Estimating the Density of Larval Gypsy Moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), Using Frass Drop and Frass Production Measurements: Sources of Variation and Sample Size

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ABSTRACT At three sites on Cape Cod, Mass., successive gypsy moth, *Lymantria dispar* (L.), larval densities were estimated using simultaneous measurements of the number of frass pellets produced per larva (frass yield) and the number of frass pellets falling in the forest per unit area (frass drop). Estimated larval densities declined through the period of larval development at all sites. Frass yield was positively correlated with ambient temperature. Frass drop was positively correlated with the basal area of host trees immediately around the frass trap. At a low-density population, rate of frass drop exhibited a diel periodicity similar to that of larval feeding. Mean-variance relationships of frass drop measurements and density estimates indicated that frass pellets and larvae were spatially aggregated within stands. The optimal ratio of drop to yield samples and the number of replicates of each sample necessary to obtain a given level of precision decreased with increasing density.

KEY WORDS Insecta, sample size, statistics, population dynamics

FRASS DROP MEASUREMENTS have been used to estimate larval densities of several forest defoliators (Morris 1949, Green & DeFreitas 1955, Tinbergen 1960). Liebhold & Elkinton (1988) described a technique for estimating larval gypsy moth densities using measurements of frass drop, the amount of frass falling from the canopy into a trap on the forest floor, and frass yield, the amount of frass produced per larva over the same time period. Densities were estimated as a constant (inverse of area of one frass trap) times the quotient of frass drop and frass yield. In this study, we determined what factors contribute to variation in frass yield and frass drop. Further, we used the observed levels of variation in frass drop and frass yield to estimate the numbers of samples necessary to obtain a density estimate at a given level of precision.

Materials and Methods

In order to quantify the daily rhythm of frass production by gypsy moth, we monitored periodic frass drop and yield over a 48-h period. Frass drop was monitored in 10 funnel frass traps (0.52 m diameter) located in a 10-m grid in a low-density gypsy moth population on Cape Cod, Mass. All pellets in each trap were counted at intervals varying between 3 and 9 h in length; counts were always made at dusk and dawn, so that frass falling during daylight hours could be separated from that falling during the night. During the same 48-h period, we measured the frass yield (as numbers of pellets) of 20 field-collected larvae individually caged on black oak foliage, 20 larvae collected at the beginning of the period and then starved, and 20 larvae collected just before sunset during the first 24-h period and then starved.

Simultaneous measurements of frass yield and frass drop during various stages of larval development were made at three 9-ha plots on Otis Airbase on Cape Cod. Densities of host trees and egg-masses are shown in Table 1. Frass yield was determined by collecting 40 larvae from the site and placing them individually in paper cups supplied with an oak branch terminal (ca. 4 leaves) overnight. The cut end of the terminal was sealed by dipping it in hot paraffin. Larvae were placed in cups located in a screened cage at the site in the afternoon and retrieved the following morning. Frass in the cups was returned to the laboratory and counted.

We attempted to determine the effect of ambient temperature on frass yield by simultaneously measuring temperature and frass yield. Hourly average temperature was measured using a CR-21 (Campbell Scientific, Logan, Utah) weather station located less then 3 km from each of the three sites.

At one site, we compared the frass yield of 20 larvae on each of three hosts: white oak, *Quercus alba* L., black oak, *Q. velutina* Lam., and pitch pine, *Pinus rigida* Mill. Larvae were caged in cups as described above. Each foliage sample was taken from a different tree.

Frass drop was measured by deploying 169 frass traps at each site in a 13 by 13 grid with 25 m between each trap. Traps consisted of a plastic

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Table 1. Host tree and gypsy moth egg-mass densities (±SEM) estimated from 169 subplots (10 m diameter) at three study sites on Otis Airbase, Cape Cod, Mass.

Plot	Basal area[a]				Egg-mass density[b]
	Black oak	White oak	Pitch pine	Other hardwoods	
1	2.41	0.42	1.14	0.04	149 ± 14
2	2.02	0.29	2.20	0.02	2,357 ± 134
3	0.31	2.18	2.23	0.02	826 ± 128

[a] m^2/ha.
[b] Egg-masses/ha.

funnel (0.52 m diameter) attached to a 1-m-high wooden stake. In the afternoon, 30-ml plastic cups were attached to the bottom of each funnel and were retrieved the following morning. Frass in the cups was returned to the laboratory and counted.

In order to determine the relationship between frass drop and host tree proximity, all trees within a 10-m-diameter circle about each frass trap were located and their distances from the trap and diameters at breast height (dbh) were measured. A Spearman correlation coefficient (Sokal & Rohlf 1981) between frass drop and basal area of surrounding oaks, derived from dbh measurements, was calculated on each of the trapping occasions. Separate coefficients were calculated using basal area within 10-, 7-, and 4-m-diameter circles around each trap.

Once early in the larval period (when second and third instars predominated) and once late in larval development (when fifth and sixth instars predominated), eight frass traps were placed 1.5 m apart within each of two 0.001-ha quadrats arbitrarily located in a 9-ha plot. The among-trap variation in frass drop within these quadrats was compared with the among-trap variation in drop among traps separated by 25 m throughout the same plot. The standard errors of the coefficients of variation for each set of frass drop measurements were calculated (Sokal & Rohlf 1981).

Densities were estimated (Liebhold & Elkinton 1988):

$$\text{Density} = C \times (\bar{x}_d/\bar{x}_y) \qquad (1)$$

in which $C = 1/(\text{frass trap area})$; $\bar{x}_d$ = mean drop (frass/trap); $\bar{x}_y$ = mean yield (frass/larva).

Because the density estimated using Equation 1 is the ratio between two random variables (frass drop and frass yield), calculation of the variance of the estimate is more complex than for a simple random variable (Cochran 1977). Confidence intervals for the density estimates (Equation 1) were calculated using Fieller's inequality (Fieller 1954, Buonaccorsi & Liebhold in press), modified by the removal of the covariance term:

$$\text{CI} = \frac{C(a_2 \pm \sqrt{b})}{a_1} \qquad (2)$$

Table 2. Costs associated with estimating gypsy moth density using frass drop and frass yield measurements[a]

	Cost ($/replicate)
Frass drop measurements	
Collector materials	1.50
Collector assembly and disassembly—5 min	0.42
Collector deployment—4 min	0.33
Preparing collectors—3 visitations, 2 min per visit	0.50
Counting pellets—3 visitations, 5 min per visit	1.25
Total	4.00
Frass yield measurements	
Materials	0.03
Preparing larva, foliage, and cage—3 times, 30 sec per visit	0.12
Counting pellets—3 times, 3 min per visit	0.75
Total	0.90

[a] Based on making three estimates in one year.

in which: $a_1 = 1 - z^2 s^2_y/n_y\bar{x}^2_y$; $a_2 = \bar{x}_d/\bar{x}_y$; $a_3 = a_2^2 - z^2 s^2_d/n_d\bar{x}^2_y$; $b = a_2^2 - a_1 a_3$; $z = 1 - \alpha/2$ percentile of N (0, 1) (e.g., $z = 1.96$ for $\alpha = 0.05$); s^2_d = drop variance; s^2_y = yield variance; n_d = number of drop samples; n_y = number of yield samples.

The variances of density estimates can be approximated using standard Taylor series methods (Mood et al. 1974):

$$s^2_{\bar{x}} = (1/\bar{x}_y^2)[(s^2_d/n_d) + (\bar{x}_d/\bar{x}_y)^2(s^2_y/n_y)] \qquad (3)$$

The unit costs per replicate for drop, C_d, and yield, C_y, measurements were calculated (Table 2). Given these costs, the proportion of drop samples, $P = n_d/(n_d + n_y)$, that minimizes variance given fixed costs or minimizes cost given a fixed variance, V_0, can be estimated (Buonaccorsi & Liebhold in press):

$$P = 1/(1 + \sqrt{(\bar{x}_d^2 s^2_y C_d)/(\bar{x}_y^2 s^2_d C_y)}) \qquad (4)$$

Using this proportion, the total number of samples ($n_T = n_d + n_y$) required to obtain a density estimate that is within d units from the true density at a given probability (we assumed 95%) was calculated (Buonaccorsi & Liebhold in press):

$$n_T = (s^2_d/P + (\bar{x}_d/\bar{x}_y)^2(s^2_y/(1 - P)))/(\bar{x}_y^2 V_0) \qquad (5)$$

in which $V_0 = d^2/z^2$.

Results and Discussion

At endemic population densities, late-instar gypsy moths feed in the canopy at night. At daybreak they move down stems to seek cryptic resting sites such as bark crevices and bark flaps where they spend their daylight hours (Forbush & Fernald 1896). Many larvae seek resting sites in the litter. As would be predicted, we observed a distinct rhythm in frass drop at a low-density site during

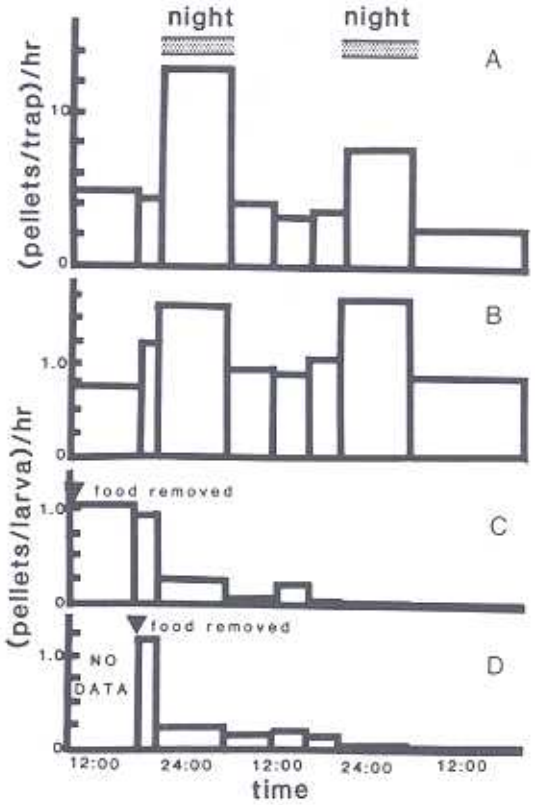

Fig. 1. Frass drop and frass yield measurements made over a 48-h period during the late-instar period at a stand harboring a low density gypsy moth population on Cape Cod. A. Frass drop: mean numbers of pellets per trap falling into 10 frass traps per hour. B. Frass yield: mean numbers of pellets produced per hour per larva individually caged on host foliage. C. Frass yield: mean numbers of pellets produced per hour per larva starved at 0930 hours EST. D. Frass yield: mean numbers of pellets produced per hour per larva starved at 2030 hours EST.

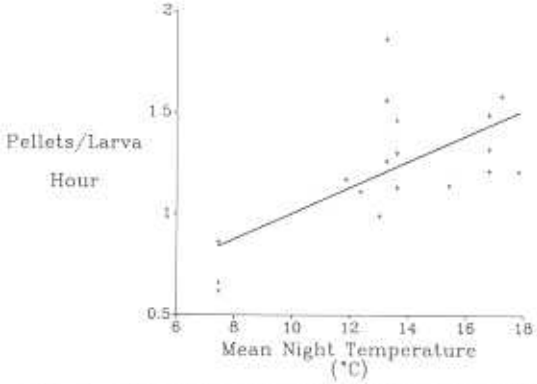

Fig. 2. Relationship between evening temperature (mean temperature from 1900 hours until 0900 hours EST) and mean hourly frass yield at three sites on Cape Cod, Mass. Each point represents the mean for one night.

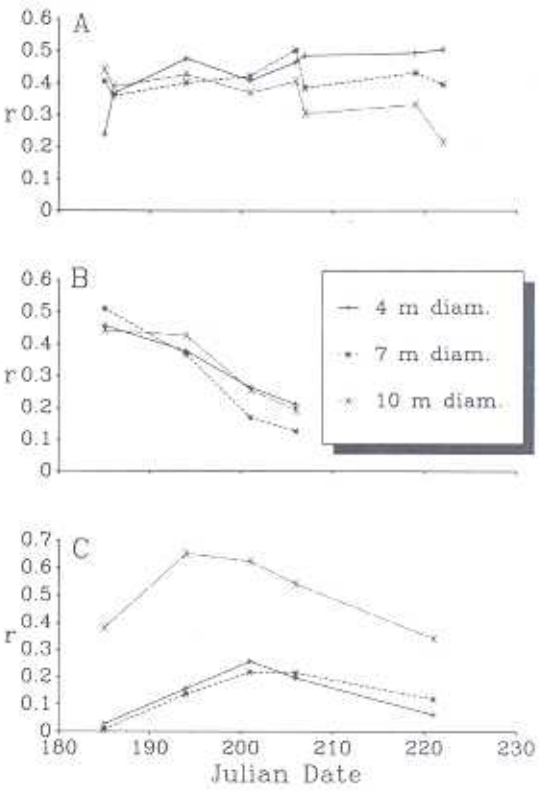

Fig. 3. Relationship of date of frass drop measurement and Spearman correlation coefficient between frass drop and oak basal area within a 10-, 7-, and 4-m circle about each frass trap. A. Plot 3 (low density). B. Plot 2 (high density). C. Plot 1 (low density).

the late-instar period. The rate of frass drop was higher during scotophase, although a significant amount of frass fell during the day (Fig. 1A). Similarly, we observed that frass yield of caged larvae was greater during scotophase, though again some pellets were released during the day (Fig. 1B). Even when food was removed from larvae, both in the morning and the afternoon, caged larvae continued to yield substantial numbers of pellets (Fig. 1 C and D). These results indicate that even when larvae are away from their food supply, such as when they seek daytime resting sites, they continue to yield frass. This finding has implications for the use of frass drop measurements to estimate larval densities; if drop measurements are taken in an above-ground trap, such as the ones used here, those pellets released during the day by larvae resting in the litter will not be measured. This would cause a consistent underestimation of frass drop and, thus, larval density. Although we do not know the magnitude of this potential bias, we recommend that frass drop and yield measurements be taken mainly during night hours to minimize this effect.

The amount of frass produced per individual per unit time of several defoliating insects has been

Table 3. Frass yield (±SEM) of fifth- and sixth-instar gypsy moth on three host species

Date	Host species	Pellets/larva[a]
2 July	White oak	19.1 ± 2.4a
	Black oak	19.2 ± 1.7a
	Pitch pine	10.9 ± 3.2b
3 July	White oak	24.5 ± 2.63a
	Black oak	17.1 ± 2.40a

[a] Treatments within each date followed by different letters were significantly different using a Mann-Whitney *U* test ($\alpha = 0.018$). Experiment $\alpha \leq 0.05$ using Sidak's inequality (Sokal & Rohlf 1981).

shown to be affected by ambient temperature (Green & DeFreitas 1955, Volney et al. 1983). Similarly, we found that ambient temperature was significantly correlated with daily frass yield of gypsy moth larvae caged in the field, although there was a great deal of unexplained variation (Fig. 2) ($R^2 = 0.437$; partial $t = 3.33$; probability of a greater $t = 0.0028$).

Another potential source of variation in frass yield is foliage quality (Barbosa & Capinera 1977). We found a significant difference in frass yield of larvae fed oak versus pitch pine, but no significant difference between larvae fed black versus white oak (Table 3). Thus, we suspect that foliage quality is not the most important factor affecting variation in frass yield, because pitch pine is not a primary host. Other factors that may also contribute to variation in frass yield are disease, parasitism (Friden 1958), and population quality (Leonard 1966). The result of this variation in frass yield is that measurement of frass drop alone provides an unreliable relative measure of population density. Also, the complexity of the above factors affecting the insects/frass ratio makes it unlikely that the ratio could be predicted at any one site without directly measuring frass yield. The larvae and foliage used in such measurements should be a random sample. Furthermore, it is advisable that frass production be monitored in the stand where a density estimate is sought to avoid any bias brought about by differences in temperature.

At the high-density site (plot 2), there was a consistent decrease over time in the correlation between frass drop and the oak basal area around each trap (Fig. 3B). Early in the season, there was a relatively high correlation between drop and oak basal area, but as development progressed, this correlation decreased. We suspect that two related factors may have contributed to this phenomenon. Larvae became less host specific as they developed (Barbosa 1978); thus, in early instars, larvae may have fed on oak exclusively, but in later instars they fed on less preferred hosts such as pitch pine. Some portions of the stand were defoliated, so that larvae may have moved out of these areas (even though the host basal area was high) to adjoining areas where foliage remained.

Table 4. Coefficients of variation for simultaneous estimates of frass drop over a 9-ha plot ($n = 169$) and over 0.001-ha subplots within the plot ($n = 8$)

Date	Plot		Subplot	
	Mean (pell./trap)	CV (±SE)	Mean (pell./trap)	CV (±SE)
3 April	317.2	64.4 ± 4.7	598.7	16.5 ± 4.3
			671.6	13.7 ± 3.5
2 July	40.8	88.2 ± 6.47	46.8	22.4 ± 6.7
			42.7	37.7 ± 11.3

Table 5. Frass drop, frass yield, density estimates, and confidence intervals from 16 sets of measurements made at three sites on Cape Cod

Plot	Date	Mean frass yield (pell./trap)	Mean frass drop (pell./larva)	Density estimate (larvae/m²)	Bounds of confidence interval	
					Lower	Upper
1	31 May	22.6	317.2	66.7	54.0	85.0
1	8 June	21.0	184.7	42.0	31.4	60.7
1	15 June	28.9	69.0	11.4	9.6	13.3
1	20 June	25.1	73.4	13.9	11.1	17.9
1	21 June	17.9	43.6	11.6	9.1	15.1
1	2 July	15.7	40.8	12.4	9.9	15.6
1	5 July	21.1	49.6	11.2	9.1	14.0
2	30 May	13.8	335.2	115.4	88.0	163.3
2	8 June	20.1	287.2	68.2	52.7	93.5
2	15 June	30.0	289.6	46.0	40.0	53.2
2	20 June	21.4	239.5	53.1	40.9	73.9
3	30 May	14.7	30.6	9.9	6.2	14.2
3	8 June	31.5	103.3	15.7	9.1	23.9
3	15 June	30.5	39.2	6.1	4.0	8.7
3	20 June	27.9	26.3	4.5	2.8	6.6
3	4 July	24.2	21.4	4.2	2.6	6.1

At one low-density site, the correlation between drop and oak basal area remained nearly constant throughout larval development (Fig. 3A). At the other low-density site the correlation was more variable through time (Fig. 3C). At this site, there was generally a lower correlation between drop and basal area within 4 and 7 m of the trap than between drop and basal area within 10 m. This suggests that there is a significant contribution of frass into the traps by larvae feeding in trees whose stems are as far away as 5 m from the trap. Because oak crowns in these and other stands on Cape Cod rarely reach 5 m in radius, these data indicate that there is at least a small amount of horizontal drift between the time a frass pellet is expelled by a larva and the time it hits the ground.

Coefficients of variation (CVs) of frass drop among frass traps located in the 9-ha plot were greater than CVs among traps located within 0.001-ha quadrats at the same site (Table 4). Much of the variation in frass drop is because of spatial heterogeneity, specifically heterogeneity in host tree density. It is likely that there is more variation in host density among points located 25 m apart than among those located 1.5 m apart.

Table 6. Parameters of Taylor's power law regression of frass drop and larval density estimates[a]

Measurement	a	b	R^2
Frass drop	13.9 ± 2.0	1.39 ± 0.14	0.859
Density estimate	0.004 ± 1.61	1.22 ± 0.15	0.818

Mean density was calculated using Equation 1 and density variance was estimated using Equation 5. Standard errors of coefficients follow parameters.

[a] Southwood 1978; $\log s^2 = \log a + \log x$.

Changes in larval density estimates between successive samples at the three sites were within the range expected from a survivorship measurement: densities decreased at all three sites (Table 5). Frass pellets produced by early instars are less distinct from other defoliators than those produced by late instars (Prota 1976; Liebhold & Elkinton 1988). For this reason, frass drop density estimates may be less reliable during the early instar period.

The slopes (b parameter) of Taylor's power law regression, $\log s^2 = \log a + b \log \bar{x}$, were significantly greater than 1 for drop and density estimates (Table 6). These results indicate that the populations of both frass and larvae were aggregated (Southwood 1978). Deviations from random distributions may be explained partially by spatial heterogeneity of the habitat (i.e., spatial variation in host density).

Calculation of P (the ratio of drop samples to total samples) from the successive measurements taken at each site (Table 5) revealed that this ratio is related to estimates of larval density (Fig. 4). From this data, we generalize that at low densities (<15 larvae/m²) it is advisable that the number of drop measurements should exceed the number of yield measurements (i.e., $P > 0.5$) and at high densities (>15 larvae/m²), the number of yield measurements should exceed the number of drop measurements (i.e., $P < 0.5$) (Fig. 4). This relationship most likely results because the number of yield measurements necessary to obtain a given level of precision of yield is probably largely independent of density, but as density decreases, the number of drop measurements necessary to obtain a given level of precision in drop increases.

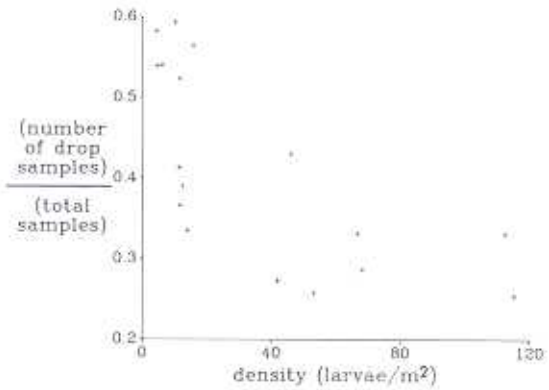

Fig. 4. Plot of proportion drop samples to total samples, P, vs density estimates.

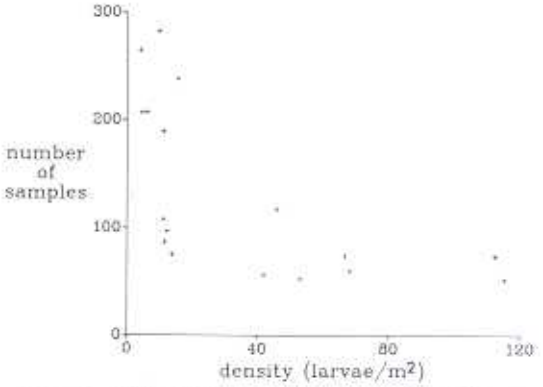

Fig. 5. Plot of total samples, N_t ($=N_z + N_y$), necessary to obtain a density estimate within 50% of the true mean at a probability of 95%, vs density.

Similarly, as density decreases the total number of measurements ($n_t = n_z + n_y$) required to obtain a given level of precision increases (Fig. 5). From this relationship, we generalize that at low densities (<15 larvae/m²), the total number of samples (n_t) required to obtain an estimate that is within 50% of the true mean 95% of the time, is 150 or more; at high densities (>15 larvae/m²), only 75–100 samples are necessary (Fig. 5). In this study, we did not collect data from sites with extremely low densities (<1 larva/m²). We expect that at these densities, almost 1,000 samples may be necessary to obtain a reasonable level of precision. Such a large number of samples would be labor-intensive, and thus for these densities we suggest using other techniques. The scale of the spatial heterogeneity in frass drop (Table 4) indicates that a smaller number of samples and a lower proportion of drop to yield samples is necessary when measuring density over a smaller area.

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