Efficacy of Pheromone Trapping of the Sweetpotato Weevil (Coleoptera: Brentidae): Based on Dose, Septum Age, Attractive Radius, and Mass Trapping

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ABSTRACT Pheromone dose, effective trapping distance, and longevity of the rubber septa loaded with sex pheromone of *Cylas formicarius* (F.) (Coleoptera: Brentidae) were evaluated for their impact on the efficacy of mass trapping of the insect in sweet potato fields in Guam in 2012–2013. The number of adults caught at different distances (10–100 m) was significantly different. Catches declined with increasing release distance from the trap in both downwind and upwind directions. While the maximum radius of attraction of pheromone-baited trap for *C. formicarius* in the field was 80 m, the effective distance for recapturing marked adults in the pheromone-baited Unitraps was 60 m. Pheromone lures were able to capture adults of *C. formicarius* after being stored in the laboratory for up to 98 d. The number of catches per trap per week was highest when lures were 0–14- and 15–28-d-old, and longer storage of septa led to a progressive reduction of catches. Pheromone traps baited with 100-μg lures captured significantly more adults compared with those loaded with 10-μg lures. In addition, effectiveness of pheromone trapping on damage to sweet potato was tested at two locations. Number of trapped adults, damage level at different times after trap installation, and yield production were evaluated. The number of *C. formicarius* adults collected in traps at both locations fluctuated dramatically among sampling dates and peaked on 13 September 2013, after which time the number of captures noticeably declined. This decrease was correlated to the increasing age and depletion of the pheromone lures. Pheromone traps significantly reduced feeding damage caused by weevils (<1 feeding hole per root in treatment; up to 38 feeding holes per root in the control) at both locations. Being consistent with damage levels, sweet potato yields in fields with traps were higher than those in untreated controls. We conclude that pheromone-baited traps are effective in reducing damage due to *C. formicarius*.

KEY WORDS pheromone dose, age of septa, maximum radius of attraction, mass trapping, *Cylas formicarius*

The sweetpotato weevil, *Cylas formicarius* (F.) (Coleoptera: Brentidae), is the most important pest of sweet potato, *Ipomoea batatas* (L.) Lamarck (Convolvulaceae), in the Pacific Islands, the southern United States, and throughout the tropics and subtropics (Talekar 1982, Reddy et al. 2012a). The pest species may produce several generations per year in the tropical climates, and does not undergo winter diapause but moves to alternative hosts of *Ipomoea* spp. (Sherman and Tamashiro 1954). The adults are active throughout the year and can fly distances of at least 1.6 km. The weevils are generally nocturnal, but may also fly in response to the pheromone source (Reddy et al. 2012b) and be found on *Ipomoea* plants during daytime (Sakuratani et al. 1994, Shimizu and Moriya 1996). According to Bourke (1985), the weevil caused economic damage in areas with a marked dry season or in unseasonably dry years. Also, damage by *C. formicarius* is a problem wherever the crop is grown and often worse during dry times. Both *C. formicarius* larvae and adults cause damage to the tuberous roots, leaves, and vines of *I. batatas* and other *Ipomoea* spp. (Chalfant et al. 1990). The weevil causes damage in the field and in storage, so the export and import of sweet potatoes are restricted in some places, thus making quarantine a significant issue (Hansen et al. 1992). Any import or export of sweet potatoes is required to go through quarantine regulations in some states, except the U.S. territories such as Mariana Islands. Even low levels of feeding by *C. formicarius* induce a chemical reaction that imparts a bitter taste and terpene odor to the tubers (Roberts 1952). In addition to damage caused directly by tunneling, larvae also cause indirect damage by facilitating entry of soil-borne pathogens (Uritani et al. 1975), which renders the product unsuitable for consumption. Losses can be severe both in storage facilities and in the field,

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where yield losses due to *C. formicarius* may range from 60 to 97% (Jansson et al. 1987, Ray and Ravi 2005, Reddy et al. 2012a).

The development of improved control methods is hampered because the immature life stages of *C. formicarius* generally go unnoticed. Eggs are laid in cavities just below the skin of the root (Sutherland 1986). Larvae are cryptic in nature and can complete their life cycles in either tubers or vines (Strong 1983). Traditionally, broad-spectrum insecticide applications are employed to manage *C. formicarius.* The below-ground feeding habits of the larvae and the nocturnal activities of the adults force growers to apply insecticides several times during the cropping season (Hwang 2000). Leng and Reddy (2012) reported that ecofriendly neem and spinosad may replace broad-spectrum synthetic neurotoxins for controlling *C. formicarius.* However, these chemicals are not readily available in the Pacific Islands. Limited work on using natural enemies has been performed (Chalfant et al. 1990), but has not yet been proven to provide effective control.

Synthetic pheromones are important components of integrated pest management programs for many insect pests (Reddy and Guerrero 2004, 2010). The pheromone for *C. formicarius* was isolated from female weevils and bioassayed in the laboratory (Coffelt et al. 1978). Heath et al. (1986) purified the active component, identified it as (Z)-3-dodecen-1-ol (*E*)-2-butenoate, and described a synthesis of the compound. The synthesis, formulation, and the field use of pheromones to manage *C. formicarius* have been reported by various workers (Jackson and Bohac 2006, Sureda et al. 2006). Pheromone traps have great potential for monitoring *C. formicarius* (Proshold et al. 1986). Hwang (2000) demonstrated a practical and inexpensive pheromone trapping method for *C. formicarius.* Furthermore, an integrated approach using pheromone-baited traps with soil insecticides in Taiwan has shown promise (Hwang and Hung 1991, 1994). These studies support the use of sex pheromones as a potential tool for monitoring *C. formicarius* (Reddy et al. 2012b).

However, traps used for monitoring alone do not provide effective control of *C. formicarius* under substantial crop damage levels (Talekar 1988, 1991). Considerable work has been done in improving the trapping efficacy (Jackson and Bohac 2006, Reddy et al. 2012b, Gadi and Reddy 2014). According to these studies, the effectiveness of trapping might be improved by combining the traps with other components. For example, our previous study suggested that pheromone-baited light red (chromameter values: L = 50.03; a = 47.6; b = 28.7; chroma = 55.6; and hue angle = 31.16) Unitraps (13 by 17.5 cm) installed 50 cm above the crop canopy effectively improved the *C. formicarius* adult catches and were recommended to use in mating, attract-and-kill, and eradication programs (Reddy et al. 2012b).

Also, in our previous study, *C. formicarius* was found to prefer light red to other tested colors, and light red Unitraps with pheromone lures caught significantly more adults than identical traps without pheromone lures, suggesting that behaviors of *C. formicarius* were influenced by both visual and olfactory cues (Reddy et al. 2012b). Gadi and Reddy (2014) reported that green pheromone-baited Unitraps significantly attracted more *C. formicarius* than other colors in indoor situations (Gadi and Reddy 2014), whereas light red Unitraps are outperformed in the field (Reddy et al. 2012b).

As a part of ongoing efforts to develop effective pheromone-based trapping techniques for *C. formicarius,* additional studies were undertaken to investigate the effects of pheromone dose, septum age, and maximum radius of attraction on the efficacy of pheromones in mass trapping of *C. formicarius* in the sweet potato fields.

Materials and Methods

Pherocon Unitraps baited with lures (Reddy et al. 2012b) were used to trap adult *C. formicarius* in sweet potato fields in Inarajan and Yigo (Guam). Trapped adults were taken to the laboratory, placed in small groups in collapsible cages (12 by 10 by 10 cm), and fed with sweet potato leaves and pieces of tubers. Fresh sweet potato plant materials were supplied weekly. The colony was maintained at 22 ± 2°C, 70–80% relative humidity, and a photoperiod of 16:8 (L:D) h. The insects were reared in the laboratory beginning in 2011. All adult insects used in experiments were 3–4-wk-old, and had been reared for multiple generations with sweet potato tubers in the laboratory colony established in 2011. Fresh tubers were provided at 2–3-wk intervals during the rearing period.

Pherocon Unitraps, frequently called bucket traps, (20.5 cm in height by 13 cm in diameter), were obtained from Trécé Incorporated (Adair, Oklahoma). Each Unitrap consisted of a funnel-shaped white plastic receptacle, with a yellow plastic lid and a holder in white color for attaching lures, mounted over a bucket to retain captured insects (Reddy et al. 2012b). Pheromone lures of 10 or 100 μg of Z3-Dodecenyl-*E*2-butenoate (purity of 90–95%) in red standard rubber septa were obtained from AgBio Inc., Westminster, Colorado. Lures were stored in a sealed impermeable bag at 4°C until use. Lures were placed in a holder inside the bucket of the trap.

Experiments were conducted in Guam at four locations: the University of Guam Agricultural Experiment Station in Yigo (13° 31′ N, 144° 52′ E, 139.5 m, a.s.l.), the Ija Experiment Station (13° 26′ N, 144° 48′ E, 74.98 m, a.s.l.), Dededo (13° 30′ N, 144° 51′ E, 96.9 m a.s.l.), and the Inarajan Experiment Station (13° 15.259′ N, 144° 43.300′ E, 35.052m, a.s.l.). In addition, morning glory, *Ipomoea triloba* (L.) (Convolvulaceae), which is a major alternative host for *C. formicarius* (Austin et al. 1991), was present on most study sites. Daily temperatures during the experiments ranged from 30.7 to 32.2°C.

Maximum Radius of Attraction of the Pheromone Septum. The effective range of capture, the optimum distance, and maximum radius of attraction of the pheromone septa were determined using Unitraps baited with a septum loaded with 10 μg of *C. formicarius* sex pheromone. This dose was chosen because of the manufacturer's decision and recommendation and also because further increase in dose would not affect the trapping distance. This experiment was carried out in sweet potato fields at the Yigo, Inarajan, and Ija experiment stations. Adult weevils used in these experiments were obtained from the laboratory culture. Using a camel's hair brush, weevils were marked on the thorax using different colors to indicate release direction and distance from the trap. Forty marked male adults were released in the late afternoon at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 m from a Unitrap baited with a lure that was positioned 100 cm above the crop. Four releases with different colors were made as replicates at the same time in both downwind and upwind directions from the trap. Wind direction at release was determined by observing the flag blowing pattern. Marked adults captured in the traps were counted in the morning following release.

Effect of Aging of Septa on Weevil Capture. Field trials were carried out at the same sites from October 2012 to January 2013 to compare the attractiveness of traps equipped with septa loaded with 10 μg of *C. formicarius* sex pheromone and exposed for varying periods. This experiment was replicated four times at each of the three locations. Freshly prepared septa previously exposed in the laboratory for 0, 14, 28, 42, 56, 70, 84, 98, and 112 d were evaluated for the same period in the field. Septa were changed at 14-d intervals in each treatment, such that throughout the trapping period of the test, there were traps containing septum ranging from 0 to 112-d-old. Traps were rotated every week to avoid possible location effects. The number of marked adults caught in each trap was recorded weekly for 18 wk.

Effect of Pheromone Dose Loaded in Septa on Weevil Capture. Unitraps were deployed at the same sites used previously and were baited with lures loaded with 0, 10, or 100 μg of the sex pheromone. These doses were suggested and recommended by the manufacturer. Traps were checked from February to May 2013. Traps were rotated every week so that each trap appeared at each place for a 1-wk period. Adults caught in each trap were recorded and removed on a weekly basis for 17 wk.

Effectiveness of Adult Weevil Trapping on Damage to Sweet Potato. Experiments to determine if pheromone-baited traps can reduce the damage caused by *C. formicarius* and increase the yield levels were conducted from June to September 2013. Twenty-five Unitraps equipped with 100 μg of *C. formicarius* sex pheromone were installed in each of the 1,012-m^2 sweet potato fields at Yigo and Inarajan. Sweet potato fields without pheromone traps at Dededo and Ija served as experimental controls. The distance between the trapping and control locations was ≈40 km, while the intertrap distance was 60 m. Traps were randomized each week at each location to avoid any location effect. All adult weevils captured in each trap were counted and removed from the trap on a weekly basis for 17 wk. Also, damage to roots (tubers) at each site was evaluated by randomly selecting eight roots from throughout the field and counting the number of feeding holes. The yield of sweet potato measured by tuber weight was recorded in each field site. Damage levels and yields from the treatment and control fields were compared to evaluate the effectiveness of pheromone traps in reducing damage from *C. formicarius.*

Statistical Analysis. Data from the pheromone dose, septum age, and maximum radius of attraction were separately analyzed using the generalized linear mixed model procedure with SAS version 9.13 (SAS Institute 2009, Cary, NC). The random factor was the variation among replicates in each treatment. Because all response variables used were counts, a one-way Poisson ANOVA model was fitted using the GLIMMIX Procedure SAS version 9.3 (SAS Institute 2009). A least square means test was used to make multiple comparisons of differences between treatments.

The number of weevils collected in the mass-trapping experiment was analyzed using a two-way ANOVA to test for significant differences among different sampling periods in repeated measures and locations. In addition, a two-way ANOVA was used to test for differences in damage reduction among sampling dates and treatments, as well as differences among sampling dates and locations with or without pheromone traps installed. Differences were considered to be significant if $P \leq 0.05$.

Results

Maximum Radius of Attraction of the Pheromone Septum. The traps placed at 10–100 m from the point of release caught 98–100% of all marked adults released in a downwind direction. Similarly, 92–95% adults released in an upwind direction were captured. Catches declined with increasing release distance from the trap in both downwind and upwind directions. When marked adults were released 60 m from

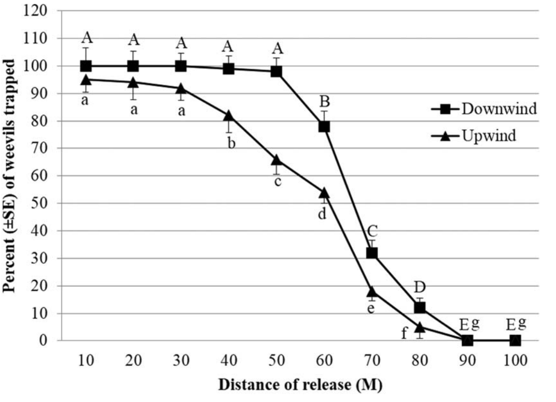

Fig. 1. Marked *C. formicarius* released at different distances from the pheromone source. Different letters indicate significant differences among treatments (one-way ANOVA with Poisson model, least square means, $P \leq 0.05$). Data were represented by means (±SE) of the marked adults (n = 40) that were released at each distance.

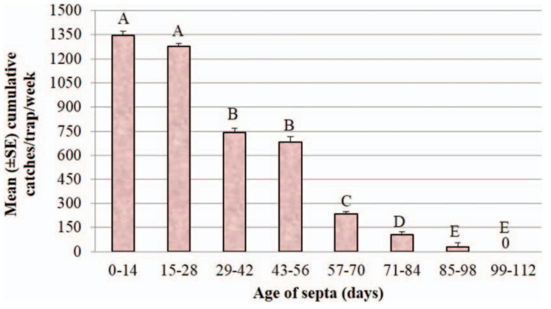

Fig. 2. Mean catches of *C. formicarius* in pheromone traps baited with septa aged for different periods. Different letters indicate significant differences among treatments (one-way ANOVA with Poisson model, least square means, $P \leq 0.05$). Bars represent means (±SE) of 12 replicates (4 replicates per location × 3 sites). (Online figure in color.)

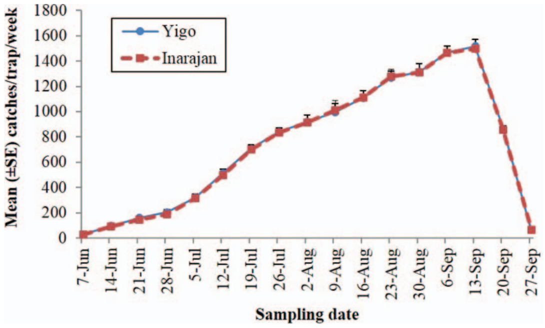

Fig. 4. Mean number of *C. formicarius* collected in traps on different sampling dates at two locations: Yigo and Inarajan. (Online figure in color.)

the pheromone source, 78% of the adults were caught in the downwind release, whereas 54% of the adults were caught in the upwind release. The number of adults caught at different distances was significantly different ($F = 12.23$; df = 9, 80; $P \leq 0.05$; Fig. 1), and most adult weevils were recovered within a radius of 60 m. Traps on the downwind side of the release points caught more adults than the traps set up on the upwind side of the release points. Although the maximum radius of attraction was 80 m, the effective distance for recapturing marked adults in the pheromone-baited Unitraps was 60 m.

Effect of Aging of Septa on Weevil Capture. Results suggest that the pheromone lures were able to capture adults of *C. formicarius* after being stored in the laboratory for up to 98 d, but there was a progressive reduction in the number of adults being caught as lures aged. The number of catches per trap per week was highest when the lures were 0–14- and 15–28-d-old, after which catches declined significantly ($F = 2.97$; df = 7, 26; $P \leq 0.05$; Fig. 2). Therefore, we recommend changing lures every 30 d to maximize attraction to the weevils.

Effect of Pheromone Dose Loaded in Septa on Weevil Capture. Traps baited with septa containing 100 μg of attractant caught more *C. formicarius* adults than those baited with septa containing 10 μg of attractant at all tested locations (Fig. 3), with significant differences observed between them ($F = 11.26$; df = 2, 97; $P \leq 0.05$). Traps without lures did not catch any adults.

Effectiveness of Adult Weevil Trapping on Damage to Sweet Potato. At both locations, the number of *C. formicarius* adults collected in traps fluctuated dramatically among sampling dates in repeated measures during the 4-mo duration of the experiment, ranging from 30.2 ± 5.6 to 1515.6 ± 57.8 per trap in Yigo and from 28.1 ± 4.9 to 1497.6 ± 68.5 per trap in Inarajan (Fig. 4). Significant differences in the number of *C. formicarius* were observed among sampling dates ($F = 283.26$; df = 16; $P < 0.0001$), but not between the two locations ($F = 0.08$; df = 1; $P = 0.7739$). No significant interaction between sampling date and location was found ($F = 0.02$; df = 16; $P = 1.000$). The peak adult trap capture occurred on 13 September 2013, after which time the number of captures noticeably declined. This decrease was correlated to the increasing age and depletion of the pheromone lures.

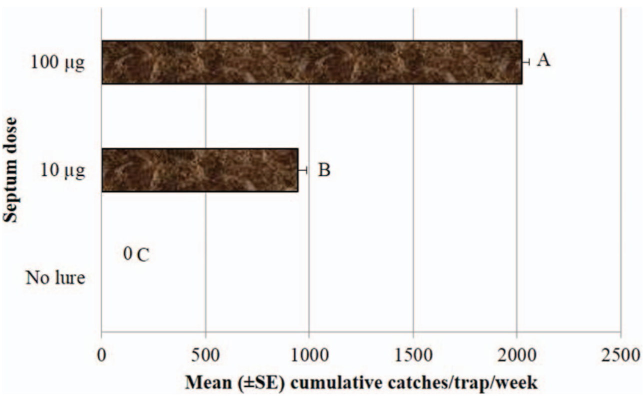

Fig. 3. Mean cumulative *C. formicarius* adults caught in pheromone traps baited with 0, 10, or 100 μg pheromone per septum. Different letters indicate significant differences among treatments (one-way ANOVA with Poisson model, least square means, $P \leq 0.05$). Bars represent means (±SE) of 12 replicates (4 replicates per location × 3 sites). (Online figure in color.)

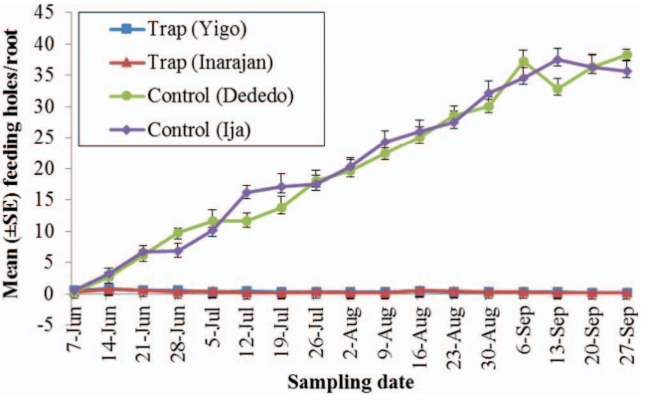

Fig. 5. Mean number of feeding holes per root (tuber) caused by *C. formicarius* in fields with pheromone-baited traps and in the control with no pheromone traps at two field locations: Yigo and Inarajan (Trapping); Dededo and Ija (Control), on different sampling dates.

The effect of traps in reducing the damage caused by *C. formicarius,* damaging holes in the treatment with traps, and the untreated control was assessed over time at two different locations. Significant differences were observed between the trap treatment and the control (F = 5793.36; df = 1; P < 0.0001), among sampling dates (F = 123.39; df = 16; P < 0.0001), and there was significant interaction between treatment and sampling dates (F = 128.45; df = 16; P < 0.0001; Fig. 5). Root damage in the treatment with traps was consistently low (<1 feeding hole per root) over the 4-mo duration of the experiment. There were no significant differences observed between field locations in fields with traps installed (F = 1.22; df = 1; P = 0.2707), among sampling dates (F = 1.56; df = 16; P = 0.0814), or in the interaction of sampling date and location (F = 0.34; df = 16; P = 0.9922). However, root damage levels in the control were high at both locations (up to 38 feeding holes per root); and there were significant differences in damage among sampling dates (F = 129.33; df = 16; P < 0.0001), but not between locations (F = 0.86; df = 1; P = 0.354). No significant interaction between sampling date and location was observed in the untreated control (F = 1.14; df = 16; P = 0.3163).

The present results indicate that pheromone-baited traps are effective in reducing the weevil numbers and the damage due to *C. formicarius.* Sweet potato yields in fields treated with traps were higher than in the untreated control, averaging 14.59 T/ha at Yigo and 13.47 T/ha at Inarajan in the trapped area, compared with 8.26 T/ha at Dededo and 7.86 T/ha at Ija in the control.

Discussion

The use of pheromone traps is a promising approach for monitoring and controlling *C. formicarius* (Jackson and Bohac 2006). However, the successful and practical use of pheromones requires an understanding of parameters such as pheromone dose, lure longevity, timing of lure replacement, and the maximum radius of attraction. From such information the best arrangement of traps and the optimal number of traps to be installed may be estimated. Factors such as wind velocity, weevil population density, and the availability of *Ipomoea* species in the intermediate area must also be considered when designing the trapping scheme.

The current study indicates that the effective distance for recapturing the marked adults in the pheromone-baited Unitraps was 60 m, even though the maximum radius of attraction of a septum containing 10 μg of *C. formicarius* attractant was 80 m (Fig. 1). However, when Sugimoto et al. (1994) used lures with the higher dosage of 100 and 400 μg in their study in Japan, the average distance for capturing *C. formicarius* was only 55 and 64 m, respectively.

The number of adults caught in traps differed in the current study when marked adult weevils were released in the downwind or upwind direction. Jansson et al. (1991) reported that males of *C. formicarius* were recaptured from all released directions with similar frequencies at night when wind direction varied considerably. Differences in the number of recaptured *C. formicarius* between upwind and downwind directions observed in this study may be explained by changes in the pheromone diffusion pattern associated with the prevailing wind direction. Impact of wind direction on captures of *C. formicarius* was also observed by Sugimoto et al. (1994).

The selection of the appropriate dose of pheromone in lures is critical for monitoring and controlling insect pests. The current study clearly indicated that a dose of 100 μg doubled the number of catches compared with a dose of 10 μg (Fig. 3). In previous reports, the response threshold dose for *C. formicarius* was ≈10 μg per septum (Jansson et al. 1991), and was shown to be adequate for monitoring weevils in sweet potato fields (Reddy et al. 2012b). However, Jansson et al. (1991) suggested that higher dosages would likely be required for mass trapping or mating disruption applications. Previous studies have shown a positive relationship between trap captures and pheromone dosages ranging from 10 ng to 1 mg (Proshold et al.

1986, Mason and Jansson 1990). However, the success of mass trapping is also dependent on the population trend in the field, and trap counts were not consistently correlated with the pheromone dose at low population levels (Jansson et al. 1991). Effect of doses was not confounded in the current study, given the high densities of *C. formicarius* (up to 2,000 catches per trap per week).

Results from this study also suggest that the pheromone lures were still able to capture *C. formicarius* adults after 98 d of laboratory storage, but were most effective within 28 d, and trap catches decreased the longer the septa were stored (Fig. 2). In India, pheromone lures for *C. formicarius* were used up to 90 d in the field (Pillai et al. 1993), and in Florida, 60-d-old rubber septa with 10 μg of pheromone performed as well as new lures (Mason and Jansson 1990). Jansson et al. (1991) reported that the sex pheromone of *C. formicarius* was moderately stable on rubber septa and that lures remained active for at least 30–64 d. However, Hwang (2000) showed that the effectiveness of lures declined after 60 d. Smit et al. (1997) reported that in Uganda, lures for the sweetpotato weevil species, *Cylas puncticollis* (Boheman) and *Cylas brunneus* (F.), showed no significant decline in attractiveness after 8 wk in the field. In addition, Hwang (2000) recommended changing lures monthly, which was supported by our results.

Effectiveness of mass trapping in reducing the damage caused by *C. formicarius* was evaluated at different locations in Guam with similar population trends before treatment, as indicated by Reddy et al. (2012a) that there was no population fluctuation on the island of Guam, although there was a difference among different islands (Guam, Saipan, Rota, and Tinian). Mass trapping of *C. formicarius* not only reduced the infestation but also almost doubled the yield compared with that of control plots. Root damage in fields with traps was significantly lower (<1 feeding hole per root), compared with the damage level in fields without traps (up to 38 feeding holes per root; Fig. 5). Our observations clearly suggest that root damage may be significantly reduced by traps. This is the first study on the mass trapping of *C. formicarius* demonstrating that the sex pheromone traps reduced the damage level and thereby increased yield of *I. batatas* in the Pacific Basin. Our results corroborate those of several studies carried out in different countries but in a different geographic area. For example, mass trapping using the pheromone suppressed populations of *C. formicarius* in Taiwan (Hwang and Hung 1991), India (Pillai et al. 1993), Japan (Yasuda 1995), and Indonesia (Braun and Van De Fliert 1999). In the Dominican Republic, the use of pheromone traps significantly reduced yield losses due to *C. formicarius* (Alvarez et al. 1996). The International Potato Center (CIP) is currently cooperating with several national programs to further explore the use of pheromone traps (CIP 1995).

Overall, the pheromone-based trapping provided significant control of *C. formicarius.* Although similar studies have been conducted on sweetpotato weevils, they were on different species (*C. puncticollis* and *C. brunneus;* Smit et al. 1997). The current study clearly demonstrates how mass trapping would help in controlling this borer pest. Because there are not many control options, the current study is important and vital for the growers, and also helps further studies on mating disruption.

Using pheromones for mating disruption is a promising potential alternative for controlling *C. formicarius* (Mason and Jansson 1990, 1991). The density of *C. formicarius* populations was noticeably decreased in pheromone-treated fields, possibly due to male sterile technique and mating disruption (Yasuda 1995). In future studies, we will focus on combining mass trapping or mating disruption with entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium brunneum*) and nematodes (e.g., *Steinernema carpocapsae, Steinernema kraussei, Steinernema feltiae,* and *Heterorhabditis bacteriophora*) for widespread control or possible even local eradiation of this serious pest.

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