Development of an Artificial Diet for Rearing *Conogethes punctiferalis* (Lepidoptera: Crambidae)[1]

Yan-Li Du[2], Peng-Peng Ai, Li-Xin Lang, Min-Zhao Zhang, and Shu-Ling Sun

Beijing Key Laboratory for Agricultural Application and New Technique, College of Plant Science and Technology, Beijing University of Agriculture, Beinong Road No. 7, Beijing, China 102206

J. Entomol. Sci. 50(2): 89–98 (April 2015)

Abstract *Conogethes punctiferalis* (Guenée) (Lepidoptera: Crambidae) is one of the most damaging Lepidoptera, attacking fruit in the temperate and tropical regions of Asia, Australia, and Papua New Guinea. Effective methods of control will likely remain limited until further physiological and ecological studies can be conducted, many of which will require effective means of rearing of the insect in the laboratory. To that end, this study was undertaken to develop and assess four meridic diets for rearing *C. punctiferalis*. The four diets differed in the amounts of chestnut meal, corn meal, and soybean meal. The diet containing 30 g chestnut meal, 70 g corn meal, and 70 g soybean meal per 700-ml diet yielded a larval survival rate of 94.5%, a generation developmental time of 42.4 d, mean pupal weights of 73.6 mg for males and 77.3 mg for females, and an adult fecundity rate of 97.9 eggs/female. Performance on this diet compared favorably with rearing on fresh corn.

Key Words *Conogethes punctiferalis*, meridic diets, larval development, adult reproduction

Conogethes punctiferalis (Guenée) (Lepidoptera: Crambidae), commonly known as the yellow peach moth in its native range in Asia, is one of the most damaging Lepidoptera attacking fruit grown in the temperate and tropical regions of South and East Asia, Australia, and Papua New Guinea (CABI International 2011). Larvae of *C. punctiferalis* are highly polyphagous and feed on a broad range of hosts, including peach, chestnut, durian, citrus, papaya, cardamom, ginger, eggplant, and maize (Sekiguchi 1974, Waterhouse 1993). Infested fruits usually are stunted and/or scorched, and may drop from the tree before maturation (CAB International 2011).

Because of its broad host range and its potential to be spread long distances, *C. punctiferalis* is expected to negatively impact many susceptible plant hosts. In China, for example, *C. punctiferalis* was reported to damage more than 100 species of trees, crops, and vegetables (Lu et al. 2010) and was identified as one of the most destructive pests of peach. Various methods, including application of insecticides, use of sex pheromones, biological control with *Bacillus thuringiensis* Berliner, and the use of cultural practices (e.g., bagging fruits) have been proposed for the management of *C. punctiferalis* (CAB International 2011, Du et al. 2014). However, *C. punctiferalis* larvae bore into and feed within host fruits for the entire

[1]Received 04 October 2014; accepted for publication 16 February 2015.
[2]Corresponding author (email: yanlidu@126.com).

larval stage, making most traditional management strategies, especially those directed to the larval state, largely ineffective. Development of effective management strategies for *C. punctiferalis* will likely involve intensive studies on the biology, ecology, physiology, and toxicology of the insect (Du et al. 2012). The nature of many of these studies will be facilitated by or require readily available specimens of eggs, larvae, pupae, and adults for testing. Thus, a meridic diet for rearing *C. punctiferalis* in laboratory is needed.

Over the past several decades, artificial diets have been widely used for mass rearing of insects in both commercial insectaries and scientific laboratories (Cohen 2004, Vanderzant 1974). Only slight differences in composition of a diet can impact rearing quality and efficiency, which is usually translated as higher survival rates, greater larval and pupal weight, shorter developmental time, and higher fecundity rates. Honda et al. (1979) and Utsumi et al. (1990) proposed meridic diets for rearing *C. punctiferalis*, but colonies fed on those diets had a lower larval survival rate and a larger variation in development duration than colonies fed on natural host plant materials (Utsumi et al. 1990). Therefore, efforts to define a meridic diet for rearing *C. punctiferalis* must continue.

Previous reports provide a basis for continued development of an artificial diet for *C. punctiferalis*. For example, larvae fed on chestnuts developed faster and had a lower mortality rate than those fed on peach, persimmon, or cypress (Choi et al. 2006, Honda et al. 1979), suggesting that chestnut could enhance performance on a meridic diet. Li et al. (2014) reported *C. punctiferalis* larvae fed on fresh corn and chestnut performed better than those fed on apple, pear, or plum. Our own preliminary testing showed that *C. punctiferalis* larvae were repelled by diets containing formaldehyde and sorbic acid as microbial inhibitors, whereas methyl paraben was not repellent. Furthermore, Wang et al. (2011) developed a diet to successfully rear the Oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), which has feeding habits and hosts similar to those of *C. punctiferalis*. Our objective, therefore, was to compare four meridic diets, each based on the diet recipe of Wang et al. (2011) but each with different ratios of chestnut meal, corn meal, and soybean meal, in successfully rearing *C. punctiferalis* in the laboratory.

Materials and Methods

Insects. Insects used in these studies were from a laboratory colony of *C. punctiferalis*, originally established from larvae collected on 9 October 2009 from cornfields at the Agricultural Experiment Station of Beijing University of Agriculture and maintained on fresh corn for >20 generations. The stock colony was held in an environmental incubator (RTOP-B, Zhejiang Top Instrument Co., Ltd., Hangzhou, China) maintained at 23 ± 1°C, 75 ± 2% relative humidity, and on 16:8-h light:dark photoperiod with 3,500 lux light intensity. All experiments for this study were conducted therein.

Artificial diets. Four artificial diets, designated AD-I, AD-II, AD-III, and AD-IV, were prepared for feeding *C. punctiferalis* larvae. Casein, cholesterol, glucose, citric acid, L-ascorbic acid, methyl paraben (a microbial growth inhibitor), brewer's yeast, and water were present in equal amounts in all four diets (Table 1). The amount of agar differed in only the AD-II diet. The total amount of corn meal, chestnut meal,

Table 1. Composition of meridic diets for rearing *Conogethes punctiferalis*.

	Quantity (g or ml)			
Ingredient	**AD-I**	**AD-II**	**AD-III**	**AD-IV**
Chestnut meal	30	20	10	20
Corn meal	70	75	80	80
Soybean meal	70	70	80	70
Yeast powder	30	30	30	30
Agar	10	15	10	10
Glucose	9	9	9	9
Citric acid	2	2	2	2
L-ascorbic acid	3	3	3	3
Casein	10	10	10	10
Methyl paraben	3	3	3	3
Cholesterol	0.1	0.1	0.1	0.1
Distilled water	700	700	700	700

and soybean meal combined in the AD-I, AD-III, and AD-IV diets was 170 g/L, whereas the amount of those combined ingredients in the AD-II diet totaled 165 g/L. The amount of agar added to AD-II diet was increased by 5 g/L to compensate for the smaller amount of those combined ingredients in the AD-II diet. The diets differed in the amounts of chestnut meal, corn meal, and soybean meal incorporated into each (Table 1).

Briefly, each diet was prepared by combining the chestnut meal, corn meal, soybean meal, and brewer's yeast powder, to which 300 ml distilled water was added. This mixture was then simmered over heat for approximately 30 min. The agar was suspended in the remaining 400 ml water and heated to boiling point (100°C), after which glucose and citric acid were added and mixed. This mixture was cooled to approximately 60°C, and L-ascorbic acid, casein, methyl paraben, and cholesterol were added and mixed. The two mixtures were then combined and thoroughly mixed. Each diet was allowed to cool to ambient temperature and was then stored at 4°C until used.

Rearing of insects. Forty neonate larvae obtained from the stock colony were transferred into individual glass containers (12 × 8 cm) containing four blocks of a specific diet, each measuring 25 × 50 × 50 mm. The container was covered with a transparent plastic lid with small holes for ventilation and placed in the environmental chamber. Diet was replenished every 3 days, and diet residues and wastes were removed as needed. Larval survival and development were checked daily. As larvae reached the penultimate stage, cheesecloth (150 × 100 mm) was placed over the diet blocks to provide pupation sites. After pupation, the

newly molted pupae were collected, weighed, numbered, and individually placed into 50-ml tubes with ventilated lids and placed in the chamber. Pupal survival, duration, and adult emergence were monitored daily. Each diet treatment was replicated five times with a total of 200 larvae per diet treatment.

For each diet treatment, adults (1:1 ratio of females:males) were placed in a plastic cage (350 × 270 × 250 mm). Moths were provided with a honey solution (8%, v/v) soaked in cotton in a 2.5-ml vial. The honey solution was replenished each day. Three days after placement of adults in the cages, an apple wrapped in a wet piece of cheesecloth was placed in each cage as an oviposition site for the females. The cheesecloth was replaced each day, and the numbers of eggs deposited on the cloth were counted. Collection of eggs was continued until no living females were available in the cage. Adult survival also was recorded. Cloths with eggs were dated, numbered, and placed into individual glass containers (12 × 8 cm). The number of hatched F_1 larvae was counted daily.

For this phase of the study, each diet treatment was replicated four times. A total of 40 moths (20 males, 20 females) was used for the AD-I diet treatment, while totals of only 10, 30, and 30 moths were used for the AD-II, AD-III, and AD-IV diet treatments, respectively, because of insufficient numbers of moths available for each treatment.

Fresh corn collected from a cornfield in Beijing University of Agriculture was used as a control diet treatment. Rearing conditions and experimental procedures were the same as that of the artificial diet treatments.

Data analyses. Response variables of duration of development (time required to complete specified periods in the development of the insect), preoviposition duration (time between the adult emergence and initial oviposition), generation duration (time of development from egg through moth preoviposition), pupation rate (ratio of pupae to larvae), and adult emergence rate (ratio of adults to larvae or the ratio of adults to pupae) were compared among the diet treatments using analysis of variance and the Bonferroni test with SPSS 16.0 statistical software. Selected life table parameters were calculated for each treatment according to methods of Ding (1994) and Liu et al. (2009). These also were subjected to analysis of variance and the Bonferroni test.

Results

Statistically significant ($P < 0.05$) differences among the treatments were observed for duration of the entire generation, with AD-I = corn check < AD-IV < AD-III < AD-II. These durations ranged from 42.4 d for AD-I to 63.3 d for AD-II. This same pattern of differences was reflected in the duration of the larval stages, with AD-I = corn check < AD-IV = AD-III < AD-II (Fig. 1). Larval development ranged from 20.3 d for AD-I to 41.8 d for AD-II.

Survival rates also were significantly impacted by diet. Percentage of survival of larvae from egg hatch to pupation was 94.5% on AD-I, 80.0% on the fresh corn control, 26.5% on AD-III, 25.6% on AD-IV, and 18.0% on AD-II (Table 2). Percentage of survival from egg hatch to adult emergence was 78.5% on AD-I, 71.4% on the fresh corn control, 20.9% on AD-III, 20.6% on AD-IV, and 8.0% on

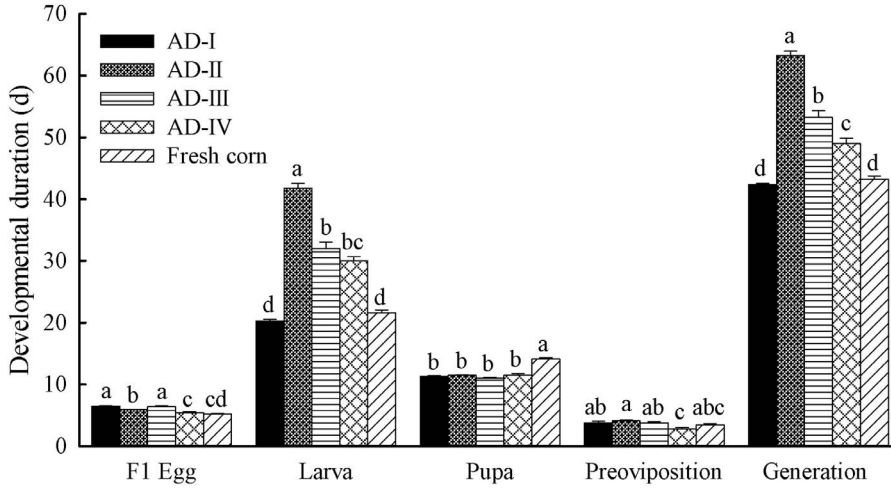

Fig. 1. Developmental duration of *Conogethes punctiferalis* reared on meridic diets and fresh corn. Bars represent means ± SE; significant differences among six host plants are indicated by letters over each bar (Bonferroni test, $P < 0.05$).

AD-II (Table 2). The hatch rate of eggs from those adults was highest for the AD-I diet (67.7%) and lowest for the AD-III (29.8%) and AD-II (26.9%) diets.

Pupal weight, adult longevity, and adult fecundity also were significantly affected by larval diet treatments. Pupal weights on the AD-I diet were greatest among all treatments (73.6 mg per male, 77.3 mg per female), did not differ significantly from weights obtained when fed on fresh corn, and were significantly greater than those observed on remaining three diets (Table 3). Adult longevity appeared to have no relationship to fecundity, with no significant differences among females developing from larvae fed on the fresh corn control, the AD-IV diet, and the AD-I diet (Table 3).

Discussion

Cohen (2004) states that relative amounts of components of artificial insect diets impact performance and fitness of the insects. We demonstrated in a previous study that the survival and reproduction of *C. punctiferalis* were impacted by the choice of host plant material incorporated in meridic diets (Li et al. 2014). In this current study, the amount of chestnut meal as an ingredient in the artificial diet was a key factor in survival, weight gain, generation time, and fecundity of *C. punctiferalis*. The AD-I diet, containing the highest concentration of chestnut meal of the diets tested, resulted in enhanced survival rate, shortened developmental duration, increased pupal weight, and increased numbers of eggs produced by females.

Furthermore, the AD-I diet compared favorably with the control diet using fresh corn as the larval food source. Larval survival and total survival rates on the AD-I

Table 2. Survival rates of *Conogethes punctiferalis* on four meridic diets and fresh corn.*

Diet	Initial No. of Larvae	No. of Pupae	Larval Survival (%)	No. of Adults	Pupal Survival (%)	Total Survival (%)
AD-I	40	37.8 ± 0.2 a	94.5 ± 0.6 a	31.4 ± 1.1 a	83.1 ± 2.1 a	78.5 ± 2.7 a
AD-II	40	7.2 ± 1.4 c	18.0 ± 3.4 c	3.2 ± 0.4 c	44.4 ± 4.8 b	8.0 ± 0.9 c
AD-III	40	10.8 ± 1.4 c	26.5 ± 3.4 c	8.4 ± 1.2 b	78.9 ± 4.7 a	20.9 ± 2.9 b
AD-IV	40	10.4 ± 1.6 c	25.6 ± 4.0 c	8.2 ± 1.3 bc	80.5 ± 3.1 a	20.6 ± 3.2 bc
Corn	40	32.0 ± 1.3 b	80.0 ± 3.2 b	28.5 ± 1.6 a	89.3 ± 3.0 a	71.4 ± 3.9 a

* Means ± SE ($n = 5$) within a column and followed by the same letter are not significantly different at $P < 0.05$ (analysis of variance; Bonferroni test).

Table 3. Pupa weight, adult longevity, fecundity, and egg hatch of *Conogethes punctiferalis* reared on meridic diets and fresh corn.*

Diet	Pupal Weight (mg)		Adult Longevity (d)		No. of Eggs per Female	F_1 Hatch Rate (%)
	Female	Male	Female	Male		
AD-I	77.3 ± 6.3 a	73.6 ± 1.1 a	12.7 ± 0.7 a	11.6 ± 1.2 b	97.9 ± 4.4 a	67.7
AD-II	58.3 ± 5.8 a	49.0 ± 0.1 b	12.7 ± 1.3 a	12.0 ± 0.1 b	14.0 ± 0.1 bc	26.9
AD-III	60.2 ± 3.5 a	59.6 ± 2.1 b	13.7 ± 1.0 a	23.3 ± 1.0 a	53.2 ± 0.1 b	29.8
AD-IV	64.1 ± 2.3 a	54.9 ± 3.9 b	8.7 ± 0.7 b	7.2 ± 1.2 b	107.1 ± 4.7 a	58.2
Corn	74.1 ± 3.6 a	65.7 ± 3.9 ab	9.9 ± 1.3 ab	7.5 ± 1.1 b	124.2 ± 20.4 a	58.3

* Means ± SE ($n = 5$) within a column and followed by the same letter are not significantly different at $P < 0.05$ (analysis of variance; Bonferroni test).

Table 4. Generation Time (*T*) and intrinsic rate of increase (r_m) of *Conogethes punctiferalis* reared on meridic diets and fresh corn.*

Diet	*T* (d)	r_m
AD-I	42.4 ± 0.05 d	0.074
AD-II	63.3 ± 0.1 a	−0.031
AD-III	53.3 ± 0.4 b	0.008
AD-IV	48.8 ± 0.2 c	0.037
Corn	43.2 ± 0.1 d	0.073

* Means ± SE ($n = 5$) within a column and followed by different letters are significantly different at $P < 0.05$ (analysis of variance; Bonferroni test).

diet and the fresh corn control were statistically similar, whereas survival rates on the remaining three artificial diets were much lower and not acceptable in terms of the diets sustaining population growth under artificial conditions. Pupal weights and numbers of eggs produced per female showed the same relative responses. Generation times (*T*) for the AD-I diet and the fresh corn control were statistically equal; both were statistically shorter than *T* values of the AD-II, AD-III, and AD-IV diets (Table 4).

The calculated natural (or intrinsic) rate of increase (r_m) for the insect cohorts fed on the different diets also demonstrated that *C. punctiferalis* performed best on the fresh corn control and the AD-I diet (Table 4). The intrinsic rate of increase is a theoretical maximum that may be reached in a given environment if the population is not resource-limited and represents the potential of the increase of a population in specified environmental conditions (Southwood and Henderson 2000). The r_m is not only dependent upon birth and mortality rates, but also on age assembly, fecundity, and developmental rate (Li et al. 2012, Niu et al. 2008). The r_m value of 0.073 for the cohort fed on fresh corn was likely due to a higher fecundity rate and survival rate, whereas the r_m value of 0.074 for the cohort fed on the AD-I diet likely resulted from high development and survival rates. The r_m value is an indicator of fitness, with a higher value indicating a higher level of fitness. In this study, the r_m values for *C. punctiferalis* larvae fed on the AD-I diet (0.074) and fresh corn (0.073) were higher than those observed with the AD-IV (0.037), the AD-III (0.008), and the AD-II (−0.031) diets. Cohorts fed on fresh corn or the AD-I diet thus had a higher level of fitness than cohorts fed on the other artificial diets.

In summary, these results demonstrate that the AD-I meridic diet as outlined herein is conducive to successfully rearing *C. punctiferalis* under laboratory or other artificial conditions. We believe that chestnut meal is a key component for the success of this diet in enhancing *C. punctiferalis* performance. We have thus adopted this as the meridic diet to be used for production of our laboratory colonies.

The AD-III, AD-IV, and especially the AD-II diets will not satisfactorily support performance of *C. punctiferalis*. All three of these diets have equivalent or higher concentrations of corn meal and soybean meal than the AD-I diet. However, the AD-I diet contains a higher concentration of chestnut meal than any of the other three

diets. Furthermore, the AD-II diet contains a higher concentration of agar than the other three. Agar is often used in artificial diets for insects and plays important roles as a solidifying agent and in increasing water-holding capacity. A higher concentration of agar might impede the boring activity and thus the survival of *C. punctiferalis* neonates.

Acknowledgments

This research was funded by the Science and Technology Fund of Beijing Municipal Commission of Education (KZ201210020019) and the National Natural Science Foundation of China (NSFC31040074).

References Cited

CAB International. 2011. *Conogethes punctiferalis* datasheet. *In* Crop Protection Compendium. CAB International, Wallingford, UK.

Choi, K.S., K.S. Han, I.K. Park, J.I. Hong, C.S. Kim, Y.J. Chung and S.C. Chin. 2006. Difference of developmental time, survival rate and sex ratio of *Dichocrocis punctiferalis* (Lepidoptera: Pyralidae) on three hosts. J. Korean For. Soc. 95: 174–176.

Cohen, A.C. 2004. Insect Diets: Science and Technology. CRC Press, Boca Raton, FL. 312 pp.

Ding, Y.Q. 1994. Mathematical Ecology of Insects. Science Press, Beijing, China. 170 pp.

Du, Y.L., H.M. Guo, S.L. Sun, M.Z. Zhang, A.H. Zhang, J.B. Wang and L. Qin. 2012. Effects of temperature on the development and reproduction of yellow peach moth, *Conogethes punctiferalis* (Guenée). Acta Entomol. Sinica 55: 561–569.

Du Y.L., M.Z. Zhang, Y.Q. Ma, S.L. Sun, J.B. Wang, J.L. Liu and L. Qin. 2014. Formulation screening of sex pheromones and field trapping tests for the yellow peach moth, *Conogethes punctiferalis* (Guenée). Acta Phytophyl. Sinica 41: 187–191.

Honda, H., J. Kaneko, Y. Konno and Y. Matsumoto. 1979. A simple method for mass-rearing of the yellow peach moth, *Dichocrocis punctiferalis* Guenée (Lepidoptera: Pyralidae), on an artificial diet. Appl. Entomol. Zool. 14: 464–468.

Li, D.X., X.H. Lei, Z. Li, L.W. Gao and Z.R. Shen. 2012. Effects of different host plants on the development and reproduction of the peach fruit borer, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae). Acta Entomol. Sinica 55: 554–560.

Li, D.Y., P.P. Ai, Y.L. Du, S.L. Sun and M.M. Zhang. 2014. Effects of different host plants on the development and reproduction of yellow peach moth, *Conogethes punctiferalis* (Lepidoptera: Crambidae). Austr. Entomol. doi: 10.1111/aen.12105.

Liu, J.L., B. Yang, Y.Y. Lu and S.S. Huang. 2009. An improvement on life table of experimental population: example from *Cyrtorrhinus lividipennis* Reuter (Hemiptera: Miridae). Acta Ecol. Sinica 29: 3201–3208.

Lu, J.Q., Z.Y. Wang, K.L. He and Y. Liu. 2010. Research history, progresses and prospects in the yellow peach moth, *Conogethes punctiferalis*. Plant Prot. 36: 31–38.

Niu, Y.H., C.Y. Zhou and L. Hua. 2008. Establishment and analysis of life table for experimental population of *Tetranychus urticae* Koch on four host plants. J. Northwest Agric. For. Univ. (Nat. Sci. Ed.) 36: 166–170.

Sekiguchi, K. 1974. Morphology, biology and control of the yellow peach moth, *Dichocrosis punctiferalis* Guenée (Lepidoptera: Pyralidae). Bull. Ibaraki-ken Horticult. Exp. Sta., Special Issue.

Southwood, T.R.E. and P.A. Henderson. 2000. Ecological Methods: With Particular Reference to the Study of Insect Populations. 3rd ed. Blackwell Science, Oxford, UK.

Utsumi, K., E. Aihara and N. Watanabe. 1990. Artificial diet of the yellow peach moth, *Conogethes punctiferalis* (Guenée) using corn seeds (Lepidoptera: Pyralidae). Res. Bull. Plant Prot. Serv. 26: 65–67.

Vanderzant, E.S. 1974. Development, significance, and application of artificial diets for insects. Annu. Rev. Entomol. 19: 139–160.

Wang, Y.R., M. Huang, X.W. Liu, J.X. Wu and D. Zhao. 2011. Artificial diet for larvae of the oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae). J. Northwest Sci-Tech Univ. Agric. For. Univ. (Nat. Sci. Ed.) 39: 161–166.

Waterhouse, D.F. 1993. The Major Arthropod Pests and Weeds of Agriculture in Southeast Asia: Distribution, Importance and Origin. ACIAR Monograph No. 21. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia.