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Source: Florida Entomologist, 93(1) : 39-44

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.093.0105>

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EFFECT OF IRRADIATION ON DEATH-FEIGNING BEHAVIOR IN THE MALE SWEETPOTATO WEEVIL *CYLAS FORMICARIUS* (COLEOPTERA: BLENTIDAE)

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ABSTRACT

The sterile insect technique (SIT) is widely used to suppress or eradicate target insect pest populations. The effectiveness of SIT depends on the ability of the released sterile males to mate with and inseminate wild females. Irradiation is effective for sterilizing mass-reared insects, and the negative impacts of this procedure are not limited to reproductive cell damage. In this study, we evaluated the death-feigning that is considered as an antipredator behavior of male *Cylas formicarius* (Summers) (Coleoptera: Blentidae) irradiated with 200 Gy, which is the dose used in the SIT program in Okinawa Prefecture. Irradiated male insects were observed in the laboratory for 10 d after irradiation. On d 0, fewer irradiated males feigned death than nonirradiated males. The number of irradiated and nonirradiated males who feigned death and the duration of their death-feigning were about the same from 2 d after irradiation. On the basis of our results, we discuss the optimal release time of irradiated male *C. formicarius*.

Key Words: sterile insect technique, predatory avoidance, tonic immobility, thanatosis, gamma radiation

RESUMEN

La técnica del insecto estéril (TIE) es usado ampliamente para suprimir o erradicar poblaciones de plagas insectiles seleccionadas. La efectividad de TIE depende de la habilidad de los machos estériles liberados a aparearse con hembras salvajes inseminadas. La irradiación es efectiva para esterilizar insectos criados en masas, pero los impactos negativos de este procedimiento no son limitados al daño de la células reproductivas. En este estudio, evaluamos el comportamiento de muerte fingida que es considerado como un comportamiento antedepredador del macho de *Cylas formicarius* (Summers) (Coleoptera: Blentidae) irradiados con 200 Gy, la cual es la dosis usada en el programa de TIE en Okinawa Prefecture. Los machos irradiados fueron observados en el laboratorio 10 días después de la irradiación. En el día 0, hubieron menos machos irradiados que fingieron la muerte que los machos no irradiados. El número de machos irradiados y no irradiados que fingieron la muerte y la duración del período de fingir la muerte fueron aproximadamente el mismo a los 2 días después de la irradiación. En base a nuestros resultados, comentamos sobre el tiempo óptimo para la liberación de machos de *C. formicarius* irradiados.

The sterile insect technique (SIT) is widely used to suppress or eradicate target insect pest populations (Dyck et al. 2005). Because the effectiveness of SIT depends on the ability of the released sterile males to mate with and inseminate wild females (Knipling 1955, 1979), information on mating performance and the competitive ability of sterile males is important information for the success of eradication programs. These factors have been focused in previous research (Calkins & Parker 2005). Especially, irradiation for sterilization negatively impacts not only reproductive cells but also somatic cells (Sakurai et al. 1994, 2000a, b; Bakri et al. 2005; Calkins & Parker 2005; Lance & McInnis 2005).

In nature, the survival of insects depends on their ability to escape from predators (Edmunds

1974; Ruxton et al. 2004). If gamma radiation reduces the antipredator behaviors of released insects, the effectiveness of SIT will have declined considerably. For example, gamma radiation damage to the midgut leads to malnutrition, which is partly responsible for the short life span of irradiated insects including the sweetpotato weevil (Sakurai et al. 1994, 2000a, b; Bakri et al. 2005). Condition-dependent resource allocation toward behavioral traits is widespread in various animal species (Hunt et al. 2004, 2005). Theoretical and empirical studies have shown that individuals with low-condition (i.e., low residual reproductive value) invest more resources in reproduction than in survival (Williams 1966; Charlesworth & Leon 1976; Clutton-Brock 1984). If the hunger of the irradiated insects increases with

time, the insects will reduce investment in anti-predator traits (Miyatake 2001b). Consequently, few survive because of the increased risk of predation in the wild. Comparing of the antipredator traits of nonirradiated insects with those of irradiated insects will provide important information that can be used to improve SIT programs. However, the antipredator traits of irradiated insects have not been widely studied. In the present study, we focused on the death-feigning behavior (thanatosis, tonic immobility) exhibited by the sweetpotato weevil *Cylas formicarius*; this trait was used as a model of antipredator behavior. Recently, death-feigning was empirically determined to be an antipredator adaptation in the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Miyatake et al. 2004; Miyatake et al. 2009), and nymphs of the damselfly *Ischnura elegans* (Vander Linden) (Odonata: Coenagrionidae) (Gyssels & Stoks 2005). Honma et al. (2006) showed that the characteristic posture during death-feigning can prevent predation gape-limited predators in the pygmy grasshopper, *Criotettix japonicus* (De Haan) (Orthoptera: Tettigidae). Furthermore, this behavior is used by the nursery web spider, *Pisaura mirabilis* against cannibalistic mating partners (Bilde et al. 2006; Hansen et al. 2008).

Cylas formicarius, which exhibits death-feigning behavior (Reinhard 1923; Sherman & Tamashiro 1954; Miyatake 2001a, b), is a good model for examining the effect of mass-rearing on predator avoidance. Wasps, mites, and mice prey on the weevils (Reinhard 1923; Cockerham et al. 1954; Sherman & Tamashiro 1954). In Okinawa Island, ants, stinkbugs, geckos, and wandering spiders have been observed to prey on the weevils (K. Yasuda, personal communication; D. Haraguchi, unpublished data). These predators, except for mice, rely on visual stimuli when hunting. When adult *C. formicarius* are startled by a strong stimulus, they fall from the leaves of host plants (e.g., sweetpotato *Ipomoea batatas*, railroad vine *Ipomoea pes-caprae*, and blue morning glory *Ipomoea indica*) to the ground and feign death by becoming extremely rigid. Since it is difficult to locate a death-feigning weevil on the ground because of its dark body, death-feigning is considered a defensive strategy that has evolved in *C. formicarius* to distract its predators and enable escape (Miyatake 2001a). The present study aims to clarify the effects of irradiation on the intensity of death-feigning behavior of *C. formicarius*. The proportion of death-feigning weevils and the duration of their death-feigning (i.e., recovery time) are compared between nonirradiated and irradiated males. We predicted that the intensity of death-feigning is reduced in irradiated male weevils because starved male *C. formicarius* reduced the intensity of death-feigning (Miyatake 2001b).

Cylas formicarius as well as *Eusecepes postfasiciatus* are important pests of sweetpotato, *Ipomoea batatas* (L) Lam., which is cultivated in the South Pacific, Caribbean Basin, and some countries of Central and South America (Chalfant et al. 1990; Yasuda & Kohama 1990; Jansson & Raman 1991; Yasuda 1993). *Cylas formicarius* was first detected in Japan in 1903 (Nawa 1903), and since then, it has spread throughout the southwestern islands of Japan (Moriya 1997). Lower level pre- and postharvest infestations can reduce both the quality and marketable yield of sweetpotato, rendering the storage roots unfit for human and livestock consumption since weevil feeding leads to the production of toxic sesquiterpenes (Uritani et al. 1975). Introducing weevils and their host plants, including sweetpotato, from infested areas to uninfested areas is strictly prohibited by the Japanese Plant Protection law. In 1994, an eradication program targeting *C. formicarius* by means of SIT and male annihilation by trapping flies with the use of sex pheromones was initiated on Kume Island, Okinawa Prefecture (Kohama et al. 2003).

MATERIALS AND METHODS

Insects and Radiation

We used mass-reared virgin adult *C. formicarius* in this study. Adults collected from Yomitan village, Okinawa Island, in Oct 1997 were reared at $25 \pm 1^\circ\text{C}$ and a photoperiod of 14:10 (L:D) h (0400-1800 h) on sweetpotato roots for 75 generations. Rearing was performed at the Okinawa Prefectural Plant Protection Center (OPPPC), Naha, Okinawa, Japan. Approximately 50 d after inoculation with weevils, we dissected the sweetpotato roots and collected the newly emerged adults. These adults were maintained in the laboratory at $25 \pm 1^\circ\text{C}$ under a photoperiod of L14:D10. The males and females were kept in separate meshed plastic containers (15 × 21 × 8 cm) and fed a diet of sweetpotato root (≈ 200 g) throughout the experiment period. Experiments were conducted in the laboratory from Apr to May 2009. The newly emerged adults were considered to be 0 d old. Weevils ($n = 150$) aged 10-14 d were placed in a 200-ml cup and irradiated with 200 Gy in the irradiation facility of OPPPC, which is designed by Radiation Application Development Association, Takasaki, Gunma, Japan. This facility contained 2.315-2.325 PBq cobalt-60 source that produced an air-absorbed dose rate of 524.9-526.3 Gy/h at 120 cm distance in the experimental period. Environmental conditions during the irradiations were 26.0-27.0°C, normal atmosphere. The sterilization procedure followed was approximately the same as that used in the SIT program of *C. formicarius* in Okinawa Prefecture (Kohama et al. 2003). The day of irradiation was considered

as d 0. To control for the effect of filling a small container during irradiation, 150 weevils that were assigned to treatment of nonirradiation were placed in a 200-ml cup while the weevils that were assigned to treatment of irradiation were irradiated. The irradiated and nonirradiated weevils were kept in separate meshed plastic containers (15 × 21 × 8 cm) and fed a diet of sweet-potato root (≈200 g). We replicated the experiments twice by using weevils from 2 separate containers.

Observation of Death-feigning Behavior

We evaluated the changes in the duration of death-feigning of sterile male weevils over time. The irradiated and nonirradiated males were observed on days 0, 2, 4, 6, 8, and 10. The sample size was 32 each d, except for treatment of nonirradiation at 6 d ($n = 31$). Each weevil from 2 treatments was placed in a well of a 24-well multiplate (AS ONE Corporation, Osaka, Japan) 5 h before to avoid disturbance by other weevils (Miyatake 2001a). Death-feigning was induced by picking up a weevil with tweezers and then dropping it back into the well. The duration of death-feigning was recorded for 120 min by using a digital video camera (GR-DF590; Victor Company of Japan, Limited (JVC); Yokohama, Japan). The duration of death-feigning was defined as the time between the transfer of the weevils into the well and the detection of the first visible movement. If the weevil failed to respond to a stimulus and ran away, the same stimulus (picking up and dropping) was repeated once or even 3 times. If the weevil failed to respond after 3 tries, it was considered non-responsive (Miyatake 2001a, b). We observed that the duration of death-feigning in a few weevils was more than 120 min (11/383 = 2.9%), and the durations of the weevils were recorded as 7200 s. All the trials were performed at 25 × 1°C and 60-80% RH, and started at 1400 h. This time was chosen because it corresponded to late afternoon in the field (i.e., 4 h before lights were switched off), which is when weevils will become active.

Statistical Analyses

A generalized linear model (GLM) with binomial error structure and logit link function (Crawley 2005) was used to determine the relationship between irradiation and the number of male weevils exhibiting death-feigning with R 2.60 (R Development Core Team 2007). To compare the duration of death-feigning by the nonirradiated and irradiated weevils, a Cox proportional-hazards regression was used (Dalggaard 2002). Treatment type, body weight, day, replicate, and interaction between treatment type and day were the independent variables.

RESULTS

The GLM results showed that irradiation had a significantly negative effect ($\beta = -0.0047 \pm 0.0024$ (SE), $z = 1.95$, $P = 0.051$) and day had a positive effect ($\beta = 0.15 \pm 0.076$, $z = 1.99$, $P = 0.047$) on the proportion of weevils exhibiting death-feigning. The interaction between treatment type and day was not significant ($\beta = 0.00071 \pm 0.00054$, $z = 1.32$, $P = 0.19$). Figure 1 shows that the more nonirradiated weevils feigned death than irradiated males on the day of irradiation (d 0); however, this difference between the nonirradiated and irradiated males decreased over 2 d. When males showing no death-feigning behavior were removed, irradiation did not affect the duration of death-feigning (Fig. 2; $\beta = 8.78e^{-5} \pm 1.10e^{-3}$, $z = 0.079$, $P = 0.94$), day ($\beta = -8.22e^{-3} \pm 2.21e^{-2}$, $z = 0.37$, $P = 0.71$), replicate ($\beta = -2.30e^{-2} \pm 9.90e^{-2}$, $z = 0.23$, $P = 0.82$) or the interaction between treatment type and day ($\beta = -4.82e^{-5} \pm 1.82e^{-4}$, $z = 0.27$, $P = 0.79$). Body weight did not affect either the proportion of males exhibiting death-feigning or the duration of their death-feigning (proportion: $\beta = -0.27 \pm 0.21$, $z = 1.29$, $P = 0.20$, duration: $\beta = -7.82e^{-2} \pm 6.16e^{-2}$, $z = 1.27$, $P = 0.21$).

DISCUSSION

Fewer irradiated males feigned death than nonirradiated males on d 0. This result suggests that *C. formicarius* is more active just after irradiation because its activity is negatively correlated with the intensity of death-feigning exhibited by other insect species (King & Leach 2006; Ohno & Miyatake 2007; Miyatake et al. 2008).

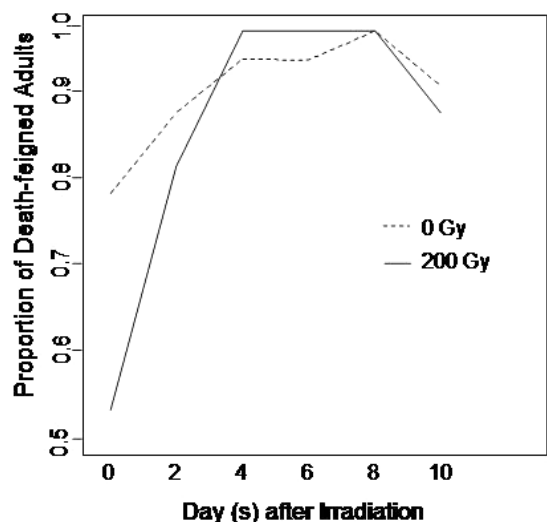


Fig. 1. Effect of irradiation on the number of male weevils exhibiting death-feigning. Dashed lines, nonirradiated (control) males; solid lines, irradiated males.

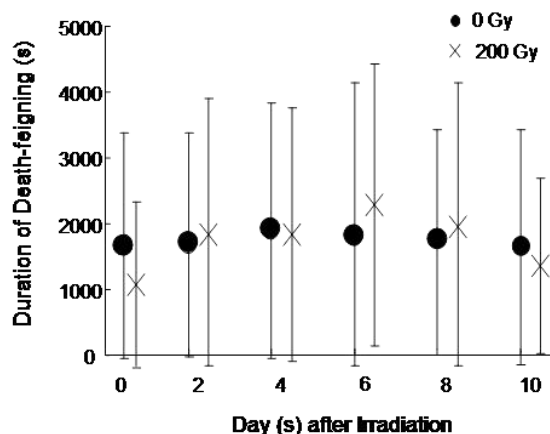


Fig. 2. Effect of irradiation on the duration of death-feigning of male weevils. Circles, nonirradiated (control) males; crosses, irradiated males. The vertical bars represent the mean \pm SD values.

Our results are consistent with those of Kumano et al. (2007): irradiated male *C. formicarius* have high dispersal ability immediately after irradiation. When irradiated weevils are released into the wild, they face high risk of predation because the intensity of their death-feigning (antipredator behavior) is reduced. It seems suitable to release sterile males into the wild 2 d after irradiation because the number of irradiated and nonirradiated male exhibiting death-feigning and the duration of their death-feigning were about the same for 2 d after irradiation. However, Kumano et al. (2008) showed that the mating ability of irradiated males did not differ from that of nonirradiated males for about a week after irradiation but drastically decreased thereafter. Therefore, irradiated males are useful for pest eradication or control for a short time after irradiation (7 d), and delaying their release is wasteful.

Alternatively, sterile weevils can be released into the wild in the evening because lower adult *Cylas formicarius* feign death during the night than daytime (Miyatake 2001a). Two reasons may explain why weevils tend to not feign death at night (Miyatake 2001a). First is the effect of predation pressure. Wasps, mites, and mice prey on weevils (Reinhard 1923; Cockerham et al. 1954; Sherman and Tamashiro 1954). Predatory stinkbugs, wandering spiders, and ants also prey on weevils on Okinawa Island (D. Haraguchi, unpublished data and K. Yasuda, personal communication). Most predators are daytime hunters, and thus, death-feigning by weevils during the night may not be useful. The second reason is the mating behavior of *C. formicarius*. Weevils copulate in the evening and through the night (Cockerham et al. 1954; Sakuratani et al. 1994); they search for mates at night. Weevils tend to not feign death at night because such behavior would reduce their

mating fitness (Miyatake 2001a). Furthermore, flight and walking activity also increase at night (Sakuratani et al. 1994), so weevils mainly disperse at night. In conclusion, it would be appropriate to release irradiated weevils in the evening based on weevil's predator avoidance, mating, and dispersal behaviors. Future studies that comparison of the fitness of the weevils between when weevils are released at daytime and at night should be needed.

Gamma radiation damages the epithelial tissue of the midgut, affecting alimentation (Sakurai et al. 1994). Malnutrition caused by the damage to the midgut leads to starvation, which accounts, in part, for the shortened lifespan of the irradiated insects. Miyatake (2001b) showed that fewer starved male *C. formicarius* feigned death and that the duration of their death-feigning is shorter. Although fewer males feigned death on d 3 of starvation (Miyatake 2001b), our results did not show definite reductions in the proportion of male feigning death and the duration of their death-feigning over 10 d. The survival rate of irradiated weevils did not differ from that of nonirradiated weevils for 7 d after irradiation (Kumano et al. 2008). These results suggested that irradiation does not result in the complete starving of *C. formicarius*, and then it is not supported our prediction that irradiation reduces the intensity of death-feigning.

Our study (and perhaps, other studies that explored the adaptive significance of death-feigning) assumed that the duration of death-feigning affects predator avoidance. To our knowledge, however, the relationship between the 2 variables has not been examined rigorously. When attacked by a predator, an insect usually falls from the branch and is out of the predator's line of vision (Miyatake 2001a). The dropped insect frequently feigns death (Ruxton et al. 2004; Ruxton 2006; Ohno & Miyatake et al. 2007). The dark body of the insect blends with the ground, causing the predator to lose sight of it (Miyatake 2001a). The predator may lose interest in the insect and give up searching for it. Therefore, we hypothesize that the duration of death-feigning is matched with the giving-up time of natural enemies. To examine the hypothesis, first, we must examine that the death-feigning behavior actually increase survival rate in the field condition. Second, major predators of *C. formicarius* in the field and their giving-up times should be examined. Such studies will improve the understanding of the adaptive significance of death-feigning behavior.

ACKNOWLEDGMENTS

We thank Y. Nakamoto and Y. Sokei (OPPPC) for irradiating of *C. formicarius*. We also thank the staff of OPPPC and Ryukyu Sankei Co. Ltd. for assistance. Dr. Yasuda provided us with information on the natural en-

emies of *C. formicarius*. We borrowed a video camera from Plant Disease and Insect Pest Management Section at Okinawa Prefectural Agricultural Research Center. We thank Dr. H. Hirayama, N. Shimada, Dr. G. Sakurai, Dr. T. Uehara and T. Yamamoto for help with collecting references. The manuscript was greatly improved by the valuable comments of 3 anonymous reviewers.

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