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HIGH POPULATION DENSITY AND EGG CANNIBALISM REDUCES THE EFFICIENCY OF MASS-REARING IN *EUSCEPES POSTFASCIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) is a major pest of sweetpotato *Ipomoea batatas* (L.) Lam (Solanales: Convolvulaceae) in some countries. In order to improve mass-rearing for an eradication program employing the sterile insect technique (SIT), optimal population density of *E. postfasciatus* in an artificial diet was examined. Six population densities (1000, 4000, 7000, 10000, 13000, and 16000 individuals per container with 200 g of artificial diet) were compared for effect on the number of eggs collected and hatchability. The total number of eggs collected after 24 d increased with an increase in population density and reached a saturation level at 13,000 individuals, whereas hatchability was not affected by population density. The results indicated that optimal population density in mass rearing was 13,000 individuals on 200 g of artificial diet. Furthermore, we examined cannibalism by adult weevils in the presence of other diets. The result suggested that egg cannibalism may be a major reason for the low rate of egg collection in the mass rearing of *E. postfasciatus*.

Key Words: West Indian sweetpotato weevil, artificial diet, sterile insect technique, density effect, mass rearing

RESUMEN

El picudo de camote de las Antillas Occidentales, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae), es una plaga mayor del camote, *Ipomoea batatas* (L.) Lam (Solanales: Convolvulaceae) en algunos países. Para mejorar la cría en masa en un programa de erradicación empleando la técnica del insecto estéril (TIE), se examinó la densidad de población óptima de *E. postfasciatus* usando una dieta artificial. Se compararon seis densidades de población (1000, 4000, 7000, 10000, 13000 y 16000 individuos por recipiente con 200 g de dieta artificial) para su efecto sobre el número de huevos recolectados y su habilidad para eclosionarse. El número total de los huevos recolectados después de 24 días incremento con el aumento de la densidad de la población y llegó al nivel de saturación a los 13,000 individuos, mientras que la habilidad para eclosionarse no fue afectada por la densidad de la población. Los resultados indicaron que la densidad óptima de la población para la cría masiva fue de 13,000 individuos con la dieta artificial de 200 g. Además, examinamos el canibalismo por los adultos de los picudos en la presencia de otras dietas. El resultado indica que el canibalismo de los huevos puede ser una de las razones mayores para la baja tasa de la colección de huevos en la cría masiva de *E. postfasciatus*.

Sweetpotato *Ipomoea batatas* (L.) Lam (Solanales: Convolvulaceae) is one of the world's major food crops, especially in developing countries (Jansson & Raman 1991). The presence of the West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) and the sweetpotato weevil *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) together have prevented growers from expanding production of sweetpotatoes into tropical and subtropical regions (Jansson & Raman 1991). Lower levels of pre- and postharvest infestations can reduce both quality and marketable yield and render sweetpotato storage roots unfit for consumption because of toxic sesquiterpenes that are produced by roots

in response to weevil feeding (Uritani et al. 1975). Much of the research has been conducted on control of *C. formicarius* (Jansson & Raman 1991; Miyatake et al. 2000; Kumano et al. 2008), but few studies have been done on *E. postfasciatus*.

Euscepes postfasciatus is the major pest of sweetpotato in the South Pacific, Caribbean basin, islands of southwestern Japan, and some countries of Central and South America (Sherman & Tamashiro 1954; Kohama 1990; Raman & Alleyne 1991; Yasuda 1993). The presence of this weevil on South Pacific islands and southwestern Japan (Okinawa) has resulted in strict quarantine regulations, including banning the export of sweetpotato from these islands (Raman & Alleyne

1991; Kuba et al. 2003). Because the adults of *E. postfasciatus* do not seem to produce sex pheromones, pheromone traps cannot be used to suppress the weevils (Kuba et al. 2003). However, sterile insect technique (SIT) is an effective method for suppression or eradication of weevils (Kuba et al. 2003).

Establishing an insect mass-rearing technique is a basic requirement for implementing SIT. Sterile insect technique involves introducing a high proportion of sterile matings into a natural population in order to reduce reproduction to a level below population maintenance (Knippling 1979). In past successful SIT projects, millions of insects per week were produced in mass-rearing facilities (e.g., 280 million per week of the tephritid melon fly *Bactrocera cucubita*es (Conquillet); 6 million per week of the Old World screwworm *Chrysomya bezziana* Villeneuve; and 14 million per week of the codling moth *Cydia pomonella* (L.) (cf., Dyck et al. 2005). In *E. postfasciatus*, a large number of weevils can be reared on sweetpotato roots (one million per week; Yamagishi & Shimoji 2000), but rearing involves some challenges. It is very expensive to procure large quantities and maintain stable quality of the sweetpotato roots throughout the year. Furthermore, weevils that are reared on sweetpotato roots face a high risk of infection with the protozoan *Farinocystis* sp. (undescribed species) at the Okinawa Prefectural Plant Protection Center (OPPPC) (Morita et al. 2007). The weevils were infected with the protozoan orally through the sweet potato root. Infection remarkably reduces the fecundity and longevity of adult *E. postfasciatus* (N. K. & D. H., unpublished data). Therefore, the development of an artificial diet for mass-rearing *E. postfasciatus* is needed.

Shimoji & Kohama (1996a) and Shimoji & Yamagishi (2004) developed an artificial larval diet, and Sakakibara (2003) developed an artificial diet for adult *E. postfasciatus* to feed and oviposit. The larval diet contains mainly sweetpotato powder, agar, cellulose, protein, sugar, dried yeast, and water. The adult artificial diet consists of the larval diet in addition to an antibiotic (chloramfenicol) and KOH. However, optimal population density for mass-rearing has not been examined. Generally, in mass-rearing the insects are reared at higher than optimal density for economical reasons. For example, a mass-rearing facility may have limited space and budget, and insects may be reared with a limited number of containers and diets. The high density is expected to have negative effects on the insects' biological performance because of resource shortages and accumulation of weevil waste (Pearl & Parker 1922; Ito et al. 1969). Furthermore, high density may promote egg cannibalism by adult insects. Females of *E. postfasciatus* excavate the surface of either the sweetpotato root or the artificial diet,

oviposit in the cavity, and then seal the eggs in the cavity with a plug (Raman & Alleyne 1991; Shimoji & Kohama 1994). The buried eggs near the surface of the artificial diet are at a high risk of being cannibalized by adult weevils intentionally or accidentally at high density. Egg cannibalism by adult insects is expected to occur frequently under mass-rearing conditions, and results in a reduction in the number of eggs collected from the mass-rearing procedure. In this study, we examined the optimal population density for egg collection in large-scale mass-rearing experiments, and the number of eggs that were cannibalized in small-scale laboratory experiments. Our results provide useful information for establishing an artificial mass-rearing system for use in an SIT strategy against this weevil.

MATERIALS AND METHODS

Insects

The West Indian sweetpotato weevils used in our study were from a colony that was originally established from insects collected in Yaese Town, Okinawa, in Nov 2004. They were cultured on sweetpotato roots at $25 \pm 1^\circ\text{C}$, 50-90% Relative humidity (RH), and a photoperiod of 14: 10 (L: D) for 18 to 20 generations at OPPPC in Naha, Okinawa. The experiments were conducted from Mar to Aug 2008. The artificial diet for adult weevils was modified from Sakakibara (2003) by replacing sweetpotato powder with the powder of railroad vine, *Ipomoea pes-caprae* (L.) (Solanales: Convolvulaceae). Other components of the artificial diet (agar, cellulose, protein, sugar, and antibiotic, etc.) were the same as Sakakibara (2003).

Effect of Population Density on Fecundity and Hatchability

Population Density

Subsequent to emergence from the sweetpotato roots, adult weevils (ages 2-9 d old) were placed in a mesh container and fecundity of the weevils was measured for 24 d. Most adults stayed in the sweetpotato roots until sexual maturation, which was about 10 d after emergence (Kohama & Shimoji 1998). The weevils were maintained on sweetpotato roots during the period between emergence and the setting up of our experiments. To determine the effect of population density on fecundity, 1,000 (5 replications), 4,000 (5 replications), 7,000 (5 replications), 10,000 (5 replications), 13,000 (5 replications), and 16,000 (4 replications) adults were introduced into each meshed container ($357 \times 287 \times 120$ mm) with 200 g of artificial diet cut in the shape of a square pillar ($5 \times 5 \times 40$ mm). When greater amounts of the artificial diet was supplied to each container, it became damp and then

spoiled (T. K. unpublished data). Numbers of weevils per container were measured by using the mean body weight from samples of 50 weevils. The sex ratio was assumed to be approximately 1:1 on the basis of Raman & Alleyne (1991). The containers were kept at 25 ± 1°C, 50-80% RH, and a photoperiod of 14:10 (L: D).

Egg Collection

Egg collection procedure in the experiment was based on the technique of egg extraction for the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Roberson 1984), which exhibits similar oviposition behavior. After the setup of the mass-rearing containers, eggs were collected 9 times in 24 d (Table 1). We rinsed the container, weevils, and diet all together with running tap water to remove the eggs that were oviposited on the artificial diet as well as at the bottom of the container. First, the weevils were scooped out of the suspension with a 1.0-mm mesh net (see weevil collection section). The large pieces of diet were rubbed vigorously by hand in water for 15 min in order to free the eggs buried in the diet. The remaining suspension was filtered through a 0.5-mm meshed sieve to separate the eggs from the diet and waste particles. The mixture of eggs and remaining waste particles were mixed with 14.6% salt-water in a glass (100 mL), and freshwater was gently added to the glass to separate the eggs from the waste (Ohno et al. 2005). After a few min, we collected the eggs from the boundary between the salt-water and the freshwater by pipette. The number of eggs per container was estimated by the method from Ohno et al. (2006). When there were very few eggs and the number could not be estimated by this method, we counted the eggs directly. For surface disinfection, the eggs were dipped in 70% ethanol solution for 40 min, and then rinsed with sterile water (modified from Ohno et al. 2004). The hatchability of the eggs was then determined (see hatchability section). From the first egg collec-

tion, the collected eggs were discarded because some fungi and mites adhered to the weevils and containers. After this treatment, few fungi and mites were observed on the weevils and containers.

Weevil Collection

After being removed from the suspension, the weevils were rinsed in water, and dried with an electric fan for 3.5 h. They were then re-introduced to containers with 200 g of fresh artificial diet to continue the egg-collection experiment. We did not record the status of individuals (dead or alive) for logistic reasons. This procedure was conducted 9 times during a 24-day period for each replication.

Hatchability

To examine the effects of population density on hatchability, we randomly chose 200 eggs that had been oviposited 7 d after the initial setup of each treatment. One hundred disinfected eggs were then placed on a nylon cloth (block) on wet filter paper in Petri dishes (9 cm in diameter × 2 cm in height) (2 blocks per density treatment). The Petri dishes were kept at 25 ± 1°C, 50-80% RH, and a photoperiod of 14:10 (L:D). Previous studies showed that the mean egg-hatching period was about 7-9 d (Sherman & Tamashiro 1954; Raman & Alleyne 1991). We recorded the number of hatched eggs at 6, 7, 10, 12, and 14 d after the day of egg collection. The experiment was replicated 3 times.

Egg Cannibalism

To examine the incidence of egg cannibalism, we conducted 2 small-scale laboratory experiments. In one we examined whether food shortage caused egg cannibalism, and in the other experiment whether adult weevils cannibalized eggs even in food-rich conditions. To examine whether

TABLE 1. SCHEDULE OF EGG COLLECTION. EGGS WERE COLLECTED 9 TIMES FOR EACH REPLICATION. WEEVILS WERE RE-INTRODUCED TO NEW CONTAINERS AFTER EACH EGG COLLECTION WITH FRESH ARTIFICIAL DIET UNTIL THE NINTH EGG COLLECTION.

	Monday	Wednesday	Friday
Week 1			Set up mass rearing
Week 2	Discard collected eggs due to contamination.	First egg collection (5 d after setting up).	Second egg collection.
Week 3	Third egg collection.	Fourth egg collection.	Fifth egg collection.
Week 4	Sixth egg collection.	Seventh egg collection.	Eighth egg collection.
Week 5	Ninth egg collection (24 d after setting up).		

egg cannibalism was influenced by the artificial diet, we used both sweetpotato root and artificial diet for these experiments. We used weevils aged 1 d after emerging from the sweetpotato, and we selected only male weevils because of the potential problem of identifying original eggs from the newly oviposited ones during the experimental periods.

Experiment 1. Effect of Starvation on Egg Cannibalism

This experiment was intended to clarify the relationship between food shortage and egg cannibalism by adult male weevils. The number of eggs cannibalized by either starved or unstarved adults was measured in the absence of other food sources. In the starved condition, each male was kept individually in a well of a 24-well multiplate (AS ONE Corporation, Osaka, Japan). The males were given neither food nor water for 7 d. Each unstarved control male was set in a small plastic container (200 mL) with either a small piece of sweetpotato root (60 mg) or artificial diet (60 mg) for 7 d as well. The diet was changed daily. After the starvation procedure, each male and 5 eggs were placed at the bottom of a well of a 24-well multiplate, and we counted the number of remaining eggs 5 d after setting up the treatment.

Experiment 2. Effect of the Presence of Other Diets on Egg Cannibalism

This experiment examined whether male *E. postfasciatus* cannibalized eggs when other food sources were available. The numbers of cannibalized eggs were compared in the presence or absence of a small piece of sweetpotato root or artificial diet. Each male was kept individually in a well of a 24-well multiplate with no diet or water for 3 d. We then divided the individual weevils into 3 treatments: (1) 5 eggs and no diet, (2) 5 eggs and a small piece of sweetpotato root (5 mg), and (3) 5 eggs and a small piece of artificial diet (5 mg) at the bottom of a well of a 24-well multiplate. For each group, we counted the number of lost eggs 2 d after setting up the experiment.

Statistical Analyses

Generalized linear mixed models (GLMMs) were performed by using R 2.60 (R Development Core Team 2007) in our analyses (Schall 1991; Crawley 2005). When female fecundity was a dependent variable, we used GLMM with Poisson error structure and a log link function. When the hatching rate or the number of cannibalized eggs was a dependent variable, we used GLMM with binomial error structures and logit link functions. When the effect of population density on female fecundity and hatching rate was examined, the dependent variable was fecundity or hatching

rate, fixed independent variables were population density and quadratic term (population density²); and the random effects were replication and blocks (hatching rate only). In experiment 1, the dependent variable was the number of cannibalized eggs, fixed independent variables were the diet treatment (i.e., no diet vs. sweetpotato root), and the random effects were the identities of the focal individuals. In experiment 2, the dependent variable was the number of cannibalized eggs, the independent variable was the diet treatment and the random effects were identities of the focal individuals. A Wald test was used to test the statistical significance of each coefficient in the model. To examine the relationship between fecundity per female and population density, we used the non-parametric Jonckheere-Terpstra test for ordered alternatives (Hollander & Wolfe 1999) because the error structure of fecundity per female was not able to be determined. In egg cannibalism experiments, the *P*-values were corrected by Tukey's all-pairwise comparisons (R add-on package "multcomp," Hothorn et al. 2008). We used the two-tailed test for statistical significance.

RESULTS

Effect of Population density on Fecundity and Hatchability

The peak of oviposition was 10 d after setting up the experiment; the number of eggs then decreased over time (Fig. 1). The fitted GLMM equation indicated the saturation curve (quadratic term (population density)² = $-8.39e^{-9} \pm 1.7e^{-9}$ ($\pm$ SE), $z = 71.45$, $P < 0.001$; linear term (population density) = $2.83e^{-04} \pm 2.34e^{-06}$, $z = 120.85$, $P < 0.001$).

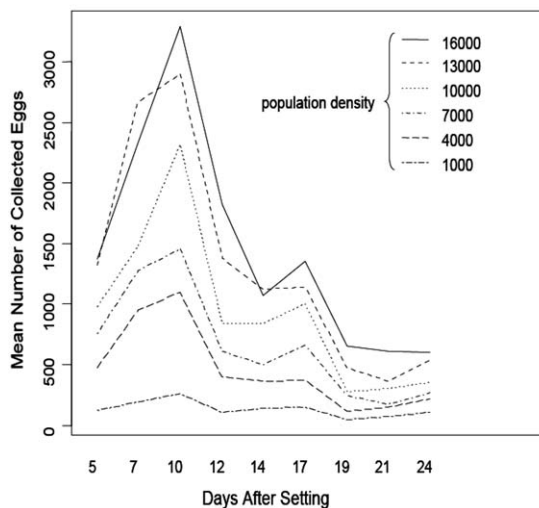


Fig. 1. Oviposition pattern of *Eusepes postfasciatus* at different population densities.

The result suggested that the total number of eggs collected per 24 d increased with the increase of population density, and reached saturation at 13,000 individuals (Fig. 2a). No significant relationship was observed between fecundity per female and population density (Fig. 2b, $J^* = 249$, $k = 6$, $P = 0.084$).

Hatchability was 57.5% with the treatment of 10,000 to 13,000 individuals, and no significant differences in hatchability among the treatments were observed (Fig. 2c, the quadratic term; $-4.88e^{-09} \pm 5.20e^{-02}$, $z = 9.38e^{-08}$, $P = 1.0$, linear term; $1.07e^{-04} \pm 9.33e^{-02}$, $z = 1.14e^{-07}$, $P = 1.0$).

Egg Cannibalism

Experiment 1. Effect of Starvation on Egg Cannibalism

Starved males cannibalized a significantly larger number of eggs than unstarved males did during 5 d (Fig. 3 and 4, starved vs. artificial diet; $b = 1.76 \pm 0.50$ (SE), $z = 3.55$, $P = 0.0011$, starved vs. sweetpotato root; $b = 1.07 \pm 0.46$, $z = 2.35$, $P = 0.050$). No significant differences were observed between treatments containing artificial diet or sweetpotato roots ($b = 0.69 \pm 0.53$, $z = 1.32$, $P = 0.38$). The b values indicated contrast coefficients (Crawley 2005).

Experiment 2. Effect of the Presence of Other Diets on Egg Cannibalism

No significant differences in the number of eggs cannibalized were apparent among the diet treatments (Fig. 4, no diet vs. artificial diet; $b = 0.28 \pm 0.44$, $z = 0.64$, $P = 0.80$, no diet vs. sweetpotato root; $b = -0.11 \pm 0.43$, $z = 0.25$, $P = 0.97$, and artificial diet vs. sweetpotato root; $b = -0.39 \pm 0.45$, $z = 0.87$, $P = 0.66$).

DISCUSSION

The total number of eggs collected over 24 d increased with population density, but leveled off above 13,000 individuals, although we did not investigate the treatment of more than 16,000 individuals. However, hatchability and fecundity per female were not affected by population density. These results implied that optimal population density in mass rearing lay around 13,000 adult individuals with 200 g of artificial diet. Few eggs were collected after 19 d. In the treatment of 13,000 individuals, a female laid 0.08 eggs per day. Raman & Alleyne (1991) reported that weevils reared on sweetpotato roots oviposited 1.0 egg per day per female. This large difference in the number of eggs collected was not likely due to only damage to weevils by the rinsing procedure or to the quality of artificial diet.

Egg cannibalism was considered to be a major reason for the low rate of egg collection. With some insects, eggs or immature larvae are canni-

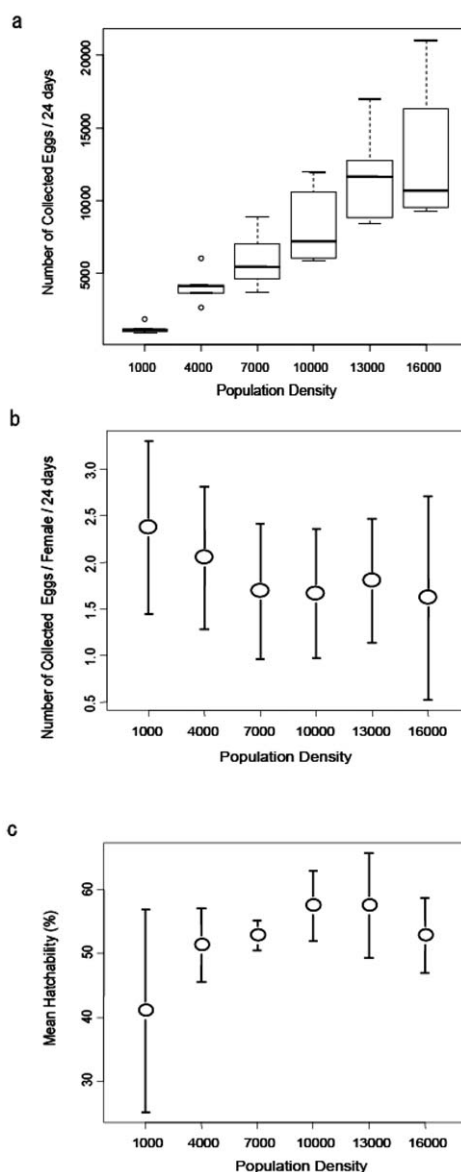


Fig. 2. (a) Relationship between population density of *Euscepes postfasciatus* adults in cages with 200 g of artificial diet and number of eggs collected. The horizontal bar in the middle of each box denotes the median number of eggs. The top of the box (above the median) denotes the 75th percentile, and the bottom of the box (below the median) denotes the 25th percentile. The vertical dashed lines indicate the maximum and minimum values of the y axis except for the outliers. Open circles represent the outliers. Outliers were values more than 1.5 times the interquartile range above the 75th quartile or below the 25th quartile. (b) Relationship between population density of *E. postfasciatus* adults and number of eggs collected per female. Vertical bars represent $\pm$ SD. (c) Relationship between population density of *E. postfasciatus* adults and hatched larvae observed on the nylon cloth in Petri dishes. Vertical bars represent $\pm$ SD.

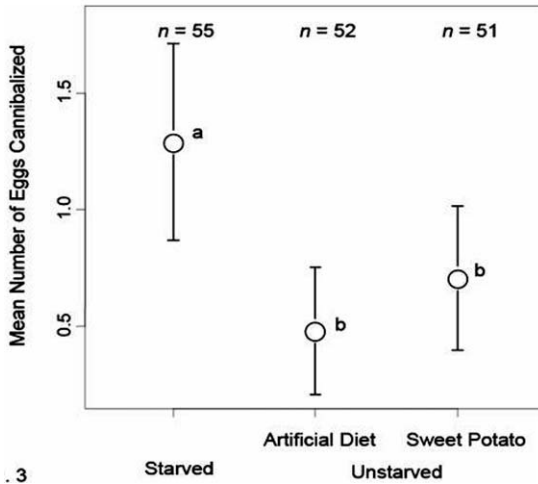


Fig. 3. Egg cannibalism among groups of starved and unstarved *E. postfasciatus* adults in the absence of other food sources. Different letters indicate a significant difference in the number of cannibalized eggs. Vertical bars represent $\pm$ SD.

balized by other individuals (Banks 1956; Fox 1975; Kinoshita 1998). In the present study, the starved male weevils cannibalized eggs more frequently than the well-fed ones did. This result implied that cannibalism due to food shortage contributed to the reduction in the number of eggs collected. Furthermore, adult weevils cannibalized some of the eggs even in the presence of available diet. This finding suggested that egg cannibalism may be a major cause of the low rate

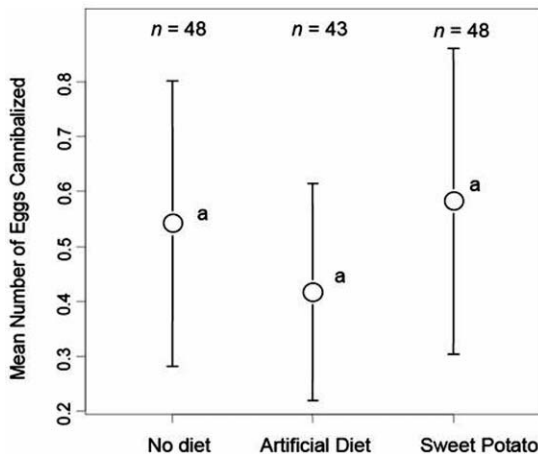


Fig. 4. Egg cannibalism by *E. postfasciatus* adults in the presence or absence of other food sources. No significant differences among the treatments were observed, as indicated by the same letters for each treatment. Vertical bars represent $\pm$ SD.

of egg collection in the mass-rearing of this species. Based on our data that each weevil cannibalized an average of 0.4 eggs during a 2-day period, we estimated that 13,000 weevils cannibalized 2,600 eggs per day. We assumed that if egg cannibalism were fully prevented, a total of 74,400 eggs would be collected in 24 d with the treatment of 13,000 individuals (in the present state, 12,000 eggs were collected). Therefore, if a method to prevent egg cannibalism is developed, the rate of egg collection would change dramatically. For example, Shimoji & Kohama (1996b) and Shimoji & Yamagishi (2002) used an aluminum meshed container that was set in a plastic cup to collect eggs easily. Eggs laid into the container fell through the mesh and into the bottom of the plastic cups. Because a mesh container separates the eggs from the adult weevils, this method would also be useful in preventing egg cannibalism. Furthermore, hatchability would probably be improved because this method does not involve rinsing the eggs, which can damage them.

Food shortages and an accumulation of weevil waste also may cause a reduction in female performance. Therefore, a greater frequency of food replacement and cleansing of containers might increase the number of eggs collected. If eggs had been collected daily instead of every 2-3 d, numbers of eggs collected may have increased. However, this approach would not be practical because labor reduction is essential in mass-rearing (Parker 2004). More fruitful approaches to increasing egg output may involve improvement of shape and water content of the artificial diet. The artificial diet sometimes became moldy during mass-rearing. If water content in the diet can be decreased, growth of the mold may be suppressed. Furthermore, small changes in diet composition can often lead to large changes in insect survival and reproduction (Cohen 2004). For example, Fisher & Bruck (2004) found that larval black vine weevil *Otiorhynchus sulcatus* survival was 23% higher and body weight was 1.6 times higher on an improved diet with 0.8 g decrease in methyl paraben and replacement of sorbic acid with potassium sorbate. Therefore, experiments should be conducted to determine whether alteration of artificial diet components increases the reproductive performance of *E. postfasciatus*. For example, reduction in antibiotic content may increase the performance of the weevil because microorganisms are symbiotically associated with *E. postfasciatus* (T. Hosokawa, personal communication).

In conclusion, we demonstrated an optimal population density around 13,000 individuals of adult *E. postfasciatus* with 200 g of artificial diet. Under mass-rearing conditions, we conclude that egg cannibalism was a major cause of the low rate of egg collection in the artificial diet treatment compared with the treatment of sweetpotato roots. Improvement of egg collection techniques

and artificial diet is urgently needed for the mass-rearing of *E. postfasciatus*.

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