

Nutrient and Energy Assays of Larval Provisions and Feces in the Black and Yellow Mud Dauber, *Sceliphron caementarium* (Drury) (Hymenoptera: Sphecidae)

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ABSTRACT: Larval foods (nest cell provisions) and larval feces of the black and yellow mud dauber, *Sceliphron caementarium* (Drury), were assayed for fresh and dry weight, % water, total protein, digestible and indigestible carbohydrates, lipids, ash, and caloric value. Provision and fecal mass dry weights are smaller in *S. caementarium* than in the organ-pipe mud dauber, *Trypoxylon politum*, but caloric values for unashed samples of provisions and feces are similar to published values for *T. politum*. The use of ash-free dry weight is, however, recommended for calculation of caloric values. Protein, lipid, and carbohydrate data are not available for other wasp provisions and feces; corresponding data for foods and feces of final instar house cricket nymphs are briefly reviewed.

Cross et al. (1978) noted that the organ-pipe mud dauber, *Trypoxylon* (*Tripargilum*) *politum*, and many other wasp species (e.g., the black and yellow mud dauber, *Sceliphron caementarium* (Drury)) offer a unique natural system for the determination of energy fluxes within a species. Some of the advantages inherent in these species are: their food is mass-provisioned by the parent and is natural in both kind and amount; it is normally completely consumed; in a healthy cell, it loses no energy through biotic decomposition; the products of egestion and of protein metabolism accumulated during the entire feeding and growth stages are deposited in a single mass inside the cocoon (Cross et al., 1978).

This study was undertaken to quantify selected aspects of larval nourishment in *Sceliphron caementarium*. Cross et al. (1978) report calorimetric measurements of provisions and larval feces of *T. politum*. Similar tests plus biochemical analyses of larval foods are reported here for *S. caementarium*. Larval provisions were quantified for fresh and dry weight, percentage water, caloric content, ash percentage, total protein, digestible and indigestible carbohydrates, and lipids. The larval feces, which are voided as a single mass prior to pupation, were similarly assayed.

S. caementarium nests are constructed of mud in sheltered, dry sites; buildings are frequently used as placement sites. A single nest cell is constructed, provisioned, and sealed by a female before work begins on another nest cell. Typically, more than one nest cell is constructed to form an aggregate nest. Nest cells are stocked with spiders; the prey taxa utilized by *S. caementarium* have been detailed by Rau (1935), Muma and Jeffers (1945), Davis (1963), and Dorris (1970). There is a substantial body of descriptive literature on the nesting biology of *S. caementarium* (Cross et al., 1975; Rau, 1915, 1928; Fabre, 1919; Davis, 1963; Eberhard, 1970; Paetzl, 1974; Schafer, 1949).

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Materials and Methods

Our studies of *S. caementarium* were made during 1980 at Washington University's Tyson Research Station near Eureka, St. Louis County, Missouri. Nests were collected there and at several houses and a barn in Crescent, Missouri. The sites at Tyson Research station were checked daily. Nests were given an identifying number, and the number and condition of the cells were recorded. Once nest cell construction and provisioning activity had ceased, a nest was removed. All cells in the nest were opened, and their contents were numbered and recorded. All spiders from each cell were counted, weighed, and frozen in a scintillation vial. The wasp developmental stage in the cell was noted, and if a larva or prepupa was present, its length was measured. Larval fecal masses were collected from cells containing prepupae and were weighed and frozen. In the laboratory, spider samples and fecal masses were dried to a constant weight in a vacuum desiccator over CaCl_2 , and dry weights were obtained using a Mettler H20T analytical balance.

Total protein content in samples of larval provisions and feces was determined by a trichloroacetic acid precipitation followed by the procedure of Lowry et al. (1951) using crystalline bovine serum albumin (Sigma Chemical Co.) as a standard. Samples were homogenized in 1 ml of 0.1 N NaOH in glass tissue grinders, brought to a volume of 5 ml with 0.1 N NaOH, and centrifuged at $1000 \times g$ for 3 minutes. The supernatant was then diluted 1:2 to 1:8 (depending on sample weight) with distilled water. One-tenth ml of each diluted sample (including BSA solutions) was combined with 0.1 ml 100% TCA and 0.8 ml H_2O to obtain a final concentration of 10% TCA. This was vortexed and refrigerated for 15 minutes, followed by centrifugation for 15 minutes in a Beckman Model TJ-6 centrifuge at $1500 \times g$. The supernatant was decanted, and a colorless precipitate remained on the bottom of the test tube. The pellet was resuspended in 0.9 ml of 0.1 N NaOH with vortexing. This was followed by the procedure of Lowry et al. (1951). Absorbances were read in a Beckman Model 24 Spectrophotometer at 660 nm.

Total lipids in all samples were determined using the Bligh and Dyer (1959) method of extraction. Dried spider and feces samples were weighed and homogenized with glass rods in 0.1 ml of 2:1 chloroform : methanol solvent. Solvent was added (2.9 ml), and the sample vials were placed in a 60°C water bath for 1 hour. The solution was then filtered into a weighed vial using 4.25 cm Whatman #1 filter paper discs. Vials were left overnight for solvent to evaporate and then were reweighed.

Digestible carbohydrate content was determined by the phenol-sulfuric acid method of Dubois et al. (1956). Spider and feces samples were dry weighed and homogenized in 2 ml H_2O in tissue grinders. The final sample volume was raised to 10 ml with H_2O , centrifuged at $1000 \times g$ for 3 minutes and placed in an 80°C water bath for 30 minutes. The supernatant was removed, and the pellet was resuspended to 10 ml with H_2O , homogenized, heated, and centrifuged again. One ml samples of the 2 different supernatants were taken, to which 1 ml of 5% phenol was added with vortexing. Five ml of concentrated H_2SO_4 were added vigorously, and samples were allowed to cool for 30 minutes. A glucose standard solution was used. Absorbances were read in a Perkin Elmer Junior III Spectrophotometer at 490 nm.

To determine indigestible carbohydrate content, samples were treated with 30% KOH, so that proteins, reducing sugars, and some glycogen would be destroyed. Pellets were obtained as described above, treated with 1 ml 30% KOH, and heated in a water bath at 100°C for 30 minutes. Trehalose standards were also treated with 30% KOH. Aliquots of 0.1 ml to 0.3 ml of sample in thick-walled test tubes were treated with 5% phenol and concentrated H₂SO₄ as described above. Absorbances were read at 490 nm.

For calorimetry, samples were dry weighed before being burned in a Parr model 1421 Semimicro Bomb Calorimeter, using the standard procedures outlined in the Parr instruction manual. Platinum fuse wire and sample holder were used; a standard fuse correction of 1.13 was therefore necessary. The calorimeter was standardized using benzoic acid pellets; the energy equivalent of the calorimeter was then calculated using the recommended H value of 6318.

The ash content of spider and feces samples was determined using a Thermolyne Type 1400 muffle furnace. The samples were dry weighed and ashed according to the procedure of Reiners and Reiners (1972).

Results

Data from the protein, lipid, and carbohydrate analyses are presented in Table 1.

Caloric content of 11 unashed samples each of spiders and feces gave mean values of 5142 ± 314 cal/g and 4680 ± 308 cal/g, respectively. The mean ash content of 19 spider samples was $7.8 \pm 2.3\%$ and for 20 feces samples was $7.6 \pm 2.0\%$. Ash content data were used to calculate the caloric values of *S. caementarium* larval provisions and feces in terms of calories/ash-free grams, following the procedure of Hubbell et al. (1965) and Prus (1975). These results are \bar{x} provisions = 5576 cal/ash-free g and \bar{x} feces = 5062 cal/ash-free g.

In a small sample ($n = 6$) of nest cells containing spiders and an egg of *S. caementarium* (all other examined cells contained a larva or prepupa and so a concomitantly reduced mass of spiders), the spiders had a mean fresh weight per nest cell of 315.7 ± 119.2 mg, mean dry weight per nest cell of 138.9 ± 34.1 mg, and, mean per cent water content of $52.6 \pm 11.1\%$.

Discussion

The mean dry weight of provisions per cell and of fecal mass for *S. caementarium* are both smaller than corresponding data recorded for *T. politum* by Cross et al. (1978) (for *T. politum*, \bar{x} provisions = 209.4 ± 10.8 mg; \bar{x} feces = 52.2 ± 2.2 mg). Cross et al. (1978) found, however, that the mean caloric values were 4998.8 ± 40.8 cal/g for provisions and 4637.9 ± 81.7 cal/g for feces; these values are not significantly different from those reported above for unashed provision and feces samples for *S. caementarium*.

Reiners and Reiners (1972) and Cummins and Wuycheck (1971) strongly recommend the muffle-furnace technique for close approximations of absolute ash content; studies by Reiners and Reiners (1972) indicated that ash results based on bomb-combustion data consistently underestimated the muffle-furnace results. The muffle-furnace technique was utilized here, and the results of ashing both spider samples and fecal masses indicated mean per cent ash contents that were very similar and that were greater than 7%. Cross et al. (1978) noted that the

Table 1. The mean percentage protein, lipid, and carbohydrate in the spider prey and larval fecal mass of *S. caementarium*. Sample size in parentheses.

	% of dry spider	% of dry larval fecal mass
Protein	33.4 ± 7.4 (9)	11.0 ± 1.2 (4)
Lipid	25.8 ± 6.1 (21)	11.5 ± 2.4 (17)
Digestible carbohydrate	19.0 ± 7.1 (17)	38.8 ± 10.7 (13)
Undigestible carbohydrate	6.3 ± 2.8 (12)	10.4 ± 0.6 (6)
Unidentified*	15.5	28.3
Totals	100%	100%

* The remaining percentage to make 100% of total dry weight.

burning of *T. politum* larval food (spiders) and feces in a bomb calorimeter produced no significant ash residue. They only employed the muffle-furnace technique to measure ash content in cocoons. Cummins and Wuycheck (1971) pointed out that comparisons of the caloric content of organisms should be made on an ash-free dry weight basis. Cross et al. (1978) did not express the caloric values for *T. politum* larval foods and feces in terms of ash-free dry weight because of their assumption of insignificant ash residue upon bombing the samples. In the present study we, too, observed insignificant ash residue after bomb calorimetry. However, muffle-furnace results showed significant ash content to be present in both food and feces. When these results were used to calculate caloric values for *S. caementarium* larval foods and feces in terms of ash-free dry weight, those values were considerably higher than values determined by us and by Cross et al. (1978) for *T. politum* on simply a dry weight basis.

No data corresponding to the protein, lipid, and carbohydrate analyses reported here are available, to our knowledge, for provisions or feces of other aculeate Hymenoptera. The data of Woodring et al. (1979) on house cricket (*Acheta domestica*) final instar nymphs provide, however, some interesting comparisons. The per cent protein reported here for *S. caementarium* provisions is higher than that reported for laboratory prepared house cricket nymphal food (21.8 ± 0.1%), but the values for per cent protein in *S. caementarium* feces and in house cricket nymphal feces (13.6 ± 0.6%) are very similar. The values reported here for lipids are higher than those reported by Woodring et al. (1979) for house crickets (dry food = 6.2 ± 0.2%; dry feces = 4.8 ± 0.1%). The high lipid percentages in the naturally occurring larval foods of *S. caementarium* may reflect Dadd's discussion of the importance of lipids as a reserve energy source, especially at physiological stages of development preceding nonfeeding periods such as pupation and diapause. The values we determined for per cent digestible carbohydrate and per cent undigestible carbohydrate in *S. caementarium* larval foods were lower than those determined by Woodring et al. (1979) for cricket nymph dry food (\bar{x} digestible = 47.1 ± 1.4%; \bar{x} undigestible = 11.5%). Our result for *S. caementarium* feces for digestible carbohydrate, however, was higher than that for cricket feces (17.6 ± 0.6%), while for undigestible carbohydrate the result was lower than that for cricket feces (20.8%). The fact that some protein, lipid, and especially digestible carbohydrate appeared in *S. caementarium* feces and in house cricket nymphal feces suggests that either some digested material was egested or that digestion of ingested foods is incomplete.

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