AN ARTIFICIAL DIET FOR THE BUTTERFLY PHYCIODES PHAON (LEPIDOPTERA: NYMPHALIDAE)

Authors: Genc, Hanife, and Nation, James L.

Source: Florida Entomologist, 87(2) : 194-198

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2004)087[0194:AADFTB]2.0.CO;2

AN ARTIFICIAL DIET FOR THE BUTTERFLY *PHYCIODES PHAON* (LEPIDOPTERA: NYMPHALIDAE)

HANIFE GENC AND JAMES L. NATION
Department Of Entomology & Nematology, University of Florida, Gainesville, FL 32611-0620

ABSTRACT

We reared newly hatched Phaon Crescent butterfly larvae to the adult stage on a completely artificial diet. About 37% of first instars survived to the adult stage. Addition to the diet of freeze-dried host plant leaves equal to 10% by weight of dry ingredients produced up to 66% survival to the adult stage. Survival of larvae and production of adults on the artificial diet without host plant leaves was increased to equal that of diet with host plant leaves by adding 5% glucose or 5% Beck's salt mix. Although the ovaries of females produced on host-free artificial diet appeared to be mature at emergence and contained mature-looking eggs, we never obtained viable eggs from them. In contrast, females produced on the artificial diet containing at least 10% by weight of freeze-dried host plant leaves laid viable eggs, and four successive generations were reared on the artificial diet with 10% freeze-dried host plant leaves. Males produced on the artificial diet without host plant tissue displayed abnormalities in the shape of the testes and parts of the vas deferentia, compared to males reared on the diet with freeze-dried host leaves or on living host plants. The role of host plant tissue in nutrition and reproduction of both male and female Phaon crescents remains to be determined.

Key Words: artificial diets, butterflies, Phaon crescent, Nymphalidae, insect-plant interaction, *Phyla nodiflora*, ovary, testes

RESUMEN

Nosotros criamos larvas de la mariposa cresciente Phaon recién eclosionadas hasta la estadia adulta sobre una dieta artificial. Aproximadamente, 37% de las larvas de la primera estadia sobrevivieron hasta la estadia adulta, pero los adultos no se aparearon ni reproducieron. La adición de hojas congeladas y secadas de la planta hospedera a la dieta igual a 10% del peso seco de los ingredientes produjieron una sobrevivencia de 66% hasta la estadia adulta, y los adultos se aparearon y pusieron huevos que eclosionaron. La sobrevivencia de las larvae y la producción de adultos sobre una dieta artificial sin hojas de la planta hospedera fue aumentada para ser igual que la sobrevivencia con hojas de la planta hospedera por añadir 5% de glucosa o 5% de la mezcla de sal de Beck, pero los adultos no se apareararon a menos que la dieta tenia hojas de la hospedera. Los ovarios de las hembras producidas sobre una dieta artificial sin la planta hospedera aparecieron ser maduras al emergir y tenian huevos maduros, igual como los ovarios de las hembras criados sobre la planta hospedera. Ningún abnormalidad en las estructuras reproductivas internas de las hembras producidas sobre la dieta artificial fue detectada. Los machos producidos sobre la dieta artificial sin el tejido de la plant tenian abnormalidad en las estructuras reproductivas internas. Asi, ambos sexos criados sobre una dieta artificial evidentemente tienen algunas abnormalidad funcionales para prevenir el apareamiento y el éxito reproductiva. El papel del tejido de la planta hospedera en influenciar el comportamiento y la reproducción de los crescientes Phaon es desconocido.

Many moths, beetles, crickets, grasshoppers, and other insects, but only two or three butterfly species, can be reared on artificial diets (Singh 1977). The cabbageworm butterfly (*Pieris rapae* (L.)) (Webb & Shelton 1988) and the painted lady butterfly (*Vanessa cardui* (L.)) have been reared on artificial diets. Semiartificial diets that contain host-plant material have been published for rearing the Monarch butterfly. The need to study and control pest insects probably has contributed to the development of artificial diets for many insects, but most butterflies are not pests on economic crops and little effort has been devoted to developing artificial rearing media for them. Butterflies tend to be restricted to one or only a few host plants as larvae, and possibly they are very sensitive to the balance of nutrients and/or presence of specific feeding cues in their host plants. A practical difficulty in working with butterflies is that many are active only part of the year, and their larval host plants are often seasonal.

The Phaon crescent, *Phyciodes phaon* (Edwards), is a small butterfly present over much of the southeastern United States. A number of factors make the Phaon crescent a suitable butterfly for study, including year-round distribution of the butterfly and its host plant in Florida. The adults do not diapause, and they mate and lay eggs in the laboratory. Females lay their eggs on the underside of leaves of the host plant *Phyla nodiflora* (L.) Greene in the family Verbenaceae (Minno & Minno 1999; Emmel & Kenny 1997; Genc 2002). In preliminary trials with several published diets for rearing butterflies and moths, Genc (2002) found that the only diet formulation that allowed a few adults to be produced was the pinto bean (PB) diet developed for certain moths (Guy et al. 1985). Survival on the PB diet was poor, however, and adults produced did not mate. Our objectives in this paper are to describe (1) diets that improve survival of Phaon crescent larvae and adult production, (2) diets that promote mating and reproduction of adults, and (3) female and male internal reproductive systems of Phaon crescents reared on completely artificial diet with those reared on the host plant.

Materials and Methods

The Butterfly and Host Plant

A colony of *Phyciodes phaon* was started from females collected on the University of Florida campus. The host plant was collected from the campus and vicinity, and maintained in containers and small outdoor plots. Leaves of the host plant were frozen in liquid nitrogen, ground while frozen in a mortar, and freeze-dried. The freeze-dried host plant leaves were stored at -20°C until needed. Adults were allowed to lay eggs on the leaves of the living host plant, and newly hatched first instars were removed and placed on diets. A breeding colony of the butterfly was maintained in the laboratory on host plants, and adults were provided with 10% honey in water.

Preparation of the Pinto Bean Diet from Components

The pinto bean (PB) diet was prepared from individual components purchased from BioServ (One 8th Street, Frenchtown, NJ 08825, USA). We mixed pinto bean meal (19 g), wheat germ (14 g), torula yeast (8 g), casein (7 g), gelcarin (3 g), methyl paraben (0.5 g), and sorbic acid (0.3 g) and added the mixture to 182 ml cold water with stirring by a mechanical mixer. The aqueous mixture was heated slowly (requiring about 15 minutes) on a hot plate to 70°C with continuous stirring. Formaldehyde (1 ml) was added and mixing was continued for about 3 minutes without further heating. Ascorbic acid (0.9 g) was added and mixing was continued an additional 3 minutes without heating, and finally tetracycline (0.01 g), BioServ Vitamin Mix #F8095 for Lepidoptera (0.8 g), and propionic acid (0.3 ml) were added with additional mixing for 2-3 minutes. The mixture was poured into paper cups, allowed to cool and gel at room temperature, and stored in a refrigerator until needed. When ground, freeze-dried plant leaves were added to the pinto bean diet, addition was made with the vitamin mixture to minimize heat damage to the host plant material.

Diet Modifications

Diets were formulated by incorporating 1% glucose, 5% glucose, 1% Beck's salt mixture, 5% Beck's salt mixture, 1%, 5%, 10%, or 20% ground, freeze dried host plant leaves into the PB diet. Diets were tested by placing 25 newly hatched larvae on each of 3 replicates of each diet. The criteria for evaluating a diet were number of adults reared and whether the adults mated and females laid viable eggs.

Dissection

The abdomen of adults was brushed with a camel's hair brush dipped in 70% ethyl alcohol to remove scales, and then opened ventrally from the first to the terminal abdominal segment. The terminology used by Dong et al. (1980) was used to describe the internal reproductive structures.

Statistics

For comparing the number of adults produced on modifications of the PB diet, we used binary logistic regression analyses (Harrell 2001; Hosmer & Lemeshow 2000). Chi Square tests were used to determine the statistical significance of the model parameters and an overall Chi Square test assessed the hypothesis of no overall treatment difference. When a significant Chi Square value was obtained, the means for adult production on each tested diet were transformed from non-linear function to linear function and least square estimates of the diet-specific probabilities, *P*, of survival to the adult stage were obtained by inverting the log odds model.

Results

Diet Modifications

Survival to the adult stage was statistically higher on PB diet with 10% or 20% freeze-dried host plant leaves than with only 1% or 5% leaves (Table 1). Moreover, adults from the diets containing 10% and 20% leaves reproduced and enabled us to maintain a colony, but adults produced with 1% or 5% leaf tissue in the PB diet did not reproduce. Addition of 5% glucose or 5% Beck's salt mix to the PB diet produced adults in numbers statistically equal to numbers of adults produced with 10% host plant leaves in the PB diet, but none of the adults from glucose or salt modified diets reproduced. Numbers of adults produced on diets with 1% host leaves, 5% host leaves, 1% glucose, 1% Beck's salt mix, or the original PB formula were not statistically different from each other.

TABLE 1. PRODUCTION OF ADULTS ON PB DIET OR PB DIET WITH AN AMENDMENT. NEWLY HATCHED LARVAE (25) WERE PLACED ON EACH OF THREE REPLICATES OF EACH DIET.

PB diet + Amendment	Mean (± SD) number of adults produced per replicate	Percent adults
1% host plant leaves	8.0 ± 0.0 a	32
5% host plant leaves	12.0 ± 1.4 a	48
10% host plant leaves	16.5 ± 0.7 b	66
20% host plant leaves	13.5 ± 2.1 b	54
PB diet	8.5 ± 0.7 a	34
1% glucose	10.0 ± 1.4 a	40
5% glucose	17.5 ± 2.1 b	70
1% Beck's salt mix	11.5 ± 0.7 a	46
5% Beck's salt mix	16.5 ± 0.7 b	66

Values in a column followed by the same letter are not significantly different from each other at $\alpha = 0.01$ level.

Anatomy of Phaon Crescent Internal Reproductive Structures

The structure of the internal reproductive system of a 10-day-old female produced on the host plant is shown in Figure 1A and the ovary of a newly emerged female adult from the PB diet is shown in Figure 1B. Adult females produced on both food sources appeared to have mature or nearly mature eggs in the terminal follicles of each ovariole at emergence, with four ovarioles in each of two ovaries. Although a large amount of fat body associated with the ovaries makes counting individual egg chambers very difficult, one newly emerged female was determined to have approximately 49 egg chambers in each ovariole. Not enough observations were made, however, to determine an average for number of egg chambers per ovary or eggs laid. The lateral oviducts guide eggs to a common, medial oviduct leading to the genital chamber (the bursa copulatrix). Paired lateral accessory glands are each connected to the median oviduct.

The structure of the internal reproduction organs from a male produced on the host plant is shown in Figure 2A, and those from a male produced on the PB diet is shown in Figure 2B. Fused testes form one testicular body in males. The testicular body is round and dark reddish brown in males produced on the host plant and on PB diet

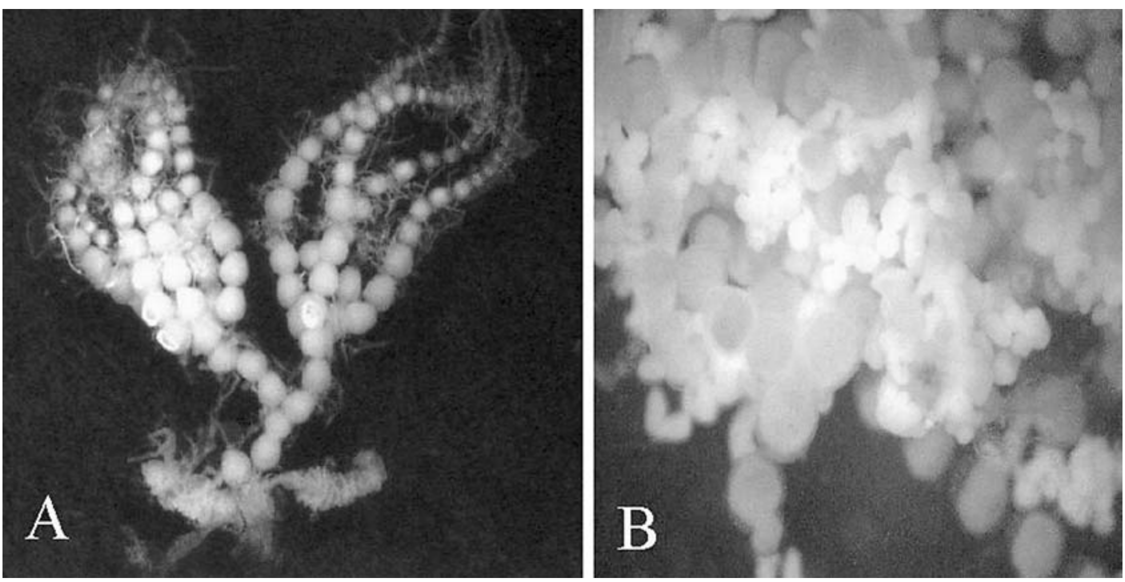

Fig. 1. Internal reproductive structure of *Phyciodes phaon* female. A. The ovary was dissected from a 10-day-old female reared on the host plant. The fat body has been almost entirely used up in production of eggs. The four ovarioles per ovary, and individual egg chambers can be seen. B. Ovary dissected from a newly emerged female produced on PB diet. Mature-looking eggs are present surrounded by large amounts of fat body, which is characteristic of newly emerged females produced on host plant, PB diet with 10% leaves, or PB diet.

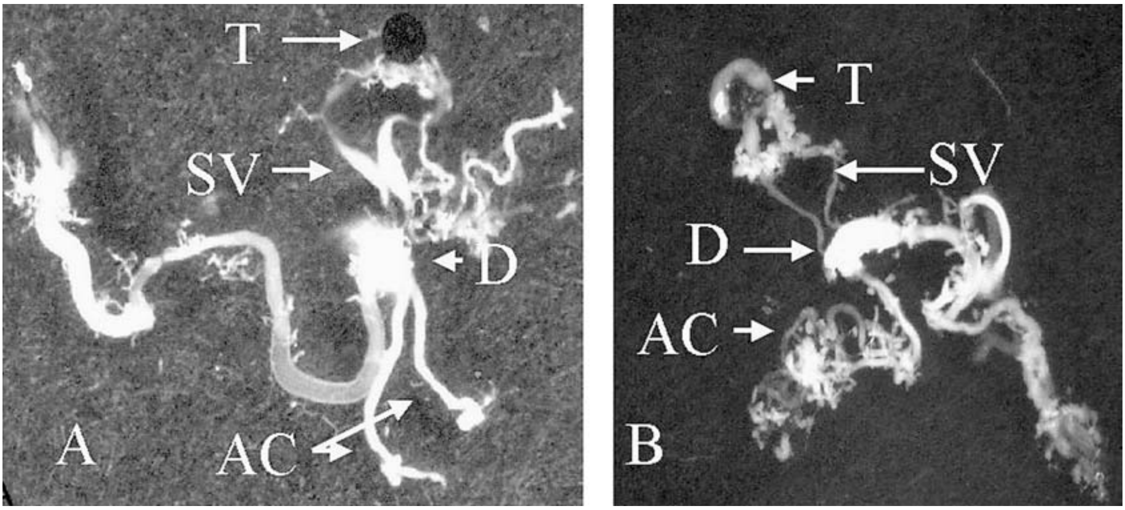

Fig. 2. Internal reproductive structures of *Phyciodes phaon* male. A. Internal structures dissected from a newly emerged male produced on the living host plant. The fused testes (T) seminal vesicle (SV) leading to the ductus ejaculatorius duplex (D) and accessory glands (AC). The duplex loops join to form the ductus ejaculatorius simplex leading to the aedeagus. B. The photograph shows the fused testes and related internal structures from a newly emerged male reared on PB diet. Note larger, discolored testes (T) and atrophied seminal vesicles (SV).

with 10% freeze-dried leaves. In males produced on the PB diet, the testicular body is not uniformly colored as in males from the host plant. There are differences also in the appearance of the vas deferentia of the males. The vas deferentia of males produced on the host plant or PB diet with 10% freeze-dried leaves have swollen vas deferentia near the midlength, forming the seminal vesicles. The seminal vesicles of males produced on the PB diet are not swollen and appear to be atrophied.

Discussion

The PB diet designed for moths clearly is not satisfactory as a diet for the Phaon crescent. As originally formulated, it allows only about 37% of newly hatched larvae to become adults, and the adults do not reproduce. Thus, a colony cannot be maintained on the artificial diet. We improved the diet with respect to both survival and ability of adults to reproduce by adding freeze dried host plant leaves equal to 10% by weight of dry ingredients of the PB formula. This improved diet produced from 66% up to 78% adults in some experiments from first instars started on the diet, and the adults mated and reproduced, maintaining the colony. Although we also improved the PB diet to give good production of adults by addition of 5% glucose or 5% Beck's salt mix, the adults did not reproduce. Glucose in the PB formula may be a feeding stimulant, and/or a readily available carbohydrate energy source. The original PB formula does not include a simple carbohydrate, nor does it include a salt mixture. Lepidopterans, most of which are phytophagous, typically have a relatively high K^+/Na^+ ratio in the hemolymph, in contrast to omnivorous and some carnivorous feeders which have low K^+/Na^+ ratios. Beck et al. (1968) developed a salt mixture (now sold as Beck's salt mixture) relatively high in K^+ and Mg^{2+} and low in Na^+ and Ca^{2+} and showed that it improved the growth and survival of the European corn borer, *Ostrinia nubilalis* (Hübner). Wesson's salt mix often has been used in insect diets (Singh 1977), but it was developed for vertebrate animals, and it has high Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios suitable for vertebrates. Although it works for some insects, probably it is not optimal for phytophagous insects.

Dethier (1954) and Fraenkel and Blewett (1943) emphasized that host plant selection is determined by the presence or absence of nonnutritive secondary plant substances that act as feeding deterrents or stimulants. Various imbalances of the nutrients in a diet can stress insects, and reduce growth and survival (House 1965; House 1969). The small amount of host leaves in the artificial diet may aid digestion and assimilation of nutrients, and may help balance some of the nutrients in the PB diet formula.

Newly emerged females reared on the host plant and on the PB diet with added host plant leaves have mature ovaries with apparently mature eggs at the time of emergence. In this respect they are similar to the cecropia moth *Hyalophora cecropia*, the silkmoth, *Bombyx mori*, and the fall armyworm *Spodoptera frugiperda*, all of which develop the ovaries and eggs during some part of the pupal stage (Tsuchida et al. 1987; Sorge et al. 2000). In contrast, the noctuid moth *Heliothis virescens* (Zeng et al. 1997) and the monarch butterfly *Danaus plexippus* (Pan & Wyatt 1971) have a relatively immature ovary at adult emergence.

Female Phaon crescents have four ovarioles in each of 2 ovaries, and each ovariole contains about 49 egg follicles, with apparently mature eggs ready to be fertilized and laid a few days after emergence. Thus, a female might be able to lay about 400 eggs, and we found that one individual did lay 434 eggs. The failure of females produced on the host-free PB diet to lay eggs may be due to a failure to mate. Despite substantial time in observations, we never observed mating in butterflies produced on the PB diet, whereas observations of mating were common in butterflies produced on PB diet with 10% freeze-dried host leaves or those produced on living host plants. Mating is a stimulus for oviposition and oogenesis in some insects. For example, oviposition in the Australian field cricket, *Teleogryllus* sp., and the onion fly, *Delia* sp., is enhanced as a result of mating (Chapman 1998). Males of some lepidopterans transfer prostaglandins or prostaglandins-synthesizing chemicals to the female during mating and these stimulate oviposition (Stanley-Samuelson 1994).

Male Phaon crescents produced on host plants have a mature reproductive system and mate within 2-3 days after emergence. The male system includes fused testes, vas deferentia, paired accessory glands, and an ejaculatory structure and duct. The enlarged regions of the vas deferentia that serve as a sperm reservoir and seminal vesicle in males produced on the host plant or PB diet with 10% freeze-dried host plant leaves appear to be atrophied in males produced on PB diet. The testes from PB diet reared males are larger (swollen) and light red in color, compared to those reared on living host plant or PB diet with 10% freeze-dried host leaves. These defects observed in the internal reproductive system of males produced on the PB diet, coupled with the failure to get any reproduction from sexes produced on the PB diet suggest that these males may not produce viable sperm.

Although no apparent abnormalities were detected in the internal reproductive system of females produced on PB diet, they could have physiological defects in the reproductive system that are not evident from simple dissections.

ACKNOWLEDGMENTS

We thank Drs. Norman Leppla, Grazyna Zimowska, Jeff Shapiro, and Simon Yu for helpful criticism of earlier drafts. We thank Kathy Milne for technical assistance in the laboratory. We thank Dr. Kenneth Portier for assistance with statistical analyses. Hanife Genc received support from the government of Turkey. Florida Agricultural Experiment Station Journal Series No. R-09650.

REFERENCES CITED

BECK, S., D., G. M. CHIPPENDALE, AND D. E. SWINTON. 1968. Nutrition of the European corn borer, *Ostrinia nubilalis*. VI. A larval rearing medium without crude plant fractions. Ann. Entomol. Soc. Am. 61: 459-462.

CHAPMAN, R. F. 1998. The Insects: Structure and Function. 4th Edition. Cambridge University Press, UK. 770 pp.

DETHIER, V. G. 1954. Evolution of feeding preferences in phytophagous insects. Evolution 8:33-54.

DONG, NGO, T. C. CARLYSLE, H. L. CROMROY, AND D. H. HABECK. 1980. Morphological Studies on the beet armyworm *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuiidae). Technical Bulletin 816, Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.

EMMEL, T. C., AND B. KENNEY. 1997. Florida's Fabulous Butterflies. Tampa, FL, World Publications. 96 pp.

FRAENKEL, G., AND M. BLEWETT. 1943. The sterol requirements of several insects. J. Biochem. 37:692-695.

GENC, HANIFE. 2002. Phaon crescent, *Phyciodes phaon*: Life cycle, nutritional ecology and reproduction, Ph.D. dissertation, University of Florida, Gainesville, FL 32611, USA.

GUY, R., N. C. LEPPLA, J. R. RYE, C. W. GREEN, S. L. BARRETTE, AND K. A. HOLLIEN. 1985. *Trichoplusia ni*, pp. 487-493. *In* P. Singh and R. F. Moore [eds.], Handbook of Insect Rearing Vol. II, Elsevier Science Publishers.

HARRELL, F. E. 2001. Regression Modeling Strategies: with Applications to Linear Model, Logistic Regression, and Survival Analysis. Springer-Verlag, 568 pp.

HOSMER, D. W., AND S. LEMESHOW. 2000. Applied Logistic Regression. Wiley & Sons, NY, 373 pp.

HOUSE, H. L. 1965. Effects of low levels of the nutrient content of a food and of nutrient imbalance on the feeding and the nutrition of a phytophagous larva, *Celerio euphorbiae* (Linnaeus) (Lepidoptera: Sphingidae). Can. Entomol. 97: 62-68.

HOUSE, H. L. 1969. Effects of different proportions of nutrients on insects. Ent. Exp. Appl. 12: 651-669.

MINNO, M. C., AND M. MINNO. 1999. Florida Butterfly Gardening: A Complete Guide to Attracting, Identifying, and Enjoying Butterflies of the Lower South. University Press of Florida, Gainesville. 210 pp.

PAN, M.-L., AND G. R. WYATT. 1971. Juvenile hormone induces vitellogenin synthesis in the monarch butterfly. Science 174: 503-505.

SINGH, P. 1977. Artificial Diets for Insects, Mites, and Spiders. IFI/Plenum, New York. 594 pp.

STANLEY-SAMUELSON, D. W. 1994. Prostaglandins and related eicosanoids in insects. Advances in Insect Physiology, 24: 115-212.

SORGE, D., R. NAUEN, S. RANGE, AND K. H. HOFFMANN. 2000. Regulation of vitellogenesis in the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J. Insect Physiol. 46: 969-976.

TSUCHIDA, K., M. NAGATA, AND A. SUZUKI. 1987. Hormonal control of ovarian development in the silkworm, *Bombyx mori*. Arch. Insect Biochem. Physiol. 5: 167-177.

WALDBAUER, G. P., AND S. FRIEDMAN. 1991. Self-selection of optimal diets by insects. Annu. Rev. Entomol. 36: 43-63.

WEBB, S. E., AND A. M. SHELTON. 1988. Laboratory rearing of the imported cabbageworm. New York's Food and Life Sciences Bulletin 122: 1-6, N.Y. State Agricultural Experiment Station, Geneva.

ZENG, E., S. SHU, AND S. B. RAMASWAMY. 1997. Vitellogenin and egg production in the moth, *Heliothis virescens*. Arch. Biochem. Physiol. 34: 287-300.