Effect of population density of *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) on the progeny populations¹

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Abstract

Adults of the sweet potato weevil, *Cylas formicarius*, were allowed to lay eggs on a 150 g tuberous root of sweet potato for 3 days under conditions of 27°C, 60% RH, and 14L:10D. The number of progeny increased with the increase of parental density, and reached the saturation level. The number of progeny per parental female showed a peak at 30–100 pairs of parents. The density response mode of this insect characterizes it as the Allee type. At very low density, the mortality of eggs and larvae was high, which might have been due to the unfavorable conditions of the tuberous root, the quality of which had not yet been improved by the frano-inducing factor secreted during feeding by the larval and adult weevil. Comparatively more eggs were laid on the tuberous root conditioned by adult-feeding or oviposition. Size of adult progeny became smaller with the increase of parental density. The duration of their development was shortened, and variation of the time period until their emergence from the tuberous root became smaller with the increase of parental density. Optimal parental density for mass-rearing was found to be 65 pairs per tuberous root of 150 g for 3-days oviposition.

Key words: *Cylas formicarius*, density effect, optimal density, mass rearing

INTRODUCTION

The sweet potato weevil, *Cylas formicarius* (Fabricius), a renowned pest of sweet potato, is distributed in the South West Islands of Japan. The adults lay eggs in the tuberous roots of the sweet potato, and the larvae and adults feed on the tuberous root, which provides both habitat and food resource in the process of development (Sutherland, 1986; Chalfant et al., 1990).

A program to eradicate this insect was carried out by using the method of sterile insect release (Sweet Potato Weevil Research Group, 1992). For this project, it was essential to produce an enormous number of insects (Knipling, 1979). Over the years, many methods of mass production have been developed in various insect species. For this purpose, high quality individuals, whose reproductive ability is able to overcome that of the wild populations, must be obtained. Generally, in mass production, the insects are reared at high density for economical reasons. However, high density may negatively effect the quality of the insects (Utida,

1941). An artificial diet for this weevil has not yet been developed, and the current preferred diet is tuberous root of sweet potato. This study intends to clarify the effect of density of adults on their progeny populations, and to apply conditions of mass production for effectively obtaining sterilized males.

MATERIALS AND METHODS

All experiments were carried out under the conditions of 27°C, 60% RH, and 14L:10D in the laboratory of Kinki University, Nara, Japan. The sweet potato weevils used were reared for several years in our laboratory under the plant quarantine permission. A plastic container (310×310×110 (depth) mm), filled with styrofoam chips for ensuring the uniform arrangement of eggs laid on the surface of the tuberous root (Kamikado et al., 1993), was used for rearing the weevils. The number of replications at each density level (pair) is shown in the parenthesis.

Density effect on progeny. Matured adults of

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1(5), 5(4), 10(3), 30(3), 65(2), 100(2), 200(2), and 300(2) pairs were introduced into each container with a tuberous root of sweet potato of 150 g. The adults were allowed to mate and oviposit for 3 days, then a tuberous root that had been kept in another container under the same condition was provided until the progeny adults emerged. The number of progeny that emerged was counted, and their body weight was measured after they had been dried for 5 h at 70°C by electric hot-air drier. The number of individuals measured was 9♂, 8♀ at 1 pair; 14♂, 15♀ at 5 pairs; and 20♂, 20♀ at the other density level, respectively.

Developmental process. Matured adults of 1(10), 10(5), and 50(1) pairs were allowed to oviposit on the tuberous root of 150 g for 3 days. On the 13th, 17th, 24th, and 31st days after oviposition, we examined the mortality and the developmental stage of the progeny in the tuberous roots of each replication by dissection.

Conditioning of the tuberous root by infestation of adults. To determine the difference in the number of eggs laid between non-infested tuberous root and tuberous root infested by adults, an oviposition experiment was performed. Given that most eggs are laid in the epidermis of the tuberous root (Sweet Potato Weevil Research Group, 1992), a tuberous root with a definitive epidermis area (3.5×8.0 cm) was prepared for oviposition. Matured adults of 3(10) or 15(10) pairs, or 6(10) or 30(10) adult males were introduced in a container with the prepared tuberous root, and allowed to feed or oviposit for 3 days. Then the oviposition punctures were marked with a felt pen to distinguish the punctures made in the later oviposition from those made in this oviposition. Matured adults of ten pairs were introduced into each container with this tuberous root and with a fresh tuberous root used for control, and allowed to oviposit for 3 days. The number of eggs laid on each tuberous root was counted.

RESULTS

Relationship between parental density and the number of progeny

Figure 1 shows the relationship between the density (pairs) of parental adults and the number of progeny emerged from the tuberous root. The number of progeny increased with the increase of

Fig. 1. Relationship between parental density of *Cylas formicarius* per 150 g tuberous root and number of progeny emerged. Each bar indicates $\pm$ SD.

Fig. 2. Relationship between parental density of *Cylas formicarius* per 150 g tuberous root and mean individual dry weight (reciprocal) of progeny emerged. Each bar indicates $\pm$ SD.

parental density up to 100 pairs per tuberous root, leveling off at about 200 male and 200 female progeny. At low parental density, variation of the number of progeny among replications was large, and several containers with 1 pair of adults did not yield any progeny.

The number of progeny per female reached a peak of 4.0–4.4 progeny per parental female at 30–100 pairs.

Body size of progeny

The dry weight of progeny is shown in Fig. 2. At 1–10 pairs, it was 3.0 mg, but as the density increased further, it decreased to 1.2 mg for both males and females. There was a significant negative correlation between parental density and mean weight ($1/x$, x : mean weight) (δ : $r=0.957$, $n=8$, $p=0.0002$; δ : $r=0.942$, $n=8$, $p=0.001$).

Daily number of adults emerged from tuberous root at each density

The duration of development from egg to adult emergence from the tuberous root was shortened with the increase of parental density (Fig. 3). The median duration was 33–34 days at the highest

Fig. 3. Adult emergence pattern of progeny of *Cylas formicarius* at different parental densities.

density; 39–42 days at the lower density; and a significant negative relationship was found between parental density and the median duration of development from egg to adult emergence (δ : $\tau = -0.741$, $n=8$, $p=0.012$; φ : $\tau = -0.786$, $n=8$, $p=0.007$, τ : Kendall's rank correlation coefficient). The value of 75 percentile–25 percentile tended to be larger at low parental density than at higher density (δ : $\tau = -0.694$, $p=0.021$; φ : $\tau = -0.619$, $p=0.044$). At high density, most progeny emerged within 3–4 days, including the peak. Female adults emerged earlier than male adults at the parental densities of 30, 65, 200, and 300 (Kolmogorov-Smirnov test: $p < 0.05$).

Developmental process

Figure 4 shows the process of development of this weevil on and inside the tuberous root at various parental densities. With one pair, the percentage of unhatched eggs remaining in the oviposition punctures was higher than that at high density ($\chi^2 = 58.201$, d.f.: 2, $p < 0.001$). On the 13th–17th day, the percentage of live larvae in the tuberous root was lower at lower densities ($\chi^2 = 32.661$ – 120.117 , d.f.: 2, $p < 0.001$). On the 24th day, the percentage of dead larvae was higher at lower densities ($\chi^2 = 46.138$, d.f.: 2, $p < 0.001$), and many progeny pupated at 50 pairs ($\chi^2 = 64.722$, d.f.: 2, $p < 0.001$). On the 31st day, most larvae pupated, and at the highest density many progeny emerged to adults ($\chi^2 = 34.892$, d.f.: 2, $p < 0.001$). Thus, at lower density many progeny died as eggs or as young larvae in the oviposition punctures, and the development of the surviving progeny was delayed

Fig. 4. Relationship between parental density of *Cylas formicarius* and developmental process on and inside the tuberous root.

Table 1. Number of eggs laid on tuber conditioned by adults of *Cylas formicarius*

No. of adults used for conditioning		No. of repli-cations	No. of eggs (mean±SD)		Significance (Wilcoxon signed rank test) <i>p</i>
Male	Female		Conditioned	Control (No adult)	
3	3	10	26.2±9.9	24.3±8.5	0.542
15	15	10	39.7±8.5	5.8±4.3	0.005
6	0	10	18.0±12.7	23.6±15.3	0.516
30	0	10	26.2±7.8	16.0±10.3	0.075

as compared with that at the higher density treatment.

Conditioning

Significantly more eggs were laid on the tuberous root infested by 15 pairs of adults than on the healthy tuberous root (Wilcoxon signed rank test, $T=0$, $p=0.005$) (Table 1), however, this difference was not found in the other cases.

DISCUSSION

Under the conditions of limited space and food resources of the tuberous root of the sweet potato, the progeny population size of *C. formicarius* increased with increases of parental density, but the increase ceased at more than 100 pairs of parental population, showing a saturation growth curve (Fig. 1). However, the number of progeny may be expected to decrease at a higher density level than that used in the present experiment. Kamikado et al. (1993) reported that the number of progeny decreased when the oviposition duration was prolonged. Because the female adult continues ovipositing throughout her life span (Mullen, 1981; Sugimoto et al., 1996), the number of eggs laid increases, leading to a decrease of the number of progeny (Kamikado et al., 1993). In this case, a condition of competition arose in the tuberous root and the size of the progeny decreased (Fig. 2).

This species of insect oviposits on the surface of the tuberous root (Sweet Potato Weevil Research Group, 1992), which makes the area of the tuberous root surface a relevant factor (Kamikado et al., 1993). On the other hand, the larvae feed inside the tuberous root, which makes the volume of the tuberous root a relevant factor. The reproduction

and growth of this insect occur in different spaces; for example, the surface and the inside of the tuberous root, as in the case of *Callosobruchus chinensis* (Utida, 1941), which infests azuki bean. At low parental density, the mortality of the larvae of *C. formicarius* on the surface of the tuberous root was higher than that inside (Fig. 4). This indicates that the survival of the larvae may depend on successful invasion into the tuberous root. However, high density of the larvae burrowed into the tuberous root may have led to competition in that habitat.

The number of progeny per parental female showed a peak at a parental pair density of about 65. This effect of density characterizes this insect as belonging to the Allee type (Utida, 1975). At low adult density, the mortalities of eggs and larvae were higher in the oviposition punctures than those at higher density (Fig. 4). It is possible for very low density to decrease the chances of mating, and that, consequently, some unfertilized eggs are laid (Reinhard, 1923). Further, sweet potato is known to produce furano-terpenoids when infested by sweet potato weevil larvae or adults (Sato et al., 1981, 1982). Terpenoid-inducing factor may be excreted by the sweet potato weevils in the course of infestation, and may be a useful nutrient of the larvae while killing the host cells (Uritani et al., 1975). At high weevil density, the tuberous root may be tenderized by cell-killing, and such an environmental condition is suitable for development of the weevils, whereas it may be too difficult for larvae to feed at low density where the level of terpenoid-inducing factor may be lower. The infested tuberous root of sweet potato stinks, and is made bitter by the production of ipomeamaron (Akazawa et al., 1960). The females may be attracted to the stink and oviposit more eggs on tuberous roots infested by many larvae or adults (Table 1). On the other hand, at high adult density, an intraspecific competition would occur in the tuberous roots. The space and food resources of the sweet potato weevil are restricted, because the life of this insect is conducted in a tuberous root from the egg stage to that of emergence of adults, as is also the case for *C. chinensis* (Utida, 1941), which feeds on the azuki bean.

The body size of the progeny was small at high adult density (Fig. 2). Small individuals of the weevil species have a low fecundity (Sweet Potato

Weevil Research Group, 1992). Therefore, such individuals are not appropriate for mass rearing to obtain large numbers of normal males for sterilization by gamma radiation (Dawes et al., 1987), and may also be inferior at behaviors such as flying and mating than are wild adults.

The duration of development was shortened with increasing parental density (Fig. 3). This shortening of development may be primarily related to decreased size and body weight of the progeny (Fig. 2).

At low parental density, the degree of dispersion of emergence time of progeny was larger than that at high parental density. In the mass rearing of insects for irradiation, it is essential to establish a uniform developmental process (Kamikado et al., 1993). Rearing at low adult density, therefore, is not desirable.

Our results indicate that the optimal parental density lies at 65 pairs of adults with a tuberous root of 150 g for a 3-day oviposition period.

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