

EXTRACTION OF HOUSE FLY (DIPTERA: MUSCIDAE) LARVAE FROM POULTRY MANURE USING BERLESE/TULLGREN FUNNELS

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Accurate determination of the numbers of late instar house fly (*Musca domestica* L.) larvae in samples of poultry manure is the first step in developing density estimators for populations of pupae and emerging adult flies. These density estimators can be used to anticipate fly abatement needs on agricultural facilities and to improve the efficiency of releases of pupal parasitoids in augmentation and inundation-based biological control programs for *M. domestica*. Presently, there is no practical method for determining the density of house fly larvae in a sample of poultry manure. Estimates of this factor (Geden & Stoffolano 1987; Stafford & Bay 1987) have been deduced mainly from extraction data using Berlese/Tullgren funnels (Brydon & Fuller 1966). However, the accuracy of these devices for separating house fly larvae from samples of poultry manure is not well understood.

This study was made to assess four parameters whose influence on the mean and variance of numbers of third instar house fly larvae extracted from samples of poultry manure placed in Berlese/Tullgren funnels is unknown. These parameters are: (1) the size of the manure sample, (2) the number of larvae in the sample, (3) the cumulative extraction rate of larvae from the manure sample over time, including the relation between extraction rate and temperature change within the manure sample, and (4) the effect of enclosure of larvae and manure in sample containers (for ≤ 4 h) on the accuracy of larval extraction.

Berlese/Tullgren funnels were constructed according to the design of Brydon & Fuller (1966). Poultry manure for the experiments was collected for 24 h in trays suspended beneath commercial laying hens. The manure was stored at -20°C then allowed to thaw to ambient temperature 8 h before use.

A 4×4 factorial design was used to determine the effect of larval density and manure volume on the accuracy of larval extraction. Each treatment combination was replicated four times ($n = 64$) and comprised one of four volumes of manure (100, 200, 300, and 400 cc) and one of four levels of larval density (50, 100, 300, 500). Two hours before a test began, known numbers of 72-hour-old early third instar larvae reared at 26°C on the Gainesville house fly diet (Hogsette 1992) were placed on, and allowed to penetrate, each manure sample. Individual samples were placed onto a single layer of cheesecloth on top of the 5 mm mesh screen (Brydon & Fuller 1966) in each funnel and shaped to a thickness of ≈ 40 mm. Larvae exiting the samples, after funnel covers were positioned and the lights (100 watts) turned on, dropped into 70% ethanol in water in 0.25-liter glass jars at the bottom of each funnel. Jars were replaced at 12 h intervals until no larvae were collected for 2 consecutive intervals.

To determine the cumulative number of larvae extracted from a manure sample over time, nine samples of 100 larvae in 400 cc of manure were prepared as described above and placed in funnels at 0 h. Collection jars were removed from funnels at 6, 24, 30, and 48 h. Temperature change at the top, middle, and bottom positions in three manure samples was monitored continuously, and reported hourly, using an Omnidata[®] EL-820 data logger (Omnidata, P.O. Box 3489, Logan, UT 84321) and ES-060 temperature probes. The experiment was repeated four times ($n = 36$).

A 2×4 factorial design was used to determine the effect of ≤ 4 h of enclosure of larvae and manure in sample containers on the accuracy of larval extraction. Treatment factors were open/closed sample container and the length of time the container was open or closed (1, 2, 3, or 4 h). At 1 h intervals, beginning 4 h before all samples were placed in the funnels (0 h) and ending at 1 h, six samples of 100 larvae in 400 cc of manure were placed into sealable plastic containers ($10 \times 10 \times 15$ cm) and held at ambient temperature (22-26°C). Air-tight lids were placed on three of the containers and these containers labelled as closed. The remaining three unsealed containers were labelled as open. All samples were placed in funnels at 0 h and the funnels operated, as before, for 48 h.

In all tests, the percent extraction of larvae for each funnel was calculated as a ratio of the numbers of larvae in the collection jar to the numbers of larvae in the manure sample, multiplied $\times 100$. Data for percent extraction (transformed to arcsin) were analyzed using analysis of variance procedures (SAS Institute, Inc. 1988). Means separation was performed using Tukey's studentized range test at $P = 0.05$. The relationship between cumulative extraction rate (%CR) and temperature change in the manure sample at top (t), middle (m), and bottom (b) positions was determined using the regression model: %CR = $t + t^2 + m + m^2 + b + b^2$ (SAS Institute, Inc. 1988).

Percent extraction of third instar house fly larvae from samples of poultry manure in Berlese/Tullgren funnels was not influenced by the size of the manure sample or by the numbers of larvae in the sample (Table 1). The overall percent extraction of larvae in this study ($n = 118$) was 98.5 ± 1.9 .

The length of time Berlese/Tullgren funnels were operated influenced the percent extraction of fly larvae ($F_{1,35} = 39.69$, $P = 0.01$). Significantly fewer (57.7%) larvae were recovered after 6 h of operation than after 24 (90.2%), 30 (96.6%) or 48 h (96.6%). Cumulative percent extraction of larvae was related to the linear and quadratic effects of manure temperature change ($F_{2,143} = 54.68$; $R^2 = 0.437$) at the top position. There was no significant change in manure temperature at any position after 30 h of funnel operation.

The enclosure of fly larvae and manure in sample containers for up to 3 h did not influence overall percent extraction of larvae compared with the data for larvae in open containers for the same length of time ($\bar{x}_{\text{open}} = 98.8 \pm 1.8\%$; $\bar{x}_{\text{closed}} = 99.2 \pm 0.7\%$). The results at 4 h ($\bar{x}_{\text{open}} = 99.3 \pm 0.05\%$; $\bar{x}_{\text{closed}} = 64.0 \pm 55.4\%$), however, were equivocal. Of the three containers closed for 4 h, one yielded 0 larvae while percent extraction for the remaining two was 96%. The results at 4 h, for closed containers, while not statistically different from the earlier times, indicate that enclosing larvae and manure in a sample container for more than 3 h at 22-26°C (as might be necessary during transport of samples) will result in variable extraction responses.

TABLE 1. MEAN PERCENT EXTRACTION (\pm SEM) OF THIRD INSTAR HOUSE FLY LARVAE FROM POULTRY MANURE IN BERLESE/TULLGREN FUNNELS.

Manure Volume	Number of Larvae			
	50	100	300	500
100 cc	99.5 (1.0)	88.1 (18.7)	95.8 (3.9)	99.3 (0.9)
200 cc	95.5 (4.1)	100.0 (0.0)	99.3 (0.5)	98.8 (0.5)
300 cc	100.0 (0.0)	99.8 (0.5)	98.3 (2.2)	99.5 (1.0)
400 cc	99.3 (2.0)	98.3 (1.7)	98.5 (1.3)	98.1 (0.8)

SUMMARY

Berlese/Tullgren funnels provide an accurate estimate of the density of third instar house fly larvae in samples of poultry manure. Percent extraction of larvae did not vary with sample size, when the range of sample sizes was between 100 and 400 cc of poultry manure, and was unaffected by larval densities from 50 to 500 per sample. Maximum extraction of house fly larvae requires ≥ 30 h of funnel operation. The accuracy of extraction decreases when manure and larvae are enclosed together in a sample container for more than 3 h prior to placement of samples in funnels.

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