Marking of the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae) with Calco Oil Red dye

II. Effects of the dye on fecundity, longevity and sexual maturity

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Abstract

The West Indian sweet potato weevil, *Euscepes postfasciatus*, was internally marked by incorporating Calco Oil Red N1700 dye into the larval diet, and the effects of the dye and its solvent on fecundity, longevity and sexual maturity were examined. There were three types of diet used in this study: standard diet, corn oil diet (standard diet+corn oil), and Calco oil diet (standard diet+corn oil+the dye). Neither dye nor corn oil affected either adult longevity or fecundity. The pre-oviposition periods of females reared on the Calco oil and the corn oil diets were significantly longer than those of females reared on standard diet, and the age at which males started mating showed a significant difference among weevils reared on the three types of diet. Thus, the solvent oil affected female sexual maturity, whilst the dye had a negative effect on male sexual maturity. None of these effects, however, is considered sufficient to prevent use of the dye for marking released weevils for the sterile insect technique (SIT).

Key words: Calco Oil Red dye, artificial diet, sexual maturity, *Euscepes postfasciatus*, SIT

INTRODUCTION

Since 1947, when the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire), was first recorded in Okinawa, its distribution has spread throughout the prefecture (Kohama, 1990). Because the weevil, which is one of the most serious pests of the sweet potato, *Ipomoea batatas* (L.) Lam. (Raman and Alleyne, 1991), has caused a large amount of economical damage here, an eradication project is in progress using the sterile insect technique (SIT).

In using SIT to eradicate the weevil, an easy mass-marking method that also allows easy recognition of marked insects by simple examination is required. Gast and Landin (1966) were the first to effectively mark the boll weevil, *Anthonomus grandis* Boheman, by incorporating Calco Oil Red N1700® dye (American Cyanamid Co.) into the larval diet. The dye was reported to have no deleterious effects on the boll weevil (Gast and Landin, 1966; Lloyd et al., 1968; Daum et al., 1969; Lindig et al.,

1980). Thus, we examined whether Calco Oil Red dye could also be used for marking *E. postfasciatus* (Shimoji et al., unpublished). The study revealed that the addition of the dye to the artificial larval diet effectively marked the weevil and had no deleterious effects on developmental period, the number of adults yielded or body size. However, Wilkinson et al. (1972) reported that the dye produced significant differences in pupal weight, longevity and egg production of three lepidopteran species. We, therefore, investigated the effects of the dye on fecundity, longevity and sexual maturity of the weevil.

MATERIALS AND METHODS

Preparation of diet. The artificial larval diet was prepared as described by Shimoji and Kohama (1996b). Fifty-five milligrams of the dye dissolved in ca. 2 ml of corn oil was added to ca. 124 g of the diet in 100 ml distilled water (Calco oil diet). To determine the effect of the dye itself, a similar quantity of corn oil was added to the same amount of diet (corn oil diet). Un-

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treated diet (standard diet) was used as a control for the corn oil diet. Thirty grams of the diet was poured into a glass petri dish (90 mm in diameter and 20 mm in depth) and the diet was allowed to solidify. The rearing petri dishes were stored in a refrigerator at ca. 4°C until use.

Rearing of insects. Adult weevils used for collecting eggs were from a stock culture maintained since 1993 in the Fruit-Fly Eradication Project Office, Okinawa. Eggs were collected using egg-collecting devices (Shimoji and Kohama, 1996a) and kept on moist filter paper in petri dishes at $25.0 \pm 0.1^\circ\text{C}$ for 7 days. One day before hatching, the eggs were treated with 70% ethanol and 5% formaldehyde solutions for surface sterilization, and then rinsed with sterile water. Fifty eggs were seeded on the surface of the diet in each petri dish with a tiny paint brush. The seeded petri dishes were incubated at $25.0 \pm 0.1^\circ\text{C}$ and RH 70–80% under a photoperiod of 14 : 10 (L : D) h.

When the first adult eclosion was observed through the bottom of the petri dishes, the diet medium was dissected. Pupae were collected and sexed using the method described by Sugiyama et al. (1996). The adults emerging from the separated pupae were collected daily, and supplied with small pieces of sweet potato root (ca. 10 g) as food. They were kept in a laboratory at $25 \pm 1^\circ\text{C}$ and RH 70–90% under 14 : 10 (L : D) h photoperiod, and all experiments were conducted in the same laboratory.

Fecundity and longevity. To examine whether fecundity and longevity of the weevil are affected by Calco Oil Red dye or its solvent, 4-day-old females reared on the three types of larval diet were used. The females were paired with mature males reared on sweet potato roots. Each pair was placed in a plastic container (101 mm in top diameter, 80 mm in bottom diameter and 44 mm in depth) and fed on a small section of sweet potato root (ca. 1 g). Every morning the pieces of sweet potato were replaced with fresh ones. The surface of the sweet potato collected was scanned using a stereomicroscope, and the number of eggs laid each day by each female was recorded.

Every morning the number of dead weevils was also checked. As weevils sometimes feign death (Tucker, 1937; Sherman and Tamashiro,

1954), we left the weevils which we had judged to be dead in the containers and confirmed their death the next day. Dead weevils were dissected to distinguish the sex. If a dead weevil was a male, another male was introduced into the container. Data of unmated or mishandled weevils were excluded.

Longevity was also determined for males reared on different larval diets. Four-day-old males were paired with mature females reared on sweet potato. They were examined in the same manner as the females.

Male sexual maturity. We examined whether differences in larval diet affected the age at which males started mating. Twenty males derived from each type of larval diet were used. A five-day-old male was paired with a mature unmated female reared on sweet potato roots, and the pair was kept in a plastic container with a piece of sweet potato (ca. 1 g) diet. Females used were marked on their elytra several days before pairing with a small dot using a harmless white paint (Opaque color No. 551, Magic®) to distinguish the sexes. Every morning the female was removed from each container and another unmated female was introduced. The females removed were dissected, and the presence of sperm in their spermathecae was examined under a microscope.

RESULTS AND DISCUSSION

Table 1 shows the effect of each of three types of larval diet on the longevity, fecundity and female sexual maturity of *E. postfasciatus* adults. Although Calco Oil Red dye was reported to affect the longevity and fecundity of three lepidopteran species (Wilkinson et al., 1972), no significant differences were found in male longevity, female longevity and fecundity among weevils reared on either Calco oil, corn oil, or standard diet. However, there was a significant difference between the pre-oviposition periods of females reared on the different diets (One-way analysis of variance, $F=6.773$, $p<0.01$). The pre-oviposition periods of weevils reared on the Calco oil and the corn oil diets were significantly longer than those of the weevils reared on standard diet (Scheffe's multiple comparisons, $p<0.05$). Thus, the corn oil solvent, rather than the dye itself, must have

Table 1. Longevity and fecundity of *Euscepes postfasciatus* reared on three types of larval diet

Diet ^a	Males ^b		Females ^b					N
	Longevity (days)	N	Longevity (days)	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	No. eggs/♀/day	
Calco oil	163.4±20.7 a	29	86.2±7.6 a	15.1±0.3 a	68.5±7.7 a	2.6±0.2 a	1.5±0.1 a	27
Corn oil	171.6±24.5 a	26	78.5±7.3 a	15.2±0.8 a	60.2±7.3 a	3.1±0.6 a	1.5±0.1 a	23
Standard	181.8±20.8 a	25	85.0±9.7 a	12.9±0.3 b	68.8±9.8 a	3.3±0.3 a	1.4±0.1 a	25

^a Calco oil: standard diet containing Calco Oil Red N1700 dye and corn oil as its solvent; Corn oil: standard diet containing the same quantity of corn oil; Standard: standard diet as described by Shimoji and Kohama (1996b).

^b Means±SE followed by the same letters in the same column are not significantly different at the 5% level by Scheffe's multiple comparisons.

retarded the female sexual maturity.

The effect of different larval diets on male sexual maturity is illustrated in Fig. 1. Males reared on the standard, corn oil and Calco oil diets took 10.8 ± 0.3 days (mean±SE, $n=20$), 12.5 ± 0.6 days ($n=19$) and 14.1 ± 0.4 days ($n=20$), respectively, to start mating after adult eclosion. There was a significant difference in reproductive age among males reared on the three types of diet (One-way ANOVA, $F=15.68$, $p<0.001$). Scheffe's multiple comparisons test showed significant differences for all three pairwise comparisons of means ($p<0.05$). Thus, unlike with female sexual maturity, not only the solvent but also the dye might be retarding male sexual maturity. The three day delay in sexual maturity of males reared on the Calco oil diet when compared to those reared on the standard diet, is disadvan-

tageous when males reared on the Calco oil diet are used for the SIT, because only sterilised mature insects are effective in reducing the fecundity of wild females.

In this study the concentration of corn oil solvent was about 1.6% of the total amount of the diet, although in similar studies, it was only about 0.5% (Burton and Snow, 1970; Hendricks and Graham, 1970; Lindig et al., 1980) or less (Wilkinson et al., 1972). Thus, the relatively high concentration of corn oil possibly affected the sexual maturation in both females and males of *E. postfasciatus*. Because in the tobacco budworm moth, *Heliothis virescens* (F.), the use of different oils as solvents resulted in different eclosion rates (Hendricks and Graham, 1970), screening for other oils is necessary to reduce the negative effect of the solvent on the sexual maturation of *E. postfasciatus*.

The only harmful effect by the dye and its solvent on *E. postfasciatus* was in the sexual maturity of both sexes. Weevils are marked easily by incorporating the dye in the larval diet, and the recognition of marked insects is quite simple: when weevils are crushed on filter paper, marked insects leave a pink stain on the paper. Furthermore, contamination of unmarked weevils by the dye from marked individuals hardly ever occurs, because the dye is concentrated in the fat bodies of the insects (Gast and Landin, 1966). We, therefore, conclude that Calco Oil Red dye is a useful tool for mass-marking of *E. postfasciatus* and for monitoring the ratio of marked (irradiated) to unmarked (wild) weevils in SIT, albeit the solvent oil

Fig. 1. Effect of the difference among three types of diet on the age that males of *Euscepes postfasciatus* start mating. Open squares show males reared on standard diet ($n=20$). Closed and open circles show males reared on corn oil diet ($n=19$) and Calco oil diet ($n=20$), respectively.

should be replaced by a less harmful one.

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