Jpn. J. Ent., **64**(1): 30–34. March 25, 1996

Rearing Larvae of the Great Mormon Butterfly, *Papilio memnon* L. (Lepidoptera: Papilionidae), on Artificial Diet

Masanobu YOSHIO and Minoru ISHII

Entomological Laboratory, College of Agriculture, Osaka Prefecture University, Sakai, Osaka 593, Japan

Abstract Larvae of the Great Mormon butterfly, *Papilio memnon* L., were reared on 5 types of artificial diets containing a small amount (7–9% by weight) of dried leaf powder of *Citrus* sp. and/or *Zanthoxylum ailanthoides* (Rutaceae). Percentage of 1st instar larvae settling on the diets, mean larval stage and pupal weight, and photoperiodic response of the larvae reared on the 3 of these artificial diets were almost the same as those of the larvae reared on fresh citrus leaves, and adults reared from the diets were normal and fertile.

Key words: Artificial diet; dried leaf powder; photoperiodic response; diapause; *Papilio memnon*.

Introduction

Artificial diets are useful for mass rearing of phytophagous insects in the laboratory. For butterflies, several artificial diets for such species as the swallowtail, *Papilio xuthus* L. (KAMANO 1965), the large white, *Pieris brassicae* L. (DAVID & GARDINER 1965, 1966), the small white, *P. rapae crucivora* BOISDUVAL (KONO 1968), have been developed. These diets may be classified into two types with respect to the amount of dried leaf powder contained, larger (*e.g.*, KAMANO, 1965) or smaller (*e.g.*, DAVID & GARDINER 1965; KONO 1968). Development of artificial diets containing less dried leaf powder would be important for the rearing of the species the food plant of which is rare or the larvae consume a large amount of leaves. However, most of such diets were developed for *Pieris* species and the development of larvae reared on the diets tended to be delayed compared with those reared on fresh leaves. Although MORTON (1981) reported the rearing of 50 butterfly species on his artificial diet containing only 1.5% of dried plant materials, he showed no data on the larval survivorship nor development.

On the other hand, the artificial diet for *Pieris rapae* developed by SATO (1974) was satisfactory for the larval development, although it contained dried powder of chlorella, a kind of algae, to save the cabbage leaf powder. He showed the possibility of butterfly rearing on the diet with a small amount of dried leaf

powder. In 1994, we developed artificial diets for the Great Mormon butterfly, *Papilio memnon* L., whose larvae consume large amounts of citrus leaves, by replacing dried chlorella powder in the medium of SATO (1974) with other substances, and examined effects of these diets on the larval development, including photoperiodic induction of pupal diapause.

Materials and Methods

Artificial diets: The composition of our artificial diets is the one modified from SATO (1974) (Table 1). Dried citrus leaf powder was prepared by drying leaves of *Citrus* sp. in a ventilated oven at 55–60°C and grinding them with a ball mill. Dried chlorella powder in SATO (1974) was replaced by casein, and cellulose powder or the powder of dried *Zanthoxylum ailanthoides* (Rutaceae) leaves in our diets. *Z. ailanthoides* is the host plant of most Japanese *Papilio* species but not some including *P. memnon.* The powder was prepared by drying the leaves at 55 or 110°C before grinding by the ball mill. The preparing procedures of these artificial diets fundamentally followed SATO (1974): Mixture I was dissolved in distilled water and boiled. When the mixture boiled, all the rest of the constituents were added and the diet was cooled. The diets thus made up were stored in a refrigerator until use. In this experiment, 5 types of diets were tested. The diets B, C, and E contain the dried leaf powder of *Citrus* sp. and *Z. ailanthoides*, while diets A and D only that of *C.* sp.

Table 1. The composition of 5 artificial diets, A–E, tested in this experiment.

Constituents	Formula				
	A	B	C	D	E
Dried powder					
Citrus (dried at 55°C)	75 g	60 g	60 g	60 g	37.5 g
Z. ailanthoides (55°C)	—	15 g	—	—	37.5 g
(110°C)	—	—	15 g	—	—
Cellulose	—	—	—	15 g	—
Casein			30 g		
Mixture I[1)]			15 g		
Mixture II[2)]			30 g		
Distilled water			700 ml		

[1)] Mixture I (ratio of weight): Gallic acid, 25; Inositol, 25; Vitamin mixture*, 2.5; Streptomycin sulfate, 2.5; Sucrose, 100; Ascorbic acid, 50.

[2)] Mixture II (ratio of weight): Choline chloride, 6; Cellulose powder, 8; KH_2PO_4, 50; K_2HPO_4, 20; Sorbic acid, 16; Agar, 200.

* Vitamin mixture (ratio of weight): Biotin, 0.02; Folic acid, 0.02; B_1-HCl, 0.1; FMN-Na, 0.1; B_6-HCl, 0.1; Nicotinamide, 0.2; Ca-Pantothenate, 0.2; Ascorbic acid, 50; Linolic acid, 50.

Animals: Eggs of *Papilio memnon* were obtained from the progeny of females collected in Kagoshima Prefecture on May 1, 1994. Larvae that hatched were divided into 6 groups and reared on these diets (group A–E) or fresh leaves of *Citrus* sp. (group F: control) at 20°C under 2 photoperiodic conditions of 13.5 L–10.5D and 12.5L–11.5D, which are slightly longer or shorter than the critical photoperiod for induction of pupal diapause at 20°C in the Kagoshima population of this species (YOSHIO & ISHII unpublished). Three to 5 larvae were reared in a clear plastic petri dish (5 cm in diameter and 1 cm in depth) during the 1st to 2nd instar. The 3rd to 4th, and 5th (final) instar larvae were reared individually in 200 ml and 500 ml clear plastic cups, respectively. Larval stage and survivorship were recorded, and pupae were weighed 4 days after pupation. Individuals whose pupal stage lasted more than 25 days were regarded as diapausing ones.

Results and Discussion

There was a marked difference in the larval survivorship among the groups reared on the 5 diets: Most 1st instar larvae died from not settling on the diet in the groups C and E, while the percentage of 1st instar larvae settling on the diet in the groups A, B, and D was not significantly different from that in group F (control group) ($P>0.05$ by FISHER's exact probability test, Table 2). The results show that the diets C and E were not suitable for larvae of this species. It is interesting that the diet C was less available for the larvae than the diet B, though their composition was the same except for the higher temperature treatment of *Z. ailanthoides* leaves. Most larvae that settled on the diet survived to form pupae in the groups A, B, and D as well as F.

Although there was no significant difference in the mean pupal weight among the groups A, B, D, and F, the mean larval stage of group F was slightly longer than that of groups A, B, and D (Table 2). It is probable that since the experiment was made in the height of summer, the citrus leaves was not in good condition.

All the individuals that pupated under 12.5L–11.5D entered diapause, while some made adult eclosion within 20 days after pupation under 13.5L–10.5D. The incidence of diapause was not significantly different among the groups A, B, D, and F under 13.5 hr photoperiod ($P>0.05$ by FISHER's exact probability test). The results show that the artificial diets A, B, and D do not change the critical photoperiod for induction of pupal diapause.

Healthy-looking adults emerged from diapausing pupae after cooling for 2 months at 10°C as well as from non-diapausing ones, unlike the rearing of KONO (1968) where adults with crippled wings were obtained. Hand-pairing was done between males and females reared on the diet D, and fertilized eggs were

Table 2. Numbers of 1st instars put on the diet, 1st instars settling on it, individuals that pupated and pupae which entered diapause, and mean larval stage and pupal weight of *Papilio memnon* reared on 5 artificial diets and fresh citrus leaves at 20°C.

Group	Photoperiod	Diet	No. of 1st instars put on	No. (%) of 1st instars settling on	No. of pupation	Larval stage[1)] mean±S.D. (days)	Pupal weight[1)] mean±S.D. (g)	No. (%) of diapausing pupae
A	13.5L–10.5D	A	12	12 (100)	11	32.3±3.1a	2.5±0.40a	1 (9)
B		B	12	9 (75)	8	32.9±2.1a	2.6±0.27a	2 (25)
C		C	10	2 (20)	2	37.5±1.5b	2.6±0.11a	0 (0)
D		D	13	13 (100)	11	32.9±3.2a	2.8±0.41a	1 (9)
E		E	13	3 (23)	1	43	2.5	1 (100)
F		Leaf	14	14 (100)	11	36.8±3.1a	2.5±0.28a	1 (9)
A	12.5L–11.5D	A	11	10 (91)	10	30.7±2.0a	2.2±0.18a	10 (100)
B		B	11	9 (82)	8	30.6±4.4a	2.3±0.27a	8 (100)
C		C	11	2 (18)	2	32 ±4a	2.5±0.69a	2 (100)
D		D	14	14 (100)	12	31.8±2.5a	2.3±0.32a	12 (100)
E		E	14	6 (43)	4	35.3±3.1b	2.1±0.47a	4 (100)
F		Leaf	10	10 (100)	8	36.0±1.9b	1.9±0.35a	8 (100)

[1)] Means followed by the same letter in the same photoperiodic condition are not significantly different by DUNCAN's new multiple range test ($P>0.05$).

obtained.

Thus the results of this experiment show the possibility of rearing the Great Mormon, *Papilio memnon* L., satisfactorily on the diets A, B, and D. Considering the purpose of cutting down the amount of citrus leaves, diet D is the best among these diets.

Acknowledgments

We are grateful to Mr. A. NOMURA of Osaka Prefectural Experiment Station of Citrus Fruits, Dr. H. TANAKA of Osaka Prefectural Agricultural and Forestry Research Center, and Mr. R. MOCHIOKA of Osaka Prefecture University for supplying citrus leaves. Our thanks are also due to Mr. Y. NAKAGAMI of the Environmental Technology Association of Kagoshima Prefecture for collecting adults of *P. memnon*.

References

DAVID, W. A. L. & B. O. C. GARDINER, 1965. Rearing *Pieris brassicae* L. on a semi-synthetic diet. *Nature*, **207**: 882–883.

———— 1966. Rearing *Pieris brassicae* L. on semi-synthetic diets with and without cabbage. *Bull. Ent. Res.*, **56**: 581–592.

KAMANO, S., 1965. Rearing of *Papilio xuthus* larvae on artificial diets. *Jap. J. appl. Ent. Zool.*, **9**: 133–135. (In Japanese.)

KATO, Y., 1993. Artificial diet rearing of butterflies. *Nature & Insects*, **28**: 27–30. (In Japanese.)

KONO, Y., 1968. Rearing *Pieris rapae crucivora* BOISDUVAL (Lepidoptera: Pieridae) on artificial diets. *Jap. J. appl. Ent. Zool.*, **3**: 96–98.

MORTON, A. C., 1981. Rearing butterflies on artificial diets. *J. Res. Lepid.*, **18**: 221–227.

SATO, Y., 1974. Rearing *Pieris rapae crucivora* larvae on artificial diets. *Kontyû*, **42**: 467–472. (In Japanese with English summary.)

(Received April 24, 1995; Accepted June 2, 1995)