Reducing rearing cost and increasing survival rate of West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) on artificial larval diet

Yukio SHIMOJI^{*,†} and Masaaki YAMAGISHI[‡]

Fruit-Fly Eradication Project Office, Okinawa Prefectural Government; Naha, Okinawa 902–0072, Japan

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Abstract

Because the ingredients of the artificial larval diet (the basic diet) used to mass-rear the West Indian sweetpotato weevil *Euscepes postfasciatus* were expensive, so we substituted materials at 1/3 of the price (the low-cost diet). There was no difference in the survival rate of the weevil between those reared on the basic diet and those on the low-cost diet. As the quantity of artificial diet per rearing tray was increased, the survival rate of the weevil rose significantly up to 30 g of diet. The survival rate of the weevil was increased 1.6 fold by inoculating eggs in scratching lines 1 mm deep and 1 mm wide on the surface of the artificial larval diet. One hundred fifty eggs per rearing tray was probably a reasonable number because the survival rate and the actual number of surviving weevils at 150 eggs did not differ significantly from their respective maximums for other numbers of eggs. Rearing of 45,000 weevils per month was established using these methods. This represents a 4.5 fold increase compared to culturing by the conventional method. The new mass-rearing method could produce 1.6 adult weevils per gram of diet.

Key words: Artificial larval diet; reducing rearing cost; increasing survival rate; sweet potato pest; *Euscepes postfasciatus*

INTRODUCTION

The West Indian sweetpotato weevil, *Euscepes postfasciatus*, native to the West Indian Islands, is distributed in some countries of Central and South America and the South Pacific Islands. It is known as a very serious pest of the sweet potato, *Ipomoea batatas* (L) Lam. (Sherman and Tamashiro, 1954; Raman and Alleyne, 1991). Its distribution has spread rapidly since it was first detected in the Katsuren Peninsula of the Okinawa Main Island (Kohama, 1990), to the Islands of southwestern Japan. These include the Amami Islands, Yoron Island, Okinawa Islands, Miyako Islands and Yaeyama Islands in the Ryukyu Archipelago (Yasuda and Kohama, 1990; Moriya, 1997; Ohmura, 2000). This weevil is specified as an important pest under the Plant Protection Law in Japan. The strict prohibition of movement of fresh sweet potatoes from a pest-infested region to non-infested areas imposes a substantial restriction on the promotion

of sweet potato production. In order to have the law revoked, this pest must be eradicated. Application of the sterile insect technique (SIT) is being examined to eradicate this pest in Kume Island located 90 km to the west of the Okinawa Main Island (Kuba et al., 2000). Establishing a mass-rearing method for the insect is a basic requirement for implementing SIT (Knipling, 1964). Shimoji and Kohama (1996b) developed an artificial larval diet for *E. postfasciatus* that contains 45.7% dry weight sweet potato root powder as used in the artificial larval diet developed for the boll weevil, *Anthonomus grandis* by the USDA (Lindig, 1979). However, the high costs of this diet and the poor survival rate of the weevil make mass rearing difficult. The present study was conducted with the aims of cutting costs and improving performance of the artificial larval diet. Other improvements such as the egg-inoculation method, diet quantity, and the number of eggs per rearing tray were also made to increase the survival rate of the weevil.

* To whom correspondence should be addressed at: E-mail: yshimoji@k6.dion.ne.jp

† Present address: Laboratory of Okinawa Forest Ecology, Tsudajuku, 1–3–1 Ohira, Urasoe, Okinawa 901–2113, Japan

‡ Present address: Research Institute for Subtropics, 1 Kumoji, Naha, Okinawa 900–0029, Japan

MATERIALS AND METHODS

Experiment (1) Reducing costs of the main ingredients. The primary ingredients of the basic diet developed by Shimoji and Kohama (1996b) (agar, cellulose, protein, sugar, dried yeast, dried sweet potato powder, and distilled water) were replaced with inexpensive ingredients in the low-cost diet as follows (Table 1): Agar (Kanto Chemical Co., Inc.) was replaced with Ina-kanten[®] foodstuff agar (Ina Foods Industries Inc.); Avicel[®] cellulose microcrystalline for column-chromatography (MERCK) was replaced with KC FLOCK[®] as foodstuff cellulose (Nippon Paper Industries); casein (Wako Pure Chemical Industries, Ltd.) was replaced with Esusan-meat[®] skimmed soybean protein (Ajinomoto Inc.); sucrose (Kanto Chemical Co., Inc.) was replaced with raw sugar (Hokubu Seitoh Inc.); yeast extract (Oriental Yeast Industries) was replaced with AY-65 brewer's yeast (Asahi Beer Brewery); domestic dried sweet potato powder was replaced with Gold mash[®] sweet potato flake foodstuff (JA Ibusuki); and distilled water was replaced with tap water. The process of Shimoji and Kohama (1996b) was also applied to making the low-cost diet, that is, Ina-kanten[®] agar

was added to tap water and heated to 80°C. All the ingredients were added to the agar solution while it was allowed to cool to 55°C. The diet was poured into Petri dishes (9.0 cm in diameter, 2.0 cm in depth) at a rate of 25 g per dish and allowed to solidify.

Eggs collected using the method of Shimoji and Kohama (1996a) were subjected to surface sterilization with a solution of 70% ethanol and 5% formaldehyde (Sugiyama et al., 1998). Five scratch lines were made on the surface of the solid diet. Ten eggs per line, a total of 50 eggs, were inoculated using a tiny brush sterilized with 70% ethanol. We observed the eclosion of weevils in the pupal chamber made at the bottom of the Petri dish to determine the developmental period from hatching to eclosion. The basic diet was prepared as a control. The experiment was replicated five times at 25°C, 70 to 80% RH under an L14:D10 photoperiod.

Experiment (2) Relationship between the diet quantity and the survival rate. The low-cost diet was placed in five plastic rearing trays (110×75×15 mm) in quantities of 10, 20, 30, 40, and 50 g. The low-cost diet was allowed to solidify and 150 surface-sterilized eggs were then inoculated on the

Table 1. Ingredient cost ratio of the low-cost diet to the basic diet for *E. postfasciatus* larvae

Ingredients	Basic diet ^a (A)	Low-cost diet (B)	Quantities ^b	Cost ratio (B/A)
Agar	Chemical agar	Ina-kanten [®]	4.0 g	0.271
Host plant	Dried sweet potato root powder	Gold mash [®]	10.0 g	0.867
Protein	Casein	Esusan-meat [®]	2.4 g	0.117
Cellulose	Avicel [®]	KC flock [®]	1.6 g	0.070
Sugar	Sucrose	Raw sugar	4.0 g	0.122
Yeast	Yeast extract	AY-65 brewer's yeast	1.0 g	0.102
Mineral	Salt mixture	Same as basic diet	0.3 g	1.000
Vitamin C	Ascorbic acid	Same as basic diet	0.2 g	1.000
Vitamin B	B-vitamin mixture	Same as basic diet	5.0 mg	1.000
Lipogen	Choline chloride	Same as basic diet	50.0 mg	1.000
Lipogen	Inositol	Same as basic diet	40.0 mg	1.000
Lipogen	Cholesterol	Same as basic diet	80.0 mg	1.000
Antibiotics	Potassium sorbate	Same as basic diet	75.0 mg	1.000
Antibiotics	Methyl <i>p</i> -hydroxybenzoate	Same as basic diet	75.0 mg	1.000
Solubilizer ^c	Ethanol	Same as basic diet	2.0 ml	1.000
Water ^d	Distilled water	Tap water	100.0 ml	1.000
Total ratio				0.285

^a Shimoji and Kohama (1996b).

^b Quantities of ingredients composing the artificial larval diet.

^c Solubilizing cholesterol and methyl *p*-hydroxybenzoate.

^d Distilled water and tap water are regarded as about the same cost because it is difficult to calculate those costs.

glassy surface of the diet using a tiny brush. The diet was dissolved 34 days after egg inoculation, and the number of surviving weevils was counted. This experiment was repeated 20 times.

Experiment (3) Inoculating eggs in scratch lines. Ten scratch lines 1 mm in depth and width were made in the surface of 50 g low-cost diet in a rearing tray. Ten surface-sterilized eggs per line, a total of 100 eggs, were inoculated using a tiny brush. One hundred eggs were inoculated at random on the glassy surface of the diet as a control. The diet was dissolved 34 days after egg inoculation, and the number of surviving weevils was observed. The experiment was repeated 14 times.

Experiment (4) Relationship between the number of inoculated eggs and the survival rate. Forty grams of the low-cost diet was put into each of six rearing trays. Surface-sterilized eggs were inoculated in the surface of each diet using a tiny brush in quantities of 100, 125, 150, 175, 200, and 250 eggs. The surface of the diet was not scratched. The diet was dissolved 34 days after egg inoculation, and the number of surviving weevils was counted. The experiment was repeated 11 times.

Experiment (5) Mass production. Five hundred polypropylene boxes with polyethylene lids (26×33×11.5 cm) were prepared as rearing chambers in this procedure. Five hundred adult weevils with the sex ratio of 1 : 1 were put into each of the boxes and fed 600 g sweet potato roots twice a week. The adults were removed after seven days, and the roots were moved to other rearing boxes of the same size and kept in an environment of 25°C, 70±10% RH, 14L : 10D photoperiod. After seven weeks, approximately 100,000 adults that emerged from the roots were recovered. Fifty polypropylene boxes with a 30 mesh metallic net at the bottom were prepared; these were modified versions of the egg collecting device of Shimoji and Kohama (1996a), i.e. new egg collection devices (16×22.5×8 cm), and 2,000 adults (the sex ratio of 1 : 1) were set in each box. Approximately 100,000 eggs were then collected per week using the egg collection method of Shimoji and Kohama (1996a). These collected eggs were surface-sterilized and put into a test tube (10 mm dia., 90 mm long), capped with a 30 mesh lid. The test tube was turned upside down and shaken over the whole surface of the diet in the plastic rearing trays. Thus,

the eggs were planted by being sprinkled through the mesh lid (hereafter referred to as the sprinkling method). The procedure was repeated every week.

The rearing trays planted with eggs were kept under conditions of 25°C, 70±10% RH for seven weeks. The number of adults produced each week was summarized monthly and compared with that of the conventional rearing method. We will henceforth refer to the conventional method as “before modification” and the improved method as “after modification.” A comparison was made between six months preceding the modification and six months following the modification.

Before modification: An average of 500 eggs (range 200 to 1,000 eggs) per plastic rearing tray were inoculated on the glassy surface of the low-cost diet setting the diet quantity at 100 g, and using the sprinkling method.

After modification: The low-cost diet was set at 30 to 40 g. The whole surface of the diet was then lightly scratched using a 50 mm long and 10 mm wide saw blade before planting the eggs. Finally, about 150 eggs were sprinkled over the diet.

Statistical analysis. Mann-Whitney's *U*-test was used to compare the results of experiments (1) and (3). Analysis of variance with Scheffé's test was used to assess the relationship among the values of the result in experiments (2) and (4). In the above values, the hatchability and the survival rate among the inoculated eggs were transformed to arcsine square-root before statistical analysis.

RESULTS

Experiment (1) Reducing costs of main ingredients

Table 2 shows results of the tests. Significantly more adults emerged from the low-cost diet than from the basic diet. The number of hatches for the two diets did not differ significantly. In addition, the developmental period from hatching to eclosion was significantly shorter for the low-cost diet than for the basic diet. These results indicate that the low-cost diet is superior to the basic diet in rearing. The cost of ingredients was reduced to about one-third of the cost of the basic diet (Table 1).

Table 2. Development of *E. postfasciatus* on the low-cost diet and the basic diet at 25°C, 14L : 10D

	No. of inoculated eggs	No. of hatches ^a (n)	No. of emerging adults ^a (n)	Development time ^{a,b} (n)
Low-cost diet	50	39.6±1.0 (8)	27.3±0.8 (8)	32.8±0.2 (190)
Basic diet	50	39.3±1.0 (8)	23.5±1.5 (8)	33.7±0.2 (179)
Mann-Whitney's <i>U</i> -test		<i>p</i> >0.05	<i>p</i> <0.05	<i>p</i> <0.01

^a Mean±SE.^b Days from hatching to adult.

Fig. 1. Number of surviving weevils (●) and hatchabilities (□) on varied quantities of artificial larval diet of *E. postfasciatus*. Hatchabilities were transformed to arcsine square-root before statistical analysis. Means of untransformed data are reported. Means followed by the same letter are not significantly different (analysis of variance followed by Scheffé's test, $p>0.05$). Vertical lines show standard errors.

Experiment (2) Relationship between the diet quantity and the survival rate

Figure 1 shows that there were no significant differences in hatchability using different quantities of diet. However, a diet increase from 10 g to 30 g significantly increased (4.4 times) the number of surviving weevils. In addition, there were no significant differences in the number of surviving weevils with a diet increase above 30 g.

Experiment (3) Inoculating eggs in scratch lines

The number of surviving weevils on the diet with eggs inoculated in scratch lines was 54.6 ± 2.8 (mean±SE, $n=14$), which was significantly greater than the number (33.1 ± 2.8 ($n=14$)) obtained when eggs were inoculated onto a glassy surface (Table 3).

Experiment (4) Relationship between the number of inoculated eggs and the survival rate

The hatchability of inoculated eggs is shown in Fig. 2. There were no significant differences in hatches among the various numbers of inoculated eggs. The number of surviving weevils increased significantly as the number of inoculated eggs increased, however the survival rates significantly decreased (Fig. 3). Consequently, in this experiment, the intersection of the regression curve of the number of developed weevils, $y=12.645 \ln(x)+42.635$ ($r^2=0.924$) and the regression line of the survival rate, $y=-3.3057x+47.253$ ($r^2=0.931$) was at 150 eggs inoculated. Thus, it seems reasonable to inoculate 150 eggs per tray since the number of surviving weevils and the survival rate at 150 eggs do not differ significantly from their respective maximums.

Experiment (5) Mass production

Rearing the weevils for six months using a modified method based on the outcomes of experiments (2) to (4) resulted in a monthly production that reached around 45,000 weevils, compared to an average of 10,000 weevils before modification (Fig. 4). We converted these to values per gram of diet to compare production efficiency. The average of the monthly production before modification was 0.2 ± 0.1 (mean±SD, $n=6$) weevils per gram. In contrast, the monthly production by the modified method was 1.6 ± 0.1 ($n=6$) weevils, which corresponds to eight times the production of the method before modification. This increase was mainly due to the influence of the diet quantity being reduced from 100 g to 30 g.

DISCUSSION

Diet is the most important factor in rearing insects. While the natural diet that the insect con-

Table 3. The number of surviving weevils on artificial larval diet of *E. postfasciatus* with eggs inoculated in scratch lines and on glassy surface

Inoculating eggs	No. inoculated eggs	No. hatches ^a	No. survived weevils ^a	<i>n</i>
In scratch lines	100	84.3±1.4	54.6±2.8	14
On glassy surface	100	84.3±1.4	33.1±2.8	14
Mann-Whitney's <i>U</i> -test		<i>p</i> >0.05	<i>p</i> <0.01	

^a Mean±SE. Fig. 2. Hatchabilities on varied number of inoculated eggs. Hatchabilities were transformed to arcsine square-root before statistical analysis. Means of untransformed data are reported. Means are not significantly different (analysis of variance followed by Scheffé's test, *p*>0.05). Vertical lines show standard errors.

Fig. 4. Changes of the monthly production of weevils over six months before and after improving for rearing methods. The averages of productions "before" and "after" July 1997 were 9,774±1,769 (mean±SE) and 44,247±3,243, respectively.

Fig. 3. Relationship between number of inoculated eggs and number of surviving weevils (○) or survival rate (●: number of survived weevils per number of inoculated eggs). Survival rates were transformed to arcsine square-root before statistical analysis. Means of untransformed data are reported. Means along the line followed by the same letters are not significantly different (analysis of variance followed by Scheffé's test, *p*>0.05). Vertical lines show standard errors.

sumes in its natural environment is ideal, it may be difficult to supply throughout the year, and its quality may vary according to seasonal changes. An artificial diet that can be maintained and supplied in a simple manner is desirable for stable mass rearing. For example, an artificial diet with low material costs was developed and produced in a simple manner for the melon fly, which was mass-produced for SIT (Nakamori et al., 1975; Sugimoto, 1978).

Artificial diets have been developed for other weevils, such as the orchid weevil *Orchidophilus aterrimus* (Mau and Lai, 1988), the pine root collar weevil *Hylobius radicis* (Hunt et al., 1992), the Pales weevil *H. pales* (Thomas, 1969; Clark, 1973; Hunt et al., 1992), the root feeding weevil *H. transversovittatus* (Blossey et al., 2000), the palm weevil *Rhynchophorus palmarum* (Zagatti et al., 1993), the plum curculio *Conotrachelus nenuphar* (Yonce et al., 1971), and the white pine weevil *Pissodes*

strobi (Zerillo and Odell, 1973; Trudel et al., 1994). The most successful example of artificial mass rearing of weevils is that of the boll weevil (Sikorowski et al., 1983). Lindig (1979) contributed to the success of mass production of the boll weevil by saving time, energy, and labor through the development of an artificial larval diet.

The Okinawa Prefectural Government is planning to implement long-term eradication control of *E. postfasciatus* by means of SIT. One SIT prerequisite is that mass production of the insect be implemented at a low cost and in a stable quantity (Knippling, 1966). An artificial larval diet for *E. postfasciatus* has been developed (Shimoji and Kohama, 1996b), but at high material costs.

We succeeded in improving the number of surviving weevils per gram of diet by eight fold and in reducing the costs of diet materials to 30% from that of the conventional rearing method. Therefore, production of 1,000,000 weevils per week was achieved at a material cost of 187,500 yen.

The measures taken in this experiment to improve the production of *E. postfasciatus* on an artificial larval diet included the results of experiments (2) determining the quantity of diet per rearing tray (3) confirming the effects of scratching the surface of the diet on the survival of eggs, and (4) determining the number of inoculated eggs per rearing tray.

In conclusion, the rearing method for *E. postfasciatus* in this experiment using an improved artificial diet successfully reduced the material costs and improved the survival rate compared with the unimproved rearing method. An additional direction of this study for *E. postfasciatus* will be to further reduce the rearing costs without reducing their life history traits, such as fecundity and longevity, during continued mass-rearing.

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