NOTE

Estimates of Gypsy Moth (Lepidoptera: Lymantriidae) Mortality at Two Sample Heights Following Application of Gypsy Moth Nuclear Polyhedrosis Virus[1, 2]

Stephen P. Cook, Ralph E. Webb and Geoffrey B. White

USDA-ARS, Insect Biocontrol Lab, Building 402, BARC-East
Beltsville, MD 20705 U.S.A.

J. Entomol. Sci. 30(4): 507-510 (October 1995)

KEY WORDS *Lymantria dispar,* population sampling, parasitism

Gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), nuclear polyhedrosis virus is a naturally-occurring microbial agent currently being developed as an alternative control tactic for use on moth infestations. The virus has been registered as Gypchek by USDA-Forest Service for gypsy moth control and is often applied aerially (Podgwaite et al., J. Econ. Entomol. 85: 1136-1139, 1992), during the time when populations are predominantly in the first and second instars (Lewis et al., USDA For. Serv. Res. Paper NE-441, 1979). One direct method to determine the efficacy of viral applications for moth control is to collect larvae from understory foliage as early as three days after treatment and rear these larvae on artificial diet to determine mortality rates (Webb et al., J. Econ. Entomol. 82: 1695-1701, 1989). A potential problem with this evaluation technique is that viral-infected larvae may demonstrate a negative geotropic response, climbing upward into the tree before death (Murray and Elkinton, J. Econ. Entomol. 85: 1865-1872, 1992). However, appreciable larval mortality may not occur for 10-14 days after viral application (Lewis and Yendol, USDA Tech. Bull. 1584: 503-512, 1981). This delay should permit accurate estimates of population infection by collection of larvae from the understory before the negative geotropic response. Once settled, young larvae (the predominant life stage collected for treatment evaluation) tend to remain on foliage and do not exhibit the diurnal movement pattern of third and fourth instars between foliage and resting places (Leonard, USDA Tech. Bull. 1584: 9-29, 1981). The objective of our study was to examine and describe the rate and cause of gypsy moth mortality for larvae collected at two heights (understory/lower canopy foliage and mid- to upper- canopy foliage) at 4-5 days after an aerial application of Gypchek.

[1] Accepted for publication 11 July 1995.

[2] Use of trade names does not imply endorsement or criticism of the products named.

Experimental work was conducted on four sites, two untreated control sites and two sites treated with a single application of gypsy moth nuclear polyhedrosis virus (Gypchek: USDA Forest Service, Hamden, CT) at 1×10^{12} polyhedral occlusion bodies per hectare delivered in an experimental carrier (Carrier 244: Novo Nordisk Bioindustries, Danbury, CT). The Gypchek was applied aerially by helicopter from approximately 16 m above the canopy, at a delivery rate of 9.5 L per ha under a boom pressure of approximately 40 PSI. One treatment site (HAM, approximately 18 ha), located in Charles Co., MD, approximately 25 km west of La Plata, received the viral application on 2 May 1994, and the second treatment site (GR5, 30 ha) was located in Green Ridge State Forest, Allegany Co., MD, and was treated on 13 May 1994. The viral formulation was applied when local gypsy moth populations were in the late first and early second instars. The two control sites (GR1 and GR2, 10 and 30 ha, respectively) were also located in Green Ridge State Forest, the nearest being over 10 km from the treated area. All four sites were mature oak forests with open understories. The three sites in Green Ridge State Forest had a large amount of witch-hazel, *Hamamelis virginiana* L. growing as an understory shrub.

Gypsy moth larvae were collected 4 (HAM) or 5 (GR5) days after the aerial applications (6 and 18 May, respectively). Larvae were collected from the control sites on 17 May. Larvae were collected from two heights at each site. Larvae were removed by hand from the understory/lower canopy (up to a height of 2.5 m); larvae from mid- to upper-canopy oak foliage were collected by removal of branch tips with foliage using a twelve-gauge shot gun and no. 4 steel shot. All larvae were placed in individual 30-ml plastic cups with cardboard lids, approximately 25% filled with semi-synthetic diet (Bell et al., USDA Tech. Bull. 1584: 599-633, 1981) and maintained for 4 wks in an outdoor insectary in Beltsville, MD. Larval mortality was recorded weekly and cadavers were necropsied using light microscopy (X400) to determine presence of viral occlusion bodies. Any parasitoid cocoons on cadavers were recorded and removed before the necropsy procedure. Overall larval survival and mortality factor by site and collection height were calculated. Paired-t tests (SAS Institute Inc., SAS/STAT User's Guide, Cary, NC, 1988) were conducted to examine between-height differences in overall percent larval mortality and percentage viral-caused mortality.

In contrast to the constant, low mortality rate of larvae collected at the two control sites, there was an initially rapid decline in larval survival at the two sites which had been treated with Gypchek (Fig. 1). Larvae collected from the understory had similar survival patterns through the 4 wk period to larvae collected from mid- to upper-canopy foliage. Neither total mortality ($t = 1.1594$; df = 3; $P = 0.3302$) or viral-caused mortality ($t = 0.5989$; df = 3; $P = 0.5914$) were significantly different between the two sample heights (Table 1).

Larval mortality attributed to virus tended to occur during the first 2 wks after collection which is consistent with the 10 to 14 day period typically reported between viral infection and larval death. The mortality attributed to parasitoids and other factors accumulated through the 4 wk period. The two parasitoid species recovered from the collected larvae were *Phobocampe disparis* (Viereck) (Hymenoptera: Ichneumonidae) and *Cotesia melanoscela* (Ratzeburg) (Hymenoptera: Braconidae), both of which are parasitic on small larvae. The *P. disparis* were recovered from larvae collected at three of the sites, but only from larvae collected from the understory

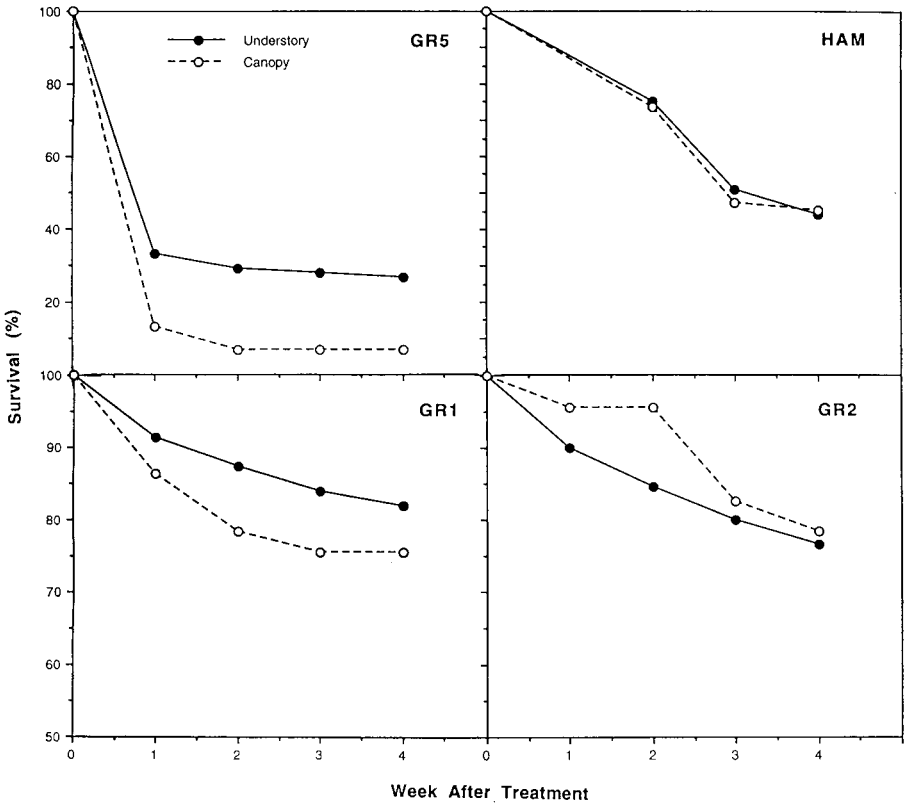

Fig. 1. Survival through the four-week observation period for larvae collected from understory vegetation or mid- to upper-canopy foliage in viral-treated (HAM and GR5) and untreated (GR1 and GR2) sites.

(Table 1). Similarly, *C. melanoscela* were recovered from larvae collected at three of the sites, but were only reared from upper-canopy collected larvae from one location (Table 1). Although differences in parasitism rates of gypsy moth larvae collected from under burlap bands and on foliage have been reported (Wilmot et al., J. Appl. Entomol. 116: 62-71, 1993), our small sample sizes did not allow such testing. Further, more extensive sampling would be required to examine a possible interaction between the virus and parasitoids as described by White and Webb (Proc. Entomol. Soc. Wash. 96: 27-30, 1994) or Reardon and Podgwaite (Entomophaga 21: 333-341, 1976).

At the time of sampling, larval infection with gypsy moth nuclear polyhedrosis virus was similar at the two collection heights (Table 1). When a resampling of older larvae was attempted, disturbed third and fourth instars often dropped from the branch tips and foliage upon disturbance and before collection. Therefore, the use of shotguns to remove branch tips and sample larvae may have dislodged

Table 1. Mortality at the end of the four-week observation period attributed to gypsy moth nuclear polyhedrosis virus (NPV), fungal infection (Fung), parasitiszation by *P. disparis* (Pd) or *C. melanoscela* (Cm) or other (Oth), undiagnosed, factors for larvae collected from Gypchek-treated (NPV) or untreated, control (CON), sites and from understory (UND) or mid- to upper-canopy (CAN) foliage.

				Larval mortality (%)					
Site	Treatment	Ht	n	NPV	Fung	Pd	CM	Oth	Total
HAM	NPV	UND	150	48.0	0.7	0.0	1.4	5.9	56.0
		CAN	53	45.3	1.9	0.0	0.0	7.5	54.7
GR5	NPV	UND	75	4.0	0.0	2.7	0.0	66.7	73.3
		CAN	15	13.3	0.0	0.0	0.0	80.0	93.3
GR1	CON	UND	150	3.4	0.0	3.3	2.7	8.6	18.0
		CAN	37	0.0	0.0	0.0	0.0	24.3	24.3
GR2	CON	UND	150	4.7	0.0	4.0	8.0	6.6	23.3
		CAN	23	8.7	0.0	13.0	0.0	0.0	21.7

early-instar larvae from the foliage and a less destructive method may be more appropriate.

Our results indicate that overall larval mortality was similar at the two collection heights, but that some mortality factors may differ between sample heights. For evaluating the efficacy of viral treatments, the 4 to 5 day larval sampling from the understory allows an accurate representation of larval survival and viral infection in the population as a whole. There may be interactions between viral infection and parasitoid presence and rates of parasitism that occur at different locations in the canopy. An overall evaluation of gyspy moth mortality factors which occur on a given site would require larval collections from several height strata and at several time intervals. A less destructive sampling method for sampling upper-canopy larvae is recommended.

We thank R. A. Bell (USDA-ARS, Beltsville, MD), F. L. Hastings (N. C. State University, Raleigh, NC), J. D. Podgwaite and T. M. Odell (USDA-Forest Service, Hamden, CT) for comments on an earlier draft of this manuscript; J. Podgwaite furnished the Gypchek used; D. Cohen and S. Hughes (Maryland Dept. of Agriculture, Annapolis, MD) located suitable gypsy moth populations and coordinated the aerial applications; T. Sukontarak assisted in field collections; the Hammel family and Green Ridge State Forest allowed us to conduct the research on these sites.