GRAVIMETRIC METHOD FOR THE MEASUREMENT OF SUGAR CONSUMPTION BY ADULT VELVETBEAN CATERPILLAR (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Determination of food consumption by adult Lepidoptera, especially noctuid species, has not been well studied, possibly because of a lack of appropriate methodology. In order to measure sugar consumption by velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, a gravimetric method was developed and is presented here together with results obtained using this method. After 24-h exposure to a moth, liquid consumption by the moth was determined by weighing the remaining solution on an electronic balance. The amount of solution consumed was calculated by converting the difference between the weight of the remaining solution and that of the unfed control to a volumetric value. Velvetbean caterpillar moths consumed a significantly greater volume of solution at lower sugar concentrations than at higher concentrations. Female moths from larvae reared on soybean foliage in field cages consumed three times that of moths from larvae reared on artificial diet in the laboratory. This method was simple, accurate, and required minimum handling of test insects. It was suitable for the measurement of food consumption of velvetbean caterpillar moths and could also be suitable for other lepidopteran species.

Key Words: *Anticarsia gemmatalis*, adult feeding, sugar consumption, compensatory feeding

RESUMEN

La determinación del consumo de alimento por lepidópteros adultos, especialmente por especies de noctuidos, no ha sido bien estudiada, posiblemente debido a la falta de una metodología adecuada. A fin de medir el consumo de azúcar por la oruga del frijol de terciopelo, *Anticarsia gemmatalis* Hübner, fue desarrollado un método gravimétrico que es presentado aquí junto a los resultados obtenidos mediante su uso. Luego de 24 horas de exposición a la polilla, el consumo del líquido fue determinado

mediante el pesaje en una balanza electrónica de la solución remanente. La cantidad de solución consumida fue calculada mediante la conversión a un valor volumétrico de la diferencia entre el peso de la solución remanente y el del testigo donde las polillas no se alimentaron. Las polillas consumieron un volumen significativamente mayor de las soluciones con baja concentración de azúcar. Las hembras de larvas criadas con follaje de frijol de soya en jaulas de campo consumieron tres veces más que las polillas de larvas criadas en dieta artificial en el laboratorio. Este método fue simple, preciso, y requirió una mínima manipulación de los insectos. El método fue adecuado para la medida del consumo de alimento de *A. gemmatalis* y podría además ser adecuado para otras especies de lepidópteros.

The adult velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), like many other noctuid species, feeds on floral and extrafloral nectars (Lukefahr & Rhyne 1960). The moths have also been observed feeding on crushed grapes (Greene et al. 1973), cut apples (X. W., personal observation), whitefly honeydew on hemp sesbania, *Sesbania exaltata* (Raf.) (Collins & Johnson 1985), and honeydew on seed heads of a few species of grasses infected with ergot fungi including Bahiagrass, *Paspalum notatum* Flugge (Greene et al. 1973) and Dallisgrass, *Paspalum dilatatum* Poiretat (Collins & Johnson 1985, Wei & Johnson 1995).

The importance of nectar sources to adult Lepidoptera has been demonstrated in studies that have shown increased numbers of eggs (Collins & Johnson 1985), increased egg-laying (Wales 1983, Adler 1989, Mason et al. 1989), increased population density (Lukefahr & Rhyne 1960, Collins 1984), insect pest outbreaks in certain agroecosystems (Burleigh 1972), and probably enhanced long-distance dispersal (Mason et al. 1989). However, comprehensive knowledge of adult food consumption is needed before we can understand how adult Lepidoptera utilize nectars in nature, and how important adult feeding is to reproduction and population dynamics, especially noctuids of economic importance. This knowledge could improve IPM programs or adult control technologies. However, adult feeding of most lepidopteran species, including noctuids, has been poorly studied (Adler 1989). One of the reasons appears to have been a lack of appropriate experimental methodology.

In measuring liquid consumption by adult Lepidoptera, the following methods have been explored: an artificial flower consisting of graduated pipette and small funnel (Alm et al. 1990), hollow plastic stoppers covered with Parafilm® to serve as a reservoir for artificial nectar (Pivnick & McNeil 1985), weighing the test insect before and after feeding (Pivnick & McNeil 1985, Adler 1989), and using a 50 or 100 *u*l microcapillary to hold sugar solution to feed test insects (May 1985a, b). Adler (1989) adopted a microcapillary method combined with spring-action clothes pins to bind the wings of *Helicoverpa zea* (Boddie) moths to restrain them while they were feeding. While the above mentioned techniques were obviously acceptable for measuring consumption in the studies cited, each one has some problem or problems which would preclude its use in measuring daily or lifetime sugar consumption by a noctuid moth. Also, previous studies with Lepidoptera, except for Alm et al. (1990) with the white cabbage butterfly, have used uptake rate as the consumption variable rather than daily consumption. In uptake rate measurement, test insects were given food once or twice a day and the liquid ingested per unit time was then calculated (May 1985a, Pivnick & McNeil 1985, Adler 1989). However, because daily consumption was not determined, the estimates to predict daily and total consumption were not suited to our objectives which were to determine the effects of sugar consumption on activity, feed-

ing pattern, lipid accumulation, and fecundity of the velvetbean caterpillar. To reach this objective we needed a technique that would give us absolute values of sucrose consumed on a daily basis. In the present study, we developed a new method to measure the daily and lifetime total nectar consumption by velvetbean caterpillar adults.

MATERIALS AND METHODS

Insect

Two sources of test insects were used in this study, lab-reared and field-reared moths. Laboratory rearing procedures were a modification of the techniques of Greene et al. (1976) and King & Hartley (1985) which had been used for maintaining the velvetbean caterpillar colony in our laboratory from 1991 to 1994 (Wei 1995). Field-reared moths were obtained from larvae reared in field cages in a soybean field at the Ben Hur Experiment Farm of Louisiana State University Agricultural Center, Baton Rouge, LA. A 1.5-ha field was planted with a soybean variety, 'Pioneer 9791', which is susceptible to velvetbean caterpillar. During the summer of 1993, six cages, 1.8 × 1.8 × 1.8 m, were set-up in the field when the soybeans were mostly at developmental stage R2 (Fehr & Caviness 1979). For ease of collection and reduction of damage, the procedures of Wei & Johnson (1994) for rearing velvetbean caterpillar larvae and harvesting their pupae from field cages were followed. The ground inside the cages was covered with one layer of saran screen (32 × 32 mesh) over which a 2-cm layer of coarse vermiculite (STRONG-LITE® Products Corp., Seneca, IL) was evenly placed at time of cage set-up. Forty lab-reared adults (20 males and 20 females) were released in each cage and removed three days later. After the larvae emerged, densities were monitored to ensure they were below levels which would result in total defoliation of soybean foliage prior to pupation. After most larvae had pupated, pupae were collected and transported to the laboratory. Pupae from either field cages or laboratory sources were sexed, and placed in individual 155 ml plastic diet cups (SOLO® Cup Company, Urbana, IL). They were held in a walk-in environmental chamber at 27 ± 1°C, 85 ± 5% RH, and a photoperiod of 14:10 (L:D) until emergence.

We performed three different experiments to examine sugar consumption by velvetbean caterpillar moths using the gravimetric method. First, we examined total liquid consumption for 10 female and 10 male field-reared moths over their entire adult life span when the insects were provided with both 30% sucrose solution and distilled water. Second, we measured daily consumption over 14 days for 10 lab-reared female moths and 10 field-reared female moths when the insects were provided with both 30% sucrose solution and distilled water. Third, we measured consumption of five concentrations of sucrose solutions (0%, 5%, 10%, 20% and 40%) in the 48 h after adult emergence. We tested 10 field-reared female moths at each of these concentrations. In each test, only moths that emerged within a 24-h period were randomly selected and transferred singly from the diet cups to 1.8 liter paper cartons (16 cm high × 13 cm in diam, Fonda® Group Inc., Union, NJ), covered with cheese cloth secured with a rubber band.

Experimental Conditions and Evaporation Monitoring

The experiments were conducted in a walk-in environmental chamber at 27 ± 1°C, 85 ± 5% RH, and a photoperiod of 14:10 (L:D). To monitor evaporation under such conditions, we measured the evaporation of distilled water and 30% sucrose solution over a 24-h period using the gravimetric method described in the present study. We also ex-

amined the effects of location (horizontal and vertical locations inside the walk-in environmental chamber) on evaporation.

Development of Methods for Measuring Sugar Consumption

When we were investigating methods to use for measuring sugar consumption by adult moths, we tested most of the available methods. We modified the method used by Pivnick & McNeil (1985) (using hollow plastic stoppers covered with Parafilm®) by weighing the plastic stoppers instead of weighing the test insects before and after feeding. However, the moths could not easily locate the food source. Weighing test insects before and after feeding not only was too time-consuming, but some test insects were damaged. More importantly, weighing the insect before and after feeding would certainly not be suitable for the velvetbean caterpillar moth which has the ability to quickly excrete liquid from both proboscis and anus during or shortly after feeding (X. W., S. J. J., & Abner M. Hammond, unpublished data). We also tested the method of using a 100 *u*l microcapillary to hold the sugar solution to feed test insects (May 1985a, b). This method was not suitable for our experiment because threading the proboscis manually into the openings of the microcapillary was not practical when measuring food consumption for 24 h per day over an extended period of time and with multiple test insects involved. We did not use the method described by Adler (1989) (using a microcapillary to hold the artificial nectar and spring-action clothes pins to bind the wings of moths to restrain them while they were feeding) because we felt that refrigerating, weighing, and pinning the wings of moths each time they were fed would cause an unacceptable amount of damage to the moths.

The method of Alm et al. (1990) (which involved the use of a small funnel connected to a graduated pipette though plastic tubing) was designed to measure the daily nectar consumption by white cabbage butterfly. However, this method was not sensitive enough to measure consumption by a velvetbean caterpillar moth. Using a similar apparatus, the difference in the liquid levels could not be detected before and after 200 *u*l of liquid were removed from a 5 ml funnel connected to a 0.1 ml pipette. Also, evaporation associated with this method would be problematic because of evaporation from the relatively large surface area of the funnel although no dimensions of the "funnel" or "graduated pipette" were given in their method.

During the development of our method, we also experimented with several kinds of containers as a reservoir for the sugar solution, including clear plastic micro tubing, plastic cells cut from blister cards (used for packing medicine by pharmacies), hollow plastic stoppers, porcelain liquid holders, and polystyrene wells. However, none of them, except for the small polystyrene wells, allowed for easy and accurate measurement without the risk of spill. The polystyrene wells, which were chosen for our method, were big enough for moths to locate the food source easily but, at the same time, small enough to minimize evaporation.

Feeding Platform

Feeding solutions were held in the 360 *u*l individual polystyrene wells (10.7 mm high $\times$ 6.9 mm in top diam) cut from EIA/RIA 8-well strips (Costar Corporation, Cambridge, MA). Two polystyrene wells were embedded 2-cm apart in a piece of styrofoam ($5 \times 4 \times 2$ cm) which formed a feeding platform.

In the first and second tests, 300 *u*l of distilled water and 300 *u*l of 30% sucrose solution (wt:vol) were separately provided in the 2 wells in each feeding platform. A 30% sugar solution was selected based on experimental results that indicated it was an ap-

propriate concentration for velvetbean caterpillar moths (X. W., S. J. J., & Abner M. Hammond, unpublished data). For the third test, only sucrose solution of the designated concentration was added to the 2 wells in a platform with 300 *u*l in each well. A feeding platform containing measured solution was then placed in each paper carton in the morning (0800, CST), and the moths were allowed to feed for 24 h. Each moth received new wells and solutions daily for the designated experimental period.

Weighing Procedure

At the end of each 24-h exposure period, wells from each feeding platform were transferred to a 32-cell weighing board made of a $10 \times 15 \times 2$ cm piece of styrofoam with rows of holes punched about the size of the outside diameter of the well. The following steps were carried out: (1) the weighing board (with wells and residual liquid) was placed on an electronic balance (precision to 0.001 g), and the balance was zeroed; (2) the liquid was pipetted from one well and the inside of that well dried with a paper towel; (3) the negative number then displayed indicated the weight of the liquid left in the well by the moth after feeding. This three-step procedure was repeated for each well in succession until all wells in a board were weighed. One of the advantages of the weighing procedure was that handling of each individual well was unnecessary, reducing the risk of spill while weighing and also increasing handling efficiency. The residual weight was subtracted from the weight of the appropriate control (distilled water or sugar solution) included for monitoring evaporation. The volumetric estimate of sugar solution or distilled water ingested was calculated by converting the solution weight to volume.

Statistical Analysis

All statistical analyses were performed using SuperANOVA version 1.11. Tukey-Kramer's test (Gagnon et al. 1989) was used for means separation.

Results and Discussion

Evaporation Monitoring

The monitoring of evaporation was very important to the accuracy of the consumption measurement. We found: (1) there was no significant difference (F= 0.0514; df= 1, 46; P = 0.8217) between distilled water and 30% sucrose solutions in the volume evaporated [see Alm et al. (1990)]; (2) the environmental chamber was uniform in effects on evaporation when relative humidity was high (85 ± 5%) and air circulation was maintained as evidenced by the finding that there was no significant difference in evaporation among various locations (F= 0.1209; df= 1, 22; P = 0.7314); (3) the average daily evaporation from a well was 24.87 ± 1.7 *u*l (8.3% water evaporated). Sugar concentration increased about 3% due to water loss by evaporation during a 24-h period, which was not considered significant in altering the sucrose concentration [velvetbean caterpillar moths were observed to feed on an extremely wide range of sugar solutions, ranging from 1% concentration to even sugar crystals (X. W., S. J. J., & Abner M. Hammond, unpublished data)]. However, in order to monitor evaporation closely, we included 2 controls (same as treatment except with no test insect) for each treatment group (usually 10 cartons) during all experiments. The mean weight of the liquid of these unfed controls served as the post-evaporation reference from which we

subtracted the residual liquid weight obtained after feeding by a moth for 24 h. Volumetric consumption data were then obtained by converting the resulting weight (the difference in weight between fed and unfed) to a volumetric value.

Consumption

Using this gravimetric method, we obtained the daily food consumption pattern of male and female velvetbean caterpillar moths. Both male and female moths consumed heavily during the first 2 days after emergence, with peak consumption occurring on the first day. Females consumed 314.1 ± 72.8 *u*l in the initial 2 days when provided with 30% sucrose solution and distilled water, which represents 37% of the total consumption over their adult lifetime (19.8 ± 8.0 days at 27°C). Males consumed 356.9 ± 69.8 *u*l in the initial 2 days representing 36.9% of their total food consumption. After day 2 they consumed a nearly constant daily volume of 25.6 ± 8.1 *u*l for females and 25.8 ± 5.1 *u*l for males. The differences in consumption between day 2 and day 3 were found significant for both male (F= 16.6798; df= 1, 18; P= 0.007) and female moths (F= 6.7824; df= 1, 18; P= 0.0179). On average, the total liquid consumption for the entire life span of a female moth was 849.3 ± 328.9 *u*l. Males consumed a total of 964.0 ± 189.6 *u*l of liquid during their entire life of 27.7 ± 6.4 d. There was no significant difference (F= 0.9128; df= 1, 18; P= 0.3520) in total liquid consumption between sexes. Even with the presence of 30% sucrose solution, distilled water was still taken up daily by moths until death, at an almost constant volume: 8.1 ± 4.0 *u*l for females and 7.6 ± 3.7 *u*l for males. A total of 150.4 ± 68.2 *u*l and 211.0 ± 57.4 *u*l distilled water was consumed per female or male moth, representing respectively, 17.6% and 21.9% of their total liquid consumption. Total sucrose consumed in dry weight was 0.21 ± 0.08 g for a female and 0.23 ± 0.05 g for a male moth when they were provided with both 30% sucrose solution and distilled water.

The daily total liquid consumption of field- versus lab-reared velvetbean caterpillar female moths is compared in Fig. 1. Females from larvae reared on soybean foliage in field cages consumed 644.2 ± 176.3 *u*l liquid in the initial 2 weeks compared with 204.8 ± 93.1 *u*l for females from the laboratory. The consumption by field-reared moths was 3 times that of lab-reared moths, showing a highly significant difference (F = 48.58; df= 1, 18; P= 0.0001) in consumption between the 2 sources, although there was no statistical difference in pupal weights between these 2 groups (F = 1.767; df= 1, 398; P= 0.1844). The lab-reared moths consumed much less, even during the peak consumption period which occurred in the first 2 days after emergence. Furthermore, distilled water constituted 22.5% of the total liquid consumption by the lab-reared moths while distilled water represented only 16.8% of the total liquid consumption by field moths. These results suggest that laboratory insects may have more stored energy carried over from larval to the adult stage and thus do not depend as heavily on feeding for their reproductive needs. Mason et al. (1989) found much more adult lipid when larvae of soybean loopers, *Pseudoplusia includens* (Walker), were reared on artificial medium in the laboratory than in adults from larvae reared on plants in the field. Also, this finding suggests that laboratory insects may be inappropriate experimental subjects for food consumption studies because their consumption is not representative of the consumption of natural populations. This could be especially important in research on adult feeding stimulants.

Moths consumed significantly more solution when they fed at low concentrations than at high concentrations (Fig. 2*;* F= 61.54; df = 4, 44; P= 0.0001). As the sugar concentration increased, the volume consumed decreased dramatically. In the initial 48-h feeding period after emergence, they consumed 657.5 ± 151.4; 526.4 ± 134.4; 346.6

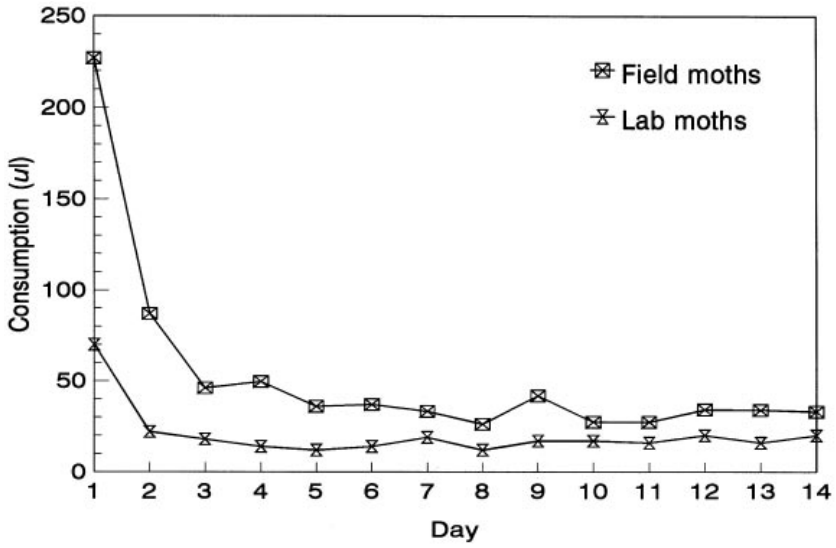

Fig. 1. Daily liquid consumption by 10 females from larvae reared on soybean foliage in field cages and 10 females from larvae reared on artificial diet in the laboratory. Moths were provided with both 30% sucrose solution and distilled water.

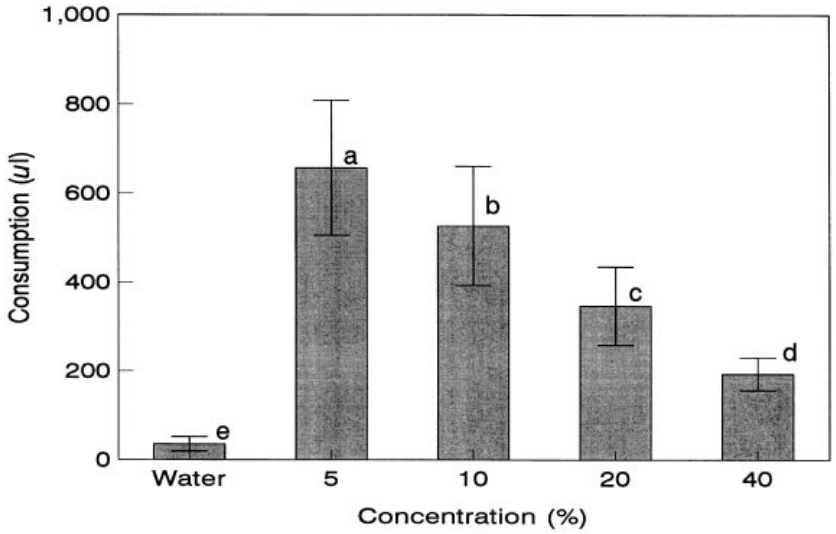

Fig. 2. Consumption by female velvetbean caterpillar moths during the initial 48-h period after emergence when provided with various concentrations of sucrose solution. Ten field-reared moths were tested at each concentration. Different letters shown by each solid and standard error bar indicates significant difference (a=0.05, Tukey-Kramer's test) in consumption.

± 88.3; and 193.2 ± 36.8 *u*l, respectively, when they were fed with 5, 10, 20, or 40% sucrose solutions. Only 35 ± 16.6 *u*l of distilled water was taken up during the same period when the insects were provided with only distilled water. All pairwise comparisons of consumption between concentrations were highly significantly different from each other. When moths were fed on 5% sucrose, they consumed 3 times as much as they did on 40%. This is an example of compensatory feeding by which moths consumed a greater volume of solution at a lower sugar concentration than at a higher concentration.

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