Techniques for Estimating the Density of Late-Instar Gypsy Moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), Populations Using Frass Drop and Frass Production Measurements

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ABSTRACT A technique was developed for estimating larval gypsy moth, *Lymantria dispar* (L.), densities using simultaneous measurements of the amount of frass produced per larva (frass yield) and the amount of frass falling in the forest per unit area (frass drop). The technique was tested in a postseason experiment in which 6,000 larvae were released in a stand. Frass yield was measured by individually caging several larvae in the field on cut host foliage. The most reliable and efficient method of measuring frass drop was the deployment of several large funnel-shaped frass traps near the forest floor. Number of pellets was found to be superior to frass weight as a unit for quantifying frass yield and drop, because it was not strongly influenced by instar distribution. Density estimates obtained using this method closely matched those expected from the number of larvae released. Frass width measurements provided a method of estimating instar distributions.

KEY WORDS Insecta, frass trap, frass size, artificial augmentation

AN ECONOMICAL yet accurate method for estimating larval gypsy moth, *Lymantria dispar* (L.), densities would be invaluable to studies of gypsy moth population dynamics. Sampling gypsy moth larval populations is difficult because larvae are mobile and often aggregate in unpredictable ways. Previous sampling methods (for example, timed walks [Connola et al. 1966], counts per twig terminal [Doane & Schaefer 1971], and counts of larvae under burlap bands [McManus et al. 1980]) were limited by their precision in estimating larval densities. Other methods, such as insecticidal spraying (W. E. Wallner,[1] personal communication; J. Gould,[2] personal communication), mark-recapture (Weseloh 1985), and whole-tree sampling (J. Gould,[2] personal communication), appear to provide more accurate density estimates, but are labor-intensive.

Frass drop measurements have been used to estimate larval densities of several forest defoliators (Morris 1949, Green & DeFreitas 1955, Tinbergen 1960, Zhang et al. 1986). Densities were estimated by relating the rate of frass fall over a given area to the rate of frass production per individual insect. Furthermore, size distribution of frass can be used to estimate instar distribution (Bean 1959). In this study, we compared several methods of measuring frass drop for estimating larval gypsy moth population densities and instar distributions. Liebhold & Elkinton (1988) describe sources of variation and statistical properties of frass drop and frass yield measurements.

Materials and Methods

On 13 August 1984 (after eclosion of the endemic gypsy moth population), 6,000 third- to sixth-instar (mostly fifth and sixth instar), laboratory-reared gypsy moth larvae were released in a stand of predominately *Quercus velutina* Lam. on Otis Airbase, Cape Cod, Mass. Larvae were released by stapling one or two paper cups, each containing 50 larvae, to every tree in a 20 by 20 m area. Twenty-four hours after release, frass traps were deployed in a 5 by 5 grid (3 m between grid points). Five frass trap designs were tested. The "funnel" trap consisted of a polyethylene funnel (8.0 cm radius) inserted into a section of tygon tubing (1.0 cm diameter by 5 cm long) with mosquito netting glued over the bottom. The "tarp" trap consisted of a canvas sheet (82 by 82 cm) stretched across a wooden frame. The "cheesecloth" trap was a cheesecloth sheet (63 by 63 cm) stretched across a wooden frame. The "disk" frass trap consisted of a molded plastic disk (8.0 cm radius) with an acrylic sticker on top. The "disk + cylinder" trap was a disk trap modified by placing a sheet metal cylinder (10 cm high) around it to prevent frass particles from bouncing sideways off the trap. The funnel, disk,

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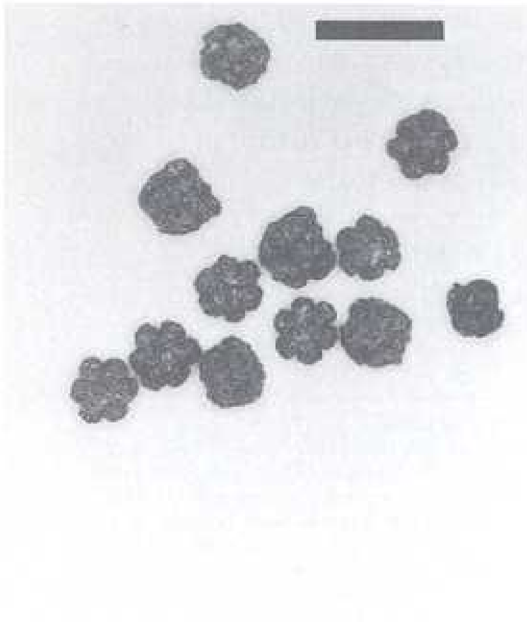

Fig. 1. Several frass pellets produced by a sixth-instar gypsy moth (bar = 5 mm).

and disk + cylinder traps were attached to the top of a 1-m wooden stake, and the tarp and cheesecloth traps were placed on the forest floor after removing low-growing shrubs. Traps were deployed for 5 d using a Latin square design. Trap positions were rotated daily, and all gypsy moth frass pellets in the traps were collected and counted (sticky disks were replaced daily for the disk and disk + cylinder traps). During the period of trap deployment, 25 lab-reared larvae were held on cut foliage in individual cages located in the field. Once each day, frass pellets produced by each larva were collected and counted and the foliage replaced. Gypsy moth frass was separated from the other debris in trap samples and oven dried prior to weighing to the nearest 0.1 mg. Additionally, the width of all pellets collected from caged larvae was measured (after drying) to the nearest 0.01 mm using an ocular micrometer.

Density estimates for each day were calculated for each of the five trap types using the following equation:

$$\text{Larvae/ha} = C \cdot \text{frass/trap} \cdot \text{larvae/frass}, \quad (1)$$

Table 1. Analysis of variance of the number of pellets per trap per day

Source of variation	Degrees of freedom	Mean square	F	Probability of a greater F
Residual	108	13,536		
Day	4	66,197	4.89	0.001
Row in grid	4	11,714	0.87	0.487
Column in grid	4	9,252	0.68	0.605
Trap type	4	112,004	8.27	0.00001

where C = 1/area of one trap (measured in ha). C was determined by measuring the horizontal area of the trap. The frass/trap (frass drop) term was the mean amount of frass per trap. Amount of frass was expressed both as weight and as numbers of pellets. The larvae/frass term was the inverse of the mean amount of frass produced per caged larva for a given day (frass yield). Again, amount of frass was expressed both as weight and as numbers of pellets.

Results and Discussion

Frass produced by late-instar gypsy moth larvae is easily distinguished from that of other forest insects. The pellets are characteristically star shaped in cross-section (Fig. 1). Few other forest defoliators produce similarly shaped frass (Prota 1976).

Analysis of variance indicated that both trap type and day significantly affected the numbers of frass pellets per square meter (Table 1). The significance of the day effect reflects a trend of decreasing frass drop over the 5-d study period. The significance of the trap type effect indicates that not all trap types caught the same number of pellets per unit area. Separation of means revealed that the disk trap caught significantly fewer pellets per unit area than the other trap types (Table 2). Among these other trap types there were no significant differences in collection efficiency. We observed that frass pellets often bounced off the disk traps rather than adhering to the sticky surface. This explanation of the observed reduction in pellet count is confirmed by the finding that the disk + cylinder trap caught significantly more pellets. Apparently, the cylinder deflected many of the bouncing pellets back onto the sticky surface. Based upon trapping efficiency and ease of use, we recommend a funnel trap for large-scale use.

The number of pellets produced per larva each day (Table 3) was not significantly different between fifth- and sixth-instar larvae. However, the number of pellets per larva varied greatly among days. In contrast to the number of pellets, the mean weight of frass produced per larva per day varied greatly among instars (Table 3). Sixth-instar larvae produced heavier frass pellets.

Density estimates based on measurements of frass pellet drop and daily frass pellet production are

Table 2. Mean numbers of pellets per square meter of trap area trapped per day in each of the five trap types

Trap type	Trap size (m^2)	Pellets/m^2 per day[a]
Funnel	0.020	185.1 ± 35.1b
Tarp	0.672	165.0 ± 13.1b
Cheesecloth	0.379	151.0 ± 30.2b
Disk	0.020	15.9 ± 6.2a
Disk + cylinder	0.020	145.3 ± 32.8b

[a] Mean ± SE. Means followed by a different letter are significantly different (Tukey's honestly significant difference method, $\alpha = 0.05$).

Table 3. Mean numbers and dry weights (±SEM) of frass pellets produced per day per larva caged in the field

Day	Pellets per larva			mg per larva		
	Fifth instar	Sixth instar	All larvae	Fifth instar	Sixth instar	All larvae
1	15.0 ± 2.7	15.1 ± 2.1	15.6 ± 1.8	29.8 ± 5.2	49.8 ± 6.9	32.6 ± 4.4
2	23.7 ± 3.0	19.4 ± 3.3	21.6 ± 2.3	43.8 ± 5.7	72.4 ± 17.2	50.3 ± 8.1
3	13.5 ± 1.8	11.4 ± 1.3	12.1 ± 1.3	25.2 ± 4.6	45.2 ± 6.8	30.8 ± 4.4
4	13.0 ± 1.8	14.0 ± 2.2	12.2 ± 1.5	26.0 ± 4.3	59.2 ± 9.7	35.3 ± 5.9

given in Table 4. Over the 4-d period there was a steady decrease in larval density estimated from funnel trap counts. Larval densities probably did decrease due to mortality and emigration from the area of release. Because there was no naturally occurring population in the surrounding area, no compensatory immigration occurred. Density estimates based on pellet drop measurements using the funnel trap were within the range of actual densities to be expected after a release of 6,000 larvae (that is, densities steadily decreasing below 6,000). Estimates based on the disk trap were consistently low, reflecting the decreased efficiency of this device.

Density estimates based on weight of frass in traps and weight of frass produced per insect were more variable than estimates based on pellets (Table 5). Density estimates were erratic within any trap type and within any day. We feel that density estimates based on numbers of pellets are more reliable than those based on frass weight because of these results, and because estimates based on numbers of pellets per insect are less likely to be affected by differences in instar distribution. Because larvae of different instars produce vastly different weights of frass (Table 3), any deviation of the instar distribution of the cohort being monitored for frass production from the instar distribution of the wild population would greatly affect the density estimate. Large larvae would contribute a larger proportion to the insect/frass estimate used in Equation 1 than would smaller larvae. In contrast, estimates derived from pellet counts are less likely to be influenced by differences between instars (Table 3). Higashiura (1987), in a similar but considerably smaller study, did not detect a statistically significant difference between larval density estimates calculated from frass pellet counts and from estimates calculated from frass weight.

Campbell (1967) used frass drop measurement to estimate larval densities in a study of the population dynamics of dense gypsy moth populations. He monitored frass fall over 24 h in 50 traps and simultaneously monitored frass production per larva by caging 200 larvae together on foliage and collecting their frass. His calculation of larval densities was based on the volume of frass produced. Frass volume is presumably a function of frass width, which varied markedly among instars in our study (Fig. 2). We suspect that volume of frass would behave in a similar fashion to frass weight, in that estimates are likely to be strongly affected by deviations of the instar distribution of the caged cohort from the instar distribution of the wild population. However, if a large larval sample, such as 200, is selected without bias, then this effect would be negligible.

Connola et al. (1966) found that both weight and numbers of pellets of frass dropping were only moderately correlated with egg mass densities the following year. One explanation for the lack of a better correlation is that pupal mortality can be substantial and may have been greater in some plots than in others. Another reason for their poor correlation is that they used only one trap per plot. The deployment of many smaller traps is likely to give a better estimate of frass drop for a given area. Although Connola et al. (1966) did not estimate densities using frass drop measurements, it is likely that their correlation with egg mass densities would have been improved by monitoring frass yield at each site (Liebhold & Elkinton 1987).

Measurement of size of frass trapped in the field may be of use in estimating the instar distribution of resident larvae. Analysis of covariance (using day as a covariate) indicated that instar was the effect explaining most of the variation in frass width (Table 6). Day of measurement was a significant source of variation; means indicated that frass width, for a given larva, increased with age within an instar. The significance of the effect of larva nested within instar indicates that there was a significant larva-to-larva source of variation. Distribution of frass widths (Fig. 2) indicated that although there was overlap, frass size can be used as a tool for differentiating larval instars. Based on these measurements, we feel that the following ranges of

Table 4. Density estimates (larvae/400 m^2) based on numbers of pellets following a release of 6,000 larvae

Trap type	Day 1	Day 2	Day 3	Day 4
Funnel	5,740	4,975	4,605	2,284
Tarp	5,380	3,852	3,148	3,356
Cheesecloth	5,052	2,930	2,798	3,931
Disk	765	369	0	0
Disk + cylinder	3,508	3,317	2,302	2,851

Table 5. Density estimates (larvae/400 m^2) based on frass weight

Trap type	Day 1	Day 2	Day 3	Day 4
Funnel	3,639	4,004	9,030	930
Tarp	5,134	2,940	2,521	1,693
Cheesecloth	5,129	2,496	1,894	2,218

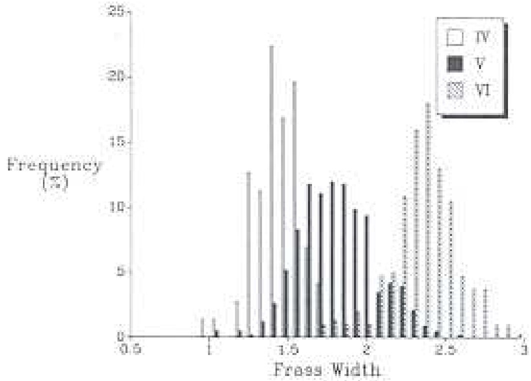

Fig. 2. Frequency distributions of frass pellet width for fourth- to sixth-instar gypsy moth individually reared on oak foliage.

widths should be used to segregate instars: fourth, 1.0–1.5 mm; fifth, 1.5–2.1 mm; sixth, 2.1–3.0 mm. However, these measurements may vary with host quality, population density, or population quality, all of which affect the size of individuals (Lance et al. 1986). Prota (1976) found slightly different frass widths for gypsy moth larvae collected in Italy.

We feel that using frass drop and yield measurements to estimate larval gypsy moth densities and age structures holds great promise as a tool for studying the population dynamics of this species. This method, along with methods to estimate densities of egg mass and pupal densities (Campbell 1967, Wilson & Fontaine 1978), could be used to generate survivorship curves for most life stages within a generation. Such survivorship information is valuable to the study of the regulatory nature of various mortality sources. This method can also be applied to field studies of insecticide efficacy by measuring pre- and postspray densities.

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Table 6. Analysis of covariance of pellet size with day

Source of variation	Degrees of freedom	Mean square	F	Probability of a greater F
Within cells (error)	761	21		
Regression (day)	1	1,095	51.7	0.0001
Instar	2	27,469	1,297.3	0.0001
Larva within instar	25	489	23.1	0.0001

References Cited

Bean, J. L. 1959. Frass size as an indicator of spruce budworm larval instars. Ann. Ent. Soc. Am. 52: 605–608.

Campbell, R. W. 1967. The analysis of numerical change in gypsy moth populations. Forest Science Mon. 15.

Connola, D. P., F. B. Lewis & J. L. McDonough. 1966. Experimental field techniques used to evaluate gypsy moth, *Porthetria dispar*, control in New York. J. Econ. Entomol. 59: 284–287.

Doane, C. C. & P. W. Schaefer. 1971. Aerial application of insecticides for control of the gypsy moth with studies of effects on non-target insects and birds. Conn. Agric. Exp. Stn. Bull. 724.

Friden, F. 1958. Frass-drop frequency in Lepidoptera. Almqvist & Wiksells, Uppsala, Sweden.

Green, G. W. & A. S. DeFreitas. 1955. Frass drop studies of larvae of *Neodiprion americanus banksianae*, Roh. and *Neodiprion lecontii*, Fitch. Can. Entomol. 87: 427–440.

Higashiura, Y. 1987. Larval densities and a life-table for the gypsy moth, *Lymantria dispar*, estimated using the head-capsule collection method. Ecol. Entomol. 12: 25–30.

Lance, D. R., J. S. Elkinton & C. P. Schwalbe. 1986. Components of density-related stress as potential determinants of population quality in the gypsy moth (Lepidoptera: Lymantriidae). Environ. Entomol. 15: 914–918.

Liebhold, A. M. & J. S. Elkinton. 1988. Estimating the density of larval gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), using frass drop and frass production measurements: sources of variation and sample size. Environ. Entomol. 17: 385–390.

McManus, M. L., D. R. Houston & W. E. Wallner. 1980. The homeowner and the gypsy moths: guidelines for control. U.S. Department of Agriculture Home Gard. Bull. No. 227.

Morris, R. F. 1949. Frass-drop measurement in studies of the European spruce sawfly. University of Michigan School of Forestry and Conservation Bulletin 12.

Prota, R. 1976. Contributi alla conoscenza dell'entomofauna della Quercia da sughero (*Quercus suber* L.). IX. Interralazioni tra quantia e forma dei coproliti ed eta larvale in *Porthetria dispar* (L.). Boll. della Soc. Sara Sci. Nat. 16: 33–38.

Tinbergen, L. 1960. The natural control of insects in pinewoods. I. Factors influencing the intensity of predation by song birds. Arch. Neerl. Zool. 13: 266–343.

Weseloh, R. M. 1985. Dispersal, survival, and population abundance of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae determined by releases and mark-recapture studies. Environ. Entomol. 14: 728–735.

Wilson, R. W., Jr., and G. A. Fontaine. 1978. Gypsy moth egg sampling with fixed- and variable-radius plots. U.S. Department of Agriculture Agriculture Handbook 523.

Zhang, X., Xiao G., Lu C. & T. Li. 1986. Studies on relationship between the frass-drop of the pine caterpillar as well as the related factors and population density. Scintia Silvae Sinicae 22: 252–259 (in Chinese).

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