment of eggs irradiated at 50 Gy.

Replication	50 Gy						Control					
	Pathogen		Parasitoids		Together		Pathogen		Parasitoids		Together	
	No.	%	No	%	No	%	No.	%	No	%	No	%
1	12	48.0	2	8.0	14	56.0	2	8.0	4	16.0	6	24.0
2	10	40.0	3	12.0	13	52.0	3	12.0	6	24.0	9	36.0
3	9	36.0	0	0.0	9	36.0	0	0.0	4	16.0	4	16.0
4	17	68.0	1	4.0	18	72.0	5	20.0	3	12.0	8	32.0
Σ	48	48.0	6	6.0	54	54.0	10	10.0	17	17.0	27	27.0

Unfortunately, females of *G. liparidis* exhibited low activity in searching for host larvae and therefore the parasitism rate was lower than expected (Table 4). We also conclude that the high mortality caused by pathogens in the irradiated group probably was responsible for the low rate of parasitism in that group. Larvae killed by pathogens died before parasitoids were able to complete their development.

There were no statistically significant differences between irradiated and control groups in the development of *G. liparidis* larvae and duration of the cocoon and adult stages (Fig. 1).

We observed some differences in the characteristics of cocoons and adults of *G. liparidis* that developed from irradiated and non-irradiated larvae. The numbers of fresh cocoons and total cocoons produced from the non-irradiated (control) groups of larvae was higher than the numbers produced from irradiated larvae. The mortality of cocoons was quite similar in both treatments. Cocoons from non-irradiated larvae produced hatched larger adults, but these differences were not statistically significant (Table 5).

Host selection experiments can be influenced by other factors, (Hoch et al. 1999). Consequently we conducted additional tests to investigate the effect of microsporidian infection in the host on parasitoid performance.

Natural Enemy Complex of *L. dispar* in Field Experimental Plots

The population density of *L. dispar* in the study plot was low during the experiment, which corresponds to the early progradation period of the pest. Of 750 larvae collected at all sites in 2001,

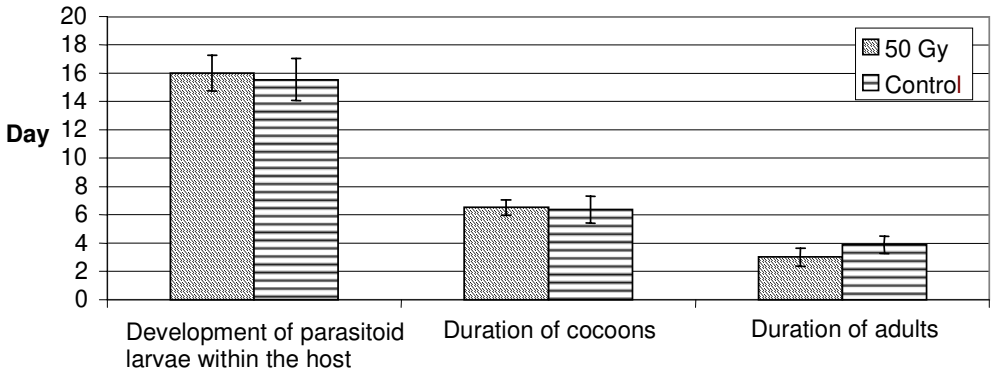

Figure 1.—Mean developmental time (means ± S.D.) of parasitoid larvae within the host, duration of cocoons, and duration of adult *G. liparidis*

Table 5.—*G. liparidis* - weight of cocoons, number and weight of male and female adults, and mortality of the cocoons for each treatment separately. Significant at P<0.05

	Weight of the fresh cocoons (g)	No. of cocoons		No of adults		Adult Weight(g)		Mortality during the cocoon stage (%)
		X	Total	M	F	M	F	
50 Gy	0.002600	29.17	175	130	14	0.000292	0.000371	17.71
Control	0.002940	33.81	541	388	63	0.000294	0.000376	16.63
Statistical differences	NS	NS	-	-	-	NS	NS	-

NS= not significantly different at P ≤ 0.05 by Tukey HSD.

Table 6.—Causes of mortality (pathogens, parasitoids and unknown) in irradiated and non-irradiated gypsy moth larvae.

Cause of mortality	Irradiated (25 Gy + 50 Gy)		Control		Irradiated and control together		Rank
	No. of larvae	%	No. of larvae	%	No. of larvae	%	
G. liparidis	5	1.0	1	0.4	6	0.8	4
C. melanoscela	10	2.0	5	1.6	15	2.0	3
G. porthetriae	6	1.2	2	0.8	8	1.0	5
Meteorus sp.			1	0.4	1	0.1	
Compsilura concinnata	16	3.2	16	6.1	32	4.2	2
Drino incospicua	1	0.2	1	0.4	2	0.2	
Blepharipa pratensis	1	0.2	1	0.4	2	0.2	
Carcelia gnava	1	0.2			1	0.1	
Zenilia libatrix			1	0.4	1	0.1	
Ichneumonidae	63	12.8	29	11.1	92	12.2	1
Total parasitoids	103	21.0	57	21.9	160	21.3	
Path + unknown	322	65.7	86	33.1	408	54.4	
Total larvae	490	100	260	100	750	100	

21.3% (160 of 750) were killed by parasitoids; the larval mortality caused by pathogens and unknown causes was 54.4% (408 of 750).

The irradiated larvae showed a very high sensitivity to the field conditions that resulted in very high mortality. Pathogens and unknown agents caused high mortality of the youngest larvae (L1+L2) and almost 100 % mortality in older larvae (L3-L5). Mortality caused by pathogens and unknown causes was much less among the control larvae (Table 6).

Parasitoids were very important as mortality agents in all experimental groups. We identified nine species of parasitoids belonging to the families Braconidae and Tachinidae. The parasitic fly *Compsilura concinnata* was the most prevalent parasitoid species. *C. concinnata* parasitizes mostly L3-L4 instars. We did not determine significant differences among species nor between the rate of parasitism between irradiated and non-irradiated larvae. The rate of parasitism was extremely similar in medium age larvae and varied from 21.7% to 26.7%. We recognized the highest diversity of parasitoids (nine species) in the control group of older larvae (L5+L6).

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References Cited

Bell, R. A., Owens, C. D., Shapiro, M., and Tardif, J. R. 1981. Development of mass rearing technology. In: Doane, C. C. and McManus, M. L. (Eds.): The Gypsy Moth: Research Toward Integrated Pest Management. U.S. Dep. Agr. Tech. Bull. 1584. 599-633.

Hoch G., Schopf, A., Maddox, J.V. 2000. Interaction between an entomopathogenic Microsporidium and the endoparasitoid *Glyptapanteles liparidis* within their host, the gyspy moth larva. J. of Invert. Pathol. 75: 59-68.

Knipling, E. F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. J. Econ. Entomol. 48: 459 – 462.

Mastro, V. C., Schwalbe, C. P. 1988. Modern insect control: nuclear techniques and biotechnology. In: Proceedings of an international symposium on modern insect control, IAEA, Vienna, p. 15-40

Reardon, R. C., Mastro, V. C. 1993. Development and status of the sterile insect technique for managing gypsy moth. USDA, NA-TP-13-93. 17 p.

Reardon, R. C., McManus, M., Kolodny-Hirsch, D., Tichenor, R., Raupp, M., Schwalbe, C., Webb, R., Meckley, P. 1987. Development and implementation of a gypsy moth integrated pest management program. J. Arbor. 13: 209-216.

Turcek, J. F. 1956. Mniska velkohlava. SVPL, Bratislava. 57pp.

Zúbrik, M. 1998. Contribution to the morphology of pupae of Tachinid (Diptera: Tachinidae) parasites of gypsy moth *Lymantria dispar* (Lepidoptera: Lymantriidae) in Slovakia [Ger.]. Forestry Journal. 44 (4): 275-285.