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Studies on the biology, host specificity, and feeding behavior of *Acythopeus cocciniae* O'Brien and Pakaluk (Coleoptera: Curculionidae) on *Coccinia grandis* (L.) Voigt (Cucurbitaceae) and *Zehneria guamensis* (Merrill) Fosberg (Cucurbitaceae).

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Abstract—The invasive plant ivy gourd *Coccinia grandis* (L.) Voigt (Cucurbitaceae) first appeared on Guam in the 1980s and subsequently spread to about 200 ha in the absence of natural enemies. Following its successful introduction as a biological control agent in Hawaii, the weevil *Acythopeus cocciniae* O'Brien and Pakaluk (Coleoptera: Curculionidae) was brought to Guam under quarantine for host specificity testing against the endemic cucurbit, *Zehneria guamensis* (Merrill) Fosberg (Cucurbitaceae) and for further biological characterization. *A. cocciniae* preferred *C. grandis* over *Z. guamensis* in single- and two-host choice tests. No larval feeding was ever observed on *Z. guamensis* plants in two-host tests, and the single instance of *A. cocciniae* feeding on *Z. guamensis* in single-host tests was likely to avoid starvation. In 2004, *A. cocciniae* at the initial field release site in northern Guam dispersed primarily downwind in accordance to the prevailing northeasterly trade winds. However, only a single vine with a few adult weevils, larvae, and pupae were found at this site four years later in January 2008. This decrease was attributed to suppression by *A. cocciniae*. Since its initial release in 2004 *A. cocciniae* has established and dispersed throughout Guam.

Introduction

Coccinia grandis (L.) Voigt (Violales: Cucurbitaceae), commonly known as ivy or scarlet gourd, is native to East Africa (Singh 1990, Chun 2001) and has been naturalized in parts of Australia, Asia (Jeffrey 1967), the Pacific, the Caribbean and the southern United States (Telford 1990, Linney 1986, McConnell and Muniappan 1991, USDA 2004). *C. grandis* is a rapidly climbing dioecious vine that may grow to several meters in length (Telford 1990). Originally described by Linnaeus in 1776 as *Bryonia grandis* L. (Linney 1986),

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synonyms of this species are *C. cordifolia* auct. non (L.) Cogn. and *C. indica* Wight & Arn. Nom. Illeg. (USDA 2004). While still taxonomically unsettled, the genus *Coccinia* contains about 30 species native to the Old World (Correll and Johnston 1979, Singh 1990).

C. grandis moved from East Africa to Southeast Asia several hundred years ago, making its way to Hawaii in the 1960s where it was declared an invasive noxious weed (Chun 2001), and later to the Marianas in the 1980s (McConnell and Muniappan 1991). The estimated area of infestation in 2000 in Guam, Saipan and Rota was 202 ha, 607 ha, and 2 ha, respectively (Muniappan et al. 2002).

In Hawaii and Micronesia, *C. grandis* has infested residential neighborhoods and farmland, frequently forming a thick canopy that covers existing vegetation, buildings and power lines (Chun 2001, Murai et al. 1998). *C. grandis* also serves as a reservoir for a number of insect pests of cucurbits, including the melon fly, *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae) (Uchida et al. 1990), the pumpkin caterpillar, *Diaphania indica* Saunders (Lepidoptera: Pyralidae), the pumpkin beetle, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae), the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), *Liriomyza* leafminers (Diptera: Agromyzidae), the black leaf-footed bug, *Leptoglossus australis* (F.) (Hemiptera: Coreidae), and *Bemesia* white flies (Hemiptera: Aleyrodidae).

Because *C. grandis* serves as an alternate host for so many cucurbit pests, Hawaii, Guam, and the Commonwealth of the Northern Mariana Islands (CNMI) have initiated biological control programs to import and release the weevil *Acythopeus cocciniae* O'Brien and Pakaluk (Coleoptera: Curculionidae) on *C. grandis* on these islands. Host specificity studies conducted by the Hawaiian Department of Agriculture (HDOA) on *A. cocciniae* were used as a template for similar studies in Guam and the CNMI. However, Because of extensive host specificity testing conducted in Hawaii, USDA-APHIS and the USFWS recommended that only the endemic cucurbit, *Zehneria guamensis* (Merrill) Fosb. (Cucurbitaceae) be tested in Guam prior to issuing a field release permit *A. cocciniae* on Guam. This paper presents basic biological information on *A. cocciniae*, reports results of host specificity tests of *A. cocciniae* against *Z. guamensis*, and describes the establishment and dispersal of *A. cocciniae* on Guam.

Materials and Methods

HOST SPECIFICITY TESTING

Biological observations were conducted in the Western Pacific Biocontrol Quarantine Laboratory (WPBQL) at the University of Guam under a 14:10 light-dark photoperiod, 65 – 80% relative humidity and a room temperature of 28 ± 2 ˚C.

C. grandis was propagated from field collected cut stems in 25 cm diameter plastic pots filled with a mixture of 50:50 Guam soil and Sun Gro® Sunshine Mix # 4 (Sun Gro Horticulture Canada Ltd., Vancouver, BC). Plants were maintained in an outdoor nursery under shade cloth, watered every other day, and fertilized weekly with 15-30-15 Miracle-Gro® fertilizer (Scotts Miracle-Gro Company, Marysville, OH). Test plants were moved into cages indoors when they were 50 cm tall and had approximately 30 leaves.

A 100 mm × 15 mm Petri dish was used to confine the weevils and individual leaves (Fig. 1). A 5 mm x 5 mm incision was made on the top and bottom rims of the Petri dish to allow insertion of a *C. grandis* leaf. Foam weather stripping was inserted as a seal over the opening and on the dish bottom to confine the weevils. Ventilation was provided by small holes in the bottom and cover of the dish. Leaves were placed in the sealed dishes while still attached to the vines.

Five pairs of newly emerged and mated weevils were released onto the enclosed leaves and removed after 24 h. Eggs laid in the leaf laminae were counted and measured under a binocular stereomicroscope, and leaves were examined daily to ascertain the onset of eclosion, duration of larval stadia, and the onset of pupation.

Observations on adult weevil longevity were made on ten newly emerged adults. The weevils were placed into individual 26 mm × 67 mm plastic tubes covered with fine mesh cloth and were fed daily with young *C. grandis* leaves until all 10 adults died.

Host specificity experiments were conducted using *C. grandis and Z. guamensis* propagated as described previously. *Z. guamensis* originated from seeds collected at Hilaan Point in northwestern Guam. *Z. guamensis* test plants were moved into indoor cages when they were about 50 cm tall and had approximately 30 leaves as was *C. grandis*.

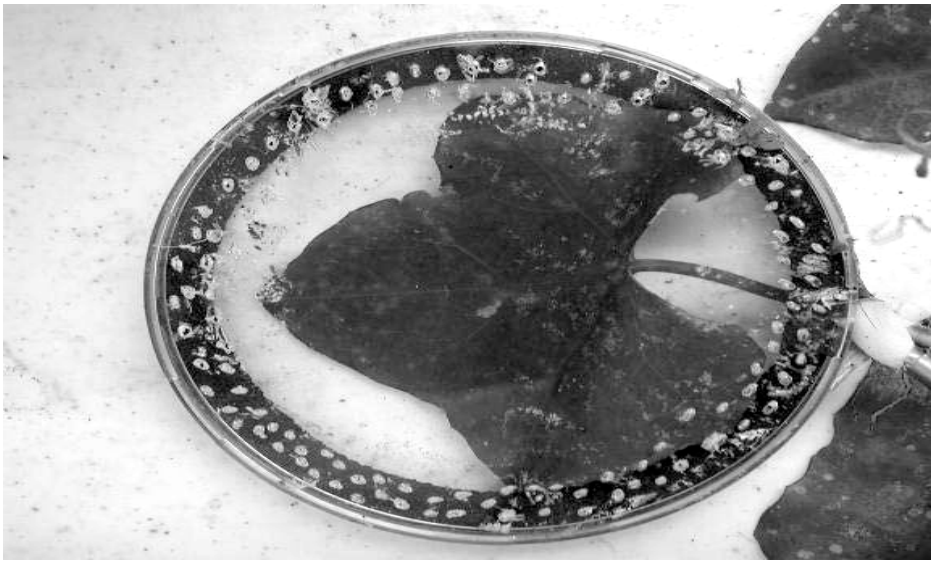

Figure 1. Rearing container with *C. grandis* leaf.

Insects were confined in 60cm × 60cm × 60 cm aluminum cages (BioQuip Products, Rancho Dominguez, CA), with the back and top fitted with Plexiglas® (Rohm and Haas, Philadelphia, PA) to allow exposure to light and permit direct observations. The upper 30 cm of front of the cage was also fitted with a Plexiglas® panel and the lower 30 cm fitted with two muslin cloth sleeves to allow secure access. The cage bottom consisted of a metal sheet, and the remaining two sides were covered with muslin cloth. Cage edges were caulked.

Single-host specificity tests consisted of a single *C. grandis* or *Z. guamensis* plant placed in the middle of a cage. In two-host tests, a single *C. grandis* and *Z. guamensis* plant were randomly placed inside a cage. Single- and two-host tests were replicated eight times. For each individual test, five pairs of newly mated weevils were released into a cage and observed on days 1, 4, 7, 14, and 21 following weevil release into the cage, which allowed sufficient time for adult leaf feeding, larval mining, and pupae formation while the test plants were still vigorous (Murai et al. 1998). Data collected included 1. the number of days required for *A. cocciniae* adults and larvae to begin feeding on test plants, 2. the number of larval mines and pupae formed 21 days after the introduction of *A. cocciniae*, 3. the nature and size of feeding damage made by *A. cocciniae*, and 4. the number of "holes" and "windows" made by the weevils after 21 days of exposure to the test plants. "Window" damage consisted of minimal feeding on the leaf by the adult insect with the upper epidermis left intact. "Hole" damage consisted of visible feeding holes in the leaf. The size of feeding holes was generalized as: ≤1 mm, 1 – 3 mm, >3 mm.

POPULATION DISPERSION

Six hundred eighty five *A. cocciniae* adults of mixed sex were released along the edge of the heavily infested Chalan Tan Rosita Road in Yigo on 27 May 2003. Plants at the initial release site in a single 1 m × 30 m swath along the roadside were sampled four times per month for 12 months from June 2003 to May 2004. The total number of *A. cocciniae* adults, larval mines, and pupae were counted on plants within a 1 m^2 quadrat positioned 1 m above the ground at the initial release site and then at 5 m intervals east and west of the initial release site.

Subsequent sampling was performed throughout Guam in January 2008 from the initial release site in Yigo and along the edge of *C. grandis* infestations identified from the road. These sites were sampled by counting the total number of *A. cocciniae* adults, larval mines and pupae within a 1 m^2 wood quadrat positioned 1 m above the ground. *C. grandis* infestations within 56 1 m^2 quadrats were sampled throughout northern Guam and seventeen 1 m^2 quadrats throughout southern Guam. Seven of these were located at the initial *C. grandis* release site in Yigo (Fig. 2).

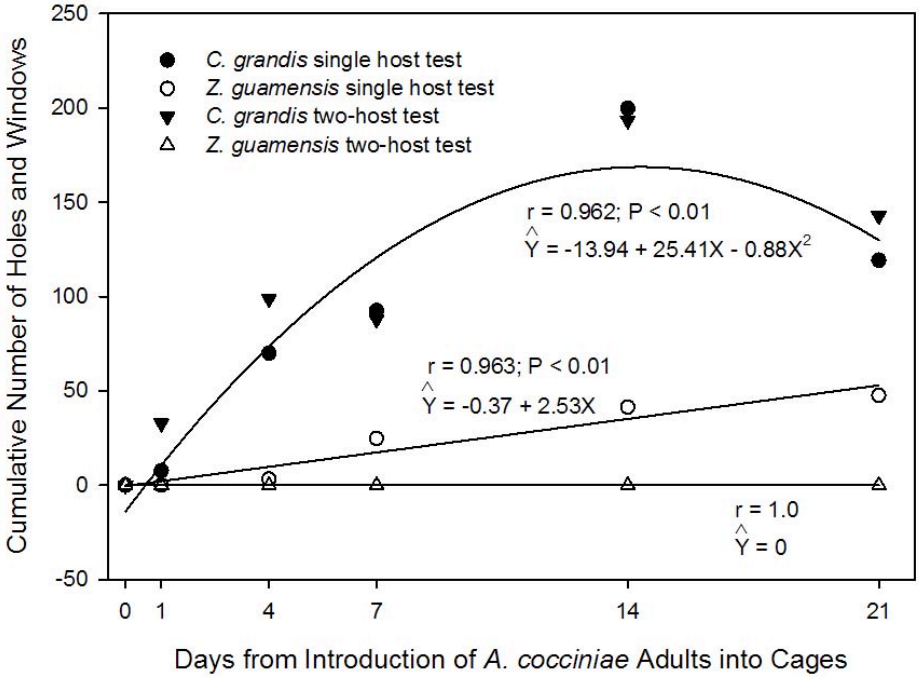

Figure 2. Total number of holes and windows on *C. grandis* leaves caused by *A. cocciniae* adults in single-host and two-host tests.

Additional observations in January 2008 were also made on *Z. guamensis* at Hilaan Point. Insects associated with *Z. guamensis* were collected and identified in the field, as well as reared from *Z. guamensis* vines kept in laboratory cages.

STATISTICAL ANALYSIS.

Data obtained in single and two-host specificity tests were analyzed using paired-sample t-tests. Repeated-measures analyses of variance were used for comparisons of adult populations among sites and years, with years as the repeated measure. Statistical analyses were performed using NCSS for Windows 12.0 (NCSS, LLC. Kaysville, Utah.). Results were considered significant if $P \leq 0.05$. Graphic analyses were performed using SigmaPlot®10 (Systat Software 2006).

Results

Neonate larvae of *A. cocciniae* tunneled into the leaf epidermis of *C. grandis*, causing serpentine leaf mines that became obvious 7 -10 days after the plants were exposed to the beetles. The larvae, which are bright yellow when mature, developed in the leaves for about two weeks, after which the last instar

pupated within the epidermis. Egg size and duration of larval stadia are shown in Table 2.

Table 2. Egg size and stadia duration of *A. cocciniae* reared on *C. grandis*.

Egg Dimensions		
	Size	*n*
Length	11.52 μm ± 0.19 μm	25
Width	8.58 μm ± 0.20 μm	25
Mean Duration of stadia (±SE)		
	Days	*n*
Eggs	5.80 ± 0.15	51
Larvae	7.32 ± 0.10	25
Pupae	14.65 ± 0.31	23
Adults	339.80 ± 37.21	10

A. cocciniae larval mines in single-host tests first appeared on *C. grandis* an average of nine days from the time of adult weevil release onto plants in single-host tests (Table 3). However, adult beetles consistently began feeding on *C. grandis* within one day. In contrast, adult weevils delayed feeding on *Z. guamensis* for nearly 6 days. Furthermore, there was only one instance of larval mining on *Z. guamensis* in single-host tests, which was first observed after 14 days. This larva died prior to pupation. In two-host tests, *A. cocciniae* larval mines first became apparent on *C. grandis* nine days after weevils were released onto *C. grandis* in two-host tests, and adults began to feed within a single day. No adult feeding or larval mines were ever observed on *Z. guamensis* plants in two-host tests.

Table 3. Mean number (±SE) of days required for *A. cocciniae* adults to begin feeding larval mines to appear in single-host and two-host tests following introduction of weevils cages.

Single-host Tests				
Life Stage	*C. grandis*	*Z. guamensis*	*n*	*t*
Adults	1.00 ± 0.0	6.63 ± 0.46	8	12.22* *
Larval mines	9.00 ± 0.19	14[1]	8	-
Two-Host Tests				
Adults	1.00 ± 0.00	---[2]	8	-
Larval mines	9.13 ± 0.13	---[2]	8	-

* * means are significantly different at $P \leq 0.01$.

[1]observation based a single instance of larval feeding

[2] no feeding damage occurred.

There were obvious differences in adult weevil feeding behavior on leaves of *C. grandis* and *Z. guamensis* (Fig 2.). The cumulative number of feeding holes and windows observed on *C. grandis* in single- and two-host tests was consistently much higher than that observed on *Z. guamensis*, and peaked at Day 14. After Day 14 the effect of weevil feeding on the entire *C. grandis* plant became visibly apparent. Leaves began to desiccate and drop from the vines, accounting for the reduction in total feeding holes observed on Day 21. In contrast there were no feeding holes or windows observed on *Z. guamensis* in single-host tests until day 21, when only a single feeding hole was observed. *Z. guamensis* leaves remained healthy and attached to the vines during all tests.

POPULATION DISPERSION

From 2003 through 2004 there was an increase in the number of adult weevils at the initial release site in Yigo in June and July 2003 (Fig. 3). A slight decrease from August through October 2003 was likely due to removal of *C. grandis* along the road by the Yigo Mayor's Office. Plants had regrown and increases in *A. cocciniae* activity again noted by February 2004.

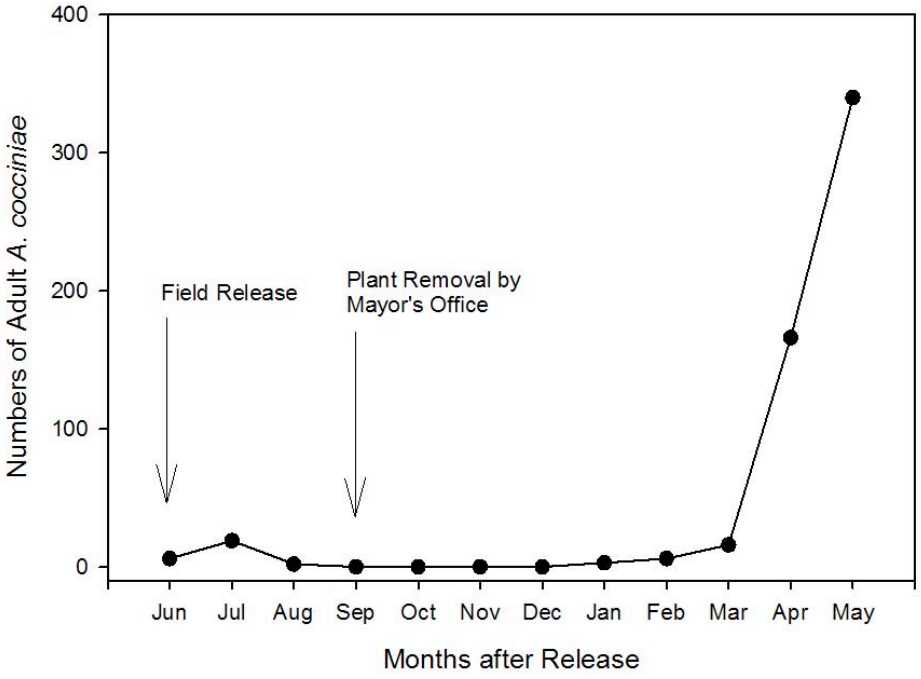

Figure 3. Change in the number of adult *A. cocciniae* weevils from 9 June 2003 - 8 May 2004 at the initial *A. cocciniae* release site in Yigo.

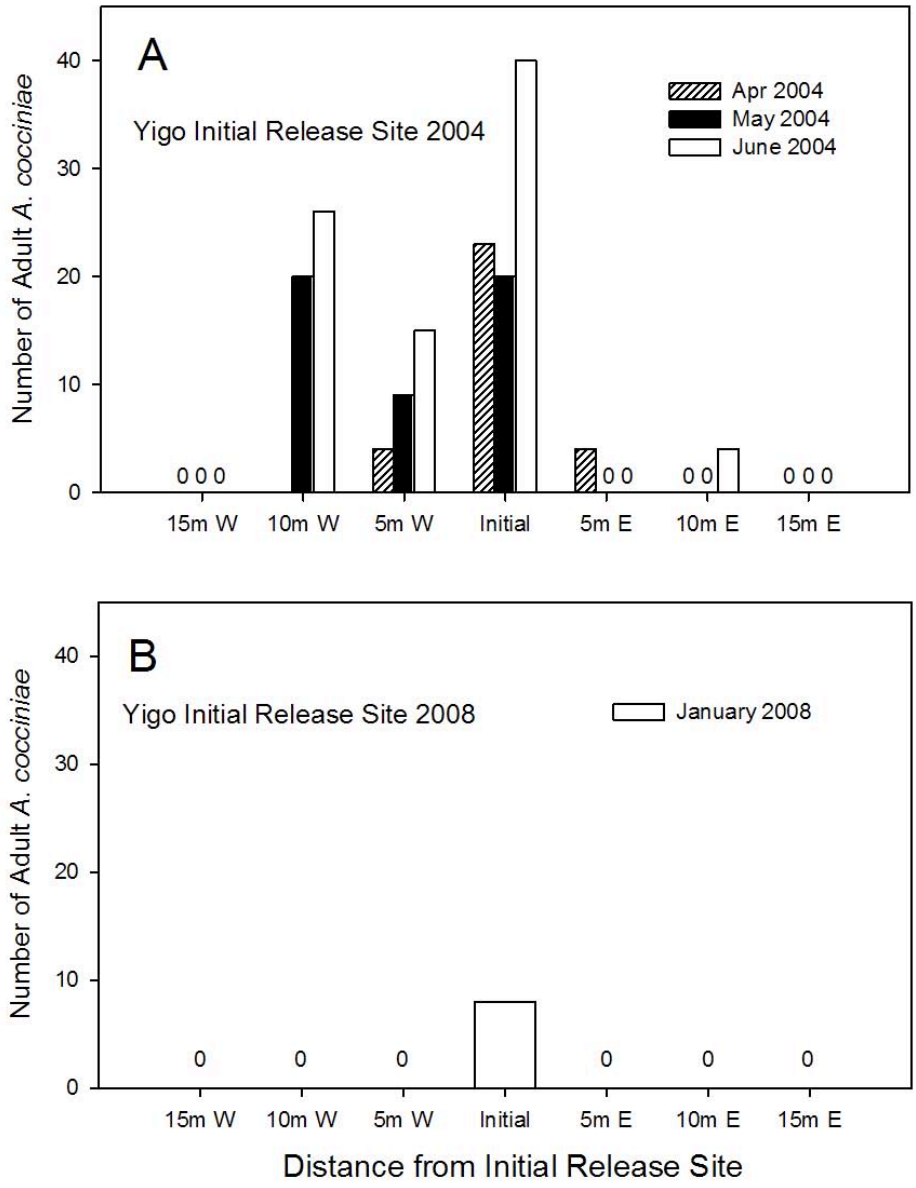

Figure 4. The number of *A. cocciniae* adults at the Yigo release site in (A) April, May, and June of 2004 and (B) January of 2008.

In 2004 *A. cocciniae* distribution was skewed toward the west, downwind of the initial release site (Fig. 4). When the initial release site was re-sampled in January 2008, a few adult weevils were found on a single remaining *C. grandis*

vine. There were no *C. grandis* vines growing in any of the sites that had been sampled previously.

The January 2008 survey at Hilaan Point in Northwestern Guam revealed the hemispherical scale *Saissetia coffeae* Walker (Homoptera: Coccidae) and an unidentified mealybug (Hemiptera: Pseudococcidae) feeding on of *Z. guamensis*.

Discussion

Biological control of *C. grandis* began in 1992 when Robert Burkhart from the Hawaii Department of Agriculture (HDOA) collected insects feeding on *C. grandis* in Kenya. In East Africa *C. grandis* is distributed along the Indian Ocean coastal region south of Mombassa and around the inland Lake Victoria Basin (Chun 2001). After initial host specificity testing in Kenya, a stem boring moth, *Melittia oedipus* Oberthür (Lepidoptera: Sesiidae), and *Acythopeus burkhartorum* O'Brien and Pakaluk, a stem boring, gall forming weevil, and *A. cocciniae* were imported to the HDOA Insect Quarantine Facility in Honolulu for further testing.

In the course of the HDOA investigations, 36 plant species or varieties, representing 15 families, including six species endemic to Hawaii, were examined using Wapshere's (1974) centrifugal phylogenic method of selection (Table1). After tests showed that the insects fed only on *C. grandis,* permits were issued by USDA-APHIS for field release of A. *cocciniae* in Hawaii. The HDOA screenings served as a template for *A. cocciniae* host specificity trials on Guam and identified *Z. guamensis* as the sole Guam candidate for host specificity testing against A. cocciniae. The recognition of possible non-target host plants is an integral part of the screening process to determine the potential of the biocontrol organism to complete its life cycle on target and any non-target organisms (McEvoy 1996). This strategy has generally been highly effective in delineating the host range of natural enemies released for biological control of weeds (Kaufman and Landis 1999).

Observations by Murai et al. (1998) showed that *A. cocciniae* eggs are laid within the laminae of mature leaves and hatch in about eight days. The length of the larval stage ranged from nine to ten days, the pupal stage lasts about 15 days, and adults lived up to 203 days. In the present study, eggs hatched two days earlier than reported by Murai et al. (1998), and the larval stage was two days shorter. However, *A. cocciniae* adults lived for twice as long as reported by Murai et al. (1998), with one adult living up to 480 days.

Feeding damage caused by *A. cocciniae* to *Z. guamensis* in the single-host tests likely resulted from *A. cocciniae* feeding to avoid starvation. Sands and Van Drieche (2000) reported that positive responses to non-target host plants in single-host tests may develop after several days of food deprivation which they called the "desperation effect". The "desperation effect" may have been exhibited during *Z. guamensis* single-host tests in this study. Santamour (2001) questioned the validity of host specificity and feeding behavior laboratory tests conducted on the Japanese beetle, *Popillia japonica* Newman (Coleoptera:

Scarabaeidae), believing that results from their tests may have represented worst case scenarios rather then real world situations. This might also have been the case with the results of *Z. guamensis* single-host tests in this study. However, Santamour (2001) felt that even though host specificity studies may be inherently flawed, they are still necessary since "natural" feeding preferences during peak insect infestations are seldom observed.

Sands and Van Drieche (2000) suggested that in the case of arthropod biocontrol agents, confinement often occurs with other organisms not normally found together. A similar rational may be true for *Z. guamensis* on Guam. Only two other insect species were found on *Z. guamensis* on Guam, and *Z. guamensis* on Guam is restricted to only a single isolated site where the probability of being discovered by *A. cocciniae* is low.

Based on data from the single and two-host specificity tests, USDA-APHIS issued a permit to release *A. cocciniae* into the field on Guam on 27 May 2003. Observations at the release site showed that *A. cocciniae* caused severe defoliation of *C. grandis* vines because of adult and larval feeding. Burkhart (1993) found extensive leaf mine damage caused by *A. cocciniae* in Kenya, and observed up to ten pupae/leaf on *C. grandis* vines. The 2004 Yigo observations showed that as *A. cocciniae* fed on *C. grandis,* infested leaves dried up and forced the movement of the weevils to vines with healthy leaves.

The density and extent of *C. grandis* infestations along the roadside in Yigo dramatically decreased between June 2003 and January 2008 (Fig. 5). *A. cocciniae* was present in widely separated villages throughout Guam, and suggests that the weevils have established and dispersed throughout the island where they may exert considerable pressure on *C. grandis* populations in concert with other released natural enemies of C. grandis, including *A. burkhartorum* and an African vine borer, *Melittia Oedipus* Oberthur (Lepidoptera: Sessidae) (G.V.P. Reddy, CNAS-WPTRC, University of Guam, unpubl. data).

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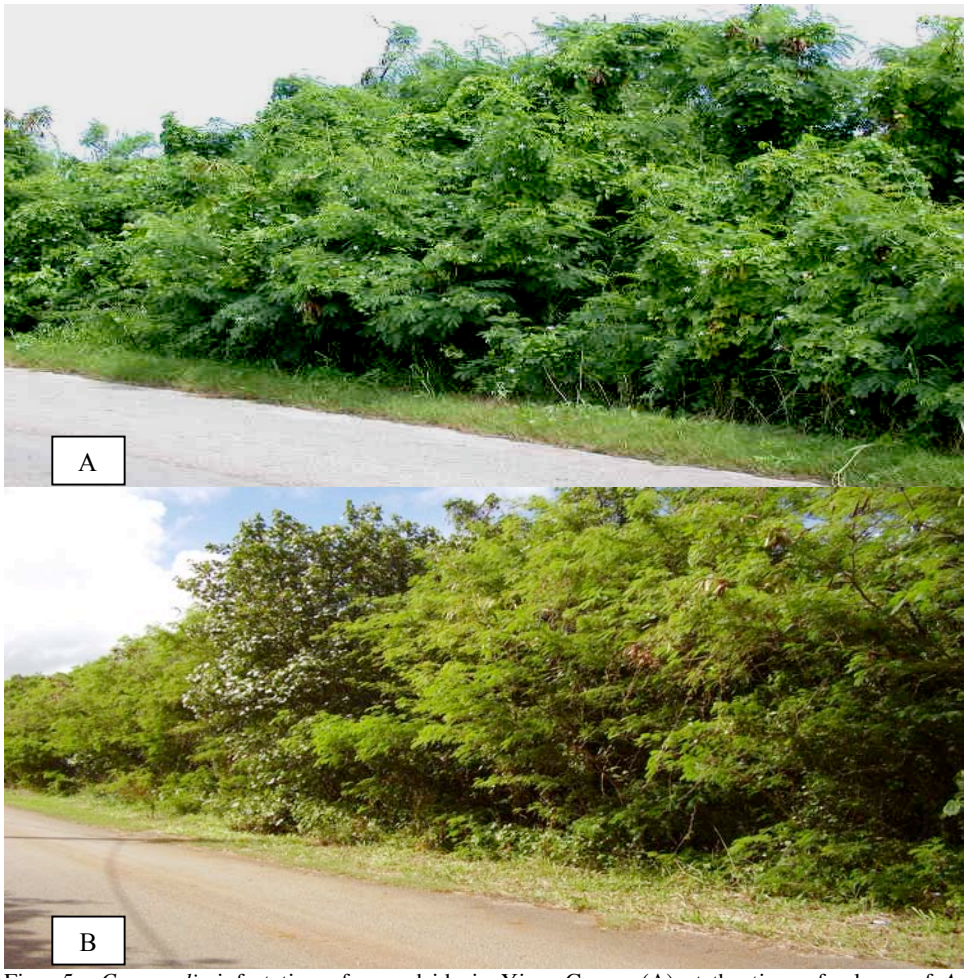

Fig. 5. *C. grandis* infestation of a roadside in Yigo, Guam: (A) at the time of release of *A. cocciniae* in June 2003, (B) January 2008.

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