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SHORT COMMUNICATION

Moisture during Egg Incubation Adversely Affects Hatching of the West Indian Sweetpotato Weevil, *Euscepes postfasciatus* (Coleoptera: Curculionidae), When the Eggs are Incubated En Masse

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Abstract We compared the egg-hatching of *Euscepes postfasciatus* (Fairmaire) with and without moisture treatment to test whether moisture is necessary during egg incubation when a large number of eggs are gathered into a mass. Moisture treatment exhibited significant undesirable effects on hatching (reduction of hatchability, delay of hatching, and increase of the variance of hatching date) compared to no moisture treatment. In addition, moisture treatment significantly increased the incidence of fungus on the egg surface, which can subsequently contaminate the larval artificial diet. Based on these results, we concluded that moisture is not necessary for incubating *E. postfasciatus* eggs. Two possible explanations for the undesirable effects of moisture on hatching were discussed: a direct effect by preventing respiration of the eggs and an indirect effect through fungal infection of the eggs.

Key words Artificial diet, Dryness, Fecal plug, Mass-rearing, Quarantine pest, Sterile insect release

Introduction

Euscepes postfasciatus (Fairmaire) (Coleoptera: Curculionidae), a weevil species native to the West Indian Islands, is a serious pest of the sweet potato, *Ipomoea batatas* (L.) Lam. (Solanales: Convolvulaceae) (Sherman and Tamashiro, 1954; Raman and Alleyne, 1991). The species invaded Okinawa Island in the middle of the 20th century and rapidly expanded its range throughout the Ryukyu Archipelago south of Amami Island, southwestern Japan (Kohama, 1990; Yasuda and Kohama, 1990). This weevil is listed as a quarantine pest under the Plant Protection Law of Japan, which prohibits movement of the host plants of *E. postfasciatus* from a pest-infested region to non-infested regions. To eradicate *E. postfasciatus* from Okinawa using the sterile insect technique, Okinawa Prefectural Government is currently trying to develop a mass-rearing technique for the weevil using artificial diets (Yamagishi and Shimoji, 2000). To obtain a continuous mass-rearing technique, we must develop or improve techniques at each step of the rearing process (e.g., egg collection, egg incubation, egg-seeding on the diet, preventing bacterial and fungal contamination of the diet, and larval rearing; see Shimoji, 2004). The incubation of *E. postfasciatus* eggs is a necessary step because the egg period is somewhat long; the eggs of the species start to hatch seven days after oviposition at 25℃ (Shimoji and Kohama, 1996a). We are currently seeking to determine environmental conditions during egg incubation that have no undesirable effect on hatchability and egg period (Ohno *et al.*, 2005b). The present study focuses on the effect of moisture during incubation on the hatching of *E. postfasciatus* eggs.

Shimoji and Kohama (1996a) showed that the hatchability of *E. postfasciatus* eggs incubated on dry filter paper did not differ significantly from that of eggs incubated on moistened filter paper at 25℃ and 70 to 80% r.h. This result suggests that moisture is unnecessary for incubating *E. postfasciatus* eggs when humidity is kept at an adequate level. However, Shimoji and Kohama's (1996a) egg-incubation experiment was conducted under conditions in which a small number of eggs (50 per Petri dish) were placed separately from each other within a single piece of filter paper (Y. Shimoji, personal communication). In the practical mass-rearing of *E. postfasciatus*, a large number of eggs should be incubated en masse because separating and arranging many eggs by hand is extremely time- and labor-consuming. Therefore, the effect of moisture on the hatching of *E. postfasciatus* eggs

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under conditions of mass-incubation was examined to determine whether moisture is in fact necessary for incubating eggs of this species.

Materials and Methods

The weevils used in this study were originally collected at Yomitan-Village, Okinawa Island, in August 1994 (approximately 17,000 individuals) and cultured on sweetpotato root at Fruit Fly Eradication Project Office, Naha-City, Okinawa Is., at 25±1℃ and 50 to 90% r.h., and with a photoperiod of 14L:10D (five generations per year, >7,500 individuals being used for producing the next generation), by the method described in Yamagishi and Shimoji (2000). The eggs were obtained from May to June 2003, using an artificial diet developed for collecting eggs of *E. postfasciatus* [a modified version of Shimoji and Kohama's (1996b) larval diet; M. Yamagishi, unpublished]. The adults were fed the artificial diet for one night, and eggs oviposited around the diet were washed out along with other debris and subsequently separated from the debris by the brine-flotation technique (see Ohno *et al.*, 2004 for details).

The collected 0-day-old eggs were submersed in water, and approximately 20,000 of them were sampled by the egg-number estimation method using measuring pipettes (Ohno *et al.*, 2006). With this method, eggs are aspirated into the pipette together with water; the total volume of the eggs is determined; then the number of eggs contained therein is predicted by a regression equation. These eggs were divided into two groups each of which contained about 10,000 eggs, and each group was settled on a black nylon cloth (5 cm×5 cm). One of the nylon cloths was placed on a plastic Petri dish (9 cm W, 1.5 cm H) lined with dry filter paper (ADVANTEC No. 1, Toyo Roshi, Japan) (hereafter the without-moisture treatment). The other cloth was placed on a Petri dish lined with filter paper soaked with distilled water (moisture treatment). Before the lid was closed, the Petri dish of the moisture treatment experiment was tilted, and any excess water was discarded. Both Petri dishes were kept in an incubator (25±1℃, 70±10% r.h., 14L:10D) for six days. To remove any uncontrollable environmental differences within the incubator, the positions of the Petri dishes in the incubator were randomly switched every day until the end of the experiment. To retain moisture for the moisture treatment, the condition of the filter paper was inspected visually every day. When the filter paper started to dry out, distilled water was supplied to the filter paper and any excess water was discarded again as above. During the incubation period, the surface of the mass of eggs was not wet regardless of the addition of water (personal observation).

After the six-day incubation, hyphae of unidentified fungal species occasionally occurred on the egg surface (see Results). Therefore, we checked for the presence of fungal hyphae on the egg for each treatment under a binocular microscope. On the same day, eggs of both treatments were wetted with distilled water and gently disentangled with a tiny paintbrush because the eggs were stuck to each other and to the nylon cloth. For each treatment, 100 eggs were randomly sampled using the paintbrush, and placed separately in a single Petri dish lined with moistened filter paper and nylon cloth (same as above). In this condition, each of the eggs became moist only where it was in touch with the nylon cloth (personal observation). The eggs subjected to without-moisture treatment were also put on wet filter paper because we were considering a situation in which the eggs are inoculated on an artificial diet with a wet surface. The Petri dishes were returned to the same incubator as indicated above. Daily, from the next day to the seventh day (7 to 13 d after oviposition), hatchlings were counted and removed from the dishes. The remaining eggs were discarded on the eighth day after confirming that there was no hatchiling. In addition to studying hatchability, we calculated the mean egg period (days from oviposition to hatching) and the coefficient of variation (CV, standard deviation divided by the mean) of the egg period as an indicator of the degree of dispersion of hatching dates, neither of which was treated in Shimoji and Kohama (1996a).

The above procedures were replicated 14 times using eggs oviposited on different days; thus, we could obtain 14 paired estimates for each of the presence of fungus, the hatchability, the mean egg period, and the CV of the egg period. The difference in the frequency of fungal occurrence between the moisture and without-moisture treatments was tested with Fisher's exact probability test. For the remaining three estimates, a comparison was made between the two treatments using Wilcoxon signed-rank test as has been done previously by us (Ohno *et al.*, 2005b, c). To test the mean egg period, we did not use a parametric statistical method because the equality of variance of the egg period between the two treatments was violated (see Results). When the 14 replications within a singe treatment could be separated into 'fungus' and 'non-fungus' groups, each of the three estimates were compared between the two groups within the treatment using Mann-Whitney U test.