An Artificial Larval Diet for the West
Indian Sweet Potato Weevil, *Euscepes*
postfasciatus (FAIRMAIRE)
(Coleoptera: Curculionidae)¹

Yukio SHIMOJI² and Tsuguo KOHAMA

Fruit-Fly Eradication Project Office, Okinawa Prefectural
Government, 123 Maji, Naha, Okinawa 902, Japan

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The West Indian sweet potato weevil, *Euscepes postfasciatus* (FAIRMAIRE), is a serious pest of the sweet potato, *Ipomoea batatas* (L) LAM, in Okinawa, Japan (YASUDA and KOHAMA, 1990). Larvae develop in the vines and tubers. Furano-terpenoids are produced in the sweet potato tubers in response to larval feeding, and these chemicals add an unpleasant taste to the tubers. Thus, the tubers are not suitable for human consumption (AKAZAWA et al., 1960; URITANI et al., 1975). The Okinawa Prefectural Government is planning to eradicate *E. postfasciatus* from Okinawa Prefecture using the sterile insect technique (SIT). For successful application of SIT to *E. postfasciatus*, it is necessary to establish a mass rearing method for the weevil; to accomplish this, development of an artificial diet is essential. Although artificial larval diets for the boll weevil, *Anthonomus grandis* (LINDIG, 1979), the orchid weevil, *Orchidophilus aterrimus* (MAU and LAI, 1988), *Hylobius radialis* and *H. pales* (HUNT et al., 1992) and the palm weevil, *Rhynchophorus palmarum* (ZAGATTI et al., 1993) have been developed, no artificial diet for *E. postfasciatus* has been reported as yet. We report here an artificial diet for larvae of *E. postfasciatus*.

MATERIALS AND METHODS

The weevils used in this study were the strain maintained in the Fruit-Fly Eradication Project Office (SHIMOJI and KOHAMA, 1994).

The composition of the artificial diet for larvae is

Table 1. Composition of artificial diet for
E. postfasciatus larvae

Agar	4.0 g
Dried sweet potato powder ^a	10.0 g
Casein	2.4 g
Cellulose	1.6 g
Saccharose	4.0 g
Salt mixture ^b	0.3 g
Ascorbic acid	0.2 g
B-vitamin mixture ^c	5.0 mg
Coline chloride	50.0 mg
Inositol	40.0 mg
Cholesterol	80.0 mg
Potassium sorbate	0.1 g
Methyl <i>p</i> -hydroxybenzoate	0.1 g
Yeast extract	1.0 g
Ethanol	2.0 ml
Distilled water	100.0 ml

^a Sweet potato was dried at 55 ± 1°C.

^b Composition of salt mixture (g): KH₂PO₄ 16.94; MgSO₄ 4.0; Ca(H₂PO₄)H₂O 2.0; NaCl 1.25; MnSO₄H₂O 0.125; FePO₄2H₂O 0.5; ZnCl₂ 0.125; CuSO₄5H₂O 0.0625.

^c Composition of B-vitamin mixture (mg): thiamine HCl 125; niacinamide 500; pyridoxine HCl 125; folic acid 125; calcium pantothenate 500; riboflavin 250; biotin 10; vitamin B₁₂ 1.0.

shown in Table 1. Preparation of the diet was as follows. First, agar was added to 100 ml of distilled water and the water was heated to 80°C. After the agar solution was boiled, the following substances were added: casein, cellulose, saccharose, salt mixture, potassium sorbate, yeast extract powder, and a mixture of cholesterol and methyl *p*-hydroxybenzoate dissolved in ethanol. The compounds were thoroughly mixed and a mixture of ascorbic acid, B-vitamin mixture, coline chloride, and inositol dissolved in 1 ml of water and dried sweet potato powder was added after the temperature of the mixture was allowed to cool to 55°C. The diet was poured into Petri dishes (80 g per dish; 9.0 cm diameter, 2.0 cm depth) and was allowed to solidify.

Eggs of *E. postfasciatus* were collected using an egg-collecting device made of an aluminum mesh container (SHIMOJI and KOHAMA, 1996). These eggs were

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² Present address: Ryukyusankai Co., Ltd., 123 Maji, Naha, Okinawa 902, Japan

Table 2. Development of *Euscepes postfasciatus* at $25 \pm 0.1^\circ\text{C}$ on artificial larval diet and sweet potato tuber^a

	Artificial larval diet	Sweet potato tuber
No. of inoculated eggs	50	50
No. of emerging adults (mean, range) ^b	28.4 (14–37)	30.8 (20–39)
Length of right elytra (mean $\pm$ SD mm) ^c	2.6 ± 0.1	2.7 ± 0.1
Day of adult emergence (mean $\pm$ SD) ^d	48.8 ± 3.9	45.9 ± 2.0

^a 5 replications.^b Means are not significantly different at the 5% level by MANN-WHITNEY's *U*-test.^c Means are significantly different at the 1% level by a STUDENT's *t*-test.^d Means are not significantly different at the 5% level by a STUDENT's *t*-test.

kept on a moist filter paper in the Petri dishes at $25 \pm 0.1^\circ\text{C}$ for 7 days. Fifty eggs were transferred with a tiny brush to the surface of the artificial diet 1 day before hatching. As a control, the same number of eggs was inoculated into tiny holes (ca. 1.0 mm diameter) on a sweet potato tuber (ca. 80 g) punctured with a probe. The rearing Petri dishes and the sweet potato tubers were kept at $25 \pm 0.1^\circ\text{C}$, 70–80% RH, and under conditions of continuous darkness.

Adults of this weevil emerge from the larval diet several days after emergence; therefore, the number of new adults appearing was recorded daily, instead of the total of larval and pupal period. The size of the newly-emerged adults was measured as the length of the right elytra. The experiment was replicated 5 times. A mated 20-day-old female reared on the artificial diet was kept in a plastic cup (70 mm top diameter, 57 mm bottom diameter and 38 mm depth) with a piece of sweet potato tuber (ca. 10 g). A mated 20-day-old female reared on the sweet potato was prepared in the same way as a control. The eggs laid in the sweet potato tuber were counted every day for 3 days. The number of hatched eggs was recorded after incubation for 8 days at $25 \pm 0.1^\circ\text{C}$ and 70–80% RH. This experiment was repeated 10 times.

RESULTS AND DISCUSSION

The mean numbers of adults emerging from the artificial diet and sweet potato tuber were 28.4 and 30.8, respectively (Table 2). There was no significant difference in the mean number of emerging adults between the artificial diet and the sweet potato tuber (MANN-WHITNEY's *U*-test, $p > 0.05$). The results indicate that weevil larvae can successfully develop on the artificial diet. The mean days at which adults emerged from the artificial diet and the sweet potato tuber were 48.8 ± 3.9 and 45.9 ± 2.0 , respectively; there was no significant difference between these val-

ues (*t*-test, $p > 0.05$). The mean lengths of the right elytra of adults emerging from the artificial diet and the sweet potato tuber were 2.6 ± 0.1 mm and 2.7 ± 0.1 mm, respectively, and this difference was significant (*t*-test, $p < 0.01$).

Other biological attributes which are useful in evaluating artificial diets are fecundity and egg viability. Females reared on the artificial diet and on the sweet potato laid 7.2 ± 2.3 (mean $\pm$ SD) eggs and 7.8 ± 2.5 (mean $\pm$ SD) eggs, respectively, over 3 days. There were no significant differences between the average numbers laid eggs (MANN-WHITNEY's *U*-test, $p > 0.05$). The hatchabilities of the eggs of female reared on the artificial diet and on the sweet potato were 90.6 ± 9.8 (mean $\pm$ SD)% and 95.8 ± 7.2 (mean $\pm$ SD)%, respectively. Since the hatchabilities were not significantly different (MANN-WHITNEY's *U*-test, $p > 0.05$), adults reared on the artificial diet should have the same reproductive ability as those reared on sweet potato.

Our results showed that there were no obvious differences in the number of emerging adults and in the days of adults emerging between the artificial diet and the sweet potato. Furthermore, the hatchability of the eggs laid by females reared on the artificial diet was high.

Adults reared on the artificial diets were slightly smaller than those reared on the sweet potato, however, the reasons for this remain unclear. In practical artificial rearing, fecundity and viability must be more important parameters than minor differences in size between adults reared on artificial and natural diets.

An artificial diet for the boll weevil proposed by LINDIG (1979) has successfully produced subsequent generations of the weevils. Since the artificial rearing of the West Indian sweet potato weevil from egg to adult was successful on the artificial diet and eggs

were successfully collected from the adults, it is highly likely that mass production of *E. postfasciatus* can be achieved.

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Comparative Analysis of *Bombyx mori* (Lepidoptera: Bombycidae) Densovirus Type 2 Polypeptides Synthesized *In Vivo* and *In Vitro*¹

Hisaya SOTOSHIRO, Motoko IKEDA and
Michihiro KOBAYASHI

Laboratory of Sericulture and Entomoresources, School of
Agricultural Science, Nagoya University, Chikusa-ku,
Nagoya 464-01, Japan

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Bombyx mori densovirus type 2 (BmDNV-2) is a member of the genus densovirus within the family Parvoviridae. In the densovirus virions, single-stranded genomic DNA of approximately 5 kilobases (kb), with either positive- or negative-polarity, is encapsidated in an icosahedral capsid of approximately 20 nm in diameter which is composed of four species of structural polypeptides with molecular weights (MWs) in ranging from 45,000 (45K) to 100K (cf., TIJSSEN et al., 1990). BmDNV-2 does not have the same molecular feature as those of any other densovirus species thus far isolated, and consists of two single-stranded DNA molecules of 6.6 and 6.1 kb and six structural polypeptide species with MWs of

approximately 120K, 118K, 53K, 51K, 49K, and 46.5K (KAWASE et al., 1984; BANDO et al., 1992; SOTOSHIRO and KOBAYASHI, 1995). Among the BmDNV-2 structural polypeptides, four lower MW (53K, 51K, 49K and 46.5K) species share a common primary amino acid sequence (SOTOSHIRO and KOBAYASHI, 1995), suggesting an overlapped arrangement of these polypeptides in the genomic DNA. In agreement with this fact, the sum of the individual MW of BmDNV-2 structural polypeptides exceeds the coding capacity of the genome.

The mechanism for generation of BmDNV-2-specific polypeptides remains unclear. Immunoblot analysis with anti-BmDNV-2 antiserum has shown that BmDNV-2-infected midgut epithelium contains almost exclusively the 53K viral structural polypeptide, and the remaining five species are undetectable or detectable in very small quantities (SOTOSHIRO and KOBAYASHI, 1995). The 53K viral polypeptide in midgut epithelium converts to the polypeptides of 51K, 49K and 46.5K during *in vitro* incubation, and this conversion is strikingly inhibited or reduced by certain protease inhibitors (SOTOSHIRO and KOBAYASHI, 1995). Together with the observation that the relative proportion of individual structural polypeptides in purified BmDNV-2 virions varies considerably from preparation to preparation (KAWASE et al., 1984; SOTOSHIRO and KOBAYASHI, 1995), these results suggest that the BmDNV-2 structural polypeptides of 51K, 49K and 46.5K are generated posttranslationally by proteolytic cleavage.

The evidence from *in vitro* translation experiments, however, is not completely consistent with generation

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