Are Sweetpotato Weevils (Coleoptera: Brentidae) Differentially Attracted to Certain Colors?

NIRUPA GADI[1] AND G.V.P. REDDY[2,3]

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ABSTRACT The sweetpotato weevil *Cylas formicarius* (F.) (Coleoptera: Brentidae) is one of the most serious insects causing damage to sweetpotatoes in the world. Small populations of these insects can cause severe damage to tubers of the crop. Without proper and effective control, weevil populations are likely to cause a huge or complete loss of sweetpotato production in sweetpotato growing areas. Consequently, there is an urgent need for development of an eco-friendly control method for this weevil. Although some control methods are effective, toxic pesticides applications are detrimental and damaging to our environment. Although pheromone traps are currently being used, no effective control of this weevil was achieved. Therefore, studies were carried out as part of the development of a trapping system for *C. formicarius*. Previous color-choice field tests have confirmed that *C. formicarius* preferred red traps over gray, green, brown, blue, white, yellow, or black; light red was more attractive than other shades of red. The current study aimed to know whether *C. formicarius* behavior changes in indoor conditions. Among the eight colored traps tested in the laboratory, green-colored traps were more effective in attracting *C. formicarius* than all other colors. It attracted nearly double the number of weevils than standard traps presently being used by growers. Based on these results, it is recommended to use green-colored traps to mass trap weevils (by attracting and killing) from sweetpotatoes grown under indoor (storage) conditions.

KEY WORDS sex pheromone, *Cylas formicarius*, color, trap

The sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), is the most serious pest on sweetpotato worldwide (Austin et al. 1991). This weevil causes damage in the field, in storage, and is of quarantine significance. The cryptic feeding habits of the larvae and the nocturnal activity of the adults make detection and management of infestations difficult, although varieties of sweetpotato having significant levels of resistance to *C. formicarius* have not yet been exploited (Downham et al. 2001, Korada et al. 2010, Muyinza et al. 2012). Losses are estimated to range from 5 to 80% (Sutherland 1986). Although *C. formicarius* can complete its life cycle in either the tubers or vines of the sweetpotato plant, it prefers tubers (Strong 1983).

Although various control measures have been practiced to diminish the damage caused by *C. formicarius*, broad spectrum insecticide applications remain the main control method (Sutherland 1986). However, the belowground feeding habits of the larvae and nocturnal activity of the adults make it necessary for growers to apply insecticide several times during the crop growing season (Hwang 2000). This chemical control is not only expensive but also toxic to beneficial insects and causes environmental pollution (Reddy et al. 2012a,b). Leng and Reddy (2012) reported that neem and spinosad are promising candidates as ecofriendly chemicals that could potentially replace broad-spectrum synthetic neurotoxins for controlling *C. formicarius.* However, these chemicals are not practically used for controlling *C. formicarius* in the field. Although biological control is viable, as wasps such as *Bracon mellitor* Say, *Bracon punctatus* (Muesebeck), *Metapelma spectabile* Westwood (all Hymenoptera: Braconidae) and *Euderus purpureas* Yoshimoto (Hymenoptera: Eulophidae) have been reared successfully in the Southern United States (Chalfant et al. 1990), there have been no studies of the parasitoid effectiveness on *C. formicarius.* Moreover, these species seem to be sporadic.

A sex pheromone, (Z)-3-dodecen-1-o1 (E)-2-butenoate has been identified from female *C. formicarius* and reported as highly attractive to male weevils (Heath et al. 1986). Traps baited with the synthetic sex pheromone showed potential to be an effective tool for monitoring *C. formicarius* (Proshold et al. 1986). A successful formulation for this sex pheromone lure and inexpensive traps designed for mass trapping of male *C. formicarius*, which is effective in the field, has been developed (Hwang 2000). As a result, attention in recent years has focused on the use of sex pheromones as a potential component in their management. Exploration into the use of the sex pheromones for the

[1] Western Pacific Tropical Research Center, College of Natural and Applied Sciences, University of Guam, Mangilao, GU 96923.
[2] Western Triangle Agricultural Research Center, Montana State University, 9546 Old Shelby Rd., Conrad, MT 59425.
[3] Corresponding author, e-mail: reddy@montana.edu.

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Fig. 1. A trap used in the current study. (Online figure in color.)

management of *C. formicarius*, including trap characteristics and practical application techniques, are progressing and has great potential for improving current *C. formicarius* management programs. Previous color-choice field tests (Reddy et al. 2012a) have confirmed that *C. formicarius* preferred red traps over gray, green, brown, blue, white, yellow, black, or red ones; light red was more attractive than other shades of red. Because *C. formicarius* is known to attack sweetpotatoes in the field and storage, the current study aimed to know whether *C. formicarius* changes its response to colors under indoor to outdoor conditions. Therefore, we undertook studies in the laboratory to evaluate the response of *C. formicarius* to different trap colors baited with and without pheromone lures.

Materials and Methods

Origin and Rearing of Weevils. Pherocon unitraps baited with lures were used to trap adult *C. formicarius* in sweetpotato fields in Latte Heights and Yigo (Guam, USA). The trapped adults were taken to the laboratory, placed in batches in collapsible cages (12 by 10 by 10 cm), fed leaves and pieces of the sweetpotato, and maintained at 22 ± 2°C, 70–80% relative humidity (RH), and a photoperiod of 16:8 (L:D) h. Approximately 5–6 generations were completed before use for experiments. For all experiments, adults were obtained from the mass-reared in a laboratory. The adults selected for the experiments were 3–4 wk old.

Sex Differentiation of Weevils. The female *C. formicarius* differ from the males in antenna and body size (Starr and Wilson 1997). The antennae of the males are straight while those of the female are round or club-shaped. The terminal club of the female's antenna is ovoid, while that of the male is cylindrical. Typically, the females are larger than the males.

Trap Type. The Pherocon unitraps (also called bucket traps), 20.5 cm high by 13 cm in diameter were also obtained from Trécé Incorporated, Adair, OK. Each unitrap consisted of a funnel-shaped white plastic receptacle with a yellow plastic lid and holder for attached lures mounted over a bucket for retaining captured insects (Fig. 1). The traps were constructed using a white bucket, a yellow cone on the top of the bucket, and a dark green cover above the cone.

Pheromone Lures. Pheromone lures in rubber septa loaded with Z3-dodecenyl-*E*2-butenoate, sealed in an impermeable bag for shipping and storage, were obtained from Chem Tica Internacional SA (San José, Costa Rica). The lure packs, each containing 10 mg of pheromone and emitting the active ingredient at <0.01 mg/d (Material Safety Data Sheet, ChemTica Internacional, SA), were stored at 4°C until use. Lures were suspended by a wire inside the bucket of the trap.

Effect of Trap Color. The Pherocon unitraps were entirely covered with brown, black, gray, yellow, red, white, green, or blue vinyl tape and tested independently. Color characteristics of the tape were determined with a Konica Minolta CR-410 Chromometer (Minolta Instrument Systems, Ramsey, NJ) and are given in Table 1.

Experiments With Different Color Traps. To know the effect of visual and olfactory cues on the trap catches, two indoor experiments on trap color were conducted in a laboratory 10 m long by 6 m wide by 3.5 m high. One set of the experiment was carried out with pheromone lures and another set up was without pheromone lures. Because *C. formicarius* is known to

Table 1. Specifications of the colors of traps used

Trap color[a]	L*	a*	b*	Chroma (C)	Hue angle (h°)
Black	30.44 ± 0.06	0.42 ± 0.03	−1.08 ± 0.04	1.16 ± 0.05	–
Brown	35.26 ± 0.18	3.98 ± 0.03	3.94 ± 0.02	5.60 ± 0.03	44.66 ± 0.11
Gray	39.83 ± 0.11	−0.17 ± 0.02	−2.23 ± 0.01	2.24 ± 0.01	85.64 ± 0.47
Yellow	82.57 ± 0.02	−2.92 ± 0.03	84.02 ± 0.27	84.07 ± 0.27	91.99 ± 0.02
Red	42.84 ± 0.11	49.88 ± 0.28	19.44 ± 0.20	53.54 ± 0.34	21.29 ± 0.09
White	92.29 ± 0.03	1.34 ± 0.01	−2.59 ± 0.04	2.91 ± 0.03	–
Green	43.50 ± 0.08	−27.32 ± 0.03	1.72 ± 0.09	27.37 ± 0.03	176.39 ± 0.19
Blue	36.02 ± 0.10	15.19 ± 0.10	−35.82 ± 0.12	38.91 ± 0.14	292.98 ± 0.08

[a] Means (±SD) were generated from three observations.

L* indicates a measure of "lightness" that runs through the center of the color chart; 100 at the top represents white and zero at the bottom represents black.

The a* axis, which runs left to right on the color chart, indicates a red shade when greater than zero (positive) and a green shade when lower than zero (negative). Similarly, the b* axis, which runs vertically through the color chart, indicates a yellow shade when positive and a blue shade when negative (Wrolstad et al. 2005).

The hue angle is expressed on a 360° grid on which 0° = red, 90° = yellow, 180° = green, and 270° = blue.

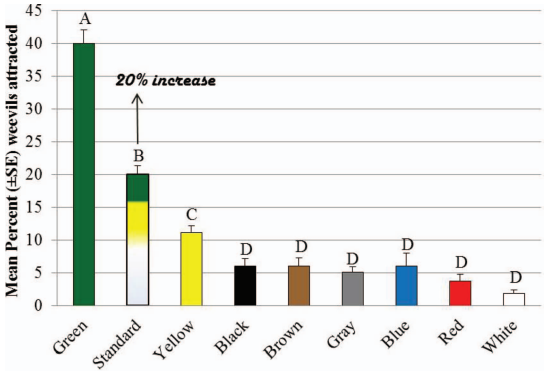


Fig. 2. Mean percent (±SE) of sweetpotato weevils attracted to unbaited-pheromone traps of different colors in the laboratory. Different capital letters indicate significant differences between treatments (one-way ANOVA with Poisson model, least square means, $P < 0.01$). Green traps outperformed all others. Bars represent means of 40 replicates. (Online figure in color.)

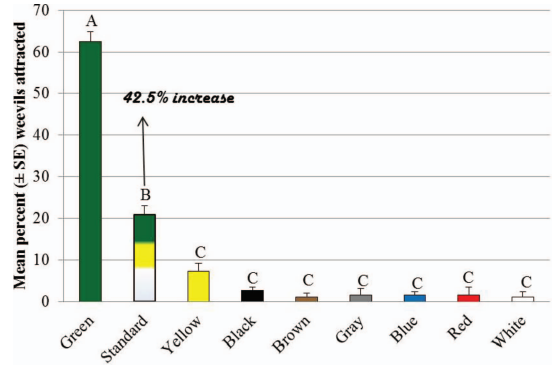


Fig. 3. Mean percent (±SE) of sweetpotato weevils attracted to pheromone-baited traps of different colors in the laboratory. Different capital letters indicate significant differences between treatments (one-way ANOVA with Poisson model, least square means, $P < 0.01$). Green traps with pheromone outperformed all others. Bars represent means of 40 replicates. (Online figure in color.)

be nocturnal (Chalfant et al. 1990), the field-collected adults were fed and reared under a reverse photoperiod for 3–4 wk before starting the experiment in the laboratory. This was to enable experiments during the daytime. The tests were run in a dimly lit (150 *lux*) laboratory between 1200 and 1730 hours with 40 by 25-cm ground traps of different colors. The traps to be tested were placed on the floor of the laboratory 2 h before the release of the adults, so that pheromone vapors could spread throughout the laboratory. The traps were placed on the floor of the laboratory. Forty adults were then released in the center of the trap circle. The inter-trap distance was 30 cm, while the distance between adults release point and traps was 1 m. The number of adults attracted by the different colored traps during the following 5 min was recorded. Each treatment was replicated 40 times. Trapped beetles were removed after capture and discarded. Uncaptured insects were removed before the next trial, and we used fresh adults for each replicate to avoid pseudoreplication.

Statistical Analysis. The data were analyzed with the generalized linear mixed model procedure of SAS Version 9.13 (SAS Institute 2009). For the reason that all response variables used were count variables, a one-way Poisson ANOVA model was fitted, by means of the GLIMMIX Procedure SAS Version 9.3 (SAS Institute 2009). The least square means test was used to make multiple comparisons for significant differences between treatments.

Results

Green traps without pheromone lures caught significantly more adult *C. formicarius* than those of any other color tested ($F = 8.13$, df = 7, 38, $P < 0.05$; Fig. 2), followed, in descending order, by standard traps and yellow traps, which are significantly different from with each other. No significant differences were observed among the responses to black, brown, gray, blue, red, and white. The green-colored traps were the most attractive to *C. formicarius* by drawing twice as many as standard traps that were currently being used.

In the second experiment trap with pheromone lure, green traps caught significantly ($F = 13.06$, df = 7, 38, $P < 0.05$; Fig. 3) more than the standard traps and all other colored traps. Interestingly, the green-colored traps with pheromone lures attracted three times more weevils than the standard traps.

Discussion

A number of reports on the attractiveness and performance of the pheromone to *C. formicarius* in the field have been described (Talekar and Lee 1989, Jansson et al. 1992, Reddy et al. 2012a and references therein). Pheromone-based trapping methods can provide a valuable and environmentally friendly control method, particularly for borer pests because control of cryptic larvae is difficult. Our previous studies indicated that *C. formicarius* responded to red-colored pheromone traps in the field; light red being most attractive (Reddy et al. 2012a). In our current study, *C. formicarius* responded to green-colored traps in indoor conditions. Moreover, it is interesting to know from the current study that catches double without the pheromone and tripled with the pheromone. Therefore, it would seem that there is some sort of interaction (i.e., the color green helps significantly more when coupled with pheromones than when not). This is clearly an interaction between visual (green-colored traps) and olfactory cues (pheromone lures; Reddy and Guerrero 2004). The sex pheromone compounds were isolated, extracted, and identified from females, which attract only males. However, females use visual or chemical cues for feeding and oviposition in plants (Reddy and Raman 2011). In this case, *C. formicarius* were using visual and olfactory cues and attracted to green-colored traps. This is the reason why green-colored traps coupled with phero-

mone lures are attracting a significantly higher number of weevils than the green-colored traps with or without a pheromone.

Numerous studies are conducted in the Okinawa Islands, Japan, on the attraction of the West Indian sweet potato weevil, *Euscepes postfasciates*, to visual and color cues (Nakamoto and Kuba 2004 and references therein). Initial studies by Nakamoto and Takushi (2002) were confirmed using root traps (pitfall trap) with blue, green, yellow, and red LED's; the root trap with the blue LED captured a higher number of *E. postfasciates* (Nakamoto and Takushi 2002) than the other colors. However, additional studies by Nakamoto and Kuba (2004) reported that the green LED trap was much more effective than the root trap (pitfall trap) for monitoring *E. postfasciates* population in the field. The authors concluded that compared with the root trap, 4- to 326-fold weevils were caught in the green LED trap during the experimental periods. Thus, the green LED trap appears much more effective than the root trap for monitoring the weevil population in the field. Our current studies are performed with different species of *C. formicarius*, and we observed somewhat different results under indoor conditions. For example, our previous color-choice field tests (Reddy et al. 2012a) have established that *C. formicarius* preferred red traps over all other colors tested. The current study clearly indicated that *C. formicarius* behavior changes in indoor conditions as the weevils preferred green-colored traps compared with all other colors tested.

In the setting where this could be applied among plants, it could be debatable that the green trap is attractive to the weevils distinct from the green vegetation, which also might have some color attraction for the *C. formicarius.* It is also believed that although green-colored traps will be somewhat attractive to the *C. formicarius* while being distinct from the green vegetation, which also might have some color attraction for the weevils. However, green-colored traps coupled with pheromone lures will have higher attraction than green traps. Nevertheless, the present results are still very useful to trap *C. formicarius* under indoor (storage) conditions where there will not be any plants.

Based on our current and previous (Reddy et al. 2012a) studies, it is recommended that green-colored pheromone traps could be used in covered spaces (i.e., warehouses, greenhouses, etc.), while light red pheromone baited traps can be used in field conditions for controlling *C. formicarius*

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