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Effect of *Ephestia kuehniella* (Lepidoptera: Pyralidae) Larval Diet on Egg Quality and Parasitism by *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae)

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Abstract

Trichogramma spp., among the most common parasitoids used for augmentation biological control, often are mass-reared on eggs of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller). To evaluate removal of nutritional components from the *E. kuehniella* larval diet and reduce production costs, colonies were maintained using one of three diets: a standard diet consisting of eight ingredients, a reduced diet containing whole wheat flour, glycerol, and Brewer's yeast, or a third minimal diet of only whole wheat flour. The standard diet sustained the fastest larval development, female pupae with the greatest mass, the highest level of adult emergence, and production of the most eggs per female. Eggs from moths reared as larvae on the standard or reduced diet had equivalent mass, length, and percent hatch. Females from larvae fed the minimal diet produced eggs with the least mass that were shorter and had the lowest percent hatch. Eggs from the three *E. kuehniella* colonies were exposed separately to *Trichogramma brassicae* Bezdenko females to determine their acceptance for oviposition. More of the eggs from the standard diet were parasitized by the females, eggs from the reduced and minimal diets being less acceptable. The percent emergence of the parasitoids was the same regardless of diet; however, the largest wasps emerged from the standard diet eggs and a greater proportion of them were females. Consequently, the standard *E. kuehniella* larval diet resulted in the highest rate of reproduction and robust eggs that produced superior *T. brassicae* wasps.

Key words: Mediterranean flour moth, egg parasitoid, insect mass rearing

Wasps in the genus *Trichogramma* are probably the most commonly mass-produced and released parasitoids of insects, primarily because of their ability to parasitize a wide range of Lepidoptera. *Trichogramma* spp. have been used for more than 100 yr as biological control agents of lepidopteran pests (Lundgren et al. 2002, Thomson and Hoffmann 2002, Luck and Forster 2003, Cônsoli et al. 2010, Wang et al. 2014). During this time, *Trichogramma* spp. have been released against at least 28 pest species on 20 crops, including cereals, vegetables, and trees, in more than 50 countries and on more than 32 million ha each year (Hassan 1993, Li 1994, Smith 1996, Sherif et al. 2008, Wu et al. 2015). There are more than 200 species of *Trichogramma*, but only 19 have been mass-reared and used effectively for biological control (Cônsoli et al. 2010). Substantial production of *Trichogramma* spp. has occurred in France, the Netherlands, Switzerland, Germany, the United States, Canada, Mexico, China, and Russia, with lesser facilities in South and Central America, Australia, southern and eastern Europe, South Africa, India, and southern Asia (Smith 1996, Davies et al. 2011).

The most common host eggs for rearing different *Trichogramma* spp. are from the Mediterranean flour moth, *Ephestia kuehniella* (Zeller), often referred to as *Anagasta kuehniella* (Zeller); rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae); and the Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) (Nagaraja 2013). The suitability of these hosts for rearing *Trichogramma* spp. depends on many factors, including characteristics of the host eggs, ease of mass rearing, storage capability, high parasitoid production (St-Onge et al. 2016), and cost (Laing and Eden 1990). Mass rearing of hosts to produce *Trichogramma* spp. tends to be expensive and labor-intensive (Wang et al. 2014). However, the Mediterranean flour moth can be reared on simple and inexpensive diets, such as wheat flour and many other foods (Stein and Parra 1987, Rodriguez-Menendez et al. 1988, Magrini et al. 1995, Filho et al. 2001, Ci et al. 2005, Locatelli et al. 2008). In an attempt to reduce the cost of host production, larvae of the rice moth have been reared on relatively inexpensive cereals, such as finger millet, *Eleusine coracana* L. Gaertn; soft white wheat, *Triticum aestivum* L.; short-grained white rice, *Oryza sativa* L.; and durra sorghum, *Sorghum bicolor* L. (Nathan et al. 2006). Parasitism of *C. cephalonica* eggs by *T. chilonis* did not vary due to larval diet but significantly more parasitoids survived to emerge and lived longer when the eggs were produced by moths derived from larvae fed finger millet. Since there is no yolk in the eggs of many parasitoid wasps, such as *Trichogramma* spp., the host eggs must provide all the essential nutrients for wasp embryonic and larval development (Farahani et al. 2016). Also, the host must have a high rate of reproduction with a short life cycle (El-wakeil 2007, Nagaraja 2013).

The challenge for mass-producing parasitoids, such as *Trichogramma brassicae* Bezdenko, is to maintain wasp quality while reducing production resources and associated costs (Leppla 2003). In a tritrophic system involving an artificial diet, insect host, and parasitoid, the first step is to formulate an affordable larval diet and design an efficient rearing system for the host. The host stage that is utilized for production must be acceptable to the parasitoid for oviposition, sustain larval development, and yield effective adults. Required characteristics of the parasitoid females include adequate size, fecundity, longevity, and level of parasitism (Williams and Leppla 1992). Postproduction quality control guidelines based on these characteristics have been developed for at least 13 hymenopteran parasitoids, including three *Trichogramma* spp. (van Lenteren 2003). These guidelines and field evaluations of target pest populations help to assure that the parasitoids are effective when released (Bigler et al. 1994, Penn et al. 1998, Liu and Smith 2000, Hassan and Zhang 2001, Thomson and Hoffmann 2002). The objective of this research was to determine if increasingly less complex and expensive *E. kuehniella* larval diets could be used to produce viable adults that oviposit a high number of eggs that are acceptable for parasitism by *T. brassicae* females and produce high-quality parasitoids.

Materials and Methods

Rearing *Ephestia kuehniella*

Ephestia kuehniella eggs were obtained from a colony that had been maintained for several years at the USDA, Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology in Gainesville, FL using modified rearing methods for stored-product insects (Davis and Bry 1985). The 24-h-old eggs were placed in each of four 13.5 cm long × 7.5 cm wide × 9.5 cm high standard plastic rearing containers with 400 eggs and 400 g of diet in each container. The infested containers were covered with plastic mesh lids and held in a reach-in growth chamber (Percival Model I-36VL) at 26 ± 1°C, 50 ± 5% RH, and 16:8 (L:D) h. After the larvae pupated and adults emerged in the containers, 200–400 1-d-old moths were transferred to a 15 cm diameter × 13.7 cm high polypropylene funnel covered with plastic mesh over the mouth and held in the growth chamber for mating and oviposition. The first generation eggs traveled down the neck of the funnel and were collected on wax paper. After 24 h, the eggs were poured onto filter paper to separate them from the moth scales and used to establish two new colonies with the different larval diets.

The colonies were maintained on either a standard or reduced diet, and eventually a minimal larval diet (Table 1). The respective diets contained 10.98, 12.20, and 12.58% crude protein; 5.80, 2.29, and 1.73% lipid; and 83.77, 89.23, and 90.63% dry matter. Protein was measured by the Kjeldahl method (AOAC 920.87), lipid by the Soxhlet method (AOAC 920.39), and dry matter by the standard method (AOAC 942.05) (Helrich 1990). The standard diet was prepared by mixing the dry and wet ingredients separately before combining them, and then the honey and glycerin were premixed and added (Davis and Bry 1985). The finished diet was covered with paper toweling and air-dried in the laboratory for 4 d, ground with a hammer mill, and stored for 5 d in a freezer at −18°C. A second colony was maintained on a previously used and less complex larval diet (Cox et al. 1984). The reduced diet was prepared by mixing glycerin thoroughly with wheat flour and yeast. After three generations, there were no significant differences in some of the rearing parameters for the first two colonies, so a third colony was added and maintained on a minimal diet consisting of only whole wheat flour. *Ephestia kuehniella* is a major pest in flour mills (Trematerra and Gentile 2010). Fresh diets were prepared for each generation and placed in the standard plastic rearing containers. Initially, separate colonies were established for the standard and reduced diets by sprinkling 100 eggs on each diet in three larval rearing containers per colony. Eggs for the minimal diet colony were derived from the third generation of the colony reared on the reduced larval diet, 100 eggs being added to each of three larval rearing containers. Larvae from the reduced diet eggs would undergo less change in diet ingredients than larvae from standard diet eggs. Thus, the standard and reduced diet colonies were reared for seven generations and the minimal diet colony was limited to four generations.

Life History of *Ephestia kuehniella*

The life history parameters determined for each of the three *E. kuehniella* colonies were as follows: immature development time

Table 1. The ingredients and percentage by weight of the standard, reduced, or minimal larval diet for rearing *Ephestia kuehniella* larvae[a]

Ingredients[b]	Grams	%
Standard diet		
Laboratory rat chow	930	9.77
Rolled oats	382	3.98
Toasted wheat germ	240	2.50
Brewer's yeast	487	5.07
White cornmeal	2,497	25.99
Whole wheat flour	2,220	23.11
Honey	1,500	15.62
Glycerol	1,350	14.06
Reduced diet		
Whole wheat flour	348	87
Glycerol	40	10
Brewer's yeast	12	3
Minimal diet		
Whole wheat flour	400	100

[a]The standard diet was developed to maintain research colonies (Davis and Bry 1985), as was the reduced diet (Cox et al. 1984). The whole wheat flour diet was considered minimal but adequate because *E. kuehniella* is a major pest in flour mills (Trematerra and Gentile 2010).

[b]Purina Rodent Laboratory Chow (http://multipurina.ca/en/rodents/products), Quaker Old Fashioned Oats (http://www.quakeroats.com), Kretschmer Toasted Wheat Germ (http://www.kretschmer.com/wheatgerm-products), MP Biomedicals Brewer's Yeast (http://www.mpbio.com), white cornmeal (http://www.quakeroats.com), organic whole wheat pastry flour (http://www.bobsredmill.com), honey (http://www.publix.com), Glycerol, Anhydrous, J.T.Baker (https://www.fishersci.com). Glycerin from Bulk Apothecary ($1.46 per lb) was used for the cost calculations (https://www.bulkapothecary.com/).

(days), female pupal mass (mg), adult emergence (%), female longevity (days), eggs oviposited per female (n), mass of eggs (µg), egg length (mm), egg width (mm), and egg hatch (%). The three containers for each new larval diet colony were held in the same growth chamber as the original colony. Immature development time was measured by recording days from egg hatch to adult emergence. The mass of 10 randomly selected female pupae per container from each colony and generation was determined with an electronic balance (Mettler Toledo, AL204 Analytical Balance). Adult emergence was recorded every morning. Female and male longevity was from emergence to the last day the moth survived. To measure fecundity, 20 pairs of 1-d-old male and female pupae were transferred randomly from each colony into 20 separate 115 ml funnels (Thermo Scientific Nalgene Polypropylene Powder Funnel) with the mouth of each covered with plastic mesh. A 100 mm × 15 mm Petri dish bottom lined with wax paper was positioned under the neck of each funnel to collect the eggs. The wax paper supporting the eggs was removed and replaced daily and the less than 24-h-old eggs were poured onto filter paper and counted using a stereomicroscope (Leica Microsystems, Inc. Buffalo Grove, IL). Egg quality was assessed based on the mass, size, and percent hatch of eggs oviposited by females from the three colonies. Six samples of 100 eggs from each container and colony were weighed individually with an electronic balance (Mettler XP6 Microbalance). To measure egg length and width, a small wet brush was used to stick 20 randomly sampled eggs per colony and generation onto a piece of cellophane tape that was transferred to a microscope slide. Images were obtained with a dissecting microscope (MZ125, Leica Microsystems) fitted with a camera (KY-F70B, JVC Americas Corp., NJ) and stored using commercial software (Auto-Montage v.5.02.0096, Synoptics Ltd., United Kingdom). The percentage of eggs that hatched was recorded for 20 randomly sampled eggs from each container and colony. The *E. kuehniella* life history data for percent adult emergence and percent egg hatch was arc-sin transformed for statistical analysis and means were compared using analysis of variance (PROC GLM, SAS 9.4) and Tukey-HSD.

Rearing of *Trichogramma brassicae*

A *T. brassicae* colony was established at the University of Florida using several shipments of parasitized *E. kuehniella* eggs provided by the Beneficial Insectary, Inc. (Redding, CA). This stock colony was maintained in the growth chamber for about 1 mo by exposing the wasps to *E. kuehniella* eggs from the standard diet colony. The host eggs were attached to 5 cm × 1 cm pieces of filter paper using 20% honey solution. Each standard egg card supporting 200–400 eggs was placed into an individual 13 mm × 100 mm glass tube and exposed to 20–40 newly emerged wasps for 6–7 h. After 4 d, the eggs became black as the parasitoids developed. The emerged host larvae were removed daily to prevent them from feeding on the remaining eggs.

Life History of *Trichogramma brassicae*

Eggs from the second *E. kuehniella* generation reared on the standard and reduced diets were used to initiate the *T. brassicae* life history study. Individual *T. brassicae* females were exposed to 24-h-old *E. kuehniella* eggs derived from each of the two larval diet colonies to obtain data from subsequent generations. Female wasps were placed into individual 10 cm high × 1.5 cm diameter glass tubes each containing a standard egg card with 70 host eggs. The tubes were closed with cotton plugs and placed in the growth chamber. After a 24-h exposure to *T. brassicae*, the host egg cards were removed and placed into separate fresh tubes. Each *T. brassicae* female was provided with a second egg card to determine the percent parasitism of 140 eggs. The number of parasitized eggs and male and female wasps that emerged were recorded from each colony, including the minimal diet colony beginning in the fourth *E. kuehniella* generation. The size of the wasps was determined by measuring the length of the left hind tibia of 10 randomly selected females newly emerged from eggs derived from each colony. Images were obtained with the dissecting microscope used to measure the *E. kuehniella* eggs. The *T. brassicae* life history data for percent emergence and percent females was arcsin transformed for statistical analysis and means were compared using analysis of variance (PROC GLM, SAS 9.4) and Tukey-HSD.

Results

Life History of *Ephestia kuehniella*

Immature *E. kuehniella* reared on the standard larval diet developed significantly faster than those fed the reduced or minimal diets (Table 2). The development time for immatures reared on the minimal diet increased significantly during generations 6–7. The mass of female *E. kuehniella* pupae from larvae reared on the standard diet was significantly greater than the mass of pupae from the reduced diet in generations 2–3, and for both of the other two diets in generations 4–7 (Table 2). Additionally, for generations 4–7, female pupae from the reduced diet had a greater mass than those produced on the minimal diet. The mass of female pupae from the standard diet increased significantly in the fourth generation from 26.4 ± 0.3 to 29.0 ± 0.2 mg. The percent emergence of adult *E. kuehniella* from larvae reared on the standard, reduced, or minimal diet decreased significantly in that order for all generations. By generation 7, females from the standard diet lived for significantly fewer days than females from the reduced and minimal diets. The standard diet produced significantly more eggs per female than the reduced diet, except for the first generation. The lowest number of eggs per female was produced by the minimal diet colony. The number of eggs peaked in generation 4 before decreasing significantly by generation 7 for females from all three diets.

The mass of *E. kuehniella* eggs from the standard diet was significantly greater than eggs from the minimal diet but not the reduced diet in generations 4–7 (Table 3). The reduced diet egg mass was not different than the mass of minimal diet eggs. Eggs from the standard diet were not significantly longer than eggs from the reduced diet after generations 4 but were longer than eggs from the minimal diet. The width of eggs was equivalent for the three diets. The percentage of egg hatch did not differ significantly from standard and reduced diets for all generations; however, it was greater for the standard diet than the minimal diet in generations 4–7. Also for these generations, there was no significant difference in the percent hatch of eggs from the reduced and minimal diets. Egg mass, length, and hatch did not vary significantly within diets for all generations, but egg width was variable for the standard and reduced diets.

Life History of *Trichogramma brassicae*

The number of *E. kuehniella* eggs from the standard diet that were parasitized by *T. brassicae* females increased significantly in generation 5 and was greater than the other two diets in generation 7 (Table 4; Fig. 1). There was no change in the number of parasitized eggs for the reduced and minimal diets during generations 4–7. After generation 4, wasps emerged from about 95% of the parasitized eggs regardless of *E. kuehniella* larval diet. Female wasps from the standard diet eggs had a significantly longer average tibia length

Table 2. Life history of *Ephestia kuehniella* larvae, pupae, and adults produced using three larval diets

Diet	Generation[a]							
	1	2	3	4	5	6	7	*F*
				Immature development time (days)				
Standard	39.3 ± 0.1bAB	39.0 ± 0.3bABC	39.8 ± 0.1bA	39.5 ± 0.2cAB	38.9 ± 0.3cBC	38.2 ± 0.0cC	39.3 ± 0.3cAB	6.9
Reduced	41.7 ± 0.0aABC	40.5 ± 0.4aC	42.0 ± 0.4aAB	42.8 ± 0.1bA	42.8 ± 0.3bA	41.6 ± 0.1bABC	41.1 ± 0.4bBC	9.5
Minimal	—	—	—	51.1 ± 0.6aB	49.9 ± 0.1aB	53.5 ± 0.2aA	53.3 ± 0.1aA	28.7
F-value	455.9	11.1	39.8	250.1	471.2	6217.3	812.9	
				Female pupal mass (mg)				
Standard	24.8 ± 0.2C	25.9 ± 0.3aBC	26.4 ± 0.3aB	29.0 ± 0.2aA	29.0 ± 0.3aA	28.8 ± 0.4aA	28.9 ± 0.3aA	36.1
Reduced	24.3 ± 0.2C	24.2 ± 0.3 bC	25.4 ± 0.3bB	25.4 ± 0.3bB	26.6 ± 0.2bA	26.6 ± 0.3bA	26.2 ± 0.2bAB	15.8
Minimal	—	—	—	24.1 ± 0.3cC	25.7 ± 0.2cA	24.2 ± 0.2cBC	25.1 ± 0.3cAB	7.8
F-value	3.2	17.7	6.2	80.4	41.4	51.5	50.9	
				Adult emergence (%)				
Standard	84.0 ± 1.9aAB	82.5 ± 1.6aB	84.8 ± 1.7aAB	91.8 ± 1.5aA	86.0 ± 1.1aAB	83.0 ± 1.1aAB	87.3 ± 3.6aAB	2.7
Reduced	73.3 ± 2.2bAB	72.8 ± 1.7bB	73.8 ± 1.1bAB	79.0 ± 1.3bA	76.5 ± 0.7bAB	71.3 ± 0.5bB	73.5 ± 1.0bAB	3.9
Minimal	—	—	—	68.5 ± 1.9cA	68.3 ± 2.1cA	65.8 ± 1.0cAB	61.5 ± 0.9cB	4.3
F-value	14.0	18.5	30.6	55.6	38.5	94.7	34.6	
				Female longevity (days)				
Standard	8.3 ± 0.2A	8.0 ± 0.2AB	7.8 ± 0.2AB	7.3 ± 0.2bBC	6.9 ± 0.2bC	7.8 ± 0.1bAB	8.4 ± 0.2bA	9.1
Reduced	8.7 ± 0.1AB	8.6 ± 0.3AB	8.1 ± 0.3BC	8.1 ± 0.3abBC	7.5 ± 0.2bC	8.8 ± 0.3aAB	9.6 ± 0.3aA	6.2
Minimal	—	—	—	8.9 ± 0.6aA	9.0 ± 0.4aA	8.6 ± 0.3aA	10.0 ± 0.3aA	2.2
F-value	3.3	3.3	0.6	4.0	16.3	5.0	9.1	
				Eggs per female (n)				
Standard	279.5 ± 8.3D	295.9 ± 6.5aDC	321.2 ± 8.6aABC	351.5 ± 8.8aA	348.7 ± 8.9aAB	326.6 ± 10.0aABC	312.2 ± 10.1aBCD	8.9
Reduced	264.5 ± 9.7AB	265.1 ± 5.9bAB	277.3 ± 8.4bAB	287.2 ± 10.8bA	266.8 ± 6.8bAB	263.3 ± 5.2bAB	249.6 ± 10.6bB	1.9
Minimal	—	—	—	240.9 ± 8.2cA	239.0 ± 9.0cA	225.8 ± 7.9cAB	210.9 ± 5.0cB	3.3
F-value	1.4	12.2	13.4	35.5	47.7	41.1	32.8	

[a]For each life history parameter, means (±SE) in columns followed by different lowercase letters and in rows followed by different capital letters are significantly different ($P < 0.05$).

of 0.150 ± 0.002 mm (mean ± SE) in generation 7 compared with wasps from reduced diet eggs (0.145 ± 0.001 mm) and minimal diet eggs (0.145 ± 0.002 mm). The sex ratio of wasps from the standard diet eggs was not statistically different from wasps produced by the reduced diet eggs but the proportion of females was greater than for the minimal diet eggs. The proportion of female wasps that emerged from eggs produced by the standard, reduced, and minimal diets remained consistent within diets across generations.

Discussion

Ephestia kuehniella were reared successfully for multiple generations on a standard, reduced, or minimal larval diet; however, the immatures developed fastest on the standard diet, the female pupae had the greatest mass, and more adults emerged. Previously, larvae reared on durum wheat flour compared to soft wheat flour also had a shorter development time and the adults produced more progeny (Locatelli et al. 2008). This was attributed to a higher protein content and quantity of water-soluble and lipo-soluble vitamins in the durum wheat flour-based diet. Larvae of the closely related almond moth, *Cadra cautella* (Walker), also exhibited superior development on high-protein diets (LeCato 1976, Allotey and Goswami 1990). In our study, *E. kuehniella* females from the standard diet had a shorter life span, but oviposited more eggs that had a somewhat greater mass and length, and a higher percent hatch. It has been determined that larval feeding is prolonged if diets have lower protein and carbohydrate content, and the adults produce fewer eggs (Ayvaz and Karabörklü 2008). We determined that the number, mass, and length of eggs, and percent hatch, were intermediate for the reduced diet and lowest for the minimal diet. A high level of fecundity of *E. kuehniella* moths also was observed if larvae were fed a nutritionally rich diet containing 43.5% maize meal, 43.5% whole meal wheat flour, 10% glycerin, and 3.0% yeast (Xu et al. 2007). An *E. kuehniella* larval diet enriched with yellow maize also supported greater fecundity (Magrini et al. 1995). In our study, significantly increasing the lipid in the larval diet was correlated with a higher rate of reproduction in *E. kuehniella* and their eggs were larger and more viable.

The *T. brassicae* oviposited more eggs per female in the largest eggs that were from *E. kuehniella* reared on the standard larval diet. *Trichogramma* spp. assess host eggs based not only on size but also shape, color, age, chorion thickness, nutritional content, and previous parasitism (Schmidt and Smith 1986, Bai et al. 1992, Schmidt 1994, Nurundah et al. 1999, Roriz et al. 2006). In our study, wasps emerged from about 95% of the parasitized eggs produced by all three larval diets but the standard diet eggs yielded a higher proportion of females. It is known that more and larger *Trichogramma* spp. wasps emerge from larger more nutritious host eggs and a greater proportion of them are females (Bigler et al. 1987, Luck et al. 2001, Pratissoli et al. 2004, Farahani et al. 2016). Thus, the mean *T. brassicae* female:male sex ratio of 69:31 could have been due to oviposition of more fertilized female eggs in the largest *E. kuehniella* eggs from the standard diet. Also, the size and number of progeny could have been affected by different nutritional or environmental conditions of the larvae or pupae. A sex ratio of 56:44 has been reported for commercial *T. brassicae* (Heimpel and Lundgren 2000).

The relative costs of the standard, reduced, and minimal *E. kuehniella* larval diets were based on ingredient prices in Karaj, Iran during May 2019. These prices are unrealistic for mass rearing but suitable for comparing the costs of the three experimental diets.

Table 3. Characteristics of *Ephestia kuehniella* eggs produced by females from larvae reared on three diets

Diet	Generation[a]							
	1	2	3	4	5	6	7	*F*
				Mass of eggs (µg)				
Standard	26.9 ± 0.2A	26.9 ± 0.3A	27.3 ± 0.2A	27.5 ± 0.1aA	27.5 ± 0.1aA	27.1 ± 0.2aA	27.3 ± 0.2aA	1.5
Reduced	26.4 ± 0.4A	26.4 ± 0.3A	27.0 ± 0.2A	26.9 ± 0.2 abA	26.9 ± 0.3abA	26.5 ± 0.1abA	26.8 ± 0.1 abA	1.1
Minimal	—	—	—	26.5 ± 0.2bA	26.3 ± 0.1bA	26.1 ± 0.2bA	26.5 ± 0.2bA	1.3
F-value	1.5	1.5	1.7	6.3	9.0	8.6	8.5	
				Egg length (mm)				
Standard	—	0.544A	0.544A	0.544aA	0.543aA	0.543aA	0.542aA	0.06
Reduced	—	0.535A	0.538A	0.538abA	0.537abA	0.539abA	0.538aA	0.09
Minimal	—	—	—	0.527bA	0.528bA	0.530bA	0.529bA	0.2
F-value	—	1.98	1.18	4.65	4.36	3.59	5.67	
				Egg width (mm)				
Standard	—	0.303B	0.304B	0.319A	0.312AB	0.310AB	0.315A	6.4
Reduced	—	0.299C	0.308B	0.318A	0.310AB	0.300BC	0.307BC	10.0
Minimal	—	—	—	0.312A	0.312A	0.308A	0.308A	1.5
F-value	—	0.26	2.47	3.14	0.19	2.66	3.03	
				Egg hatch (%)				
Standard	94.7 ± 1.8aA	93.7 ± 2.5aA	95.3 ± 1.6aA	96.3 ± 1.4aA	99.0 ± 1.0aA	97.0 ± 1.3aA	97.3 ± 1.2aA	1.2
Reduced	92.0 ± 0.9aA	92.7 ± 2.2aA	93.0 ± 1.6aA	94.3 ± 1.2 abA	96.3 ± 1.7abA	95.0 ± 1.3abA	94.7 ± 1.4abA	0.98
Minimal	—	—	—	91.7 ± 0.6bA	91.3 ± 2.4bA	92.0 ± 1.3bA	91.0 ± 1.3bA	0.08
F-value	1.70	0.09	1.05	4.33	4.76	3.65	5.66	

[a]For each life history parameter, means (±SE) in columns followed by different lowercase letters and in rows followed by different capital letters are significantly different ($P < 0.05$).

Table 4. Parasitism, emergence, and sex ratio of *Trichogramma brassicae* from eggs of *Ephestia kuehniella* produced by females from larvae reared on three diets

Diet	Generation[a]						
	2	3	4	5	6	7	*F*
			Parasitized eggs per female (n)				
Standard	90.70 ± 6.25D	94.65 ± 6.01CD	106.25 ± 7.15BCD	127.15 ± 7.13aAB	121.30 ± 7.00aABC	136.20 ± 8.99aA	6.57
Reduced	77.85 ± 6.27B	79.95 ± 5.83B	105.25 ± 7.92A	112.05 ± 7.17abA	108.10 ± 7.61aA	101.30 ± 9.03bA	57.4
Minimal	—	—	92.35 ± 7.48A	91.40 ± 8.11bA	84.60 ± 6.98bA	96.20 ± 5.67bA	0.46
F-value	2.10	3.08	1.06	5.75	13.67	7.32	
			Emergence (%)				
Standard	92.06 ± 1.27aC	92.94 ± 1.06aCB	97.33 ± 0.43aA	95.31 ± 0.62ABC	95.77 ± 0.56AB	95.21 ± 0.66ABC	5.43
Reduced	80.98 ± 1.46bB	78.26 ± 1.29bB	93.72 ± 0.79bA	94.20 ± 0.49A	93.77 ± 0.67A	95.08 ± 1.05A	57.4
Minimal	—	—	92.12 ± 1.88bA	95.12 ± 1.23A	92.05 ± 2.05A	94.45 ± 0.61A	1.05
F-value	34.12	0.56	8.68	2.40	2.96	0.60	
			Sex ratio (% female)				
Standard	0.66 ± 0.03AB	0.66 ± 0.03AB	0.79 ± 0.03aA	0.71 ± 0.04AB	0.68 ± 0.05AB	0.65 ± 0.04aAB	2.53
Reduced	0.69 ± 0.02A	0.70 ± 0.03A	0.70 ± 0.03abA	0.61 ± 0.03A	0.63 ± 0.03A	0.60 ± 0.03abA	2.14
Minimal	—	—	0.62 ± 0.04bA	0.66 ± 0.05A	0.57 ± 0.06A	0.50 ± 0.04bA	2.08
F-value	0.53	0.75	7.50	2.12	1.35	3.92	

[a]The eggs were used to initiate the indicated *E. kuehniella* generation. For each life history parameter, means (±SE) in columns followed by different lowercase letters and in rows followed by different capital letters are significantly different ($P < 0.05$).

Glycerol was considerably more expensive than all the other ingredients, so a less expensive alternative was substituted for the cost comparisons (Table 1). This resulted in costs of $0.957, $0.692, and $0.661 for 100 g of the standard, reduced, and minimal larval diet, respectively. Larvae reared on 100 g of these diets produced 44, 37, and 31 females (50% females, Table 2) that oviposited a mean of 312, 250, and 211 eggs per female, respectively, yielding 13,728, 9,212, and 6,541 eggs at a cost of 0.070, 0.075, and 0.101 per 1,000 eggs (cost 100 g diet/total eggs produced by the females × 1,000). When 140 eggs from the three separate diets were exposed to *T. brassicae* females to determine their acceptance for oviposition, more of the standard diet eggs were parasitized, followed by the reduced and minimal diets (Table 5). After adjusting for differences in parasitism, parasitoid emergence, yield of parasitoids, and cost of host eggs, the standard diet was less expensive than the reduced and minimal diets per 1,000 parasitoids. Moreover, parasitoids from the standard diet were significantly larger and potentially would be more effective when released in the field.

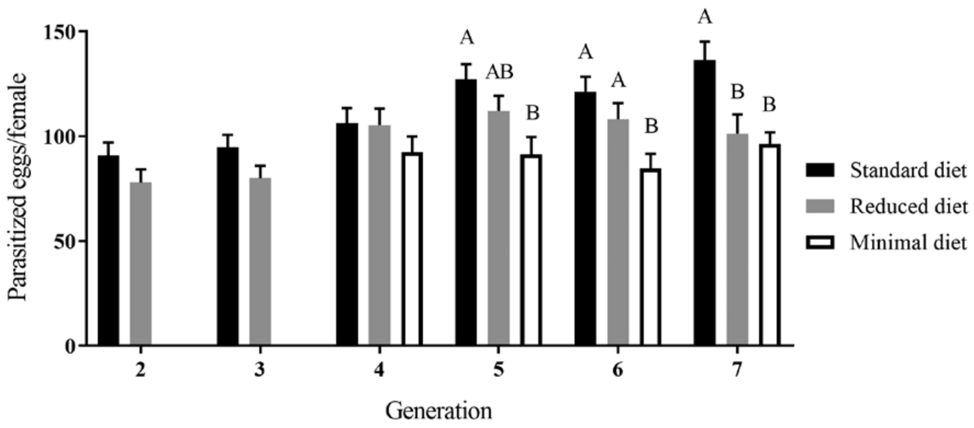

Fig. 1. Mean (±SE) number of *Ephestia kuehniella* eggs parasitized in each generation by *Trichogramma brassicae* females. The eggs were from moths reared as larvae on a standard, reduced, or minimal diet.

Table 5. Cost of 1,000 *Trichogramma brassicae* produced from *Ephestia kuehniella* eggs oviposited by females from a standard, reduced, or minimal larval diet

Diet	Eggs parasitized/ female (no.)[a]	Parasitoid emergence from eggs (%)	Parasitoids/140 host eggs (no.)	Parasitoids/1,000 host eggs (no.)	Cost/1,000 host eggs ($)	Cost/1,000 parasitoids ($)
Standard	136	95.21	129.49	924.93	0.070	0.075
Reduced	101	95.08	96.03	685.93	0.075	0.109
Minimal	96	94.45	90.67	647.64	0.101	0.156

[a]Each parasitoid female was provided with 140 host eggs over 48 h to determine the total number of eggs a female could parasitized (Table 4).

The objective of this research was to reduce the number of ingredients in *E. kuehniella* larval diet, and thereby cost, without decreasing the quantity or quality of eggs produced by females from larvae fed the less complex diets. The eggs had to be accepted by *T. brassicae* females for oviposition and yield high-quality wasps. Unfortunately, decreasing the dietary ingredients prolonged *E. kuehniella* larval development, lowered pupal mass, reduced adult emergence, and yielded fewer eggs per female that had less mass and were smaller. Fewer of the eggs from the simpler diets were parasitized by *T. brassicae* females than eggs from the standard diet and the diminished eggs yielded smaller wasps. Conversely, the standard *E. kuehniella* larval diet resulted in the production of more and larger eggs, and a greater number of them were parasitized by *T. brassicae* females than eggs from the reduced and minimal diets. The standard larval diet ultimately was less expensive to use because the resulting *T. brassicae* had a significantly higher rate of reproduction than wasps from the other host eggs.

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