Improvement of survival of the West Indian sweet potato weevil, *Euscepes postfasciatus* (Coleoptera: Curculionidae), by placing absorbent paper on the artificial diet after egg seeding and ensuring air permeability of the rearing tray

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Abstract

A remarkable reduction of the post-hatching survival rate caused by adopting the furcellaran solution (a viscous liquid, hereafter FS) as an egg-seeding agent has become a problem during development of mass-rearing methods for the West Indian sweet potato weevil (*Euscepes postfasciatus*) using an artificial diet. We hypothesized that FS physically inhibits the feeding of hatched larvae on the diet, and predicted that removing and/or drying FS after egg seeding would improve the survival of *E. postfasciatus*. To confirm this prediction, we examined the effects of two post-egg-seeding treatments (placing absorbent paper on the diet surface and ensuring air permeability of the rearing tray) on the survival and development of the weevils. Neither of the two treatments worked well individually, but they exhibited a clear synergistic positive effect on weevil survival. Their survival rate jumped from 2% to 40% when these two treatments were combined, without any adverse effects on the developmental period and the adult body size. These treatments thus enabled the FS-based egg-seeding method, which is much simpler than previous methods, clearly contributing to the establishment of a mass-rearing system for *E. postfasciatus*.

Key words: Artificial rearing; *Ipomoea*; quarantine pest; sterile insect technique; ventilation

INTRODUCTION

Euscepes postfasciatus (Fairmaire), a weevil species native to the West Indian Islands, is a worldwide quarantine pest of the sweet potato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae). This weevil has invaded several countries and seriously impacted sweet potato production (Sherman and Tamashiro, 1954; Raman and Alleyne, 1991). In Japan, *E. postfasciatus* invaded Okinawa in the middle of the 20th century and rapidly expanded its range throughout the Ryukyu Archipelago (Sakae, 1968; Kohama, 1990). This species is designated as a plant quarantine pest under the Plant Protection Law of Japan, which prohibits the transportation of fresh sweet potatoes from pest-infested regions (i.e., Ryukyus) to non-infested regions (i.e., mainland Japan). Eradication of this weevil has

been vigorously sought in Japan not only to promote sweet potato production in infested regions, but also to prevent the expansion of the weevil's range to non-infested regions.

The Okinawa Prefectural Government initiated the world's first trial to eradicate *E. postfasciatus* using the sterile insect technique (SIT) (Kuba et al., 2000). Sterile males of *E. postfasciatus* produced using fresh sweet potato tuberous roots were released in a restricted area of Kume Island, the first target area (Kuba et al., 2003). Although SIT has worked well in this restricted area (Kuba et al., 2000, 2003), the target area has not yet been extended throughout the island, due to limited production of sterile weevils (Ohno et al., 2006a). This limitation arises primarily from the high cost of fresh sweet potato tuberous roots, as well as from unexpected yield fluctuations in mass rearing using

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sweet potatoes (Ohno et al., 2006a). A mass-rearing method using an artificial diet is clearly essential for successful eradication of *E. postfasciatus*. Small-scale methods for successive rearing of *E. postfasciatus* using an artificial diet have been developed (Yamagishi and Shimoji, 2000; Shimoji, 2004). We are presently attempting to extend these methods to a mass-rearing system by developing or simplifying the techniques at each step necessary for rearing (e.g., egg incubation, egg seeding on the diet, and prevention of bacterial contamination of the diet) (Ohno et al., 2005a, b, c, 2006b, c, d, 2007; Ohno and Kohama, 2007). This study seeks to overcome the problem of the marked reduction of the post-hatching survival rate that emerged while simplifying the egg-seeding method.

The traditional procedures necessary for seeding *E. postfasciatus* eggs on the diet are tedious and inconvenient. Before egg seeding, eggs are submerged in a disinfection agent, rinsed and air-dried, and then carefully separated using a tiny paintbrush. Eggs of *E. postfasciatus* tend to stick to each other when dried, and it takes an average of 3 min to separate 30,000 eggs, even when the dried eggs are moistened (Ohno et al., 2006c). Separating the dried mass of eggs without moisture requires a much longer time. The labor for separating eggs increases in proportion to the number of eggs to be treated, thus becoming an obstacle to extending the rearing method to the mass-rearing technique. When one million eggs are used in one egg-seeding operation, it takes at least 100 min to separate them. In addition, previous egg-seeding methods per se have some disadvantages: the separated eggs are placed on the diet surface individually using a tiny brush (very time-consuming) or are carefully sprinkled onto the diet surface using a meshed test tube (Shimoji, 2004). Sprinkling eggs onto the diet surface is somewhat simpler than placing eggs using the brush, but controlling the egg-seeding density and assuring even distribution of the eggs on the diet surface are difficult.

Alternatively, we are currently testing the use of a viscous liquid, furcellaran solution (FS), as an egg-seeding agent to overcome these problems (Ohno et al., 2005a, b, 2007). Furcellaran is a type of carrageenan, a sulfated polysaccharide extracted from red algae (*Furcellaria fastigiata* L. (Lam.)) and is used as a food gelling agent (McCandless and Craigie, 1979). This egg-seeding method has

been successfully used in the mass rearing of another curculionid pest, the boll weevil *Anthonomus grandis* (Boheman), which is injurious to cotton (Sikorowski et al., 1984). This method involves mixing disinfected but non-dried eggs with FS which takes only a few minutes, and then dropping the mixture onto the diet. A clear advantage of this method is that air-drying and separation processes are unnecessary. Further, eggs are evenly suspended in FS by simply shaking the mixture. Therefore, this method has the advantages of being simple and straightforward irrespective of the number of eggs used, as well as assuring even distribution of the eggs and controlling the egg-seeding density on the diet. We demonstrated that submersion of *E. postfasciatus* eggs into FS has no detectable effect on egg hatching (Ohno et al., 2005b). Thus, the FS-based egg-seeding method was once expected to be adopted in the artificial rearing of *E. postfasciatus*; however, we recently revealed an adverse effect of FS on weevils: when *E. postfasciatus* eggs were inoculated with FS on the diet surface, the post-hatching survival rate declined to less than half that of the control (Ohno et al., 2007). Therefore, some refinements of the rearing method are necessary before FS can be adopted as an egg-seeding agent.

As a possible cause of this reduced survival, we consider that the hatched larvae were suspended in FS due to its viscosity, preventing them from contacting and feeding on the diet (Ohno et al., 2007). If this hypothesis is true, we can predict that weevils will survive at a much higher rate when hatchlings are given easier access to the diet surface by post-egg-seeding treatments to remove or dry FS. The present study examined the survival and development of *E. postfasciatus* under two treatments after egg seeding with FS: placing paper on the diet surface to absorb excess FS, and improving the air permeability of the rearing tray to accelerate evaporation of the water content of FS.

MATERIALS AND METHODS

Insects and egg seeding on the diet. The weevils used in this study were originally collected in Yomitan Village, Okinawa Island, in August 1994 and cultured on sweet potato tuberous roots at the Fruit Fly Eradication Project Office under conditions of $25 \pm 1^\circ\text{C}$, 50–90% RH and 14L10D (five

generations per year). The eggs were obtained in January 2004 as described in Ohno et al. (2004, 2006b), and incubated in a plastic Petri dish lined with dry filter paper in an incubator (MIR-253, Sanyo Electric Co. Ltd., Tokyo, Japan; $25\pm1^{\circ}\text{C}$, 14L10D) for six days. After incubation, the eggs were moistened, carefully separated using a tiny brush, and submersed in 14% saltwater. Eggs that sank in the saltwater were discarded because they were a source of bacterial contamination (Ohno and Kohama, 2007). Eggs that did not sink were rinsed with distilled water and transferred to a 5 ml measuring cylinder to estimate the number of eggs (Ohno et al., 2006d). These eggs were disinfected by submersing in 70% ethanol for 5 min and then in 5% formaldehyde for 20 min, and then rinsed with sterile water (Ohno et al., 2008). In the subsequent operations, all tools and trays used for handling the disinfected eggs and the artificial diet were sterilized by autoclaving (121°C , 40 min) or by spraying with 70% ethanol. Fifty-four glass Petri dishes (9 cm diameter, 2 cm height) were prepared, with 25 g of Shimoji and Kohama's (1996) artificial diet in each. Furcellaran powder (Ina Food Industry Co., Ltd., Ina, Japan) and distilled water were mixed at the ratio of 500:3 weight, autoclaved (121°C , 40 min), and then cooled to room temperature ($23\text{--}25^{\circ}\text{C}$). The disinfected eggs were mixed with FS at the ratio of 55 eggs per 1 ml based on the results of the above egg-number estimation using the measuring cylinder. The mixture was gently shaken to suspend eggs evenly in FS. Two milliliters of this egg-FS mixture was dropped evenly within a 5 cm-diameter circle of the diet surface of each Petri dish, using a micropipette, of which 2 mm of the plastic tip (maximum volume: 1 ml) was cut off to prevent the tip from being plugged by the eggs.

We examined the number of eggs contained in 1 ml of the egg-FS mixture and the weight of the 1 ml mixture along with the above egg-seeding operation. A plastic Petri dish (9 cm diameter, 1.5 cm height) lined with moistened filter paper and a black nylon cloth was placed on an electric balance. One milliliter of the egg-FS mixture was dropped onto the nylon cloth in the Petri dish using the same micropipette as above. After weighing, the eggs contained therein were counted and the lid of the Petri dish was closed. This procedure was replicated 20 times. The mean number ($\pm\text{SE}$) of

eggs in the 1 ml mixture was $56.4 (\pm 1.62)$, close to the predetermined number of eggs (55). The mean weight ($\pm\text{SE}$) of 1 ml of mixture was 1.01 g (± 0.005). To estimate hatchability, all Petri dishes were kept in the same incubator as above, and hatchlings were counted and removed every day from the next day to the eighth day (14 d after oviposition), as in Ohno et al. (2006b). Mean hatchability ($\pm\text{SE}$) in 20 Petri dishes was 84.8% (± 1.64). The mean number of eggs in the 1 ml mixture, weight of the 1 ml mixture, and hatchability were used for the following analyses.

Treatments for improving survival. Weevils were reared using a two-factorial design: one factor was the number of pieces of absorbent paper (0, 1 and 2 pieces) placed on the diet surface, and the other was the air permeability status of the Petri dish (not permeable, permeable for the first three weeks, and permeable throughout the rearing period), resulting in nine (3×3) experimental groups (six Petri dishes for each group). The treatment 'permeable for the first three weeks' was included because we suspect that the treatment 'permeable throughout the rearing period' may cause excessive desiccation of the diet, leading to an adverse effect on the survival and development of weevils. Kimwipes[®] S-200 (Nippon Paper Crexia Co., Ltd., Tokyo, Japan) was used as the absorbent paper after cutting into 5 cm $\times$ 6 cm rectangles and autoclaved. Eighteen of the above 54 egg-seeded Petri dishes were lidded without placing the paper on the diet surface. For each of the other 18 Petri dishes, one piece of paper was gently placed on the egg-seeded part of the diet surface. For each of the remaining 18 Petri dishes, one piece of paper was similarly placed, and another piece of paper was then placed on top. Air permeability was controlled by sealing the gap between the body and the lid of the Petri dish using 2 cm-wide clear cellophane tape (Fig. 1). Sealing the gap (Fig. 1A) is the conventional method (Shimoji, 2004), while leaving most of the gap open (Fig. 1B) is a newly tested method. In each of the three groups varying in the number of pieces of paper, the gaps of six Petri dishes were sealed, while those of the remaining 12 were left open. All Petri dishes were kept in the same incubator ($25\pm1^{\circ}\text{C}$, 14L10D) as indicated above, and the relative humidity in the incubator was monitored using a data logger (Thermo Recorder RTR-53, T&D Corporation, Matsumoto,

Fig. 1. Status of the air permeability of Petri dishes used for rearing *E. postfasciatus*. A, air not permeable. The gap between the body and lid of the Petri dish was sealed. B, air permeable. Two areas of the gap were taped for ease of handling, and others were left open. For ease of recognition, the artificial diet was not included, colored tape instead of clear tape was used for sealing, and the Petri dishes were placed upside down.

Japan) throughout the rearing period. Mean relative humidity ($\pm$ SE, range) during the period was 73.6% ($\pm$ 0.58, 52–83%). On the 21st day after egg seeding, the time when the diet surfaces of all non-sealed Petri dishes seemed to be dried in S. Ohno's visual inspection, six non-sealed Petri dishes from each group were randomly chosen, and the gaps between their lid and body were sealed as above. The positions of the Petri dishes in the incubator were randomized at least once a week until the end of the experiment.

Weighing the content of the Petri dish. To examine the effect of air permeability on the desiccation of the contents of Petri dishes, we weighed all of the Petri dishes using an electric balance on the date of egg seeding, and on the third to the 11th week after egg seeding at two-week intervals. To calculate the weight of the Petri dish contents (diet+FS+weevils), the weight of each Petri dish without the contents and the cellophane tape used

were measured at the end of rearing (12th week from egg seeding, see below), and these values were subtracted from the total weight each week. The weight of the absorbent paper (0.073 g for one piece, mean of 20 pieces) was also subtracted from the total weight for paper-placed Petri dishes. The ratio of the weight of the contents each week to that at the date of egg seeding was calculated for each Petri dish. To test the relationship between treatments and the pattern of decrease in the ratio of the weight over time, the ratio was arcsin-square-root transformed and analyzed by repeated measures ANOVA where pieces of paper and air permeability were included as fixed effects, and weeks after egg seeding as the time-course effect.

Parameters for survival and development. We measured the number of emerging adults per Petri dish as an indicator of the survival rate, weeks from egg seeding to adult eclosion as the developmental period, and left elytron length as the adult body size. Since adult emergence of *E. postfasciatus* is usually visible in a pupal chamber made on the bottom of the Petri dish (Shimoji, 2004), newly emerging adults were counted once a week for all Petri dishes until they could no longer be observed. The mean developmental period was calculated by summing the weeks at adult emergence for all observed individuals and then dividing the sum by the number of observed individuals. At the 12th week from egg seeding, diets were dissected and all emerging adults (containing individuals of which emergence weeks could not be determined) were counted. Three adults for each sex, if available, were randomly chosen from each Petri dish, and their left elytron lengths were measured in 0.002 mm increments with the ocular micrometer of the binocular microscope.

Prior to statistical analyses, the number of emerging adults per Petri dish was square-root transformed after adding 0.5, and weeks from egg seeding to adult eclosion and left elytron length were $\log_{10}$ transformed. The number of emerging adults per Petri dish was tested by ANOVA where pieces of paper and air permeability were included as effects. When remarkable increases of emerging adults were observed, estimated post-hatching survival rates were calculated for selected experiment groups as follows: mean number of emerging adults/(mean number of eggs seeded (113) $\times$ mean hatchability (0.85)). The weeks from egg seeding

to adult eclosion was analyzed by the same ANOVA after pooling data from different Petri dishes within each experiment group. To test the effect of treatments on adult body size in relation to sex, left elytron length was analyzed by ANOVA where sex was included as an effect in addition to the above two treatment effects. For each variable, pairwise comparisons of means were made among experiment groups by the Tukey-Kramer HSD test when at least one of the treatment effects was significant at the 5% level.

RESULTS

Temporal changes of weight of the Petri dish contents

In repeated measures ANOVA on the ratio of weight of the contents of the Petri dish from three to 11 weeks after egg seeding, the effect of the status of air permeability was significant ($p < 0.001$), while the effect of the number of pieces of paper ($p = 0.18$) and interaction between the two treatments ($p = 0.83$) were insignificant. The interaction between air permeability and weeks from egg seeding was significant ($p < 0.001$), while the interaction between pieces of paper and weeks from egg seeding and the three-way interaction were insignificant (both $p > 0.6$), irrespective of the statistics used (Wilks λ or Hotelling's T^2). Although weevil survival varied greatly among the experiment groups (see below), the weight ratio was not significantly affected by the pieces of paper, suggesting that the degree of weevil survival did not affect the weight of the contents of the Petri dish or its temporal change.

Based on this result, we indicated temporal changes of the % weight (Fig. 2) by pooling the data from Petri dishes with different numbers of pieces of paper. The significant effect of air permeability indicated that the method used to ensure air permeability tested in this study contributed to drying the contents of the Petri dish. In fact, the ratio of weight in two experiment groups in which air permeability was ensured markedly decreased during the first three weeks, and was consistently lower than that of the group in which permeability was not ensured (Fig. 2). The significant interaction between air permeability and week indicated that the mode of temporal change in the ratio of weight varied depending on the status of air perme-

Fig. 2. Temporal changes in the ratio of weight of the contents of the Petri dish (artificial diet+egg-seeding agent+weevils) after egg seeding on the diet under different conditions of air permeability of the Petri dish. Air permeability was controlled by sealing or not sealing the gap between the lid and the body of the Petri dish using cellophane tape (see Fig. 1). $n = 18$ for each data point.

ability. In fact, the ratio of weight in the group in which air permeability was ensured throughout the observation period decreased more markedly than that in the other two groups (Fig. 2).

The decreased weight ratio three weeks after egg seeding in an air-permeable condition was 8.7% (mean value when data from “air permeable” and “air permeable for the first three weeks” groups were combined), corresponding to 2.3 g in actual weight. This value was almost the same as the weight of the egg-FS mixture dropped onto the diet surface (2.02 g), suggesting that weight decrease during the first three weeks was due mostly to evaporation of the water content of FS, and the decrease after three weeks was mainly due to drying of the artificial diet.

Effect of the treatments on survival and development

In ANOVA on the number of emerging adults per Petri dish, effects of both air permeability and pieces of paper and their interaction were significant (Table 1). Remarkable increases of emerging adults were observed only when “placing paper” and “ensuring air permeability” were simultaneously conducted (Table 2), apparently leading to

Table 1. ANOVA tables for testing the effects of pieces of absorbent paper placed on the diet surface and condition of air permeability of the Petri dish on survival and development of *E. postfasciatus*

Variable/Source of variance	d.f.	SS	F value	p
Number of emerging adults per Petri dish ^a				
Pieces of paper	2	37.791	16.112	<0.001
Air permeability	2	120.136	74.048	<0.001
Pieces of paper×Air permeability	4	13.082	1.967	0.009
Error	45	37.794		
Weeks from egg seeding to adult eclosion ^b				
Pieces of paper	2	0.015	2.691	0.069
Air permeability	2	0.310	54.307	<0.001
Pieces of paper×Air permeability	4	0.054	4.743	<0.001
Error	814	2.323		
Left elytron length ^b				
Pieces of paper	2	0.002	3.245	0.041
Air permeability	2	0.000	0.111	0.895
Sex	1	0.004	12.535	<0.001
Pieces of paper×Air permeability	4	0.002	1.443	0.221
Pieces of paper×Sex	2	0.000	0.840	0.433
Air permeability×Sex	2	0.000	0.806	0.448
Pieces of paper×Air permeability×Sex	4	0.000	0.313	0.869
Error	243	0.068		

Zero, one, or two pieces of absorbent paper (Kimwipes[®]) were placed on the diet surface after egg seeding. Method for controlling air permeability is the same as in Fig. 1. Six Petri dishes were prepared for each combination of pieces of paper and air permeability.

^aData were transformed to $(x+0.5)^{0.5}$ prior to ANOVA.

^bData from different Petri dishes were combined and $\log_{10}$ transformed prior to ANOVA.

Table 2. Relationships among air permeability status of the Petri dish, pieces of absorbent paper placed on the diet surface, and survival and development of *E. postfasciatus*

Air permeability	No. pieces of paper	No. emerging adults per Petri dish ^a	Weeks from egg seeding to adult eclosion ^b
Not permeable	0	1.83±0.31 a	7.42±0.30 ab
	1	3.00±0.73 ab	8.23±0.44 a
	2	5.67±1.61 ab	8.67±0.19 a
Permeable for the first three weeks	0	9.50±3.05 bc	6.83±0.12 bc
	1	29.50±5.20 d	6.60±0.07 bcd
	2	38.17±3.89 d	6.49±0.06 bcd
Permeable	0	15.83±3.41 cd	6.40±0.08 cd
	1	37.67±4.13 d	6.37±0.06 d
	2	32.00±5.24 d	6.55±0.08 bcd

^aIndicator of survival rate (mean±SE). Labels with the same letters indicate no significant difference (Tukey-Kramer HSD test on $(x+0.5)^{0.5}$ transformed data, $p=0.05$; $n=6$ for each group).

^bIndicator of developmental period (mean±SE). Labels with the same letters indicate no significant difference (Tukey-Kramer HSD test on $\log_{10}$ transformed data, $p=0.05$; n varies from 7 to 180 for each group).

significant interaction between pieces of paper and air permeability. The effect of placing paper on the diet surface when air permeability was not ensured was insignificant, while the effect of air permeability when paper was not placed on the diet surface

was significant only when air permeability was ensured throughout the rearing period (Table 2), implying that neither of the two treatments works well individually. The effects of pieces of paper (one or two) and air permeability after three weeks were

unclear because the number of emerging adults did not vary significantly among the four groups in which both placing paper on the diet surface and ensuring air permeability were conducted (Table 2). The post-hatching survival rate of the group containing the highest number of adults (two pieces of paper placed, air permeable for the first three weeks) was estimated to be 39.9%, 21-fold that of the control (1.9%; paper not placed, air not permeable).

ANOVA applied to weeks from egg seeding to adult eclosion revealed that the effect of air permeability and the interaction between air permeability and pieces of paper were significant, while the effect of pieces of paper was insignificant (Table 1). When compared with the control (paper not placed, air not permeable), reduction of the developmental period was observed only when air permeability was ensured throughout the rearing period (Table 2). In the groups in which paper was placed, the developmental period was remarkably longer in the air-not-permeable condition than in the air-permeable conditions (Table 2), which appears to be the cause of the significant interaction. Overall, it was proven that the developmental period was shortened rather than prolonged by the simultaneous operation of placing paper and ensuring air permeability.

ANOVA applied to elytron length revealed that the effects of pieces of paper and sex were significant, while the effects of air permeability and all of the interactions were insignificant (Table 1). Based on this result, we considered that air permeability did not affect elytron length, and thus compared the result for each sex by pooling the data from groups differing in the status of air permeability (Table 3). In males, elytron length when either one or two pieces of paper were placed did not differ significantly from when paper was not used, while the value was significantly larger in the two-piece group than in the one-piece group. In females, the length did not vary significantly irrespective of the number of pieces of paper. There was no evidence to suggest that placing paper on the diet surface reduced the body size of weevils.

DISCUSSION

As possible methods to overcome the reduced survival rate of *E. postfasciatus* caused by FS, we

Table 3. Relationship between pieces of absorbent paper placed on the diet surface and elytron length as the body-size indicator of *E. postfasciatus*

No. pieces of paper	Male ^a	Female ^a
0	2.394±0.016 ab	2.434±0.019 a
1	2.386±0.012 a	2.465±0.013 a
2	2.434±0.012 b	2.464±0.013 a

^a Mean±SE, in mm. Labels with the same letters indicate no significant difference (Tukey-Kramer HSD test on log₁₀ transformed data, $p=0.05$; n varies from 34 to 48 for each group).

examined the effects of two post-egg-seeding treatments on the survival and development of weevils: placing absorbent paper on the diet surface and ensuring air permeability of the rearing tray. Both treatments contributed to a remarkable (almost 20-fold) improvement of survival, confirming our prediction that removing and/or drying FS after egg seeding would raise the survival rate. The two treatments did not induce any detectable adverse effects on the development of *E. postfasciatus* (i.e., delayed development or reduced body size). Instead, in some cases they shortened the developmental period, the result of which is favorable for the mass weevil rearing. Based on these results, we concluded that the two treatments are very effective for artificial rearing of *E. postfasciatus*, promising to accelerate the development of a mass-rearing system for weevils, and thus the SIT program targeted toward the weevil.

In the present study, the maximum post-hatching survival rate of *E. postfasciatus* was 40%, which is still lower than under previous rearing methods in which FS was not used (60–73%; Shimoji et al., 1999; Ohno et al., 2004, 2007; Shimoji and Yamagishi, 2004); therefore, there is still room for further improvement of the survival rate of this weevil. This improvement may be realized by refining the methods used for removing and drying FS after egg seeding, as suggested below.

Relationships between placement of paper, air permeability, and survival and development of weevils

When air permeability was not ensured, placing paper on the diet surface did not significantly affect the survival rate (Table 2), suggesting that the effect of the paper becomes apparent only under air-

permeable conditions. When paper was not placed on the diet surface, air permeability raised the survival rate significantly, but not remarkably (Table 2). These results indicate that the effects of placing paper and ensuring air permeability on the survival of weevils were not reciprocally additive, but synergistic, thus resulting in significant interaction between the two treatments (Table 1). At present, the cause of this synergistic effect is unclear. One possible explanation is that placing one or two pieces of paper (Kimwipes®) on the diet surface was insufficient to allow the eggs to come into contact with the diet surface. In other words, space remained between the paper and the diet surface soon after egg seeding, possibly due to the viscosity of FS. Ensuring air permeability can reduce this assumed space by evaporating the water content of FS, thus helping hatchlings access the diet surface.

In the paper-placed experimental groups, the developmental period became longer under air-not-permeable conditions than under the air-permeable conditions (Table 2). This phenomenon may also be explained by the hypothesis that a space exists between the paper and the diet surface soon after egg seeding. If air permeability is not ensured, this supposed space may not be reduced and larvae cannot access the diet for a long time, thus leading to delayed development; however, further explanation is needed because the observed delayed development in the paper-placed groups was more marked than in the paper-not-placed groups (Table 2), which was confirmed by the significant interaction between pieces of paper and air permeability (Table 1). It is possible that the longer developmental period observed in the paper-placed/air-not-permeable groups resulted from the larvae being in contact with the paper, instead of the diet surface, for a long time.

The elytron length when one or two pieces of paper were placed did not differ significantly in either sex from when paper was not placed, suggesting no adverse effect of paper on adult body size; however, male elytron length was significantly larger in the two-piece group than in the one-piece group (Table 3). The cause of the size difference, as well as the reason why such a difference is observed for males but not for females, is unclear at present and needs explanation.

Air permeability in the late phase of rearing and number of pieces of paper placed on the diet surface: effects on the survival of weevils, and implications for further improvement of the survival

Although the temporary decrease in weight of the contents of the Petri dish was more marked in the “air permeable” group than in the “air permeable for the first three weeks” group (Fig. 2), the survival of weevils did not significantly vary between those groups (Table 2). This result suggests that, to improve weevil survival, air permeability is necessary only in the early phase (in this case, the first three weeks) of the rearing period. The three weeks after egg seeding is the time when the diet surfaces of air-permeable Petri dishes were considered to be dried (see Materials and Methods), and when the contents of the Petri dish lost approximately 2 g of weight, corresponding to the weight of the egg-FS mixture dropped onto the diet surface. Therefore, it is highly probable that drying FS is critical for improving the survival rate, whereas subsequent drying of the artificial diet is less important. Attempts to further accelerate FS drying (e.g., making a larger opening for ventilation in the rearing tray, or incubating the tray in a low-humidity environment), may lead to further improvement of *E. postfasciatus* survival. With respect to the labor necessary for actual mass rearing, the operation for sealing the opening of the rearing tray may be time consuming. The present results may also suggest that such a sealing procedure will become unnecessary; however, it is hard to imagine that extreme drying of the artificial diet would not affect the survival and development of weevils. Therefore, it is important to clarify the limit of diet drying that would not adversely affect the weevils.

The number of emerging adults did not significantly change with the number of pieces of paper (one or two, Table 2), suggesting that the survival of weevils was not affected by the pieces of paper, as far as we tested; however, it is unclear whether the survival rate would be improved if many more pieces of paper or other kinds of paper (such as filter paper) were used. One interesting trial would involve layering several pieces of paper on the diet surface after egg seeding, and then removing all the pieces of paper except for the bottom one soon after absorption of FS. These treatments should be tested in relation to the above attempts to acceler-

ate the drying of FS.

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