

Short Communication

Female Marking via Rubidium-Labeled Ejaculates in the West Indian Sweetpotato Weevil (Coleoptera: Curculionidae)

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Abstract

The West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire), is one of the pests of the sweet potato, *Ipomoea batatas*, in tropical and subtropical countries. Although an eradication program using the sterile insect technique (SIT) for this weevil is now underway in Japan, the lack of potent attractants such as sex pheromones for this weevil species prevents effective monitoring and hampers pest control operations. New methods to monitor the performance of sterile males using trapped wild females are needed. In this study, we tested whether the ejaculate of the labeled males that were fed an artificial diet labeled with the trace element, rubidium (Rb), is detectable in the fertilized females. We fed an artificial diet treated with Rb (1.00, 0.50, 0.25, and 0.00%) to adult male weevils for 14 d after emerging and quantified the rubidium content in inseminated females. We also examined the side effects of the artificial diet on mating performance and longevity. The advantages of monitoring the spatial level of sterility using data from females labeled with ejaculate of Rb-fed males in an SIT eradication program are discussed.

Key words: rubidium, sterile insect technique, insect ejaculation, diet marking, diet labeling

The sterile insect technique (SIT) is a species-specific and environmentally friendly pest control method (Knipling 1955) and has been used successfully for decades to control various pest species (Dyck et al. 2005). The mating frequency of sterile males elucidates changes in the target pest population dynamics directly, and the field data are monitored and analyzed regularly to comprehend the changes in the dynamics of the target population in area-wide pest management programs using SIT (Parker 2005, Vreysen 2005). However, it is difficult to confirm it directly because the effect of sterile male mating appears in the next generation.

Insect marking has been used in various ecological studies, including the monitoring of the SIT program (Hagler and Jackson 2001). A fluorescent powder dye is used to mark mass-reared insects in almost all SIT programs (Dowell et al. 2005, Parker 2005), and elytral color polymorphism is used to monitor in sweetpotato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae) control

(Shiromoto et al. 2011). However, these external markers are not appropriate for detecting the mating frequency of sterile males. Trace elements are used as useful internal marking tools in many insect species. Artificial diets containing trace elements are the most common internal, self-marking technique (Akey and Burns 1991, Hagler and Jackson 2001). Rubidium (Rb) in its chloride form (RbCl) is the most frequently used nontoxic, trace element marker for insects (Hagler and Jackson 2001). Successful trace element marking in small insects, including aphids, mosquitoes, and whiteflies (Solberg et al. 1999, Wilkins et al. 2007), implies that ejaculate labeled by dietary trace elements will be detected in unmarked females. However, high concentrations of trace elements adversely affect survival, and the natural levels of trace elements vary geographically (Van Steenwyk et al. 1992), so it is necessary to determine these variables before using elemental markers (Hagler and Jackson 2001). The objective of this study was to quantify Rb content in the ejaculate in females

inseminated by a dietary-Rb-labeled male. We also investigated the side effects of the labeled diet on male longevity and mating performance.

Materials and Methods

General Methods

We designed four Rb-dose artificial diets (1.00, 0.50, 0.25, and 0.00% Rb) and fed them to male weevils. After the feeding period, the labeled males were allowed to mate with females, and the Rb content of inseminated females was quantified. The Rb content of specimens, the possibility of the 'hitchhiking' of Rb on the body surface from the male to the female, and the side effects of feeding the Rb-labeled diet were determined. Except for the wild females, we used 14- to 18-d-old virgin, mass-reared male and female weevils that were fed the artificial diet after emergence. All the experiments were conducted in the laboratory from May to August 2014 at the Okinawa Prefectural Plant Protection Center (OPPPC) in Naha, Okinawa, Japan.

Insect Materials

The West Indian sweetpotato weevil, *Euscepes postfasciatus* (Fairmaire) (Coleoptera: Curculionidae), is one of the pests of the sweet potato, *Ipomoea batatas* (L.) Lam. (Solanales: Convolvulaceae), in tropical and subtropical regions globally, including the southern Islands of Japan (Yasuda and Kohama 1990, Raman and Alleyne 1991). Although the body size of this weevil is small (3–5 mm), the infected sweet potato roots cause serious health damage in humans and livestock because of the production of a toxic terpenoid (Uritani et al. 1975, Raman and Alleyne 1991). An eradication program using SIT for *E. postfasciatus* is now underway in Okinawa (Kuba et al. 2003) using a mass-reared strain maintained at the OPPPC. This strain based on 14 000 weevils originally collected at Yaese Town, Okinawa, Japan (26°700'N, 127°420' E), in December 2004 (see Kumano et al. 2008), and was predominantly used in this study.

Artificial Diet

An artificial diet with sweet potato powder agar is used in the mass-rearing of this weevil (Urasaki et al. 2009, Ohishi et al. 2018). We mixed 2.8, 1.4, and 0.7 g of RbCl in 200 g of the raw material of

the artificial diet to produce 1.00, 0.50, and 0.25% Rb-dose diets, respectively. The ingredients were heated to 90°C while mixing, dispensed into a plastic container (210 × 140 × 80 mm), and cooled to room temperature. The cooled artificial diets were wrapped and stored in an incubator at 5°C until use. An artificial diet without RbCl was used as a control.

Experimental Procedure

Feeding Treatment

Sweet potato roots were dissected approximately 7 wk after egg laying, and newly emerged weevils were extracted from pupal chambers. Following Kohama and Sugiyama (2000), the sex was determined under a stereomicroscope (SMZ645-1, Nikon, Japan). Fifty weevils in each sex were placed in separate 250-ml meshed plastic cup (φ 80 × 50 mm high) and reared on the artificial diet (~10 g) for 14 d. The artificial diet was changed every 2 d. Rb-dose classes were 1.00, 0.50, 0.25, and 0.00% for males and 0.00% for females (Table 1).

Mating Trial

Since males sometimes leave females without copulation after mounting in this weevil (e.g., Kumano et al. 2008), Rb hitchhiking from the labeled males to the mounted females may occur. Therefore, we obtained the mated and mounted female specimens in mating trial. In this trial, a randomly chosen virgin female was paired with one marked male fed one of the four Rb doses in a plastic Petri dish (φ 30 mm), and mating was allowed for 2 h (between 1800 and 2000) under dark conditions. Mating behavior was observed directly using a light emitting diode (LED) flashlight covered with red cellophane. The number of copulations obtained in the mating trial was used to analyze the side effects of feeding the diet containing Rb on mating performance of the males.

Sampling and Rubidium Quantification

The Rb content was quantified in four groups of females: 1) wild females, 2) virgin females not in contact with males, 3) virgin females mounted by the labeled males, and 4) females inseminated by the labeled males. The wild female specimens were obtained from the LED trap in June–July 2014 on Kume Island, Okinawa, Japan (N: 26°20'57", E: 126°44'00"), which is the target area of the eradication program. Virgin female specimens and inseminated females were chosen randomly after the feeding period and the mating trial, respectively. All the collected specimens were

Table 1. Generalized linear models of the effect of rubidium (Rb) dose on male mating performance and female Rb concentration

Treatments	Rb dose (%) of male diet	Mating performance			Rb concentration (μg/l)		
		Female status	<i>n</i>	No. of mated pairs	<i>n</i>	Mean ± SD	Range (minimum–maximum)
Wild females	—	—	—	—	17	2.23 ± 0.54a	1.42–3.56
Virgin females non in contact with males	—	—	—	—	16	2.40 ± 0.37a	1.53–3.06
Virgin mounted females	1.00	Mounted	—	—	8	2.18 ± 0.61ab	1.69–3.65
Inseminated females	0.00	Inseminated	30	12a	16	1.61 ± 0.19a	1.37–2.20
	0.25	Inseminated	65	24a	18	22.99 ± 9.62b	10.65–42.92
	0.50	Inseminated	45	28a	21	46.28 ± 37.97c	9.74–122.86
	1.00	Inseminated	60	30a	20	66.75 ± 22.97d	36.46–140.35

The analyses were conducted using generalized linear models. Different letters in the same column indicate a significant difference ($P < 0.05$).

individually placed in a 0.5-ml plastic tube and stored at -20°C until the Rb quantification. The frozen specimens washed under running water for 2 h individually and desiccated in an incubator at 60°C for one night. Each weevil was placed into a glass tube (φ 16.5 \times 45 mm height) with 100 μl of 30% hydrogen peroxide (H_2O_2) and 100 μl of 69% nitric acid (HNO_3) to mineralize. Samples were heated at 100°C for 2 h. After cooling, samples were diluted to 1 ml with deionized water, and the resulting digested material was filtered to obtain a clear solution. Rb was quantified using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7700x, USA) at the Center for Research Advancement and Collaboration in the University of Ryukyus.

Male Longevity

Male longevity was investigated for 16 d with three Rb doses (1.00, 0.50, and 0.00%) using 70 male weevils for each dose after artificial diet feeding. Weevils were maintained in 250-ml meshed plastic cups with a piece of artificial diet (~ 10 g). Mortality was recorded every 2 d, and the artificial diet was changed.

Statistical Analysis

The effects of Rb dose on male mating behavior and female Rb content were analyzed using generalized linear models. The effect of Rb dose on male longevity was analyzed with a Cox proportional hazards regression. The period between the initiation of the experiment and weevil death, and Rb dose were used as the dependent and independent variables, respectively. In the analysis of male mating performance, the copulation (or not) was the dependent variable (binomial error with a logit link function), and the Rb dose in the artificial diet was the independent variable. In the analysis of male Rb dose on the female Rb content, the latter was used as the dependent variable (Gaussian error with identity link function), and treatments were independent variables. Likelihood ratio tests and Tukey's pairwise comparisons were conducted to examine differences between treatments using the package 'multcomp' (Hothorn et al. 2008) in R. All tests were performed on R version 3.5.2 for Mac OS X (R Development Core Team 2020).

Results

General Results

Table 1 shows the results of this study. No difference in the Rb content was noted between wild females and mass-reared females not in contact with males ($\beta \pm \text{SE} = -0.177 \pm 0.162$; $t = -1.093$; $P = 0.282$; GLM). There was also no difference in the Rb content between being mounted by males and that of females not in contact with males ($\beta \pm \text{SE} = -0.217 \pm 0.202$, $t = -1.074$; $P = 0.294$, GLM). Therefore, the experimental preparation and the weevil strain maintained in the OPPPC were considered to meet the preconditions for this study.

Rubidium Content

The female Rb content differed among the seven treatments in the four female groups ($\text{df} = 6$; deviance = 70,682; $P < 0.0001$; likelihood ratio test; Table 1). The multiple comparisons indicated that the Rb content of females not in contact with males and wild females did not differ from that of females inseminated by males fed a 0.00% Rb-dose diet. There was also no significant difference between virgin mounted females and females inseminated by males fed a 0.25% Rb-dose diet. The relationship between the Rb dose in the diet and the Rb content of the inseminated females was positive ($\beta \pm \text{SE} = 0.622 \pm 0.057$; $r^2 = 0.90$; $t = 10.77$; $P < 0.0001$; GLM; Table 1).

Mating Performance

Of the 200 mating trials, 94 (or 47.0%) of females were inseminated by males. The proportion of the mated males was not different between the groups fed different Rb-dose diet ($\text{df} = 3$; deviance = 2.1364; $P = 0.5446$; likelihood ratio test).

Longevity

The survival rate at 16 d was 0.92, 0.97, and 0.97 for the 1.00, 0.50, and 0.00% Rb doses, respectively. There was no difference between doses (0.50 vs 0.00%: $\beta \pm \text{SE} = 0.418 \pm 0.912$, $Z = 0.458$, $\text{df} = 1$, $P = 0.890$; 1.00 vs 0.00%: 0.936 ± 0.836 , $Z = 1.119$, $\text{df} = 1$, $P = 0.500$; 0.50 vs 1.00%: 0.517 ± 0.730 , $Z = 0.709$, $\text{df} = 1$, $P = 0.757$; Cox regression analysis and Tukey's HSD).

Discussion

Ejaculates derived from the males fed the Rb diet were quantifiably associated with the Rb dose in the diet in *E. postfasciatus*. The 1.00% Rb diet had no adverse effects on male longevity and mating performance, and the potential Rb content of the mass-reared female weevils was similar to that of wild females. Although the Rb content of mounted females was low to that of females inseminated by 0.25% Rb-dose males, there was no statistical difference. This inconsistency may be due to the sample size of the mounted females, and we believe that Rb hitchhiking is unlikely. Although the effectiveness of the monitoring trap is not currently optimal in this weevil (Kumano 2014), Rb may be a tool to monitor the spatial level of sterility in an eradication program. To our knowledge, this is the first study to quantify the content of trace elements in females inseminated by labeled males via ejaculate transfer in insects.

The Rb content of females inseminated by 0.50% Rb-dose males was larger than that inseminated by 0.00% Rb-dose diet males, virgin mounted females, and wild females. This suggests that a 0.50% Rb-dose diet is sufficient to discriminate treated females from wild ones. However, it is known that the quantity of trace element content declines over time after feeding (Gary 1971). Although the pathways for potassium leading to the ejaculate have not been well studied in insects, it is highly possible that the Rb content in an ejaculate reduce over time. In future study of SIT, we need to investigate the effect of sterilization on Rb intake into the body through the artificial diet or the chronological changes in the residual concentration of Rb in ejaculate.

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