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# Impact of food diversity on biological parameters of *Apolygus lucorum* (Hemiptera: Heteroptera: Miridae)

Yanrong Li, Zhen Li, Yuhui Yang, Qingpo Yang, Xiaoxia Liu\*, and Qingwen Zhang\*

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## Abstract

*Apolygus lucorum* (Meyer-Dür) (Hemiptera: Heteroptera: Miridae) is an important insect pest of cotton and other crops, but the species also feeds on some insect pests. In this study, the effect of 5 food condition treatments, including corn kernel, cotton leaves, eggs of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), a combination of corn + *H. armigera* eggs, and of cotton leaves + *H. armigera* eggs, on the biological parameters of *A. lucorum* was evaluated in the laboratory. Mixed foods (e.g., corn + *H. armigera* eggs) significantly increased nymphal survival, prolonged adult longevity, and improved female fecundity. The survival rate of nymphs with only *H. armigera* egg was the lowest, and development times with cotton leaves were the longest. Under mixed food conditions, nymphs preyed on more eggs in the combination with cotton leaves than with corn. These results indicate that *A. lucorum* does have a certain ability to prey on *H. armigera* eggs, but that during the growth and development of *A. lucorum*, plant food is still necessary for development.

Key Words: *Helicoverpa armigera*; predation; development; fecundity; mirid bug

## Resumen

*Apolygus lucorum* (Meyer-Dür) (Hemiptera: Heteroptera: Miridae) es una plaga importante del algodón y otros cultivos, pero la especie también es un depredador de algunas plagas de insectos. En este estudio, se evaluó en el laboratorio el efecto de 5 tratamientos de condiciones de alimentos, incluyendo: grano de maíz, hojas de algodón, huevos de *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), una combinación de maíz + huevos de *H. armigera*, y hojas de algodón + huevos de *H. armigera*, sobre los parámetros biológicos de *A. lucorum*. Los alimentos mixtos (por ejemplo, maíz + huevos de *H. armigera*) aumentó significativamente la sobrevivencia de las ninfas, prolongó la longevidad de adultos, y mejoró la fecundidad de las hembras. La tasa de sobrevivencia de las ninfas alimentadas con solamente huevos de *H. armigera* fue la más baja, y el tiempo de desarrollo de las alimentadas con hojas de algodón fue el más largo. En condiciones de alimentos mixtos, las ninfas se alimentaron de más huevos en la combinación con hojas de algodón que con el maíz. Estos resultados indican que *A. lucorum* tiene una cierta capacidad para comer los huevos de *H. armigera*, pero durante el crecimiento y desarrollo de *A. lucorum*, el alimento de la planta es todavía necesario para el desarrollo.

Palabras Clave: *Helicoverpa armigera*; depredación; desarrollo; fecundidad; chinche mirido

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The green plant bug *Apolygus lucorum* (Meyer-Dür) (Hemiptera: Heteroptera: Miridae), is a polyphagous agricultural pest that has a wide range of hosts, including crops, vegetables, fruits, and ornamental plants (Lu & Wu 2008). Both adults and nymphs may harm plants by piercing and sucking various plant tissues with the scalpellum affecting plant growth and development, which causes massive reduction of output and economic losses. In recent years, with the widespread planting of transgenic *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) (Bt) cotton, the population of *A. lucorum* increased quickly (Wu & Guo 2005; Lu et al. 2007, 2010; Lu & Wu 2008; Wu et al. 2008) and has become an important agricultural problem in cotton in East Asia (Miyata 1993, 1994; Watanabe 1999; Men et al. 2005; Lu & Wu 2008; Lu et al. 2008; Gao et al. 2012).

The feeding habits of mirids (i.e., plant bugs) are relatively complex; some are phytophagous and only feed on a variety of crops; some are carnivorous and only prey on small insects, such as thrips, aphids, and their eggs; and some are omnivorous and feed on both plants and insects (Wheeler 2001). *Lygus pratensis* (L.) (Hemiptera: Heteroptera: Miridae) is an important pest in cotton fields but also preys on *Aphis gossypii* Glover (Hemiptera: Aphididae), *Leptinotarsa decemlineata*

(Say) (Coleoptera: Chrysomelidae), and *Agromyza frontella* (Rondani) (Diptera: Agromyzidae) larvae in the field (Wheeler 1976; Cleveland 1978; Liang et al. 2013). *Apolygus lucorum* can be a damaging pest in newly planted crops but also preys on some small insects, such as aphids and aleyrodids, and on eggs of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Wang et al. 2010). Although omnivorous bugs can complete their growth and development either on plant tissue or on other arthropods, the associated differences in nutrients can affect their life history and population dynamics (Eubanks & Denno 1999). *Dicyphus hesperus* Knight (Hemiptera: Heteroptera: Miridae) could complete development and reproduction on mullein with or without *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. However, the development time was shorter and survivorship higher with eggs than without eggs, and the fecundity higher and longevity longer also (Juan et al. 2004).

In order to clarify the feeding habitats of *A. lucorum* and the impact of food diversity on its development and proliferation, we compared the influences of 5 different foods on the immature development and adult fecundity of *A. lucorum*. We also determined predation of *H. armigera* eggs alone and in combination with plant tissue. In addition,

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the immature development and adult fecundity of *A. lucorum* that fed on corn alone and in combination with eggs were recorded over 3 successive generations.

## Materials and Methods

### INSECT RESOURCES

*Apolygus lucorum* nymphs and adults were collected from cotton fields at the Shang Zhuang Experimental Station of China Agricultural University in Beijing (China) from Jul to Aug 2012. A laboratory colony was kept in 20 × 13 × 8 cm rearing containers and reared on fresh corn ears. Corn also served as the oviposition substrate, and fresh corn was provided every 2 d. Corn containing *A. lucorum* eggs was subsequently placed in rearing containers that were lined with filter paper and kept in the incubator until 1st instars emerged. Nymphs were placed in similar containers, which were covered with nylon organdy mesh to enable air circulation, and provided with fresh food every 2 d until adult emergence. Each rearing container housed about 100 nymphs or 60 to 80 adults. The colony was maintained at 25 ± 1 °C, 60 ± 5% RH, and a 14:10 h L:D photoperiod.

Cotton bollworms (*H. armigera*) were collected as larvae from cotton fields in Hebei Province, China, and reared on an artificial diet (Wu 1980) for at least 30 generations in the laboratory. For colony maintenance, adult *H. armigera* males and females were mated in a metal frame screen cage (40 × 30 × 30 cm) with a 10% honey solution provided on a ball of cotton in a glass Petri dish (9 cm diameter). Eggs of *H. armigera*, laid on sheets of blue cloth, were checked under a dissecting microscope for dehydrated or broken eggs, which were removed gently. The resulting egg cloth was then cut into small pieces (3 × 3 cm, with 100 eggs on each piece) and provided as food for *A. lucorum*.

### EXPERIMENTAL DESIGN

The method used in our present study was similar to that used in the work of Lu et al. (2009, 2010). Individual nymphs were placed separately into disposable plastic Petri dishes (9 cm diameter) and fed on 5 different types of diets, including corn kernel, *H. armigera* eggs, cotton leaf, corn kernel + *H. armigera* eggs, and cotton leaf + *H. armigera* eggs. Each individual nymph was fed with 1 corn kernel, 1 piece of cotton leaf (wet cotton ball with petiole), 1 piece of egg cloth with 100 eggs, or the respective mix foods. The food was replaced every 24 h. Each treatment consisted of 90 nymphs split between 3 cohorts of 30 nymphs as 1 replication. This experiment was conducted in environmental growth chambers (Ningbo Jiangnan Instrument Factory, Ningbo, China) maintained at 25 ± 1 °C, 60 ± 5% RH, and a 14:10 h L:D photoperiod.

### Immature Development and Survival

Nymphs treated with different foods were checked every day until the appearance of adults, and each nymph was considered to enter

the next instar when the exuvia was observed. Survival and development time of each instar were recorded. Wet weight of nymphs was measured on an analytical balance accurate to 0.0001 g (Sartorius BS 123S, Data Weighing Systems, Elk Grove, Illinois, USA).

### Adult Longevity and Fecundity

Adults of *A. lucorum* were collected as newly emerged from the 5 food treatments as described above. The adults were paired and placed into a single Petri dish with different foods for feeding and oviposition, and their foods were replaced and the amount eaten was recorded daily. The longevity of individual adults and mortality rates were determined for each treatment. Female fecundity was quantified each day. Each food regime treatment included 20 to 40 mating pairs, and each pair was considered a replicate.

### Predation on *H. armigera* Eggs

We also checked the predation of *A. lucorum* nymphs on *H. armigera* eggs in 3 treatments, including *H. armigera* eggs alone, corn kernel + *H. armigera* eggs, and cotton leaf + *H. armigera* eggs. New egg cloth was provided every day. The replaced one was observed under a dissecting microscope. Punctured and dehydrated eggs were considered preyed upon, and the number of eggs preyed upon was recorded every day.

### Effects of Diet on Three Generations

Another experiment was conducted to evaluate the effect of plant food alone and plant food with eggs on the 1st, 2nd, and 3rd generations of *A. lucorum* being fed 1 of 2 different diets. Here, the 2 treatments corn kernel and corn kernel + *H. armigera* eggs were continuously observed for 3 generations. The immature development, survival, adult longevity, and fecundity were recorded as above.

### STATISTICAL ANALYSES

The effect of different food diets on the development, body mass, predation, and survival of immature *A. lucorum*, and the longevity and fecundity of adults were analyzed using 1-way ANOVA (SPSS Inc. 1998). The effects of food diets on 3 generations were analyzed using a general linear model. Means were separated by Tukey's test ( $P = 0.05$ ). Nymphs that died before eclosion and adults that produced no eggs were excluded from analysis of nymph development and adult fecundity, respectively.

## Results

### IMMATURE DEVELOPMENT AND SURVIVAL

Nymphs could complete their development under all food conditions, but the development period showed significant differ-

**Table 1.** Mean (± SE) development time (d) of *Apolygus lucorum* nymphs that fed on different foods.

| Food type                            | 1st instar   | 2nd instar    | 3rd instar   | 4th instar   | 5th instar    | Total nymph   |
|--------------------------------------|--------------|---------------|--------------|--------------|---------------|---------------|
| Corn                                 | 3.07 ± 0.06a | 1.69 ± 0.06bc | 1.58 ± 0.06b | 1.92 ± 0.06b | 3.13 ± 0.04a  | 11.40 ± 0.09b |
| <i>Helicoverpa armigera</i> egg      | 2.75 ± 0.08b | 1.92 ± 0.05ab | 1.79 ± 0.08b | 1.79 ± 0.12b | 3.07 ± 0.05ab | 11.32 ± 0.13b |
| Cotton leaf                          | 3.33 ± 0.10a | 2.13 ± 0.07a  | 3.25 ± 0.09a | 4.00 ± 0.17a | 3.00 ± 0.17ab | 15.71 ± 0.09a |
| Corn + <i>H. armigera</i> egg        | 2.22 ± 0.09c | 1.76 ± 0.10bc | 1.68 ± 0.08b | 1.84 ± 0.07b | 3.03 ± 0.10ab | 10.53 ± 0.14c |
| Cotton leaf + <i>H. armigera</i> egg | 3.10 ± 0.10a | 1.47 ± 0.12c  | 1.50 ± 0.09b | 1.87 ± 0.06b | 2.77 ± 0.11b  | 10.70 ± 0.12c |

Means in a column followed by different letters are significantly different ( $P < 0.05$ ; ANOVA and Tukey test).

**Table 2.** Mean ( $\pm$  SE) immature survival rate, adult longevity, and fecundity of *Apolygus lucorum* on different foods.

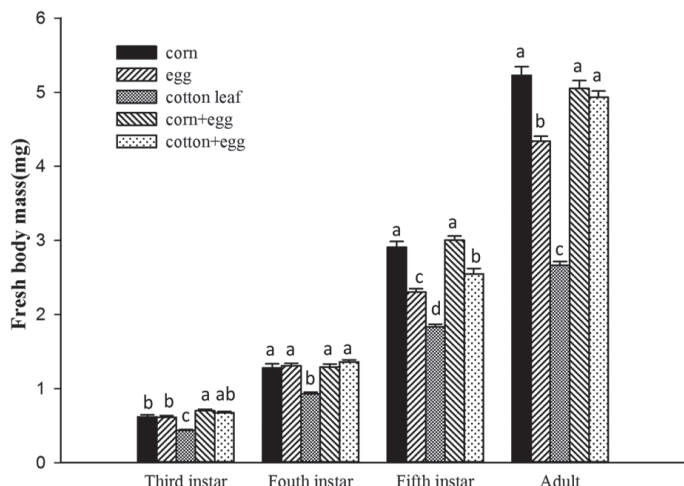
| Food type                            | Nymph survival rate (%) | Adult longevity (d) |                   | Pre-oviposition period (d) | Oviposition period (d) | Fecundity (no. of eggs) |
|--------------------------------------|-------------------------|---------------------|-------------------|----------------------------|------------------------|-------------------------|
|                                      |                         | Male                | Female            |                            |                        |                         |
| Corn                                 | 60.82 $\pm$ 3.67a       | 31.85 $\pm$ 2.54a   | 34.30 $\pm$ 1.36a | 6.33 $\pm$ 0.25b           | 20.73 $\pm$ 2.06a      | 107.87 $\pm$ 14.19b     |
| <i>Helicoverpa armigera</i> egg      | 11.43 $\pm$ 1.64c       | 2.00 $\pm$ 0.20c    | 2.27 $\pm$ 0.15c  | —                          | —                      | —                       |
| Cotton leaf                          | 27.35 $\pm$ 5.96b       | 3.13 $\pm$ 0.29c    | 4.20 $\pm$ 0.28c  | —                          | —                      | —                       |
| Corn + <i>H. armigera</i> egg        | 64.57 $\pm$ 5.93a       | 27.80 $\pm$ 0.94a   | 31.40 $\pm$ 1.09a | 5.18 $\pm$ 0.30c           | 18.73 $\pm$ 1.38a      | 170.64 $\pm$ 11.32a     |
| Cotton leaf + <i>H. armigera</i> egg | 51.37 $\pm$ 5.30a       | 16.07 $\pm$ 1.04b   | 15.47 $\pm$ 0.90b | 8.80 $\pm$ 0.25a           | 5.70 $\pm$ 0.47b       | 42.10 $\pm$ 3.59c       |

Means in a column followed by different letters are significantly different ( $P < 0.05$ ; ANOVA and Tukey test).

ences among treatments ( $F = 237.5$ ;  $df = 4, 180$ ;  $P < 0.001$ ) (Table 1). Development time was significantly shorter when nymphs were fed *H. armigera* eggs with either corn or cotton than when fed *H. armigera* eggs alone or plant tissue alone. Development time was significantly longer when nymphs were fed cotton leaves than when fed all other diets. The survival rate of nymphs was significantly higher when they were fed *H. armigera* eggs with corn compared with *H. armigera* eggs with cotton or cotton alone or *H. armigera* eggs alone ( $F = 27.75$ ;  $df = 4, 26$ ;  $P < 0.001$ ) (Table 2). When the nymphs fed only on cotton leaves, the weight of the nymphs was the lowest in each instar (3rd instar:  $F = 34.24$ ;  $df = 4, 150$ ;  $P < 0.001$ ; 4th instar:  $F = 19.99$ ;  $df = 4, 150$ ;  $P < 0.001$ ; 5th instar:  $F = 61.87$ ;  $df = 4, 150$ ;  $P < 0.001$ ), and the weight of adults was also lower than in the other treatments ( $F = 138.9$ ;  $df = 4, 150$ ;  $P < 0.001$ ) (Fig. 1). The weight gain of nymphs and adults was largest when they fed on the corn + *H. armigera* eggs diet.

**ADULT LONGEVITY AND FECUNDITY**

Adults died soon after eclosion in the 2 treatments of cotton leaf alone and *H. armigera* eggs alone, so there were no fecundity data for the 2 treatments. Longevity of females was significantly higher when nymphs were provided with corn with or without *H. armigera* eggs than when given any other diet ( $F = 229.8$ ;  $df = 4, 85$ ;  $P < 0.001$ ). In contrast, fecundity of females was significantly higher ( $F = 24.81$ ;  $df = 2, 36$ ;  $P < 0.001$ ) when nymphs were provided with both corn and *H. armigera* eggs than when given only corn (Table 2).



**Fig. 1.** Body mass ( $\pm$  SE) of *Apolygus lucorum* fed on different foods. Bars topped by different lowercase letters represent means that are statistically different among different foods (1-way ANOVA followed by Tukey test,  $P < 0.05$ ).

**PREDATION ON *H. ARMIGERA* EGGS**

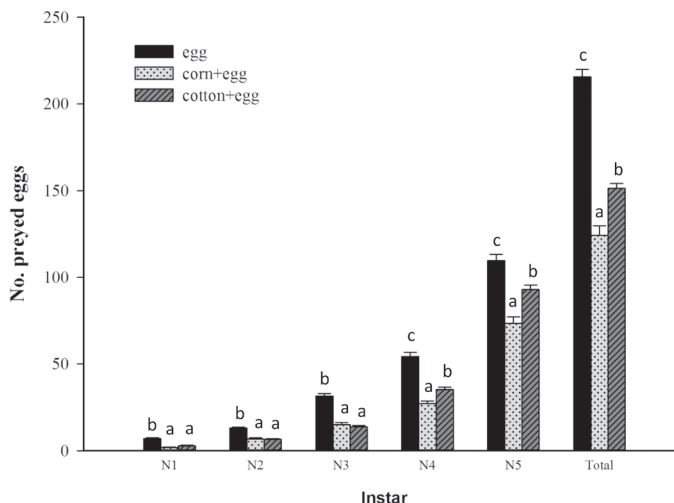
When given no choice, nymphs fed on eggs. However, when given both plant tissue and eggs, they still fed on eggs even though they consumed significantly fewer eggs when offered a plant and egg combination diet than when provided only eggs (Fig. 2). Nymphs fed on significantly fewer eggs with corn than those with cotton ( $F = 101.5$ ;  $df = 2, 80$ ;  $P < 0.001$ ).

**EFFECTS OF DIET ON THREE GENERATIONS**

In the investigation of 3 successive generations comparing the corn and the corn + *H. armigera* eggs treatments, there was no significant interaction between food and generation and nymphal survival rate (Table 3). However, the interaction between food and generation significantly affected the fecundity of females ( $P < 0.05$ ; Table 3). During 3 successive generations, the fecundity of females (Table 4) in the corn alone treatment ( $F = 8.52$ ;  $df = 2, 42$ ;  $P < 0.01$ ) declined to approximately 53% of that in the 1st generation. The fecundity of females in the corn + *H. armigera* eggs treatment ( $F = 5.79$ ;  $df = 2, 35$ ;  $P < 0.01$ ) declined to approximately 26% of that in the 1st generation.

**Discussion**

Most omnivorous bugs can complete their life cycles with various food sources, but different plants or prey will affect their life cycles and population dynamics (Eubanks & Denno 1999). This study showed



**Fig. 2.** Number ( $\pm$  SE) of *Helicoverpa armigera* eggs preyed upon by *Apolygus lucorum* for each instar. Bars topped by different lowercase letters represent means that are statistically different among different foods (1-way ANOVA followed by Tukey test,  $P < 0.05$ ).

**Table 3.** Effect of food, generation, and food\*generation on the life history parameters of *Apolygus lucorum*.

| Source of variation      | Food      | Generation | Food*generation |
|--------------------------|-----------|------------|-----------------|
| Development time         | <0.001*** | 0.085      | 0.248           |
| Nymph survival rate      | 0.863     | 0.124      | 0.827           |
| Adult longevity (male)   | 0.005**   | 0.016*     | <0.001***       |
| Adult longevity (female) | 0.032*    | <0.001***  | <0.001***       |
| Fecundity                | <0.001*** | <0.001***  | <0.038*         |

Food types: corn; corn+ *Helicoverpa armigera* egg.

Generation: the 1st, 2nd, and 3rd generations of *A. lucorum*.

\* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

that *A. lucorum* nymphs were able to complete growth and development to adulthood feeding on corn kernels, cotton leaves, or *H. armigera* eggs alone, but the examined fitness parameters improved when the nymphs fed on both corn and *H. armigera* eggs. Furthermore, we found that *A. lucorum* adults died soon and could not lay eggs when their diet was exclusively cotton leaves or *H. armigera* eggs. When *A. lucorum* was fed only eggs or cotton leaves, the lack of protein in cotton leaves and the lack of carbohydrate in eggs might have caused a nutritional imbalance. In the field, *A. lucorum* not only harm the cotton leaves but also feed on the terminal bud, flower heart, and cotton boll (Lu & Wu 2008), the nutrient contents of which are higher than that of cotton leaves. Some research has demonstrated that mixed food sources were conducive to immature development in omnivorous insects. *Macrolophus pygmaeus* (Rambur) (Hemiptera: Heteroptera: Miridae) could complete its life cycle when feeding only on plants, but when insects such as whiteflies and *Myzus persicae* (Sulzer) (Aphidomorpha: Aphididae) supplemented the plant food, the survival rate of nymphs increased and the development time shortened (Lykouressis & Perdakis 2000). By supplementing with an animal-based food source, the immature development period of *Lygus lineolaris* (Palisot) (Hemiptera: Heteroptera: Miridae) was shortened and the fecundity increased (Cohen 2000). The mirid *D. hesperus* could not complete its growth and development with only tomato leaves, and only 6% of individuals completed nymphal development on a diet of *E. kuehniella* eggs alone, but a high proportion of nymphs (97%) completed development on a diet of the two in combination (Gillespie & McGregor 2000).

In the present study, we found that *A. lucorum* had the habit of feeding on animal food in the process of growth and reproduction. When *H. armigera* eggs and corn kernel or cotton leaves were supplied simultaneously, eggs could be preyed on. Although *A. lucorum* could feed on animal tissue, plant food was more important for the

development and fecundity, which was different from other omnivorous mirids identified as natural enemies, such as *D. hesperus* (Gillespie & McGregor 2000). Corn, compared with cotton leaves, was better for nymphal development and survival. It is possible that corn kernel has more nutrients than leaves of cotton. This also suggests that *A. lucorum* has the potential to cause damage to corn, although more field investigations and further studies on confirming the ecological niche of *A. lucorum* are necessary to evaluate this potential.

In the present study, mixed foods were found more advantageous to adult reproduction, with corn kernel superior to cotton leaves. However, plant tissue was the main food source for *A. lucorum*, and the predation on *H. armigera* eggs could be considered to be supplementary nutrition. In addition, the target DNA fragment (i.e., mitochondrial cytochrome c oxidase I; *COI*) of *A. gossypii* was detected in *A. lucorum* adults (Wang et al. 2010), which proved that *A. lucorum* could prey on *A. gossypii* in a cotton field.

Mixed foods may be used effectively to retard population degradation. As far as is known, population degradation is a common phenomenon in the process of breeding insects, mainly manifesting as reduced individual quality, declined fecundity, and extended development duration. The main reason might be the simplicity of genetics and the differences between the simple artificial environment and the complex natural environment (Huggans 1970). This study was conducted by individual feeding and observation of *A. lucorum* for 3 successive generations under the conditions of feeding corn or both corn and *H. armigera* eggs. Declined fecundity of *A. lucorum* was found when diet was limited to only corn. However, the higher fecundity of females with a diet of both corn and *H. armigera* eggs remained the same for each generation. These results indicated that females were acquiring a higher quality or quantity of proteins necessary for egg development from *H. armigera* eggs than from corn alone, although he decrease in fecundity over the 3 generations provided with corn alone may be an artifact of continuous rearing in the laboratory.

The phytophagy of an omnivorous insect makes it a crop pest, and its predation potential makes it feed on other pests and play the role of a natural enemy. In our laboratory study, *A. lucorum* did have a certain ability to prey on *H. armigera* eggs, and this predation was conducive to the development and fecundity of the insect. However, during the growth and development of *A. lucorum*, plant food was still necessary, and *A. lucorum* could not complete the life cycle with only *H. armigera* eggs. In this case, *A. lucorum* should also be considered as a pest. Field investigations and further studies on confirming the ecological niche of *A. lucorum* are still necessary and might supply important clues to effective integrated management of this pest.

**Table 4.** Mean ( $\pm$  SE) development time, immature survival rate, adult longevity, and fecundity of *Apolygus lucorum* on different foods over 3 successive generations.

| Generation | Life history parameter   | Food type           |  |
|------------|--------------------------|---------------------|--|
|            |                          | Corn                | Corn + <i>Helicoverpa armigera</i> egg |
| 2nd        | Development time         | 11.69 $\pm$ 0.10a   | 10.69 $\pm$ 0.16b                      |
|            | Nymph survival rate      | 51.86 $\pm$ 3.67a   | 52.92 $\pm$ 5.04a                      |
|            | Adult longevity (male)   | 25.60 $\pm$ 1.83a   | 28.70 $\pm$ 0.78a                      |
|            | Adult longevity (female) | 27.40 $\pm$ 1.15a   | 28.10 $\pm$ 0.84a                      |
|            | Fecundity                | 122.63 $\pm$ 11.87a | 141.67 $\pm$ 7.54a                     |
| 3rd        | Development time         | 11.48 $\pm$ 0.08a   | 10.87 $\pm$ 0.11b                      |
|            | Nymph survival rate      | 62.54 $\pm$ 5.11a   | 59.94 $\pm$ 5.42a                      |
|            | Adult longevity (male)   | 19.30 $\pm$ 1.56b   | 31.25 $\pm$ 0.99a                      |
|            | Adult longevity (female) | 21.20 $\pm$ 1.63b   | 29.65 $\pm$ 0.77a                      |
|            | Fecundity                | 50.91 $\pm$ 6.90b   | 125.50 $\pm$ 9.27a                     |

Means within a row followed by different lowercase letters are significantly different ( $t$ -test,  $P < 0.05$ ).

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