Photographic Estimation of Weight of Insect Larvae[1]

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ABSTRACT

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A photographic technique for measuring insect larvae was tested on *Heliconius cydno* (Bates) (Lepidoptera: Nymphalidae) and *Trirhabda geminata* Horn (Coleoptera: Chrysomelidae). The method was found to estimate live body weight to within 12% accuracy over a range of 1.0 to 1,000 mg for *H. cydno* and 25% accuracy for *T. geminata*. The method should prove useful for: (1) measuring size and growth rate in species which are too small or delicate to handle, and (2) making accurate in situ field measurements.

Ecological and physiological investigations of insect larvae often require measurement of insect weight (Waldbaur 1968, Scriber and Slansky 1981). Live weights (Duffey and Isman 1981, Slansky 1978, Smiley 1978) or dry weights (Blau et al. 1978, Scriber and Slansky 1981) may be needed, depending on the nature and limitations of the investigation. Although weighing is most accurately accomplished with the use of a microbalance, there are many situations when this is not feasible. For example, the younger stages of many insects are highly delicate and should not be handled, particularly if the phenomenon under investigation has a behavioral component which might be affected by minor damage during handling. This has tended to discourage investigations of the earlier instars (Scriber and Slansky 1981), yet these larvae may be the most sensitive indicators of host plant or environmental quality (Isman and Duffey 1982).

Weight or size may be estimated by various techniques. Two of the most widely used are based on head capsule measurements (Dyar 1890) and body length measurement (e.g., Schoener 1980). In the latter technique, caliper measurements are used to estimate weight by solution of a predetermined regression equation, where weight is expressed as a function of the body length. Rogers et al. (1976) report a general length-dry-weight regression for insects, but these authors suggest that for greater accuracy a separate regression line should be calculated for each type of insect under investigation.

In many cases, the use of calipers is not feasible, either because the insect is small and delicate or has an unusual body posture, or because the live insect is active. In those cases, optical measurement is the most feasible solution. In this paper we discuss a photographic method for optical measurement and test its accuracy in estimating body weight. The method has the following advantages: (1) the insect is never mechanically disturbed; (2) measurement is virtually instantaneous, so that moving insects can be investigated; (3) the method is more accurate than techniques based on head capsule or body length measurements; (4) the method gives a reasonable approximation to body weight without the use of a predetermined regression equation; and (5) many insects may be photographed rapidly and easily, maximizing the rate at which data are collected. The principal disadvantage is that extra time is required to develop the film and measure the images. However, compared with the gravimetric techniques currently in use (Scriber and Slansky 1981), the photographic technique is not exceptionally time consuming.

To test the accuracy of the method, we photographed and weighed larvae of *Trirhabda geminata* Horn (Coleoptera: Chrysomelidae) and *Heliconius cydno* (Bates) (Lepidoptera: Nymphalidae) and regressed actual live weight against estimated weight. For comparative purposes, we also measured head capsule width and body length in *H. cydno*, and regressed dry and live weights against these measurements.

Materials and Methods

Larvae were photographed in the focal plane of a metric ruler attached in front of a Nikon "FE" camera. Care was taken to ensure that the insect was exactly in the plane of the ruler; otherwise the measurements would be inaccurate. A narrow-aperture setting (f16 or smaller) was used to improve focus and accuracy of the technique. With this apparatus it was not necessary to look through the camera viewfinder; focusing was automatically accomplished by aligning the insect in the plane of the ruler. An identifying label was placed on the ruler in the field of view of the camera. Since the desired result consists of a silhouette of the insect's body, it was not necessary to achieve perfect exposures.

An alternative procedure was to use a Nikon 55-mm macro-lens with magnification calibrations inscribed on the barrel of the lens. By setting the lens on the desired setting and focusing by moving the camera back and forth, it was possible to take photographs without the use of the ruler. The disadvantage of this technique was that through-the-lens focusing must be carefully achieved in each separate photograph, and that no record of scale is permanently recorded in each frame. The use of small, stick-on labels attached to the plant in each photograph somewhat obviated the latter problem.

After film development (done with standard Kodak developers in the laboratory), *H. cydno* volumes were calculated by projecting the developed film onto a sheet of paper by using a photographic enlarger. The silhouette of the insect's body was then traced onto the paper. Spines were ignored, and surface undulations in body outline were smoothed. The body was then divided into regions with constant diameter, as shown in Fig. 1. Short

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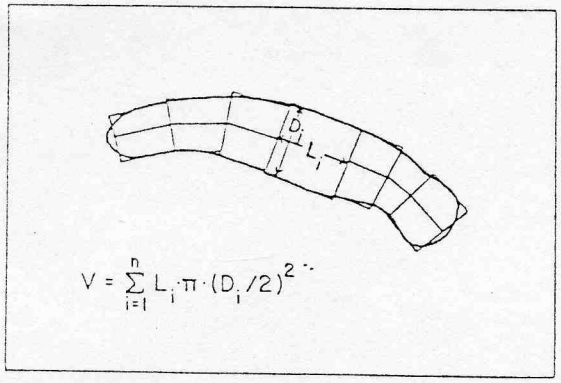

FIG. 1.—Silhouette of a *H. cydno* larvae showing the method used to estimate body volume for a larva curved on the plant surface.

sections of curved regions were treated as "curved rectangles," as shown in Fig. 1. The volume of each section was calculated as $(D_i/2)^2 \cdot \pi \cdot L_i$, where D_i is the diameter of section i and L_i is the length down the center of section i. Summing the volumes of all the sections $\sum_{i=1}^{n} (D_i/2)^2 \pi L_i$ gave the estimated total volume of the insect.

For *T. geminata*, a slightly modified procedure was used in which photographs were taken from both a top and side view. Overall length, average width (top view), and average height (side view) were then measured and used to calculate volume by assuming that the larvae were elliptical in cross section. This method of analysis was much faster in that fewer measurements and calculations were necessary. It was significantly less accurate than the technique for *H. cydno*, however, as indicated below.

The measurement and calculations given above yield estimates of volume which are proportional to the amount of magnification during photography and subsequent projection. They are therefore usable in calculations such as relative growth rates where relative rather than absolute measurements are needed. To calculate absolute volume, the relative volumes must be divided by the cube of the magnification factor. The magnification factor itself may be determined from the observed enlargement of the ruler or labels included in the photographs.

Larvae were weighed to three significance figures on a Cahn electrobalance (model 7500) immediately before or after photography. Figures 2 and 3 plot the natural logarithm of weight for *T. germinata* and *H. cydno* larvae as a function of the natural logarithm of estimated volume. Linear regression was performed on the log-transformed volumes and weights, and the SD of the residuals was calculated. This quantity (SE) estimates the amount of error expected in using the technique, and the quantity e^{SE} is about equal to the coefficient of variation in estimated weight.

A separate analysis was done on *H. cydno* to compare the accuracy of the photographic technique with estimates based on head capsule width and body length. The latter two dimensions were measured with calipers

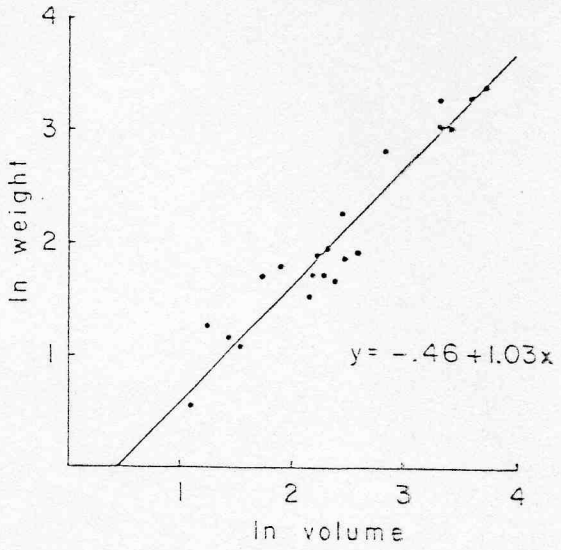

FIG. 2.—Log volume versus log weight for *T. geminata* from 1st to 3rd instars.

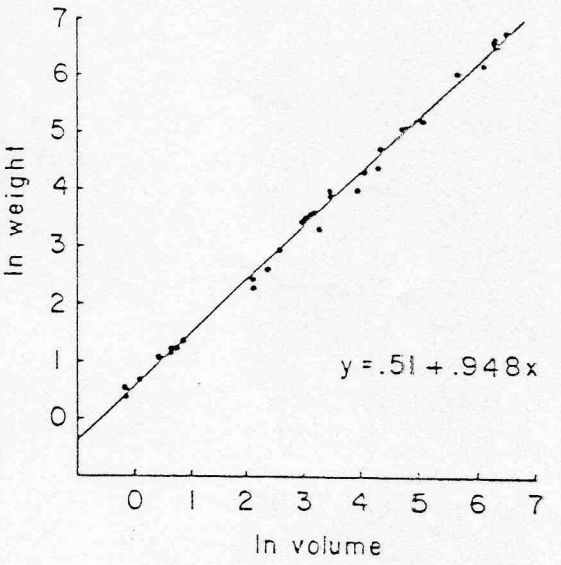

FIG. 3.—Log volume versus log weight for *H. cydno* from 1st instar to prepupae.

on live larvae, and the live larvae were weighed. The larvae were then killed by freezing and dried to constant weight at 60°C in a drying oven, after which they were reweighed. Regression analysis and calculation of SEs was performed on both live and dry weights as a function of head capsule width or body length, as indicated in Table 1.

Results and Discussion

The data from *Trirhabda geminata* indicate that when overall length is combined with width × height (Fig. 2), an estimate of volume is produced which is sufficiently accurate to measure weight differences between

individuals of different ages. The best estimate was obtained by considering width × height ($r^2 = 0.926$), but a simpler measurement based on width alone or height alone was only slightly less accurate ($r^2 = 0.890$ and 0.860, respectively). The deviation of points above and below the regression line indicate that the *T. geminata* measurement techniques yield a coefficient of variation in estimated weight of ca. 25%. This measurement technique therefore is sufficiently accurate to estimate growth rates over 3- to 4-day periods where the insects increase in size by 500 to 5,000%.

The *H. cydno* measurements (Fig. 3) were more accurate, due to the more precise measurement techniques employed (Fig. 1). Here the deviation of points around the regression line indicated ca. 12% coefficient of variation in estimated weight. Since these larvae typically double in size every 30 to 40 h, this measurement technique is sufficiently accurate to assess day-to-day changes in weight. In fact, using the method at 6-h intervals produces a reasonably smooth growth curve (Smiley, unpublished data).

As indicated in Table 1, the photographic method estimates wet weight and dry weight with ca. two to four times the accuracy of linear measurements on head capsule width or body length. The technique thus appears to be suited to investigations where accuracy is desired, such as measurement of insect growth over a short time period. Dry-weight estimation appears to be less accurate than live-weight estimation by any of the techniques, but the fact that it can be estimated reasonably accurately (<20% coefficient of variation) in very small larvae (<1.0 mg) suggests that this technique might be useful in laboratory investigations of early instars. However, we suggest that the greatest utility of the photographic method will be in field studies of larval growth rates. Here photography may be used to estimate weights on live animals whose movement and unusual body postures confound simple length and width measurement, with sufficient accuracy that growth rate can be estimated before the larva emigrates or is consumed by a predator. This should facilitate examination of how the factors affecting larval growth and survivorship interact under natural conditions.

Table 1.—SEs of weight estimates of *H. cydno*[a]

As estimated by:	Live wt	Dry wt
Head width	0.41 (51%)	0.48 (62%)
Body length	0.22 (25%)	0.25 (28%)
Photo vol	0.12 (13%)	0.18 (20%)

[a]SEs (SD of residuals) after linear regression of logarithmically transformed data. Approximate percent coefficients of variation are in parentheses.

One final application of the photographic technique is suggested for community studies of insect growth where a diversity of species and orders is being investigated. Because insect specific gravity may be assumed to be near 1.0 (Wigglesworth 1972), the photographic volume technique can be used to estimate weight without the aid of a predetermined regression equation. This is done by assuming that 1 mm^3 = 1 mg of live body weight. Thus the investigator is relieved of the necessity of carrying out a separate regression analysis on each type of insect.

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