Nutrient Content of Brown Marmorated Stink Bug Eggs and Comparisons Between Experimental Uses

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Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug (BMSB), has become a major pest. Seven experiments examined the nutrient content of their eggs in the context of female reproductive investment and use for experiments. Among 542 clusters examined, an average egg contained 23.50 ± 0.561 µg lipid, 3.17 ± 0.089 µg glycogen, and 3.08 ± 0.056 µg sugar. Mature eggs within a female’s ovary can make up 61% of her total lipid, 35% of glycogen, and 20% of sugar levels. Eggs obtained from a colony reared on a steady diet are expected to have consistent nutrient content. The age of a parental female only slightly affected the lipid level of oviposited eggs but did not affect glycogen or sugar levels. However, egg nutrient content can differ substantially by the source of the parental females; wild eggs had higher lipid but lower sugar content than colony-produced eggs. Further, the length of time that eggs are frozen influenced egg nutrient content. Freshly laid eggs had higher lipid and lower sugar levels than eggs frozen for 1 or 2 yr. Whether an egg turned grey following removal from cold storage did not affect nutrient content, nor did being frozen within 1 or 3 d of oviposition. The temperature at which eggs were left exposed did not impact egg nutrient content, but glycogen decreased and sugar increased with deployment time. This information combined with how factors affect host selection by natural enemies will help refine future experiments that use BMSB egg clusters.

Key words: biological control, Halyomorpha halys, lipids, maternal investment, sentinel

Eggs are the starting point of an organism’s life cycle. Understanding the nutrient content of eggs provides information about parental female investment in reproduction, what the progeny are provided with until they can feed, and practical comparisons of egg clusters used in experiments. This paper examines the currently unknown nutrient content of brown marmorated stink bug (BMSB), Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), egg clusters. BMSB is an invasive pest from eastern Asia and a serious pest in many important economic crops in North America and Europe (Rice et al. 2014, Kriticos et al. 2017). BMSB egg clusters are used widely to survey natural enemies in its adventive range (Abram et al. 2017), since egg parasitoids are key factors in limiting BMSB populations in its native range (Yang et al. 2009).

Female BMSB require approximately 2 wk, or 148 DD, to become reproductively mature after eclosion or exiting overwintering sites (Nielsen et al. 2008). BMSB can complete one to two generations per year in temperate and up to five generations in tropical regions (Nielsen et al. 2008, Leskey et al. 2012, Haye et al. 2014). Eggs are light green in color, ~1.3 mm in diameter, and hatch within 4–5 d (Nielsen et al. 2008). Females lay ~28 eggs per cluster with an average 224 eggs over her lifetime. First instar nymphs primarily feed on the egg casing that they hatched from which provides important gut bacteria and do not feed on other foods until molting into the second instar (Bansal et al. 2014, Taylor et al. 2014). Our first two experiments aim to quantify maternal investment and nutrients available to progeny by measuring the lipid, glycogen, and sugar content of 1) oviposited BMSB eggs and 2) previpositional eggs inside a reproductively mature female.

Factors that affect how parental females allocate nutrients to eggs have practical implications for harvesting eggs from a BMSB colony. To date, research on nutrient levels has only been conducted for BMSB adults and nymphs. Wild adults have lower body mass (Funayama 2012), and glycogen and sugar stores following diapause (Skillman 2017). BMSB nymphs that feed on mixed diets develop quickly into adults that have higher nutrient stores than nymphs fed single or suboptimal diets (Acebes-Doria et al. 2016, Acebes-Doria 2016). Clearly seasonality and diet affect nutrient stores in adults, but additional factors likely affect maternal provisioning as well. In the parasitoid wasp, Eupelmus vuilletti (Crowford) (Hymenoptera: Eupelmidae), females produce eggs with 50% more energy content
in the beginning of their reproductive cycle than at the end (Giron and Casas 2003). Our third experiment examines how the age of parental females affects nutrient allocation to her eggs.

Furthermore, understanding the nutrient content of eggs provides a practical evaluation of the quality of eggs used for experimentation. Egg quality may be especially important for egg parasitoids, since the developing parasitoid derives its nutrients only from the host. For example, host eggs with lower protein, glycogen, and triglyceride content produce Trichogramma (Hymenoptera: Trichogrammatidae) wasps with lower nutrient reserves, fecundity and longevity than higher quality host eggs (Kishani Farahani et al. 2016). A review by Abram et al. (2017) revealed that most biological control research has focused on BMSB egg clusters rather than nymphs or adults. BMSB volatile and contact kairomones likely help parasitoids find egg clusters (Hedstrom et al. 2017). Jones et al. (2014) found higher parasitism rates by endemic parasitoids on naturally laid BMSB egg clusters than on fresh colony-reared clusters placed in the field. Subsequent parasitism surveys have used naturally laid egg clusters when available (Dieckhoff et al. 2017, Jones et al. 2017, Zhang et al. 2017). While naturally laid eggs are ideal, it can be challenging to find enough wild eggs, so researchers use sentinel eggs for adequate replication. So, the fourth experiment explores the potential differences in nutrient content between wild-collected and colony-produced eggs. Differences are expected given that colony-reared BMSB are fed the same daily diet, while wild BMSB feed on a range of hosts that vary over time.

Because sentinel BMSB egg clusters are widely used in research, experiments 5–7 explore how differences in the collection, storage, and deployment of egg clusters might influence their nutrient content. Fresh sentinel egg clusters less than 24-h old are ideal because natural enemies would normally encounter fresh eggs (not frozen) and have sufficient time to attack before eggs become unsuitable. For example, the specialist parasitoid Trissolcus japonicus (Ashmead) (Hymenoptera: Scelionidae), oviposits and successfully develops on BMSB eggs less than 3-d old (Qiu et al. 2007). Laboratory studies present these ideal BMSB egg clusters to predators and parasitoids (Arakawa and Namura 2002, Abram et al. 2014, 2015, Hedstrom et al. 2017, Konopka et al. 2017). Unlike laboratory studies that can be staggered over time, field trials often require a large number of clusters at one time and use fresh eggs when fresh eggs are not available (Morrison et al. 2016, Ogburn et al. 2016, Hedstrom et al. 2017). Egg masses frozen within a month are prioritized for field experimentation leaving longer-stored egg masses to accumulate for parasitoid colony maintenance. The fifth experiment explores the difference in nutrient content between freshly laid eggs and sentinel eggs clusters frozen for 1 or 2 yr. A longer freeze duration was tested to establish if any changes occurred. Next, acquiring egg clusters less than 24-h old requires frequent harvesting from the colony, and immediate experimentation if using fresh eggs. This is not always possible, so eggs that are 24- to 72-h or 24- to 48-h old are used (Morrison et al. 2016, Dieckhoff et al. 2017). Additionally, some frozen BMSB egg clusters turn gray within an hour after removal from −80°C storage (VPS pers. obs.). Thus, the sixth experiment examined potential differences in nutrient levels of eggs frozen at less than1- or 3-d old, as well as different colored frozen eggs. Last, sentinel egg clusters are often left in the field for 2–7 d (Jones et al. 2014, Cornelius et al. 2016, Herlihy et al. 2016, Morrison et al. 2016, Ogburn et al. 2016, Dieckhoff et al. 2017, Zhang et al. 2017), where they are exposed to warm to hot temperatures from May to September. If longer exposure times and higher temperatures cause nutrient degradation, experimental protocols might be adjusted to optimize parasitism and predation. The seventh experiment compares the nutrient content of frozen sentinel eggs exposed to varying temperatures over different durations.

In all, these seven experiments address the nutrient content of BMSB eggs; experiment: 1) describes the lipid, glycogen, and sugar content of BMSB eggs; 2) describes content of preovipositional eggs; 3) examines whether nutrient allocation to egg clusters change with maternal age; 4) compares wild-collected eggs and colony-reared eggs; 5) compares freshly laid egg clusters to those that have been frozen; 6) compares age and color of eggs; and 7) compares frozen eggs deployed at different temperatures and durations.

Materials and Methods

We used a wild BMSB colony to harvest egg clusters and not to rear continuous generations. Wild BMSB were collected via beat sheet from May to October 2014 through 2016 from five holly (Ilex aquifolium L. [Aquifoliales: Aquifoliaceae]) sites throughout the Willamette Valley of Oregon. Colonies were maintained at 22°C, 16:8 (L:D) h, 60% RH (Medal et al. 2012). Each 29.5- x 29.5- x 30.5-cm Bug dorm container (BioQuip, Rancho Dominguez, CA) had ~50 adults that were given carrots, jelly beans, raw unsalted peanuts, and a water wick. Eggs were collected daily from Monday to Friday by wetting the cluster, removing it with a spatula, and placing it on filter paper. Egg clusters collected from Tuesday to Friday were less than 1-d old, while Monday egg clusters were less than 3-d old.

Nutrient Bioassay Method

The lipid, glycogen, and total sugar content was measured using a protocol developed for mosquitoes (Van Handel 1985a,b), which has later been adapted for parasitic wasps (Olson et al. 2000), phorid fly (Fadamiro et al., 2005), coccinellids (Seagraves et al. 2011), drosophila fly (Tochen et al. 2016), and BMSB adults (Skillman 2017). Some additional dilution steps were done as described here. Each egg cluster was crushed with a pestle in a 1.5-ml microcentrifuge tube and 110 µl of 2% sodium sulfate. A control that did not include an egg cluster was run during each assay set of 20 clusters. Next, 990 µl of chloroform–methanol (1:2) was added, and tube was centrifuged for 3 min at 13,000 rpm to form a glycogen precipitate at the bottom. The supernatant was transferred into a glass test tube, vortexed, and aliquoted further: 50 µl for the lipid assay and 50 µl for the sugar assay.

For the lipid assay, the supernatant was boiled at 90°C for ~2 min until evaporation. Next, 40 µl of sulfuric acid was added and heated at 90°C for 2 min. Once cooled, 960 µl of vanillin reagent was added, vortexed, and left at room temperature for 20–30 min. Absorbance was read at 525 nm on a spectrophotometer (Ultraspec 3100 pro, Amersham Biosciences, Piscataway, NJ), and lipid content was estimated from the absorbance values of lipid standards made for each vanillin reagent. To calibrate the standard, 0, 1, 5, 10, 35, and 50 µg of canola oil was reacted with vanillin as described earlier, and the relationship between the absorbance value and lipid content was calculated by a linear equation. A similar calibration was done for glycogen and sugar standards. For the glycogen assay, 975 µl of anthrone reagent was added, and the tube was vortexed until the precipitate dissolved. An aliquot of 50 µl of this mixture was transferred to a new tube to which 950 µl anthrone was added. Once vortexed, it was heated at 90°C for 10 min. Once cooled, absorbance was read at 625 nm. For the sugar assay, each tube of 50 µl was heated at 90°C for 1 min leaving ~25 µl of supernatant. Next, 975 µl of anthrone reagent was added, vortexed, and heated at 90°C for 10 min. Absorbance was taken at 625 nm to determine sugar levels.
Because only 50 of 1,000 µl of the supernatant/mixture was used to assess the different nutrient levels (lipid, glycogen, and sugar), the estimate was multiplied by 20 for calculating total levels in an individual BMSB egg cluster.

Egg nutrient content

To document the nutrient range of laid BMSB eggs, 40 egg clusters were collected from the laboratory colony of BMSB. Twenty egg clusters were collected and frozen on both 9 February 2015 and 13 June 2016. All clusters were less than 3-d old. Individual egg clusters were stored at −80°C before each cluster was weighed and the number of eggs counted. Cluster sizes ranged from 19–30 eggs, with an average of 27.5 eggs per cluster.

The average lipid, glycogen, and sugar content with standard errors were reported. Only descriptive statistics were given here, because data were collected to determine nutrient values and not to test specific hypothesis. To standardize data here and in all other sections, the value for each cluster was divided by the number of eggs in the cluster. Because numerous frozen and freshly laid egg clusters were measured in all the experiments, a second average was taken pooling egg data from experiments 1, 3, 4, 5, and 6. The egg data in experiments 2 and 7 were excluded from the pooled analysis because experiment 2 included previpositional eggs and experiment 7 eggs were set out for an extended period.

Preovipositional eggs

In total, 11 and 9 females were picked from the laboratory colony during 2015 and 2016, respectively. They were weighed and thorax width was measured before being dissected for an egg count. Eggs were carefully removed from the female and washed in deionized water. Eggs were dried on filter paper. All eggs and the corresponding female were stored in paired tubes at −80°C until nutrient assays were run. Adult BMSB were assayed with the same methods as described for the egg cluster.

The average standardized lipid, glycogen, and sugar content and standard errors were reported for dissected eggs, female body without eggs, and combined value of a given female and her egg load. Again, only descriptive statistics were given here for recording nutrient values.

Maternal age

To determine the nutrient content of eggs as females aged, wild adult BMSB that recently emerged from overwintering were collected from holly in Monmouth, OR, on 14 May 2015. They were transferred into egg laying arenas made of a 591.5-ml plastic container with a mesh lid. In all, 25 arenas were set up, each having an adult pair, one carrot, three shelled raw peanuts, and water wick. The carrot was changed and water wick resaturated weekly, and the peanuts replaced every few weeks. Arenas were held at ~22°C with ambient light.

Arenas were checked for eggs and mortality of adult BMSB. If the female died, the observation was ended. If the male died, he was replaced with a male from the colony. The arenas were checked daily from 14 May to 25 September 2015 to collect less than 1-d old eggs. After 25 September, arenas were checked only on weekdays due to limited oviposition during winter. When eggs were found, they were collected, counted, and stored at −80°C until nutrient assays were run.

Using a linear regression model, the untransformed lipid, glycogen, or sugar content of eggs was regressed with the days since trial initiation. These analyses and all others were conducted in JMP 11.0.0 (SAS 2015).

Wild versus colony eggs

In total, 17 wild egg clusters were collected during the summer in 2015 and 2016. Egg clusters that fell onto the beat sheet were frozen at −80°C. Clusters ranged from 17 to 29 eggs, with an average of 24.83 eggs per cluster. For the other treatment, 17 clusters were picked from the colony around the same dates that the wild eggs were collected and stored at −80°C. Clusters ranged from 23–33 eggs, with an average of 27.35 per cluster. All the eggs, both wild and colony collected, were of unknown age (<3 d).

The untransformed lipid, glycogen, and sugar content of egg clusters were standardized on a per-egg basis and compared between wild and colony BMSB in three separate t-tests (α = 0.05).

Fresh versus frozen eggs

Although the fresh, frozen 1-yr, and frozen 2-yr egg cluster types were laid by different females, all females were collected from the same five holly sites during late August or September 2014–2016. Fresh egg clusters were collected from the BMSB laboratory colony on 5, 9, and 12 December 2016 just before the bioassay. The 1-yr frozen egg clusters were collected from the wild laboratory colony on 1–29 December 2015, and the 2-yr frozen on 1–30 December 2014. All egg clusters were less than 3-d old, and frozen at −80°C. Thirty egg clusters of each type were compared for a total of 90 egg clusters.

The untransformed lipid, glycogen, and sugar content of egg clusters were standardized on a per-egg basis and compared in separate tests. A one-way ANOVA tested the effect of each treatment, and Tukey’s HSD compared means between the three treatments (α = 0.05).

Color and age of eggs

In total, 20 green egg clusters and 20 gray egg clusters were used. Ten clusters of each color were less than 3-d old and 10 clusters were less than 1-d old. All clusters were collected from the BMSB colony and were laid between July 2014 and September 2015, with a mix of clusters from both years in each treatment.

The untransformed lipid, glycogen, and sugar content of egg clusters were standardized on a per-egg basis and compared with a two-factor ANOVA that tested the effects of egg color, age group, and color × age interactions (α = 0.05).

Temperature and deployment duration

Eggs were held at 22.5°C to represent a warm day or 34°C to represent a hot summer day. The control eggs were not exposed to heat but were collected from the same stock of frozen eggs as those placed into the warm or hot treatments. We tested deployment for 0 (control), 4, and 7 d, as eggs are commonly deployed for 4–7 d in the field. Ten clusters were exposed to each of six treatment combinations: control warm, control hot, 4-d warm, 4-d hot, 7-d warm, and 7-d hot. The experiment was run in November and December in 2016. All clusters used were frozen <1 d of oviposition between May and August 2016 and were stored at −80°C for less than 1 yr. For the warm and hot treatments, an egg cluster adhered to filter paper was paper clipped to the underside of a leaf of a rhododendron in a 3.8-liter pot. Ten egg clusters were paper clipped to each of four plants: five clusters were collected at 4 d and again at 7 d. The warm treatment plants remained on the laboratory bench under ambient temperature. The hot treatment plants were placed into an incubator at 34.5°C. Photoperiod was 16:8 h L:D in both temperature treatments. Later, all the eggs were stored for a few days at −80°C before running nutrient assays.
The untransformed lipid, glycogen, and sugar content of egg clusters were standardized on a per-egg basis and compared in separate tests. Egg clusters from both November and December trials were pooled together for analysis. A two-factor ANOVA tested the effects of temperature, deployment duration, and temperature-deployment interactions, and Tukey’s HSD compared means between deployment durations (α = 0.05).

### Results

#### Egg nutrient content

Eggs have higher lipid content. The concentration of lipid was seven to eight times higher than glycogen or sugar among a sample of 40 and 542 egg clusters (Table 1). The majority of these eggs were harvested from a colony of wild-collected BMSB adults. Egg clusters from the first group of 40 clusters weighed on average 37.3 mg.

#### Preovipositional eggs

A reproductively active female had an average of 22.25 eggs and between 10 and 28 eggs in her ovary. Likewise, the concentration of lipids was approximately seven times higher than glycogen or sugar (Table 1). Egg nutrients comprise 37–70% of total lipid, 22–63% of total glycogen, and 7–41% of total sugar content within a reproductively active female.

#### Maternal age

In total, 340 egg clusters were laid during the experiment, with an average of 11.3 clusters laid in captivity per wild-collected female, ranging from 6 to 20 egg clusters per female. An average egg contained 22.53 ± 0.82 µg lipid, 3.29 ± 0.13 µg glycogen, and 2.78 ± 0.04 µg sugar. Overall, nutrient content of eggs laid did not vary substantially as females aged. Lipid levels of eggs increased slightly, but the relationship was weak (lipid/egg = 19.1 + 0.067*days, r² = 0.02, Table 2, Fig. 1). Interestingly, one female survived a year and laid eggs throughout her whole life span. Her final egg cluster was laid 245 days into the experiment.

#### Wild versus colony eggs

Lipid and sugar content per egg were also different between wild and colony eggs, but glycogen content was similar (Table 2, Fig. 2). Wild eggs had more lipids, while colony eggs had more sugar.

### Fresh versus frozen eggs

There were some differences observed between fresh, frozen 1-yr and frozen 2-yr eggs for lipid and sugar levels, but glycogen was similar (Table 2, Fig. 3). For lipid levels, fresh eggs had the highest levels, while both frozen eggs were lower. For sugar, frozen 2-yr eggs were highest, while fresh and frozen 1-yr eggs were lower.

### Color and age of eggs

There was no difference between the average lipid, glycogen, and sugar content per egg for any of the four egg types (Table 3, Fig. 4).

### Temperature and deployment duration

Temperature and duration × temperature interaction did not affect the lipid, glycogen, and sugar content per egg (Table 3, Fig. 5). However, duration length significantly affected glycogen and sugar levels. We observed a significant increase in sugar and a decrease in glycogen content of eggs deployed for 4 or 7 d compared to the control.

### Discussion

The nutrient profile of BMSB eggs and the factors that influence it contribute to our knowledge of BMSB biology. BMSB eggs provide offspring with an average of 23.50 ± 0.561 µg lipid, 3.17 ± 0.089 µg glycogen, and 3.08 ± 0.056 µg sugar in nutrient reserves that are crucial for their development. This requires a major investment by adult females; preovipositional eggs can comprise 61% of the total lipid content within a female, 35% of total glycogen, and 20% of total sugar. Studies that monitor nutrient levels of adult females should record the egg load of females if eggs are included in the whole-body nutrient assay or exclude eggs entirely from the nutrient assay. The second approach is time consuming, because eggs must be removed gently to prevent rupturing and body remnants must be carefully separated from preovipositional eggs. Much like adult BMSB, eggs have higher lipid content than glycogen or sugar (Skillman 2017). Lipid levels were seven to eight times higher than glycogen and sugar, suggesting that lipids are a critical nutrient for adult females during egg production. Because BMSB eggs are primarily composed of lipids, they are also an energy-rich resource for predators and parasitoids.

In some insects, maternal age influences egg production. As female cowpea weevils, Callosobruchus maculatus Fabricius
(Coleoptera: Chrysomelidae), the egg size and hatchability decrease (Fox 1993). In addition, the age of the maternal grandmother can influence offspring in *Drosophila serrata* Malloch (Diptera: Drosophilidae) (Hercus and Hoffmann 2000). Older mothers produce female offspring, which later produce eggs with lowered hatchability. In contrast, the duration of wild overwintered BMSB females held in captivity had weak to no correlation with her egg nutrient levels. Thus, a colony of spring-collected BMSB fed a constant diet is expected to produce eggs of consistent nutrient content regardless of maternal age.

Unlike maternal age, the source of BMSB egg clusters did influence their nutrient content. The lipid and sugar levels varied between wild and colony eggs, which may have been due to the diet of the parental females. The diet of the squinting bush brown, *Bicyclus anynana* Balter (Lepidoptera: Satyridae), influences its reproductive output, egg composition, and egg hatchability (Geister et al. 2008). In our experiment, the diet history of the wild females was unknown except for being collected off of holly, a known BMSB host plant. In contrast, the colony females were supplied a constant diet of jelly beans, carrots, and peanuts. Jelly beans are high in sugar, which may explain why the colony-produced eggs contained more sugar than wild-collected eggs.

Next, additional comparisons were done with frozen sentinel eggs because they are convenient to acquire and eliminate the risk of pest eggs hatching in grower fields. Also, frozen BMSB eggs can be advantageous for surveying parasitoid species in the field. Previously, native parasitoids in Maryland emerged at higher rates from frozen than fresh sentinel eggs, while the specialized parasitoid *T. japonicus* emerged at similar rates from both kinds of eggs (Herlihy et al. 2016). In our experiment, freezing eggs and the duration of cold storage affected nutrient levels. An 11.6% and 10.6% decrease in lipid levels was observed in frozen 1- and 2-yr old eggs compared to fresh eggs, respectively. This decline might be due to lipid oxidation, which has been noted in frozen meat, leading to loss of nutrient value, change in color, and the production of toxins (Soyer et al. 2010). Declining lipid levels have also been observed in human breastmilk frozen for 90 d (García-Lara et al. 2012). Next, sugar levels increased the longer the BMSB eggs were frozen, a 17.8% and 53.2% increase was observed in frozen 1- and 2-yr old eggs compared to fresh eggs, respectively. One freeze–thaw cycle of human serum increased glucose levels by 14% (Flood et al. 2002). Overall, freezing BMSB eggs can influence lipid and sugar composition, with potentially more changes the longer the egg clusters are frozen.

Some frozen BMSB sentinel egg clusters turned from their original pale green to gray after removal from the −80°C freezer. However, color change did not influence nutrient content, whether it occurred among eggs stored frozen ≤1 or ≤3 d of being laid. This suggests that color change is not associated with nutrient degradation, which

Table 2. Statistical results of nutrient content per egg with a linear regression for Experiment 3 on maternal age, *t*-test for Experiment 4 on wild and colony eggs, and ANOVA for Experiment 5 on fresh and frozen eggs

| Experiment | Nutrient | df |  F or t  |  P  | r² |
|------------|---------|----|---------|----|----|
| 3-Maternal age | Lipid  | 1, 338 | 6.887  | 0.009 | 0.020 |
|             | Glycogen | 1, 338 | 0.623  | 0.430 | – |
|             | Sugar   | 1, 338 | 0.789  | 0.375 | – |
| 4-Wild vs colony | Lipid  | 32  | 5.57  | 0.000 | – |
|             | Glycogen | 32  | 0.23  | 0.817 | – |
|             | Sugar   | 32  | 2.56  | 0.015 | – |
| 5-Fresh vs frozen | Lipid  | 2, 57  | 4.41  | 0.017 | – |
|             | Glycogen | 2, 57  | 1.26  | 0.290 | – |
|             | Sugar   | 2, 57  | 15.74 | 0.000 | – |

Fig. 1. Lipid content of BMSB eggs by days since trial initiation in Experiment 3. Each egg cluster is represented by one point.

Fig. 2. Lipid (a), glycogen (b), and sugar content (c) of BMSB eggs from the wild and colony sources in Experiment 4; average ± SE as gray diamond and bars. Asterisk indicates a difference by *t*-test.
was an initial concern when we noticed this during field studies. The reason for the color change remains unknown. Our results suggest that eggs harvested from a colony every 2–3 d have a similar nutrient content to eggs harvested daily. Although nutrient content did not differ, such eggs could differ in attractiveness and suitability for parasitoids.

The temperature of deployment (22.5 or 34.5°C) in our experiment did not impact nutrient content of previously frozen BMSB eggs, even though high temperatures are known to degrade nutritional value of fruits and vegetables (Barrett 2007). However, temperature does impact development of BMSB eggs and the parasitoids within them. BMSB eggs can develop between 15 and 33°C, with no development above 35°C (Nielsen et al. 2008). Three Trissolcus spp. parasitoids of BMSB (Hymenoptera: Scelionidae) develop successfully at 17–27°C, which is a smaller window within the developmental range of BMSB (Arakawa and Namura 2002).

The deployment time of eggs impacted their nutrient content. Glycogen content in deployed eggs decreased by 17.6 and 33.4% at 4 and 7 d, respectively, compared to nondeployed eggs. Concurrently, there was an 82.2 and 77.5% increase in sugar levels in 4 and 7 d deployed eggs, respectively, compared to non-deployed eggs. These trends might suggest that the glycogen in the eggs were breaking down into sugar; glycogen is a storage form of glucose (Arrese and Soulages 2010). More studies with varying exposure times should be conducted with eggs collected from multiple parental female stocks to ensure that such trends are consistent. Whether such changes in nutrient content of eggs affects parasitoid development or host finding...
is unknown. In this experiment and egg freezing experiment, lipid levels declined by 11%, glycerogen levels declined by 18–33%, and sugar levels increased by 18–82%. In another egg-parasitoid system, a ~37% decline in protein and ~22% decline in triglyceride levels in host eggs was associated with decreased parasitoid fecundity and longevity (Kishani Farahani et al. 2016). Nevertheless, future experiments using frozen clusters should keep deployment times consistent across treatments in case it will affect natural enemy performance.

In summary, we provide a baseline measurement of lipid, glyco- gen, and sugar content of a BMSB egg and demonstrate that eggs comprise a substantial portion of the nutrient levels in reproductive females. Subsequent studies showed that the nutrient levels of eggs did not appear to be affected by maternal age when the female was given a constant diet, by color change, by egg age (within 3 d) at the time of freezing, or temperature during deployment. While no changes in nutrient composition were observed with these comparisons, it is still important to consider whether these eggs will be equally attractive and suitable to parasitoids in studies. Nutrient content of eggs were affected by the source of BMSB that produced eggs, deployment time, and whether the egg clusters were fresh or frozen for 1–2 yr. Given that nutrient levels differed in these comparisons, future experiments should use egg clusters that are consistent in source, fresh/frozen status, and deployment times across treatments whenever possible. Future studies could examine whether nutritional differences at these magnitudes has any effect on parasitoid host selection and fitness.

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