Survival and Reproduction of Small Hive Beetle (Coleoptera: Nitidulidae) on Commercial Pollen Substitutes

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Survival and reproduction of small hive beetle (Coleoptera: Nitidulidae) on commercial pollen substitutes

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Abstract

An assay was developed to investigate the potential survival and reproduction of the small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), when provided artificial food resources in managed European honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), colonies. Supplemental feeding was done to maintain the health of the hive, initiate comb building, expand colony numbers, and promote pollen foraging. To complement the protein requirement of honey bees, commercial pollen substitutes have been developed and are available for producers. Small hive beetles also exploit the pollen substitutes when present in the hive. Adult beetles were provided with 7 different commercial pollen substitutes and allowed to freely feed and oviposit over a period of 30 d. Beetles that survived the longest on the treatments did not necessarily produce the most larvae. The supplement Bee-Pro® supported the greatest survival, yet produced very low larval numbers. Global Patties® produced the most larvae; however, adult survival was low. This result may have been due to the larvae using all of the food resources in a short amount of time, leaving little to support adult survival. Four of the 7 treatments supported 70% or greater beetle survival for the entire assay period. This study suggests that the protein supplement needs to be readily consumed by the honey bee colony if it is to be effective for pest control. Careful selection of the amount and type of honey bee supplemental diets is important in balancing nutritional needs of bees and reducing potential problems with small hive beetles.

Key Words: *Apis mellifera*, supplemental feeding

Resumen

Se desarrolló un ensayo para investigar la posible sobrevivencia y reproducción del pequeño escarabajo de la colmena, *Aethina tumida* Murray (Coleoptera: Nitidulidae), cuando se proporcionaron recursos alimenticios artificiales en colonias manejadas de la abeja europa, *Apis mellifera* Linnaeus (Hymenoptera: Apidae). Se realizó la alimentación suplementaria para mantener la salud de la colmena, iniciar la construcción de panales de miel, expandir el número de colonias y promover el forraje de polen. Para complementar el requisito de proteínas de la abeja europa, se han desarrollado sustitutos comerciales del polen que están disponibles para los productores. Los pequeños escarabajos de la colmena también explotan los sustitutos del polen cuando están presentes en la colmena. Los escarabajos adultos recibieron 7 sustitutos de polen comerciales diferentes y se les permitió alimentarse y ovipositar libremente durante un período de 30 dias. Los escarabajos que sobrevivieron el tiempo más largo en los tratamientos no produjeron necesariamente la mayoría de las larvas. El suplemento Bee-Pro® soportó la mayor sobrevivencia, pero produjo un número de larvas muy bajo. Global Patties® produjo la mayor cantidad de larvas; sin embargo, la sobrevivencia de los adultos fue baja. Este resultado pudo haber sido debido a que las larvas utilizaron todos los recursos alimenticios en un corto período de tiempo, dejando poco para apoyar la sobrevivencia de los adultos. Cuatro de los 7 tratamientos soportaron una sobrevivencia del escarabajo del 70% o mayor durante todo el periodo de ensayo. Este estudio sugiere que el suplemento de proteina necesita ser consumido inmediatamente por la colonia de las abejas si va a ser eficaz para el control de plagas. La cuidadosa selección de la cantidad y el tipo de dietas suplementarias es importante para equilibrar las necesidades nutricionales de las abejas y reducir los posibles problemas con los pequeños escarabajos de colmenas.

Palabras Clave: *Apis mellifera*, alimentación suplementaria

The honey bee, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), uses resources readily available in the environment to meet their nutritional needs. The honey bee diet consists of nectar and pollen that is collected and stored, as well as water that is used within the hive. Their diet is rich in carbohydrates, proteins, vitamins and minerals. Honey bees forage for floral nectar that is collected and taken back to the hive, where it is transformed into honey. Nectar and honey are the main carbohydrate sources for the honey bee. Water is also an important resource for the honey bee, and colonies use large amounts of water to dilute honey and regulate hive temperatures (Haydak 1970). Pollen provides bees with protein, fats, vitamins and minerals necessary for survival. These resources are fed to developing larvae and young bees for muscle growth and development. Additionally, pollen is used in the production of royal jelly, which is produced by worker bees and fed to the queen, developing queen larvae, and worker larvae (Somerville 2000).

It has been estimated that an average size honey bee colony will use 100 to 200 kilos of honey and 20 to 35 kilos of pollen per year (Standifer et al. 1977). During certain times of the year, or periods of unique environmental conditions, food resources for the honey bee may be deficient. Inadequate pollen or pollen with poor nutritional value will result in a decline in brood rearing and shortened worker

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lifespan (Kleinschmidt & Kondos 1976, 1978; Knox et al. 1971). Manning (2016) outlined the vitamin and mineral requirements that are essential to meet the nutritional needs of the honey bee. Improper overall nutrition will lead to reduced colony numbers, shorter lifespan, decrease in drone production, disease susceptibility, and death of the colony (Standifer 1980). Honey bees produce propolis, a resinous mixture of saliva, beeswax and exudates gathered from sap and other botanical sources. This is not consumed by the bee, it is used to seal cracks and openings in the hive to assist in temperature regulation and colony hygiene.

When resource quality and quantity in the environment is inadequate for the colony, beekeepers can provide artificial food resources, referred to as supplemental feeding. In managed bees, supplemental feeding is done to sustain the health of the hive, expand colony numbers, comb building, and promote pollen foraging (Somerville 2005; Standifer et al. 1977). The use of supplemental feeding has become a standard management practice in commercial beekeeping. Nectar can be replaced by providing sugar syrup, usually a thick sucrose solution. Sugar cane and beet sugars are the best carbohydrate substitutes (Barker 1977). Commercial pollen substitutes are available to complement the protein requirement of the honey bee. However, supplemental pollen is not a complete replacement for natural pollen and should only be used when natural pollen is insufficient. The use of natural pollen taken from 1 hive or another location can pose a health risk. Certain diseases such as American Foulbrood, which forms spores, can be introduced into the hive from contaminated honey and pollen. For this reason, these products should not be fed to honey bee colonies unless it has been irradiated (Hansen & Brødsgaard 1999). Commercial protein alternatives offer a safe option to natural pollen.

A requirement for a preferred honey bee pollen supplement is ready consumption of the material. If a large quantity of supplement is placed in the hive and not consumed rapidly, unwanted pests rather than the bees may be fed. Major honey bee pests, such as the small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), can exploit the commercial pollen substitutes and thus increase their reproductive potential. This pest was first reported in the United States in 1996 and has plagued beekeepers since its introduction and subsequent spread. This sub-Saharan Africa native emerges from the soil and seeks refuge in honey bee hives. Adult beetles and larvae cause destruction by consuming honey bee eggs, brood, stored pollen, and honey. The female can lay an abundance of eggs in her lifetime and can live for many months, with the larval stage causing the most damage (Somerville 2003). Depending on temperature, larvae feed for about 10 d before leaving the hive and pupating in the soil. The adult beetles introduce *Kodamaea* (*Pichia*) *ohmeri* yeast found in the beetle's gut into the honey and pollen stores (Benda et al. 2008). This causes the honey to ferment making it unsuitable for consumption by the honey bee or humans. When the larval population reaches a certain point, the queen will stop egg-laying. In an effort to save the colony, the bees will abscond from the hive. If the queen has survived, the colony may relocate; otherwise the result is death of the hive.

There are a number of commercially available pollen substitutes for supplemental feeding. Most are purchased in powder form and mixed with high fructose corn syrup or sugar syrup as recommended by the manufacturers. Some manufacturers state the product can be presented to the bees as a dry powder. The product is mixed to a stiff dough-like consistency and made into patties. The patties are presented to bees by placing them on top of the frames under the lid.

Due to the concerns about potential enhancement of small hive beetle populations in the presence of bee supplements, we compared effects of exposure to different commercial pollen substitutes on beetle survival and reproduction. The overall intent of this research was to gain knowledge and disseminate this information in regards to current cultural practices in honey bee husbandry and better management of small hive beetles.

Materials and Methods

BEETLES

The colony of *A. tumidae* was based on individuals collected from wild honey bee populations and then maintained for 2 generations at USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, USA. All beetles were reared on pollen patties (Global Patties, Butte, Montana, USA). Insects were reared in a temperature controlled chamber at 23 ± 5 °C, 60% RH, and photoperiod of 12:12 h L:D.

SURVIVAL AND REPRODUCTION

To compare the effects commercial pollen substitutes have on the small hive beetle, an assay was developed to see if they could (1) survive by directly feeding on the pollen substitute and (2) lay eggs and produce viable larvae. Each assay repetition was conducted in a clear plastic container (10 × 10 × 7.5 cm) with ventilation provided through a fine copper wire grid glued over an 8 cm circular hole in the lid. A Petri dish (60 × 15 mm) (Falcon®, 35-1007, Becton Dickinson, Franklin Lakes, New Jersey, USA) base containing 35 g of a pollen substitute treatment was placed in each container. Each treatment container contained 5 male and 5 female beetles within 24 h of eclosion and provided with water from a moistened dental wick inserted into the lid of a 30 ml cup (Richmond Braided cotton roll, Charlotte, North Carolina). Sex determination of adult beetles was done by grasping the beetle so the ventral tip of the abdomen was viewable. Gently squeezing the abdomen will cause the female to extend her ovipositor, or the male to protrude his 8th tergite (Schmolke 1974). There were 50 replicates of each treatment. Treatments were held in a temperature controlled chamber at 23 ± 5 °C, 60% RH, and photoperiod of 12:12 h L:D. Treatments were checked daily for mortality and mature larvae. Dead beetles and larvae in the crawling stage were removed daily and counted. The assay was conducted for 30 d. Statistical analyses were conducted by using SAS programming (SAS Institute Inc. 2009). Analysis of variance (PROC ANOVA) followed by means separation with the Waller test was employed to compare the mean responses to various diets.

POLLEN SUBSTITUTES AND THEIR FORMULATION

All pollen substitutes were prepared according to the manufacturer's recommendations (Table 1). Each treatment was weighed to a standard amount of 35 g and placed in the Petri dishes with each repetition receiving 1 dish. Pollen patties manufactured by Global Patties evaluated as a positive control as this was the diet and reproduction substrate used in our rearing colony.

Results

Adult beetle survival was clearly affected by the different pollen substitutes. Overall, there were 2 groups of diets based on survival, those with over 70% survival and those with less than 30% survival at day 30. Numbers of larvae produced varied significantly with the different supplements as compared to Global Patties. There was a range of 250 to 0.13 larvae produced per repetition.

Table 1. Instructions for preparing the commercial pollen substitutes.

Pollen Substitute	Mixing Instructions
Global Patties (Butte, Montana)	The manufactured Pollen Patties consist of dry sucrose, sucrose syrup, soy flour, yeast and 4% pollen. The pre-formed patties (10 cm × 28 cm) were provided from the manufacturer between 2 layers of wax paper. For the experiments, a portion of the patty was weighed and placed in the Petri dish.
Brushy Mountain Ener-G-Plus Bee Diet Pollen Substitute (Moravian Falls, North Carolina)	A soft cake was prepared by blending 453 g of dry mix with syrup made from 840 g of sucrose and 300 ml of water. The resulting mixture was covered with wax paper and allowed to rest for about12 h at room temperature until it formed a dough-like consistency. The manufacturer's instructions recommended a 2:1 sugar solution, but previous attempts resulted in a loose mixture that did not solidify. The amount of water was decreased to 300 ml, resulting in a more solid consistency.
Dadant Brood Builder™ (Hamilton, Illinois)	The diet was prepared by blending 200 g of Brood builder dry mixture with 236 ml of a 2:1 sucrose: water solution. After forming stiff dough, it was distributed in individual Petri dishes.
Bee-Pro® (Mann Lake Ltd., Hackensack, New Jersey)	The mixture was comprised of 757 ml of high fructose corn syrup (Karo® Light Corn Syrup, ACH Food Co., Inc., Memphis, Tennessee) added to 453 g of Bee-Pro® mix and 453 g sucrose. Dry ingredients were blended together using a stand mixer; the high fructose corn syrup was added until the mixture formed stiff dough.
Ultra Bee (Mann Lake Ltd., Hackensack, New Jersey)	The mixture was prepared by combining 757 ml of high fructose corn syrup (Karo® Light Corn Syrup, ACH Food Co., Inc., Memphis, Tennessee) added to 566 g of mix and 453 g sucrose. Dry ingredients were blended with the syrup using a stand mixer; high fructose corn syrup was added until the mixture formed stiff dough.
MegaBee™ (Castle Dome Solutions, Helena, Arkansas)	The diet was comprised of 413 g of dry mix that was placed in a stand mixer. To this was added 475 ml of high fructose corn syrup (Karo® Light Corn Syrup, ACH Food Co., Inc., Memphis, Tennessee) and was blended into stiff dough and placed in individual Petri dishes.
Dadant Artificial Pollen 23® (Hamilton, Illinois)	The artificial pollen was prepared by combining 453 g of Dadant Artificial Pollen 23 and 770 g of a 50% sucrose and water solution. After forming stiff dough, the mixture was distributed in individual Petri dishes.

GLOBAL PATTIES

Our results indicate a sharp decline in adult survival ($F = 6.14$; df = 6; $P = 0.0140$) and larval production at 24 d with 68% survival (Fig. 1). There were significantly more larvae ($F = 61.73$; df = 6; $P < 0.0001$) produced on the Global Patties than any other treatment. Beetles produced an average of 250 larvae per repetition over the 30 d period (Fig. 2).

BRUSHY MOUNTAIN ENER-G-PLUS BEE DIET POLLEN SUBSTITUTE

During the 30 d trial, 87% of the larvae survived ($F = 6.37$; df = 6; $P = 0.0123$) on this treatment (Fig. 1). Although survival was high, beetles produced an average of 2.5 larvae ($F = 1.52$; df = 6; $P = 0.2184$) per repetition (Fig. 2).

DADANT ARTIFICIAL POLLEN 23

There was 75% survival ($F = 0.30$; df = 6; $P = 0.5824$) on this treatment until 22 d, after which there was high mortality (Fig. 1). This treatment produced an average of 2.5 larvae ($F = 1.53$; df = 6; $P = 0.2180$) over the 30 d period (Fig. 2).

DADANT BROOD BUILDER™

This treatment had significant low survival ($F = 62.87$; df = 6; $P < 0.0001$) at 14 d with only 52% surviving the entire period (Fig. 1). There were few larvae produced ($F = 1.72$; df = 6; $P = 0.1917$), with 0.23 larvae per repetition (Fig. 2).

BEE-PRO®

Adult survival was greatest on this treatment with 90% survival ($F = 10.36$; df = 6; $P = 0.0015$) over the course of 30 d (Fig. 1). However, on average 0.13 larvae were produced ($F = 1.72$; df = 6; $P = 0.1906$) per repetition (Fig. 2).

ULTRA BEE

Adult survival was the second highest ($F = 7.15$; df = 6; $P = 0.0081$) on this treatment, with 88% over the course of 30 d (Fig. 1). There were 0.13 larvae produced ($F = 1.72$; df = 6; $P = 0.1906$) on each repetition (Fig. 2).

MEGABEE™

This treatment had the third highest survival ($F = 1.48$; df = 6; $P = 0.2250$) and the second highest larval production. There was 87% survivability on this treatment, with an average of 23.3 larvae ($F = 0.30$; df = 6; $P = 0.5864$) per repetition produced over the course of the trial.

DISCUSSION

Supplemental feeding has become a standard management practice in commercial beekeeping. The honey bee has become accustomed to receiving pollen substitutes to augment their diet. Therefore, it was anticipated that the small hive beetle would survive equally on all of the treatments provided. Of the commercial bee supplement treat-

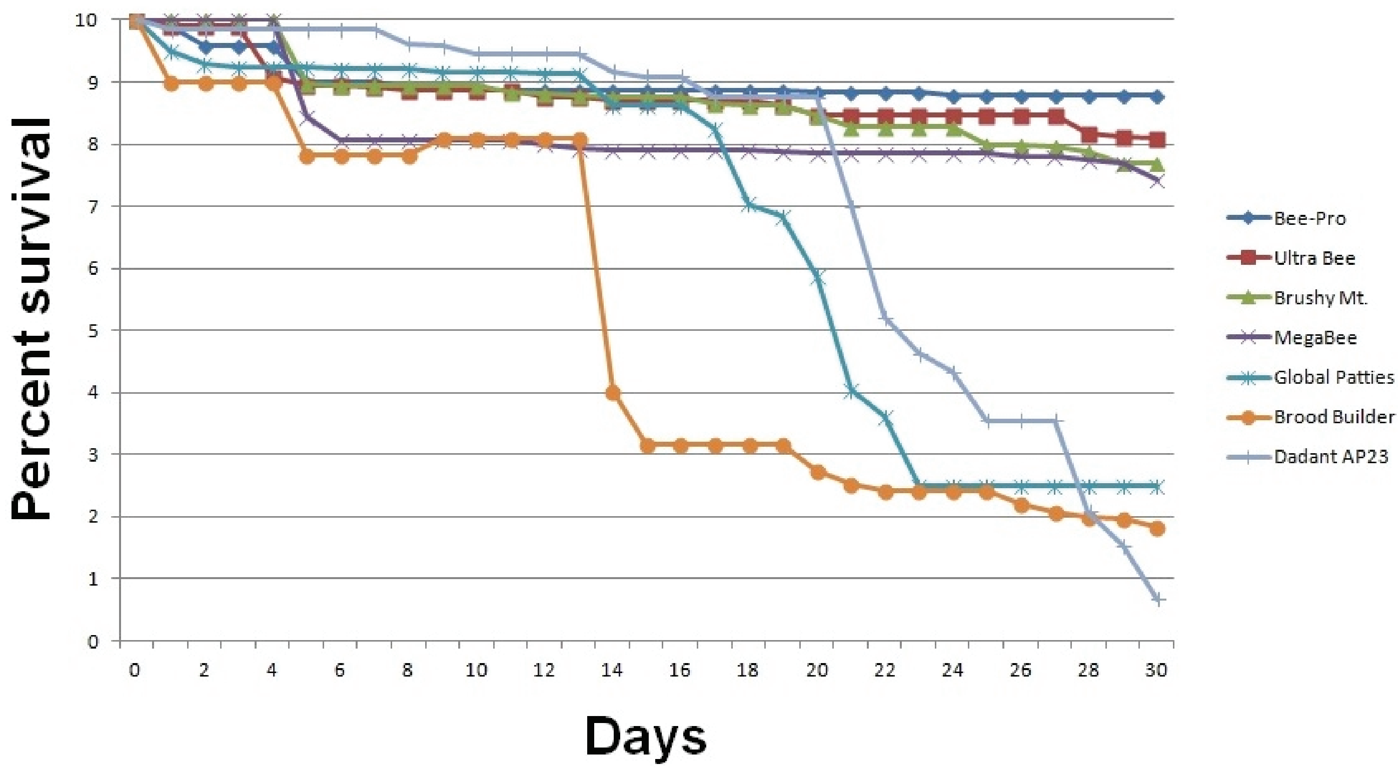

Fig. 1. Mean number of days survival of adult *Aethina tumida* on different commercial honey bee protein supplements (N = 50).

ments, it was assumed that the Global Patties would provide for the best feeding and reproduction media because this diet contains sugar, soy flour, yeast, and 4% pollen. This particular diet was used in mass rearing the beetle for the laboratory studies. Surprisingly, the survival of adult beetles was not the highest. Due to the high average number of larvae produced per repetition (250 larvae per repetition; 7751 total larvae), all the food resources were observed to be consumed by the larvae, with no food remaining to support continued survival of adults. However, in a hive the beetle has stores provided by the bees and has the ability to exploit these resources. It is unknown if previous feeding experience as a larva influences the adult beetle's behavior. If so, this would account for the high adult consumption and reproduction on Global Patties. The presence of natural pollen in the diet may have enhanced survival and reproduction in this treatment.

The commercial supplement MegaBee™, as well as the other treatment, contained no natural pollen, with protein supplied in the form of soy flour and yeast. Considering the composition of this diet, reproduction on MegaBee™ was higher than those treatments containing the same ingredients (23.3 larvae; $F = 0.30$; df = 6; $P = 0.5864$). Although not significant compared to the other treatments, if this supplement is not properly utilized in the hive, it may be cause for concern. The production of 23 larvae per repetition (722 total) in a 30 d period has the likelihood of destroying a hive. The beetles can use the artificial pollen for egg laying and initial larval feeding. Once the larvae disperse, there is the potential for them to become distributed in the honey and pollen stores throughout the hive. If supplemental feeding is being performed to assist a weak hive, the overload of beetle larvae may be detrimental.

The supplements, Dadant AP23® and Brushy Mountain Ener-G-Plus had high survivability, 75% and 87% respectively, and produced an average of 2.5 larvae, which are low in comparison to Global Patties and MegaBee™. However, any support of production of small hive beetles in hives is cause for concern. A single female beetle can lay about 2000 eggs in her lifetime (Somerville 2003). We have demonstrated high survival of adult beetles on bee supplement for at least 30 d and it is estimated that beetles live more than 12 months (Somerville 2003). Thus, continued presence of excess honey bee supplement has the potential to support exponential increases in populations within a short amount of time.

Data on adult survival (90% and 88%) and larval production on the Bee-Pro® and Ultra Bee supplements were very similar (0.13 larvae per rep). Although larval production was low, it is unknown if there would have been a greater number of offspring on these treatments if the assay was conducted for a longer period of time. It appears that the treatment is nutritious enough to sustain life, but may not contain the nutrients needed for reproduction. In contrast, the supplement Dadant Brood Builder™ had 52% survival with high mortality at 14 d and produced few larvae (0.23 larvae per repetition).

The preference of honey bees for a pollen substitute was not assessed for any of the treatments. A pollen substitute that meets honey bee nutritional needs, but which caused high mortality and low reproduction of the small hive beetle, would be the best treatment for supplemental feeding. All of the commercially available pollen substitutes are comparable in cost. Global Patties can be used immediately, the others require preparation.

Foraging for carbohydrates and other nutrients is critical for the survival and reproduction of the honey bee. Nectar provides the carbohydrate resource; pollen provides essential proteins and amino acids. While protein is critical for the survival of the honey bee, it is also a fundamental part of the small hive beetle diet as well. With parallel nutritional requirements, supplementing the honey bee's diet in the presence of the small hive beetle may present dangerous results.

Commercial and backyard beekeepers have adopted the use of supplemental feeding as standard management practice. The use of a pollen substitute assists a deficient hive and helps maintain the honey bee's protein requirement. Commercial substitutes allow for the replacement of protein without the use of natural pollen. Unfortunately,

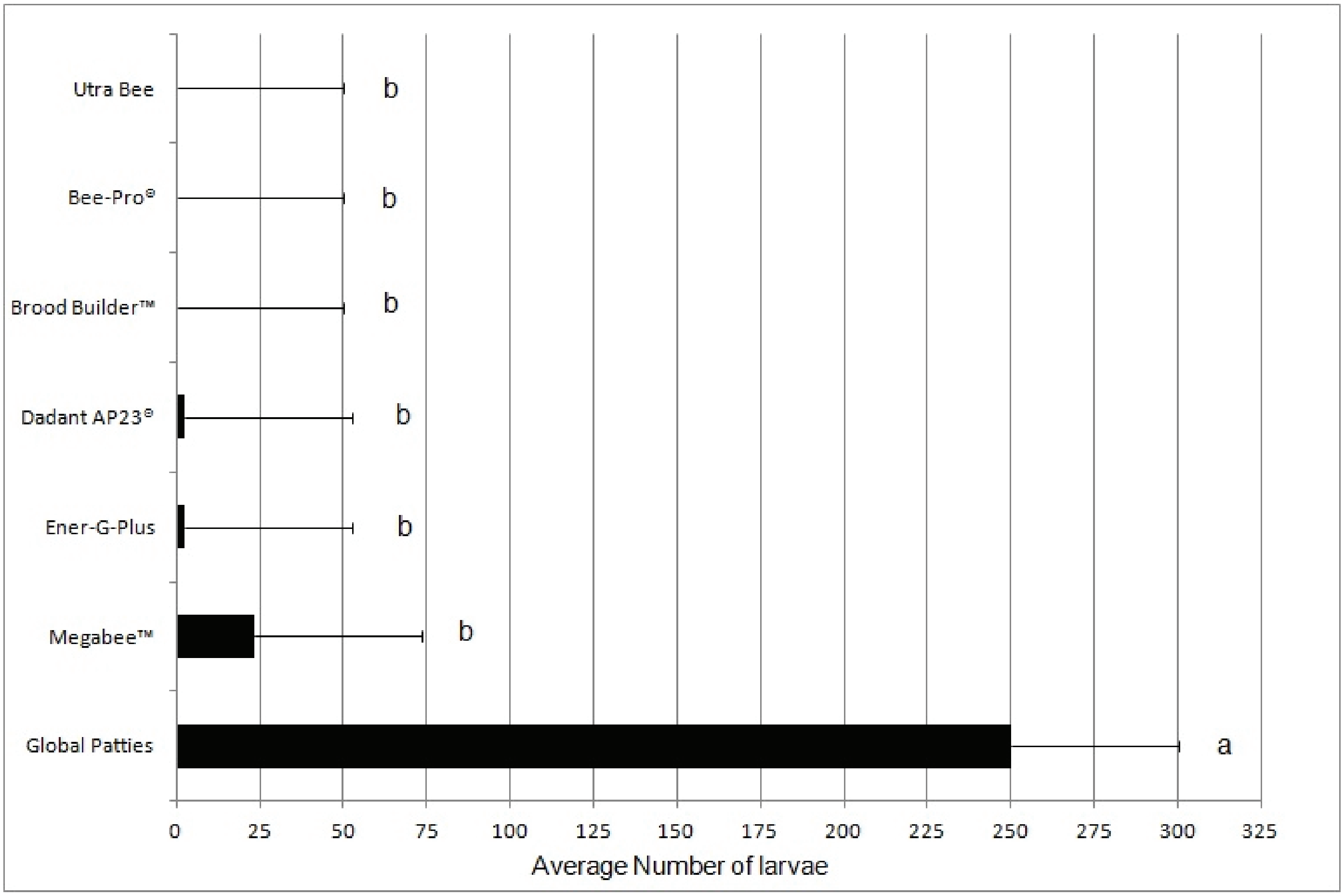

Fig. 2. Mean number (± SE) of larval *Aethina tumida* produced after access to different commercial honey bee pollen supplements for 30 d. Means that have the same letter are not statistically different (N = 50).

there is a risk of enhancing the small hive beetle population if proper management practices are not followed. The ideal practice is to optimize honey bee health and minimize beetle pests.

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