Improved Semipure Artificial Diet for *Ectropis obliqua* (Lepidoptera: Geometridae)[1]

Yong Zhang, YiRan Chen, Chan Zhang, TianYu Zhao, YanHua Long[2], YanZhuo Zhang[3], and YunQiu Yang[4]

Anhui Agricultural University, State Key Laboratory of Tea Plant Biology and Utilization, Hefei, 230036 China

J. Entomol. Sci. 54(3): 250–255 (July 2019)

Abstract An improved artificial diet for *Ectropis obliqua* Prout (Lepidoptera: Geometridae) is described. Mean growth parameters of insects fed the diet demonstrated a higher level of pupation, a greater pupal weight, and a higher level of emergence from pupation as compared to those fed a commonly used general purpose diet. The feeding effect is similar to a diet containing tea powder. This improved artificial diet is suitable for *E. obliqua* used in research and allows for the addition of any monomeric compounds to assess impacts on *E. obliqua* growth or other parameters.

Key Words geometrid, artificial diet, *Ectropis obliqua*

Ectropis obliqua Prout (Lepidoptera: Geometridae) is a major pest of tea, *Camellia sinensis* L., in China (Hu et al. 1994, Yang et al. 2016). Its larvae are defoliators that seriously affect the growth of tea plants, as well as tea production and quality. Approximately 4 million hectares of tea are infested by *E. obliqua* annually (Gao et al. 2007). In order to effectively conduct research on the biology and management of *E. obliqua*, it is necessary to produce large numbers of healthy specimens in laboratory colonies. An artificial diet is an important component of these efforts (Singh and Moore 1985).

Currently used artificial diets for *E. obliqua* contain tea powder (Liu et al. 2015, Yuan et al. 2017). *Ectropis obliqua* fed on a diet (Zhang et al. 2007) that did not contain tea powder exhibited a pupation rate of 76.2%, a larval development duration of 11 d, and an emergence rate of 73.6%, but larval and pupal weights were lower than those feeding on tea leaves or on diets containing tea powder. These lower-performing larvae are not adequate for use in assays and other studies.

Diet supplemented with appropriate amounts of tea powder can yield *E. obliqua* that grow and develop normally, but the complex composition of tea powder interacts with individual diet additives (Fang et al. 2003, Hiyama et al. 2010). It is necessary to develop a diet to overcome this problem and to identify ingredients to replace tea powder.

[1]Received 08 April 2018; accepted 29 July 2018.

[2]Anhui Agricultural University, School of Life Sciences, Hefei, 230036 China.

[3]University of Georgia, College of Agricultural and Environmental Sciences, Athens, Georgia 30602 USA.

[4]Corresponding author (email: yyq_lyh@163.com).

Table 1. Composition and proportion (%) of ingredients of selected artificial diets for *Ectropis obliqua*.

Diet Preparation Groups	Zhang et al (2007) Diet	Proposed Diet	General Purpose Tea Powder Diet
A	Agar (1.8%)	Agar (2%)	Agar (2%)
B	Wheat germ (13.8%) Casein (8%)	Wheat germ (12%) Soybean (8%) Sugar (2%)	Wheat germ (12%) Soybean (8%) Sugar (2%)
C	Wesson's salts (2.6%) Sorbic acid (0.6%) Nipalgin (0.5%) Inositol (0.8%)	Wesson salts (0.5%) Sorbic acid (0.5%) Nipalgin (0.25%) Inositol (0.035%)	Wesson salts (0.5%) Sorbic acid (0.03%) Nipalgin (0.25%) Inositol (0.035%)
D	Vitamin C (1%) Vitamin B (0.9%)	Vitamin C (0.2%) Yeast (0.2%) Choline chloride (0.05%) EGCG	Vitamin C (0.2%) Tea powder (2%)

Materials and Methods

Insect rearing. *Ectropis obliqua* larvae were maintained in a ventilated plastic boxes in the environmental chamber maintained at 23 ± 2°C with a photoperiod of 16:8 h (light:dark), and a relative humidity of approximately 75%. Larvae were fed on fresh leaves of tea, *C. sinensis* cv. Nongkangzao for several generations.

Preliminary optimization of artificial diet. The artificial diet developed by Zhang et al. (2007) was used as the control (Table 1). Artificial diets used to rear *Spodoptera litura* (F.), *Hyphantria cunea* (Drury), *E. obliqua*, and other lepidopterans (Gupta et al. 2005, Mukaiyama 1966) were used to develop a recipe for a proposed alternative diet for *E. obliqua* (Table 1). Ingredients of the general purpose artificial diet containing tea powder are listed in Table 1.

Additive concentration assay. The compound (–)-epigallocatechin-3-gallate (EGCG) is the major catechin (phenol) found in green tea (Nagle et al. 2006) and reportedly possesses antimicrobial (Matsumoto et al. 2012), anti-inflammatory, and antioxidative (Cavet et al. 2011) properties in humans. And, according to the content of EGCG in tea, we determined that the concentration of EGCG in an artificial diet for *E. obliqua* might range from 0.5% to 3% (Wan 2003). We therefore established EGCG concentrations of 0%, 0.5%, 1%, 1.5%, 2%, and 2.5%.

Briefly, the Zhang et al. (2007) diet and the modified diet (Table 1) were prepared with ingredients in proportion to those listed. For each, agar (Group A) was dissolved in an appropriate amount of distilled water and heated to 50–60°C when ingredients from Group B were added, stirred, and boiled for 1 to 2 min. These

Table 2. Mean ± SD pupation rate, larval duration, pupal weight, and emergence rate of *Ectropis obliqua* fed an improved artificial diet and the Zhang et al. (2007) artificial diet.

Diet	Pupation Rate (%)	Larval Duration (d)	Pupal Weight (mg)	Eclosion Rate (%)
Zhang et al. (2007) diet	66.7 ± 5.77 a*	18.1 ± 0.35 a	85 ± 3.1 a	63.3 ± 5.77 a
Improved artificial diet	73.3 ± 5.77 a	19.0 ± 0.36 a	102 ± 3.6 b	66.7 ± 5.77 a

* Means within a row followed by the same lowercase letter are not significantly different according to least significant difference test after one-way analysis of variance.

mixtures were then cooled to 50–60°C when ingredients from Group C were added and mixed until the temperature decreased to 20°C, when Group D ingredients were added and mixed. Each diet was then allowed to solidify as a paste and stored at 4°C. The diet supplemented with EGCG was prepared following the same method, except that EGCG at different concentrations was added with the Group D ingredients.

The prepared artificial diets were cut into thin slices and placed upside down on the inside top of the sterile petri dish. Neonates were transferred to the petri dish. The petri dish was placed directly under the light source so that larvae were phototropically attracted to the diet. Petri dishes containing larvae and diet were placed in an environmental chamber maintained as previously described. Controls consisted of the general-purpose diet, the diet containing tea powder, and the artificial diet with 0% EGCG.

In each experiment, the feeding assays were repeated independently three times; replicates included 10 larvae each. Growth and development were monitored daily. Larval duration, pupation, and pupal weight were recorded. Emergence from pupae also was recorded. Data were subjected to analysis of variance followed by a least significant difference test to separate significantly different treatment means ($P < 0.05$).

Results and Discussion

No significant differences of pupation rate, larval duration, or emergence rate were observed with the general-purpose diet or the improved artificial diet (Table 2). The effects of diet supplemented with different concentrations of EGCG are shown in Fig. 1. Larvae fed the diet containing 3% EGCG are not included in Table 2 or the figures because almost all larvae died before pupation.

Pupation increased with an increase of dietary EGCG from 0% to 2.5%.

Pupation increased by more than 35% when the larvae fed on diet supplemented with 2.5% EGCG (Fig. 1A). Duration of larval development was slightly but significantly ($P > 0.01$) extended with larvae fed on diet supplemented with EGCG; however, there were no significant differences in larval duration among different

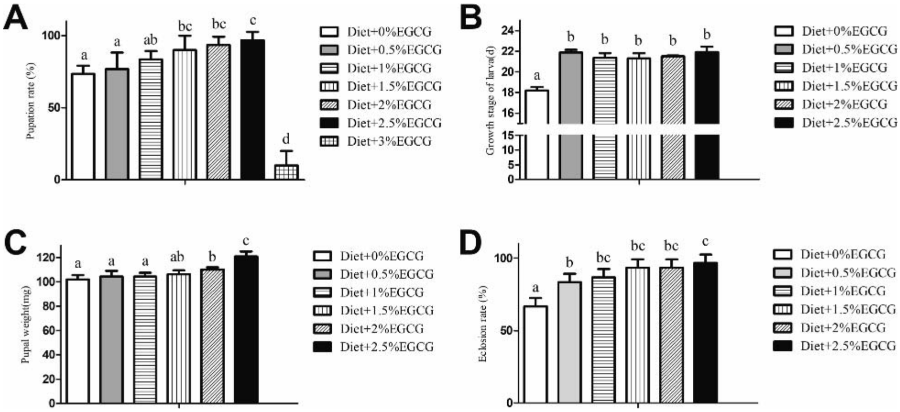

Fig. 1. The effect of diet supplemented with different concentrations of (–)-epigallocatechin-3-gallate (EGCG). Mean ± SD percentage of pupation rate (A), instar duration (d) (B), pupal weight (mg) (C), and percentage of emergence rate (D) of *Ectropis obliqua* fed diet supplemented indicated concentrations of EGCG. Bars within each graph with the same lowercase letter are not statistically significant different (least significant difference, $P < 0.05$).

EGCG additive concentrations. Pupal weight also increased with an increase of EGCG concentration from 0% to 2.5%. Diet supplemented with 2% EGCG was significantly higher than 0% ($P < 0.05$). Pupal weight increased by more than 41% when the larvae fed on diet supplemented with 2.5% EGCG (Fig. 1C). Emergence rate also increased as dietary EGCG increased from 0% to 2.5%. Emergence on diet supplemented with 0.5–2.5% EGCG was significantly higher than 0% ($P < 0.01$). Emergence increased by more than 33% when the larvae fed on diet supplemented with 2.5% EGCG (Fig. 1D).

These results demonstrate that EGCG added to the standard diet is beneficial for *E. obliqua*. The artificial diet supplemented with 2.5% EGCG appears to be the most suitable for *E. obliqua* (i.e., optimal diet) of the six diets supplemented with EGCG and tested herein. Performance increased with a dietary supplement of EGCG between 0% and 2.5%. When EGCG concentration exceeded 2.5%, performance decreased. This optimal diet (diet + 2.5% EGCG) yielded similar responses as the diet containing tea powder (Table 3). Although the average pupal weight of the improved diet was approximately 20 mg lower than the average pupal weight with the general purpose tea powder diet, significant differences were observed with pupation rate ($t = -1.000$, $P = 0.356$), pupal weight ($t = -5.113$, $P = 0.248$), and emergence rate ($t = -0.701$, $P = 0.660$).

In plants, phenols act as a defensive mechanism not only against herbivores, but also against microorganisms and competing plants (War et al. 2012). Artificial feeding experiments have shown that most plant phenols are toxic to many insects (Chan et al. 1978, Kubo et al. 2008).

Consumption of EGCG was positively correlated with epigallocatechin-3-gallate (PO) activity (an immune-related enzyme) of *E. obliqua* in a range of 0% to 2.5%.

Table 3. Mean ± SD pupation rate, larval duration, pupal weight, and emergence rate of *Ectropis obliqua* fed an improved artificial diet supplemented with (–)-epigallocatechin-3-gallate (EGCG) and the Zhang et al. (2007) artificial diet.

Diet	Pupation Rate (%)	Larval Duration (d)	Pupal Weight (mg)	Eclosion Rate (%)
Diet + 2.5% EGCG	95.0 ± 4.09 a*	21.9 ± 0.43 a	120.8 ± 3.3 a	88.8 ± 8.54 a
Tea powder diet	97.5 ± 2.89 a	22.2 ± 0.44 a	136.0 ± 5.0 b	92.5 ± 6.46 a

* Means in each row followed by the same letter are not significantly different according to least significant difference test after one-way analysis of variance.

EGCG also enhanced PO activity in the mud crab, *Scylla paramamosain* Estampador (Wang et al. 2017). Therefore, EGCG may increase the activity of the enzyme so that *E. obliqua* immunity, and therefore growth, is improved. However, when the amount of EGCG concentration reached 3**%**, the EGCG may exceed the larvae's own detoxification capacity.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (31870635 and 31300426), Anhui Major Demonstration Project for Leading Talent Team on Tea Chemistry & Health and Earmarked Fund for Modern Agro-Industry Technology Research System in Tea Industry, Anhui provincial R&D project (1804e03020320), and SKLTOF201801109.

References Cited

Cavet, M.E., K.L. Harrington, T.R. Vollmer, K.W. Ward and J.-Z. Zhang. 2011. Anti-inflammatory and anti-oxidative effects of the green tea polyphenol epigallocatechin gallate in human corneal epithelial cells. Mol. Vision 17: 533–42.

Chan, B.G., A.C. Walss, W.L. Stanley and A.E. Goodban. 1978. A rapid diet preparation method for antibiotic phytochemical bioassay. J. Econ. Entomol. 71: 366–368.

Fang, J., L. Zhu, Z.D. Yang and B.G. Zhao. 2003. Advances and problems in insect artificial feed studies. J. Sci. For. Sci. Tech. 24: 18–25.

Gao, X., X. Wan, Y. Yang, G. Zheng and Q. Shen. 2007. Studies on the biological habits of *Ectropis obliqua* Prout. Plant Prot. 33: 110–113.

Gupta, G.P., S. Rani and A.B.M. Raghuraman. 2005. Improved artificial diet for mass rearing of the tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae). Int. J. Trop. Insect Sci. 25: 55–58.

Hiyama, A., M. Iwata and J.M. Otaki. 2010. Rearing the pale grass blue *Zizeeria maha*, (Lepidoptera, Lycaenidae): toward the establishment of a lycaenid model system for butterfly physiology and genetics. Entomol. Sci.13: 293–302.

Hu, C., J.Q. Zhu and G.Y. Ye. (eds.). **1994**. *Ectropis oblique* Warren, a serious geometrid pest of tea bush in east China. Scientific and Technical.Publishers, Shanghai China.

Kubo, I., I. Hori, K. Nihei, H. Satooka, L. Carlos and J.S. Calderón. 2008. Insect growth inhibitory activity and cytotoxicity of tannic acid from Gallae Rhois. Biopestic. Int. 4: 6–14.

Liu, Q., J. Xu, C.M. Li, G.J. Han, J. Sun, J.H. Qi, C.J Wang and G.S. Liu. 2015. Growth and reproduction of ectropis obliqua fed on artificial diet. J. Tea Sci. 35: 323–328.

Matsumoto, Y., K. Kaihatsu, K. Nishino, M. Ogawa, N.K. ato and A.Yamaguchi. **2012.** Antibacterial and antifungal activities of new acylated derivatives of epigallocatechin gallate. Front. Microbiol. 3: 1–10.

Mukaiyama, F. 1966. Rearing of the fall webworm, *Hyphantria cunea* on artificial diets. J. Insect Biotechnol. Sericult. 35: 145–147.

Nagle, D.G., D. Ferreira and Y.D. Zhou. 2006. Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. Phytochemistry 67: 1849–1855.

Singh, P. and R.F. Moore. 1985. Handbook of Insect Rearing. Elsevier, Amsterdam, The Netherlands.

Wan, X.C. 2003. Tea Biochemistry. China Agricultural Press, Beijing. In Chinese.

Wang, Z., B. Sun and F. Zhu. 2017. Epigallocatechin-3-gallate inhibit replication of white spot syndrome virus in *Scylla paramamosain*. Fish Shellfish Immun. 67: 612–619.

War, A.R., M.G. Paulraj, T. Ahmad, A.A. Buhroo, B. Hussain and S. Ignacimuthu. 2012. Mechanisms of plant defense against insect herbivores. Plant Signal Behav. 7: 1306–1320.

Yang, Y., L. Zhang, F. Guo, Y. Long, Y. Wang and X. Wan. 2016. Reidentification of sex pheromones of tea geometrid *Ectropis obliqua* Prout (Lepidoptera: Geometridae). J. Econ. Entomol. 109:167–175.

Yuan, Z., J. Zhang and Q. Sun. 2017. Analysis of nutrient composition of artificial diet of tea geometrid. J. Tea Business. 39: 28–33.

Zhang, Y., B. Xu, Y. Wang and L. Qu. 2007. Artificial feeding and passage method of tea geometrid and its larval artificial diet. CN 1954669 A[P].