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A SHORT-TERM AUXILIARY DIET FOR THE PREDACEOUS STINK BUG, *PERILLUS BIOCULATUS* (HEMIPTERA: PENTATOMIDAE)

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ABSTRACT

Perillus bioculatus (F.) can be maintained in the laboratory on a diet of *Heliothis virescens* F. larvae if supplemented with Colorado potato beetle (CPB) eggs. Here we demonstrate that an artificial diet can replace the CPB eggs and maintain the colony for at least three generations. This enables us to maintain our *Perillus* colonies at high numbers independent of the normal fluctuations in our CPB colony. Survival of nymphs, adult longevity, start of ovipositioning, total number of eggs per female, total number of clutches per female, and percent hatch were equivalent between the two rearing regimes for three generations. Fecundity on the artificial diet was greatly reduced by the sixth generation, leading to the collapse of the colony during the seventh generation.

Key Words: *Perillus bioculatus,* artificial diet

RESUMEN

Perillus bioculatus (F.) puede ser mantenido en el laboratorio con una dieta de larvas de *Heliothis virescens* F. si son suplementados con huevos del escarabajo de papa de Colorado (CPB), (*Leptinotarsa decemlineata* Say), Aqui demostramos que una dieta artificial puede reemplazar los huevos de CPB y mantener la colonia por lo menos en tres generaciones. Este nos permite mantener poblaciones altas de nuestra colonia de *Perillus* independientemente de las fluctuaciones normales en nuestra colonia de CPB. La sobrevivencia de las ninfas, la longevidad de los adultos, el inicio de la oviposición, el numero total de huevos por hembra, el numero total de grupos de huevos por hembra, y el porcentaje de eclosión fueran equivalentes entre los dos regímenes de cria por tres generaciones. La fecundidad de las hembras alimentadas con la dieta artificial fué grandemente reducida en la sexta generación, causando la caida total de la colonia durante la septíma generación.

Perillus bioculatus (F.) is a predaceous pentatomid endemic to North America. *P. bioculatus* feeds on a number of insect orders under both field and laboratory conditions, but has an intrinsic preference for the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Saint-Cyr & Cloutier 1996). CPB is the major defoliator of potato (reviewed Ferro 1985, Hare 1990, Cloutier et al. 1996), tomato (Schalk & Stoner 1979), and eggplant (Cotty & Lashomb 1982; Hamilton & Lashomb 1996). With its strong preference for CPB, *P. bioculatus* has drawn attention as a possible biocontrol agent for CPB in North America (Biever & Chauvin 1992) and Europe (Jermy 1980).

Currently there is no published optimized artificial diet for *P. bioculatus*. Therefore, *P. bioculatus* must be reared on CPB or a secondary host such as *Trichoplusia ni* (Biever & Chauvin 1992) or *Heliothis virescens* supplemented with CPB (Adams 2000). Currently we are maintaining our *P. bioculatus* colony on *H. virescens* larvae, and feeding only first and second instar nymphs on CPB egg masses (Adams 2000). Even with decreased dependence on CPB, problems with the CPB colony have led to loss of our *P. bioculatus* colonies, or to insufficient numbers of CPB egg masses to undertake planned experiments. We investigate here whether a simple, inexpensive diet for another predaceous insect, *Chrysoperla rufilabris* (Cohen & Smith 1998), could replace the CPB eggs to maintain *P. bioculatus* for several generations. This would serve as a bridging diet when CPB is unavailable, while avoiding the cost of incorporating an additional prey insect colony into our research program.

MATERIALS AND METHODS

Rearing

P. bioculatus were reared as described by Adams (2000). Stock colonies were reared in a walk-in environmental chamber set to 16 h light:8 h dark, 25 ± 2°C and 65% relative humidity. Both nymphs and adults were fed 5th instar *H. virescens* larvae. The *H. virescens* larvae were frozen and stored at -25°C until needed. Approximately 300 *P. bioculatus* eggs were used to start each cage of nymphs. First and second instars received three CPB egg masses and one *H. virescens* larva daily. Third through fifth instar nymphs received

seven larvae per day. All nymphal colonies also received two water wicks with a potato leaf inserted in each.

Adults were removed daily from the nymphal colonies and placed into egging cages which had three water wicks with an inserted potato leaf. Each egging cage contained 40 females and 10 males, and 10 *H. virescens* larvae were provided daily. The females deposited their eggs on any hard substrate in the cage. Eggs were collected on Mondays, Wednesdays and Fridays and placed into hatching tubs.

Diet Preparation

The diet was prepared according to Cohen & Smith (1998), except that antibiotics were not included. The diet contained: 100 g 70% lean ground beef, 100 g beef liver, 100 g hen's eggs, 15 g sucrose, 5 g honey dissolved in 20 ml water, 10 g brewer's yeast, 5 ml 10% acetic acid and 45 ml water. Because we were not able to get the diet mixture to solidify as described by Cohen & Smith (1998), the diet was fed to stink bugs in Parafilm domes (Adams 2000).

Experimental Treatment

In the colony-hatching containers the first instar nymphs aggregated on the water wicks and fed minimally, if at all, on the CPB egg masses. Therefore, for ease of handling, all experiments were set up using unfed (water only) second instar nymphs. The nymphs were set up in plastic containers (25 cm diameter by 7 cm high, Pioneer Packaging, Dixon, KY) with a 5 cm hole in the lid covered by nylon screen (Sefar America, Kansas City, MO). The nymphs were reared at 16 h light:8 h dark and 30 ± 0.5°C in a reach-in environmental chamber (Conviron, model I25L, Winnipeg, Canada). Three replicates with 30 nymphs each were used for each treatment group.

Developmental Experiments

The control nymphal colony was given three CPB egg masses per day for the first three days, and three water wicks with potato leaves. The diet treatment nymphs received 2 parafilm domes of artificial diet and three water wicks without potato leaves. The artificial diet domes were changed every other day. All treatment groups received two *H. virescens* larvae per day for the first eight days, after which the number was increased to three per day.

Fecundity Experiments

Potato leaves were not used, because the developmental experiment showed no significant difference in development of *P. bioculatus* with or without potato leaves. The control adults received *H. virescens* larvae, three CPB egg masses per day for the first three days and three water wicks. The artificial diet-treatment group received the artificial diet, *H. virescens* larvae and three water wicks throughout their life cycle. Adults were removed daily from the rearing containers, sexed and weighed. Individual mating pairs were placed into plastic wide-mouth containers (10 × 8.5 × 9.5 cm) (Consolidiated Plastics, Twinsburg, OH), with a 6.5 cm hole in the lid covered by Nitex screen (Tetko, Lancaster, NY). Each mating container had a roll of paper (6 × 64 cm) for ovipositioning and one water wick. The adults were fed one *H. virescens* larvae per day. The cups were checked daily for eggs, which were subsequently removed, counted and placed in 1 oz. plastic cups (Fill-Rite, Newark, NJ) with lids (Stan-Pac, Lewiston, NY) to measure percent hatch.

Statistical Analysis

Proportions of adult emergence were compared using constant statements in PROC LOGISTIC, (SAS Institute 1989). Longevity, start of oviposition, total number of eggs per female, total number of clutches per female, and percent hatch were analyzed using ANOVA with the means being separated using Student-Newman-Keuls test, SAS Institute (1989). The level of significance in all tests was 0.05. The developmental rate for each treatment group was estimated by using the median adult emergence time computed from Kaplan-Meier estimates of survival produced by the surfvit function of S-Plus (S-Plus 2001). For the Fecundity Experiments the initial experimental design called for collecting data from the first, third, sixth, ninth and twelfth generations.

Results

Preliminary experiments demonstrated that the Cohen-Smith artificial diet alone could not support nymphal development (results not shown). There was a significant decrease in survival from 65% to 36% for *P. bioculatus* nymphs fed only *H. virescens* larvae (χ^2= 9.6747, n = 6, *df* = 1, $P < 0.0019$), as compared to control nymphs, which received both *H. virescens* larvae and CPB eggs. Substituting the artificial diet for CPB eggs had no significant effect upon survival of the nymphs to adult emergence, compared to controls at the 0.05 level (Table 1). Increasing the availability of the artificial diet from the first 3 days to 6 days or for all nymphal instars did not increase survival of the nymphs compared to the controls at the 0.05 level (Table 2). Nymphs fed the artificial diet had median adult emergence times equivalent to the controls (Table 3). Of nymphs receiving only *Heliothis* larvae, insufficient numbers survived to analyze their median adult emer-

TABLE 1. EFFECT OF NYMPHAL DIET ON SURVIVAL OF NYMPHS TO ADULT EMERGENCE.*

Diet	Number of Adults
Larvae only	10.8 ± 2.1[a]
Larvae + CPB eggs (control)	19.5 ± 1.4[b]
Larvae + artificial diet	17.3 ± 2.0[b]

*Data are expressed as means ±SE of six replicates of 30 nymphs each. Nymphs were fed *H. virescens* larvae only (Larvae only), or *H. virescens* larvae supplemented during the 2nd instar with either Colorado potato beetle eggs (Larvae + CPB eggs) or artificial diet (Larvae + artificial diet). Means followed by the same letter are not significantly different at the $P \leq 0.05$ level (PROC LOGISTIC).

TABLE 2. EFFECT OF DURATION OF FEEDING ON ARTIFICIAL DIET UPON NYMPH SURVIVAL TO ADULT EMERGENCE.*

Treatment	Number of Adults
Control	23.0 ± 2.5
3D	22.7 ± 1.7
6D	24.5 ± 1.4
15D	24.0 ± 1.8

*Data are expressed as mean ±SE of six replicates of 30 nymphs each. Nymphs were fed *H. virescens* larvae supplemented during the 2nd instar with Colorado potato beetle eggs (Control) for the first 3 days (3D), six days (6D) or throughout nymphal development (15D) with artificial diet. PROC LOGISTIC analysis revealed no significant effects of diet on the number of adults obtained.

gence times. It is clear from these data that the artificial diet can support a generation of nymphal development as effectively as can Colorado potato beetle eggs.

There was no significant difference in the weights of day 1 adults for the first generation. However, by the third generation there was a significant increase in the weight of females receiving the artificial diet ($F = 3.95$, n = 66, $df = 2$, $P < 0.0239$) (Table 4). There was no observed difference in weight of males in the course of these experiments. Females that were fed *H. virescens* larvae supplemented with the artificial diet throughout nymphal development showed no reduction in fecundity for three generations, compared to the controls (Table 5). There were no significant differences in female longevity, onset of oviposition, total number of eggs, number of clutches per female, or percent hatch at the 0.05 level. There was a marked decrease in the number of eggs laid by the females during the sixth generation of continuous rearing on the artificial diet plus larvae, and loss of the colony during the seventh generation.

DISCUSSION

The data presented here clearly demonstrate that a diet regime of the modified Cohen-Smith artificial diet for *C. rufilabris* (Cohen & Smith 1998) plus *H. virescens* larvae is as efficient as CPB eggs and *H. virescens* larvae for supporting *P. bioculatus* development for at least three generations. Supplementing *H. virescens* larvae with the Cohen-Smith artificial diet (Cohen & Smith 1998) produced three generations of *P. bioculatus* phenotypically indistinguishable (except for increased weight of female adults) from those on the control diet of larvae plus CPB eggs. This demonstrates that an artificial diet and a suboptimal secondary prey, which by themselves cannot support normal development, can be used in combination as a bridging diet to maintain a research colony during periods when the primary prey is unavailable.

However, while early generations seemed healthy, the experimental colony suffered from reduced fecundity by the sixth generation, and completely collapsed during the seventh. This raises intriguing questions as to what biochemical and molecular changes have occurred over the seven generations. Insects will express a particular subset of genes in order to survive environmental stresses such as heat shock (Roberts & Feder 1999), cold shock (Yocum 2000), and desiccation (Tammariello et al. 1999). As with other stresses, low food quality or quantity will induce a number of physiological and behavioral changes in insects (reviewed, Slansky & Scriber 1985; Wheeler 1996). We are currently investigating unique gene expression associated with feeding on suboptimal diets. Our bridging diet fed to *P. biocula-*

TABLE 3. EFFECT OF NYMPHAL DIET ON THE MEDIAN EMERGENCE TIME OF ADULT *P. BIOCULATUS*.*

Treatment	Nymphs[1]	Adults[2]	Median adult emergence (D)	0.95 LCL	0.95 UCL
Control	360	255	21	20	21
3D	360	243	22	21	22
6D	180	147	20	20	20
15D	180	144	21	21	22

*Data are expressed as median adult emergence times (days) ± lower (LCL) and upper (UCL) 95% confidence limits. Nymphs were fed *H. virescens* larvae supplemented during the 2nd instar with Colorado potato beetle eggs (Control) or for the first 3 days (3D), six days (6D) or throughout nymphal development (15D) with artificial diet.

[1]Total number of 2nd instar nymphs at the start of the experiment.

[2]Total number of adults that emerged.

TABLE 4. EFFECTS OF PROLONGED FEEDING ON THE ARTIFICIAL DIET ON DAY 1 ADULT *P. BIOCULATUS* WEIGHT.*

Treatment	Generations	Males		Females	
		N	Weight (mg)	N	Weight (mg)
Control	F1	18	47.8 ± 1.5[a]	27	60.1 ± 1.1[a]
Diet-fed	F1	18	47.8 ± 1.3[a]	24	63.8 ± 1.2[ab]
Diet-fed	F3	14	48.9 ± 1.9[a]	18	65.2 ± 2.1[b]

*Data are expressed as means ±SE of the weights of N number of day 1 adults. Means followed by different letters are significantly different from each other at $P \leq 0.05$ as determined by Student-Newman-Keuls test. Nymphs were fed *H. virescens* larvae supplemented with either Colorado potato beetle eggs during the 2nd instar (Control) or the artificial diet throughout development (Diet-fed).

TABLE 5. EFFECTS OF PROLONGED FEEDING ON THE ARTIFICIAL DIET ON *P. BIOCULATUS* FECUNDITY.*

Treatment	Generation	N	Longevity (D)	Initiation Oviposition (D)	Eggs/ female	Clutch/ female	% Hatch
Control	F1	17	17.4 ± 2.4	4.5 ± 0.2	109.1 ± 17.5	9.5 ± 1.0	39.1 ± 5.4
Diet-fed	F1	14	16.1 ± 2.0	4.6 ± 0.4	95.5 ± 13.8	11.4 ± 1.0	25.7 ± 5.4
Diet-fed	F3	9	21.7 ± 2.3	4.4 ± 0.3	156.6 ± 22.7	11.7 ± 1.0	39.1 ± 4.0

*Data are expressed as means ±SE of N number of females each paired with a single male. Nymphs were fed *H. virescens* larvae supplemented with either Colorado potato beetle eggs during the 2nd instar (Control) or artificial diet throughout development (Diet-fed). ANOVA analysis revealed no significant effects of diet on female longevity (days), start of oviposition, total number of eggs per female, number of clutches per female, or percent hatch at the $P \leq 0.05$.

tus will also be an excellent model system to examine how gene expression is altered over generations in response to feeding on a suboptimal diet. Our ultimate goal is to identify molecular markers to use in diagnosing deficiencies in other artificial diets under development.

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