Estimating Field Hatch of Gypsy Moth (Lepidoptera: Lymantriidae)

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ABSTRACT Field hatch of gypsy moth, *Lymantria dispar* (L.), is an important population parameter of interest to both managers and researchers. It has been problematic to quantify hatch in the field without affecting its outcome. A method for quantifying hatch after it has naturally occurred in the field is presented. This technique uses a previously developed regression model for estimating fecundity, coupled with a count of unhatched eggs, to calculate the proportion of hatch. Hatch estimates were regressed against known hatch from a laboratory population to test the performance of the method ($r^2 = 0.90$, $p = 0.0001$). The method can be used to estimate first-instar hatch on an individual egg-mass basis or for a population.

KEY WORDS Insecta, *Lymantria dispar*, egg hatch, fecundity

DENSITY ESTIMATES OF GYPSY MOTH, *Lymantria dispar* (L.), are commonly made at the egg-mass stage and used to predict the potential for forest defoliation. The number of egg masses per unit ground area is estimated using a variety of methods, including fixed- and variable-radius sample plots, random walks, or transects (Wilson 1978, Eggen & Abrahamson 1983). Previous works described how density estimates could be improved by using a rapid field estimate of fecundity based on an allometric relationship between egg-mass length and the number of eggs per mass. (Moore & Jones 1987, Jones et al. 1989). Estimates of egg density per unit ground area can then be made by multiplying egg-mass density by mean fecundity. An estimate of egg density rather than egg-mass density should provide a better population estimate because it incorporates fecundity, which can vary up to 20-fold (Leonard 1974). However, not all eggs hatch after diapause because of winter kill, parasitism, or incomplete embryogenesis. Hatch in some populations has been known to vary between 0 and 100% (Kosugi 1954). Thus, quantifying field hatch would permit estimation of the number of neonates that emerge in spring, providing a more refined tool for predicting potential gypsy moth defoliation.

Existing methods for estimating hatch are often labor-intensive and produce estimates whose relationship to field hatch is unknown. Some managers remove a sample of masses from the field and incubate them under artificial conditions (New York State Department of Environmental Conservation, personal communication). Larvae are counted for a sample of masses to determine average hatch, and the laboratory estimate is extrapolated to the field. However, even minor changes in temperature or humidity during egg incubation are known to affect hatch (Giese & Casagrande 1981), and laboratory hatch under constant or optimal environmental conditions is therefore unlikely to reflect hatch in the field. In some studies, eggs have been separated from scale hair, counted, returned to the field in mesh packets, and later reexamined after hatch has occurred under natural field conditions (Campbell 1976). Although this approach may better approximate true field hatch, it assumes that separating eggs from scale hair before hatching has no effect on egg viability. Scale hair insulates eggs (Summers 1922), and dehairing egg masses before hatching may alter the effects of temperature on hatch and increase the potential for egg desiccation. Use of either of these methods is also labor-intensive because it requires egg incubation and larval counting or multiple field visits coupled with counting and recounting eggs. In both cases, one can never be certain how these estimates compare with true field hatch.

This study reports a new method that takes an alternate approach to invasive sampling before eggs hatch. A sample of egg masses is measured in the field to estimate fecundity on the basis of length (Moore & Jones 1987), and the same masses are collected after hatch is completed. Parasitized and nonviable eggs are clearly distinguishable from hatched eggs and eggshell remnants, and their numbers can be counted in the laboratory. These values, together with the fecundity estimate, can be used to arrive at a population estimate of field hatch. Population estimates of nonviable and parasitized eggs are also obtained at the same time.

Materials and Methods

Study Population. Laboratory studies were conducted on a total of 195 egg masses over a period of 2 yr to validate the performance of the estimation technique. These laboratory-sustained masses were used to develop and test the method, and no attempt was made to extrapolate from laboratory hatch to field hatch for these particular masses. Masses were collected from the Cary Arboretum, Millbrook, N.Y. The maximum length was measured for each mass before collection. Masses were collected in fall and stored over the winter at 5°C to complete the natural period of egg diapause. One week before the time of field hatch, masses were removed from cold storage and dehaired (Moore & Jones 1987).

Egg Characteristics. Eggs with holes before the end of egg diapause were assumed to be parasitized, and these eggs were counted and removed from each mass. A portion (n = 336 eggs) of the parasitized eggs was set aside for measurement of parasitoid-emergence hole size. Masses were then incubated in individual petri dishes at 23°C, and larvae were counted and removed daily. When hatch was completed, numbers of larvae and hatched eggshells were totaled, and a portion (n = 64) of the hatched eggs was measured to determine gypsy moth larval-emergence hole size. The maximum diameter of emergence holes in parasitized and hatched eggs was measured to ±0.003 mm, using a stereomicroscope with an eyepiece micrometer calibrated at 35× magnification.

Egg emergence hole sizes were compared to determine whether eggshell characteristics could be used to distinguish eggs with gypsy moth larval hatch from those with parasitoid emergence. A comparison of mean hole size was made using a t test, and 99.9% CIs were calculated (Steel & Torrie 1980).

Integrity of Egg Remnants. Provided larval- and parasitoid-emergence holes are distinctly different, hole size can be used to determine hatch. However, if larvae destroy the evidence of egg fate (i.e., consume or damage hatched eggs), a direct count of eggs with hatch characteristics is not feasible. To test this assumption, the number of eggshells with gypsy moth emergence holes and the actual number of larvae that hatched from each mass were compared on an individual egg-mass basis. For the sample of 195 egg masses, larvae were removed from the eggs daily. Because it was not known whether larvae fragment or consume eggs before they disperse, and because the average residence time for larvae to remain on a mass is 3 d (Capinera & Barbosa 1976), an additional 10 masses were collected, and larvae were allowed to remain until hatch was completed. As with the previous sample, eggs were descaled and counted before hatching, but parasitized eggs were not removed. At the conclusion of hatch, eggs were recounted and categorized as nonviable, parasitized, or hatched. The number of larvae was also counted for a comparison with the number of eggs with gypsy moth hatch.

Estimated Versus Actual Hatch. If hatched eggs cannot be counted directly, an estimate of hatch may be a viable alternative. To explore this possibility, both actual and estimated hatch were determined for each of 195 egg masses, and a linear regression of actual versus estimated hatch was used to examine the strength of the relationship between the true value for hatch and an indirect estimate. Hatch was expressed as a proportion. Actual hatch per mass was determined by directly counting larvae. Estimated gypsy moth larval hatch was calculated per mass as: $H_e = 1 - [(p + n)/f_e]$, where p is the number of parasitized eggs, n is the number of nonviable eggs, and f_e is the bias-corrected estimate of fecundity based on egg-mass length (mm). Fecundity was estimated using the model $y = 1.58x + 0.29$, where y is $\log_{10}$ (number of eggs per mass), and x is $\log_{10}$ (egg-mass length) (Moore & Jones 1987). The antilog of y (f_e) was used in calculations requiring estimated fecundity and was multiplied by a factor of 1.1027 to correct for systematic bias in the estimate due to log transformation (Sprugel 1983): $f_e = 2.15(L)^{1.58}$, where L is egg-mass length.

Results and Discussion

A deliberate choice was made to sample across a broad fecundity range (16–828 eggs per mass) to ensure that the technique would be broadly applicable. Actual laboratory hatch ranged from 0 to 428 larvae per mass. The percentage of hatched larvae per mass for both actual and estimated hatch ranged from 0 to 100%.

Egg Characteristics. Eggs were characterized by emergence hole size in an effort to distinguish parasitized eggs from eggs with gypsy moth hatch. Holes left by exiting parasitoids differed from gypsy moth emergence holes in both size and shape. Parasitoids, which included *Ooencyrtis kuvanae* (Howard) seen on masses in the field and *Anastatus disparus* (Ruschka) found emerging in the laboratory just before gypsy moth hatch, left round, discrete holes that were significantly smaller ($P < 0.0001$) than those eggs with larval hatch (Fig. 1). Emerging gypsy moth larvae left jagged, irregular holes which often encompassed more than half of the circumference of the eggshell. The 99.9% CIs for hole size of parasitized eggs (0.575–0.612 mm) and hole size of eggs with larval hatch (1.200–1.290 mm) did not overlap, and the tails of the size distributions were nonoverlapping, with a single exception: one egg that was classified as a hatched egg fell within the upper tail of the parasitoid-

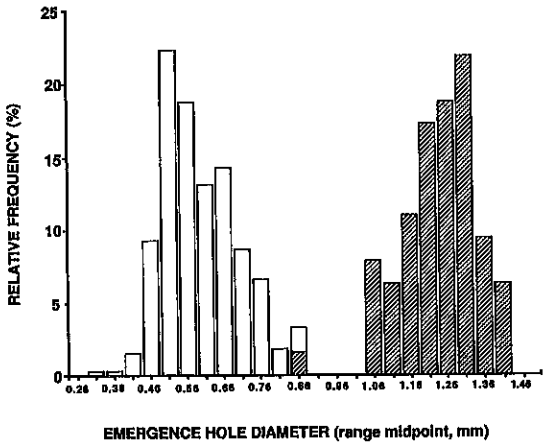

Fig. 1. Frequency histogram of size distributions of egg emergence holes (±0.003 mm) sampled from 195 egg masses. Parasitoid emergence: ☐ (n = 336 eggs); gypsy moth hatch: ▨ (n = 64 eggs).

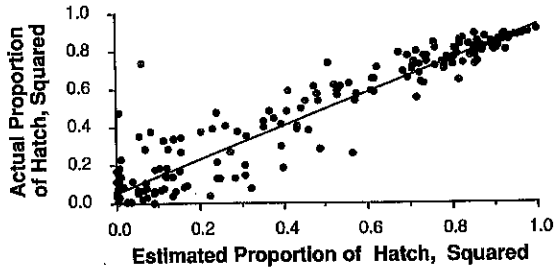

Fig. 2. Regression of the squared proportion of estimated hatch $H_e^2 = (1 - [(p + n)/f_e])^2$ versus the squared proportion of actual hatch $H_a^2 = (1 - [p + n)/f_a])^2$ for a test population observed in the lab (p, number of parasitized eggs; n, number nonviable eggs; f_e, estimated fecundity; f_a, actual fecundity). $H_a^2 = 0.95$ $H_e^2 - 0.04$, $P = 0.0001$.

emergence, hole-size distribution. Provided this sample of eggs is representative of the population, this would result in a misclassification of egg fate for only 1 in 400 eggs. Thus, whether eggs are nonviable or parasitized can be determined in masses after hatch with a high degree of confidence. Parasitized eggs can be visually distinguished from eggs with gypsy moth hatch. With experience (≈15 min), this can be done rapidly without requiring repeated measurements of hole size to make the determination of egg fate. Nonviable eggs are whole, and although they may be compressed and desiccated, they are readily distinguishable from either parasitized or hatched eggs.

Integrity of Egg Remnants. It was possible to discriminate between nonviable, parasitized, and hatched eggs when larvae were removed daily. However, if larvae destroyed the evidence of hatch when they remained on or in the mass, a direct count of hatch would not be feasible. Numbers of emerging larvae were compared with numbers of hatched-eggshell fragments. Although there was a 1:1 correspondence between numbers of larvae and numbers of hatched eggs for the 195 masses that had larvae removed daily during emergence, the additional sample of 10 masses yielded very different results. For these masses, where larvae had remained during the full course of hatch, larvae were found to damage or eat a variable number of eggshells ranging from 0 to 400 eggs per mass. There was no consumption of parasitized eggs, because counts of parasitized eggs were identical before and after gypsy moth hatch. The total number of emerging larvae plus parasitized eggs plus nonviable eggs added up to the total number of eggs before hatching for these 10 masses, so there was also no consumption of nonviable eggs during hatch. The difference between the number of larvae and hatched eggshells is attributed to either breakage and fragmentation of eggshells into smaller pieces which could not be counted, or to some larvae consuming a large part of their own or other eggshells in the process of hatching. Consequently, the number of eggshell fragments from hatching larvae cannot be considered reliable as a direct estimate of hatch.

Estimated Versus Actual Hatch. Hatch (H_e) was estimated indirectly by using 1.0 minus the ratio of unhatched eggs to estimated fecundity. The validity of the estimate was tested by regressing an analogous ratio for known hatch (H_a), on an individual egg-mass basis: $H_e = 1 - [(p + n)/f_e]$ regressed on $H_e = 1 - [(p + n)/f_a]$, where p is the number of parasitized eggs, n is the number of nonviable eggs, f_e = is the bias-corrected, estimated fecundity as defined previously, and f_a is the actual fecundity. Actual fecundity was determined before hatch by counting all eggs. The number of unhatched eggs was the sum of parasitized eggs and nonviable eggs ($p + n$). The regression of the proportion of estimated hatch squared (H_e^2) on the proportion of actual hatch squared (H_a^2) yielded an r^2 of 0.90 ($H_a^2 = 0.95$ $H_e^2 - 0.04$, $P = 0.0001$) (Fig. 2). Residuals from the unsquared regression (H_a versus H_e) indicated inequality of error variance, but squaring both dependent (H_a^2, or the squared proportion of actual hatch) and independent variables (H_e^2, or the squared proportion of estimated hatch) made the errors more normal, an assumption of regression analysis (Fig. 3).

Field Hatch Estimation. After testing the validity of the estimation method on laboratory-sustained masses, it is clear that this approach can be readily applied to the field. Although individual egg-mass estimates of fecundity and hatch can be made, it is probably more suitable to estimate mean fecundity and hatch at the population level because of the high variance associated with the fecundity estimate. Additionally, population estimates are more practical for mak-

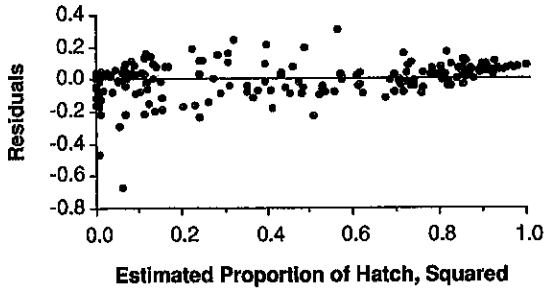

Fig. 3. Residuals from regression $H_a^2 = 0.95H_e^2 - 0.04$ (Fig. 2), where H_a^2 is the squared proportion of actual hatch and H_e^2 is the squared proportion of estimated hatch.

ing predictions of potential defoliation. The following approach is suggested for estimating gypsy moth field hatch: a sample of masses should be marked and measured in the field before hatching. Some additional masses could be marked and measured to ensure that sufficient data are collected, as there may be some attrition of masses in the sample, due either to damage during the over-wintering period or to difficulties in relocating masses after hatch. Masses should be collected as soon as possible after hatch to avoid further weathering and potential damage. Alternatively, masses can be measured and collected after hatch, but if estimates are to be made for a specific year of oviposition, there must be prior knowledge of whether old masses persisting from previous years are present. Location of masses before hatching adds sampling effort but removes any ambiguity in identifying the current generation of egg masses.

Masses should be dehaired in the laboratory, and numbers of parasitized and nonviable eggs should be counted. Mean values for fecundity ($\bar{f}_e$) based on mean egg-mass length for the sample ($\bar{L}$), mean number of parasitized eggs ($\bar{p}$), and mean number of nonviable eggs ($\bar{n}$) can then be used to estimate mean hatch, parasitism, and nonviability for the population. The antilog of the Moore & Jones (1987) fecundity regression can be used with the bias-correction factor to produce a population estimate of fecundity ($\bar{f}_e$) based on mean egg-mass length: $\bar{f}_e = 2.15(\bar{L})^{1.58}$, where $\bar{L}$ is mean egg-mass length for a population. Proportion mean estimated hatch ($\bar{h}_e$) is calculated:

$$\bar{h}_e = \sqrt{(0.95\,[1 - (\bar{p} + \bar{n})/\bar{f}_e]^2) - 0.04}\,.$$

The resulting calculated proportion of hatch can be converted to the number of neonates (i.e., first instars) by multiplying the proportion hatch by estimated fecundity for the population.

The number of masses to be sampled and the particular sampling approach (e.g., the use of fixed-area plots) are contingent on the objectives of the managers or research scientists. Based on the variation in the fecundity estimation model ($\sigma^2 = 160.15$), we calculated a sample size of 35 masses for a precision of the estimate selected at $p = 0.05$, an error of ≈75 larvae per mass, and a power of 0.80 (Welkowitz et al. 1976).

Although minimal laboratory work is required to use this method, an observer with some experience in differentiating between hatched and parasitized eggs can process masses rapidly (usually within 20 min per mass) for a reliable estimate of gypsy moth field hatch. It may be most appropriate for managers to estimate hatch when population densities are building toward defoliating levels. The method may, of course, be used by research scientists interested in determining hatch in their studies.

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