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REPORT TO THE US FISH AND WILDLIFE SERVICE – PACIFIC ISLANDS DIVISION ON A VISIT TO GUAM MARCH 3–17, 2022

OBSERVATIONS, ASSESSMENT, AND RECOMMENDATIONS FOR APPLIED BIOLOGICAL CONTROL OF THE CYCAD AULACASPIS SCALE

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I. Introduction

The cycad aulacaspis scale (CAS), *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae) (**Figure 1**), is a nearly cosmopolitan pest of cycads. All plants in the family Cycadaceae and several species of Zamiaceae are hosts of the CAS (Marler *et al*. 2021). Takagi (1977) described the species based on material collected from *Cycas* sp. in Bangkok, Thailand. The armored scale insect was first detected in the USA in 1994 on cycads in the Fairchild Tropical Botanic Garden and Montgomery Botanical Center in Coral Gables, Florida, after it was brought unaware on infested plants from Southeast Asia to the Montgomery Botanical Center (Howard *et al*. 1999; Tang *et al*. 2006). From this single introduction, the pest dispersed throughout Florida and into other southeastern USA states (Germain and Hodges 2007; R. D. Cave personal observation) and Texas (Bográn et al. 2006; Flores and Carlson 2009). Wind was certainly a factor in the dispersal of the invasive insect, but the movement of cycads from infested nurseries in the ornamental plant industry probably enhanced the spread of the insects.

The cycad aulacaspis scale was found on Oahu, Hawaii, in 1998 and soon spread to Hawai'i and Kaua'i (Heu *et al*. 2003). Five years later, the scale was discovered on ornamental king sago palms (*Cycas revoluta* Thunb.) in Guam (Terry and Marler 2005) in front of a hotel in Tamuning. Within two years, the scale reached most areas of Guam and invaded the native forests of *Cycas micronesica* K. D. Hill (Moore *et al*. 2005a), an indigenous tree unique to Micronesia. The scale insect was discovered on Rota in 2007 (Calonje 2008) and Palau in 2008 (Orapa and Cave 2010).

Figure 1. Male and female cycad aulacaspis scales, *Aulacaspis yasumatsui*. Photo by R. D. Cave

The cycad aulacaspis scale is now established in many tropical and subtropical regions and countries, including the West Indies (Étienne 2007; Segarra-Carmona and Pérez-Padilla 2007), Mesoamerica (Germain and Hodges 2007; Normark *et al*. 2017; Castillo-Gómez *et al*. 2021), Indonesia (Muniappan *et al*. 2012), Singapore (Hodgson and Martin 2001), Philippines (Lindstrom *et al*. 2008), China (Cave 2006), Taiwan (Hodgson and Martin 2001), Vietnam (Cave 2006; Germain and Hodges 2007), Africa (Germain and Hodges 2007; Nesamari *et al*. 2015; Dimkpa *et al*. 2021; Macharia *et al*. 2021), and Europe (Masten Milek *et al*. 2008; Trencheva *et al*. 2010; Malumphy and Marquart 2012; Ülgentürk 2015; Ülgentürk *et al*. 2015). The pathway responsible for this global distribution is the intercontinental commercial and private movement of potted cycad plants. The presence of CAS on the leaves is easily visible, but the insect infests microhabitats on the plant that are not easily inspected (Marler and Moore 2010).

The life cycle of CAS is typical of other armored scale insects. The sessile adult female secretes a round, white armor under which she feeds with piercing-sucking mouthparts and lays eggs. From the eggs emerge tiny nymphs, called crawlers, that have functional legs but do not feed. The crawlers walk to other plant parts or adjacent host plants or are dispersed to other plants by the wind. When the crawler finds a suitable site on the plant, it settles, inserts its mouthparts, and molts to the next life stadium, which lacks legs but has functional piercing-sucking mouthparts. While feeding, it secretes substances that form the armor covering the body. Upon the next molt, if a male, the insect produces a white, elongate, three-ridged covering (test) (**Figure 1**) under which the insect molts three times and eventually emerges as a tiny adult with one pair of wings but no functional mouthparts. If a female, she secretes additional armor material and molts to an immobile, wingless, feeding adult (**Figure 1**). Males likely locate females by detecting pheromones, as demonstrated for *Aulacaspis murrayae* Takahashi (Ho *et al*. 2014), mate with them, and then die. Females then produce eggs to repeat the life cycle.

Cave *et al*. (2009b) and Ravuiwasa *et al*. (2012) studied the development of CAS at various temperatures. The optimal temperature for female development is 28–30° C, at which female developmental time is 20–27 days. The immature stages do not develop below 20° C. Bailey *et al*. (2010) performed life table studies of the scale and observed that females may live up to 65 days and lay up to 305 eggs.

Following the discovery of CAS in Guam in 2003, populations of *C. micronesica* quickly declined (Marler and Krishnapillai 2020). Within five years, *C. micronesica* stem counts were reduced by almost 88% and continued to decrease until now only about 4% of the original pre-CAS population of *C. micronesica* remains. In some areas of Guam, the mortality rate of 100-year-old trees is 100%. In 2015, the US Fish and Wildlife Service listed *C. micronesica* as threatened under the Endangered Species Act (Guertin 2015). Marler and Lawrence (2012) predicted the extinction of *C. micronesica* from Guam by 2019, but small pockets of large trees continue to survive to this day.

Just as critical as tree loss is the loss of reproductive capability by plants that survive a CAS infestation. Unhealthy, mature *C. micronesica* plants in Guam's forests are not producing viable seeds (Marler and Terry 2011), and therefore new seedlings are rare and susceptible to damage by ungulates. Seeds from scale-infested plants (**Figure 2**) are wanting in nonstructural carbohydrates, and seed germination rates are significantly lower than for seeds from healthy plants (Marler and Cruz 2019). Moreover, Marler and Terry (2021) reported that mature male plants infested by CAS produce significantly smaller cones.

Figure 2. Seed of *Cycas micronesica* entirely covered by cycad aulacaspis scale. Photo by R. D. Cave.

The disappearance of *C. micronesica* from Guam may threaten the survival of two animal species. The Mariana fruit bat, *Pteropus mariannus* Desmarest, eats the fleshy covering of *C. micronesica* seeds. After a typhoon, the seeds may be the only food source for the bats (Haynes and Marler 2005). In 2020, the US Fish and Wildlife Service estimated that only 45 Mariana fruit bats remain in Guam in a single roost site on Andersen Air Force Base. A moth, *Anatrachyntis* sp., may play a significant role in the pollination of *C. micronesica* (Terry *et al*. 2009), and *C. micronesica* may be its only host plant.

II. Previous Efforts in Biological Control of the Cycad Aulacaspis Scale in Guam

Efforts to suppress CAS populations with biological control in Guam began in 2004 (Moore *et al*. 2005b), when about 100 adults of the lady beetle *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae) (**Figure 3**) were collected in Hawaii and shipped to Guam. This predatory beetle, commonly called the purple scale predator, was selected for introduction in Guam because it was easily collected in Hawaii, where it apparently suppresses CAS populations effectively. Beetles were mass reared and released in Guam National Wildlife Refuge at Ritidian Point beginning in February 2005. By July 2005, adult beetles were found in abundance on cycads at Urunao Beach, 1 km from Ritidian Point. Following establishment of *R. lophanthae* at Ritidian Point, more than 7,000 beetles were released on cycads at 115 sites throughout Guam.

Figure 3. Adult *Rhyzobius lophanthae*.

Survival of mature *C. micronesica* appeared to improve after establishment of *R. lophanthae*, yet the overall population of the cycad was not recovering because seeds and seedlings were being killed by the cycad aulacaspis scale and other causes (Marler and Terry 2011). Marler *et al*. (2013) showed that predation rates by *R. lophanthae* are significantly lower on plants close to the ground. The preference for mature trees is not completely understood, but Marler and Marler (2018) showed that volatile chemical cues are a contributing factor.

In August 2005, about 500 adults of the parasitic wasp *Coccobius fulvus* (Compere and Annecke) (Hymenoptera: Aphelinidae) (**Figure 4**) were taken from a laboratory colony (original stock from China) in Florida and brought to Guam (Moore *et al*. 2005b). About 250 of these wasps were placed in a cage with a CAS-infested king sago palm; the remaining wasps were released on *C. micronesica* at Marbo Cave. This site was chosen because *R. lophanthae* was not yet present in the area. An additional 250 *C. fulvus* received in September 2005 were released at Marbo Cave, as attempts to culture the parasitoid in the laboratory were not successful. Unfortunately, *C. fulvus* failed to establish in the field (Moore *et al*. 2005b).

Figure 4. Adult female *Coccobius fulvus*. Note round emergence hole in the armor of a female cycad aulacaspis scale and the elongate, carinate tests of the male scale. Photo by D. Caldwell.

In a second attempt to establish feral populations of *C. fulvus* in Guam, G. V. P. Reddy of the University of Guam (UoG) imported adults from a laboratory colony in Florida (original stock from Thailand). Attempts to establish a laboratory colony failed again. Several of the imported wasps were released on *C. micronesica* in Talofofo in 2008 (G. Reddy personal communication to Aubrey Moore, 2022), but there was no subsequent indication that the parasitoid became established.

In a third attempt to establish *C. fulvus* in Guam, A. Moore imported specimens collected in Florida by R. Cave in September and October 2014. Creation of a laboratory colony was again unsuccessful. Half of both shipments were released at Ritidian Point, but no evidence was found to verify establishment in the field. Why establishment of *C. fulvus* in the field did not occur after three attempts is unclear. Possibilities are that insufficient quantities of the parasitoid were released, or parasitized scales were eaten by *R. lophanthae*, or the released wasps were not adequately vigorous to reproduce and disperse.

About 100 adults of the parasitic wasp *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae), or a cryptic species very similar to it, arrived in Guam in 2012 (A. Moore, personal communication, 2022) from Hawaii where they had been reared from CAS collected from king sago palms. This parasitoid causes high parasitism rates that appear to significantly suppress CAS populations in Hawaii; severely infested king sago palms apparently survive the effect of the pest when the parasitoid is present (M. G. Wright personal communication, 2022). *Aphytis* poss. *lingnanensis* also attacks CAS in southern Texas (Flores and Carlson 2009), but its effect on scale populations is undocumented. In Guam, the wasps were put into a cage with scale-infested *C. micronesica* leaves. All detectable *R. lophanthae* adults and larvae were removed from these leaves, but apparently undetected beetle eggs and tiny larvae beneath scale covers consumed all the scales before any adult *A. lingnanensis* could emerge. Therefore, a laboratory colony was not established, and no field releases were made.

G. V. P. Reddy imported into Guam the parasitic wasp *Arrhenophagus chionaspidis* Aurivillus (Hymenoptera: Encyrtidae) (**Figure 5**) from a laboratory colony in Florida, and field releases were made during 2008 (G. Reddy personal communication to A. Moore, 2022). High parasitism rates at Ritidian Point were observed in February 2013 (A. Moore personal communication, 2022).

Figure 5. Cycad aulacaspis scale nymphs parasitized by *Arrhenophagus chionaspidis*. Photo by R. D. Cave

III. Activities and observations in Guam during March 3-17, 2022

Dr. Toni Mizerek of USFWS in Guam facilitated several site visits and appointments. Dr. Aubrey Moore of UoG served as my primary host while in Guam. He accompanied me during all field work and office visits.

Thursday, March 3 – Evening arrival into Guam

Friday, March 4 –

Aubrey Moore and I met Toni Mizerek, Michael Park (Natural Resource Specialist, Andersen AFB), Jill Liske-Clark (Naval Facilities Engineering Systems Command (NAVFAC) Marianas), and Mario Martinez (NAVFAC Guam) at Andersen AFB. We went to the Tarague Basin, a forested area with sporadic *C. micronesica* being tagged and seedlings flagged. Most trees are 3-4 m tall with apparently uninfested leaves, but the base of the petioles had settled crawlers, females, and males on them. There was no evidence of parasitism. Many of the lower leaves were dead (**Figure 6**), but there was no evidence that scales were on them when they were alive. Adults of a small lady beetle (Coleoptera: Coccinellidae) (**Figure 7**) were observed foraging in leaf axils, and a few individuals were collected. The beetle appears to be a member of the subfamily Coccidulinae (hereafter called "cocciduline"). The fauna of this subfamily in Micronesia is poorly known.

Figure 6. *Cycas micronesica* at Tarague Basin on Andersen Air Force Base. Photo by R. D. Cave

Figure 7. "Cocciduline" lady beetle observed on *Cycas micronesica* infested by the cycad aulacaspis scale in and near Guam National Wildlife Refuge. Note the two female scale armors with a large amount of the test destroyed by a predatory lady beetle and the female scale insect bodies gone. Photo by R. D. Cave.

We then explored a forested area next to the Andersen AFB golf course. Some trees are naturally occurring and about 2–4 m high. A few male trees had cones, and some female trees had large seeds. Other trees, 1.0–1.5 m tall, were transplanted to the site. Most of these trees had no live CAS infestation on the leaflets, but CAS was seen at the base of leaves and on crowns. No evidence predation or parasitism on these latter plant parts was seen. No infested crown samples were taken so as not to injure the plants; therefore, parasitism and predation could not be checked in the laboratory. One tree did have remnants of a high density of CAS on both sides of several leaves, but the scales had been killed by predators (**Figure 8**); no live scales or predators were evident. Adult "coccidulines" were on crowns with light infestations of CAS; five specimens were collected.

Figure 8. Cycad aulacaspis scales preyed on by lady beetles, Andersen Air Force Base. Photo by R. D. Cave

Jill Liske-Clark, Aubrey Moore, Toni Mizerek, and I had lunch together to discuss the situation of CAS on Tinian and options for biological control of the pest on that island. There needs to be done a survey of the predators and parasitoids of CAS present on Tinian.

Aubrey Moore, Toni Mizerek, and I met with three representatives of the Guam Department of Agriculture: Christine Fejeran, Forestry and Soil Resources Division Chief; Glenn Dulla, Biosecurity Division, Chief, Invasive Species Coordinator and plant health regulatory officer; and Chris Rosario, Biosecurity Division, agriculturalist. Glenn Dulla stated "no one champions the cycads", which may explain the lack of public outcry for the native plant's demise. The Forestry and Soil Resources Division has two nurseries with space for CAS biological control research and/or rearing natural enemies. The Guam Department of Agriculture personnel said there is no limit on their ability to collaborate in CAS biological control, and they can assist in the issuance of needed permits. There was a discussion about the genetic variation of CAS and *C. micronesica* and how it is a topic that requires investigation.

Monday, March 7 –

Aubrey Moore, Toni Mizerek, Jill Liske-Clark (by phone), Michael Park, Mario Martinez, and I met at Andersen AFB with Sara Diebel (Environmental Flight Chief overseeing all environmental impacts of AAFB activities), Jen Horeg (wildlife ecologist, NAVFAC Marianas), and Lauren Gutierrez (environmental manager, Guam US Marine Corps Base). All participate in the Integrated Natural Resources Management Plan (INRM) that encompasses Guam and the Commonwealth of the Northern Mariana Islands (CNMI). INRM is a loose-knit, project-oriented association with a documented agreement among environmental managers of military lands. Diverse projects are funded from INRM's budget. New projects, increased budgets, and continued funding are authorized by the Department of Defense in Washington. INRM has no action plan/budget for CAS biological control except on Tinian.

USMC is moving to nurseries about ~2,000 cycad pups and growing out seeds from habitat destroyed by the base's development. Contracted nurserymen hired to care for the plants are trained in recognizing CAS. The plants are treated with imidacloprid and an insect growth regulator. The plan is to relocate the cycads into preserved habitat in a couple of months. Biological control is needed after the plants are moved because pesticide treatments may not be feasible.

Aubrey Moore discussed the need to re-initiate the monitoring of 12 plots, many on Department of Defense lands, established by UoG horticulturalist Thomas Marler to evaluate *C. micronesica* survival and reproduction. Toni Mizerek and Jill Liske-Clark are looking to form a working group for native plant recovery in Guam and CNMI.

Aubrey Moore, Michael Park, and I went to the Tarague Triangle near the northern coast to see a fenced area (for protection from ungulates) with *C. micronesica* trees tagged and treated with granular imidacloprid. One 3.5-m tree (tag #A05614) had dead CAS on some of its new leaves, which apparently had been consumed by the "cocciduline". Several of the predatory beetle were present, and seven specimens were collected. One small, red lady beetle of the species *Novius pumila* (Weise) (= *Rodolia pumila* Weise) was taken from another tree.

Tuesday, March 8 –

From a conference room at the UoG, I delivered a 50-minute seminar about CAS biological control. Six attendees formed the audience in the room, and 47 people attended by Zoom. Afterwards, I met with Dr. Else Demeulenaere, Associate Director Natural Resources, Center for Island Sustainability, UoG. The Center has a project (funded by USFWS) to grow out 50 seeds in the nursery and eventually transplant them to designated area in southern Guam. The relocated trees will be monitored by community members. Dr. Demeulenaere mentioned that her Center may be in a position to monitor the 12 Marler plots, if funding can be obtained for such purpose.

Aubrey Moore and I met with Dr. Jim McConnell, horticulture professor at UoG. He has an agreement with the Department of Defense to monitor and care for *C. micronesica* in ungulate-free areas on Andersen AFB (Ache, Tarague Basin, AAFB golf course) and Tinian. In his nursery, his team is growing *C. micronesica* under screen for eventual transplant to habitat. Some plants have a few CAS, for which he applies Safari for control.

Wednesday, March 9 –

Aubrey Moore, Toni Mizerek, and I met with Tammy Mae Summers (Guam NWR manager) and Kawika Davis (Guam NWR biologist) at the Guam NWR. Kawika Davis monitors tagged *C. micronesica* for the McConnell team and is willing to monitor trees, form a database of tree health, and take pictures. Andersen AFB is constructing a new firing range, which will make some parts of Guam NWR off-limits for field work on days that the firing range is in use.

Kawika Davis led us along a coastal trail to find *C. micronesica*. Trees were few and widely separated. One *C. micronesica* tree, toppled by a fallen tree (**Figure 9A**), had a large population of CAS consumed by a predator, probably by the "coccidulinae", of which eight specimens were collected. Other cycad trees seen were clean. Near Star Cave, several *C. micronesica* had light populations of CAS. One small plant had one leaf heavily infested with CAS, but the other leaves were clean (**Figure 9B**).

Figure 9. *Cycas micronesica* with a population of the cycad aulacaspis scale in Guam National Wildlife Refuge. **A**) Plant with CAS and several "coccidulines". **B**) Plant with leaf in the foreground densely infested with CAS but erect leaves without scale insects. Photos by R. D. Cave.

Upon leaving Guam NWR, we stopped at the emergency ramp where there are *C. micronesica* on which Aubrey Moore released *C. fulvus* in 2014. No live CAS were seen on the plants, and no evidence of parasitism was present.

<u>Thursday, March 10</u> –

Aubrey Moore and I returned to Andersen AFB and met Mike Park to go to the unfenced control plot in Tarague Triangle, where trees are tagged and monitored. Some trees were dead, and others looked quite unhealthy due possibly to a pathogen. From one tree, I removed a bract from a live pup on a seemingly dead tree. A small larval *R. lophanthae* and a few female scales were on the inner side of the bract. Tree #A08155, about 2 m tall, had older leaves with moderate densities of old, eaten scales, but the newest leaves had a few scales (also eaten) at the base of the stem, none on the leaflets; no predatory beetles were on the plant.

The fenced area of Tarague Triangle has fewer trees that are widely spaced. No CAS were on new leaves, but there were variable densities on old leaves. One adult "cocciduline" was collected.

Aubrey Moore and I went to Mangilao Golf Course for lunch. There are four king sago palms in front of the clubhouse. The plants were densely covered with old scales. One adult lady beetle *Telsimia nitida* Chapin was collected. No live individuals of the parasitic wasp *A. chionaspidis* were seen.

<u>Friday, March 11</u> –

In the morning, I examined material that was collected in the field the previous day. On cycad pups collected at Tarague Triangle, live female CAS with eggs were found on the inner side of bracts.

In the afternoon, Aubrey Moore and I met Olympia Terral in Yona village. We hiked to an isolated area of Cotal Conservation Area to see two *C. micronesica* on the edge of a forest. Both trees had abundant CAS of all stages. No predation was evident, and no predatory beetles were observed. There were no other trees in the area. We then hiked north to see one tall *C. micronesica* that was apparently free of CAS. There were no other trees in the area. This area has seen severe deforestation and extensive reforestation with earleaf acacia, *Acacia auriculiformis* A. Cunn. ex Benth. Cycads are certainly few and far between, and the area would likely be inhospitable to predators and parasitoids because of sparse cycads and open environment.

<u>Sunday, March 13</u> –

Aubrey Moore and I went to the Ija Research and Education Center in Inarajan, where there is a group of 15–16 *C. micronesica*(?) with crowns about 1 m high (**Figure 10A**). The trees are in full sun next to tall trees of earleaf acacia. Some trees had live male, female, and immature CAS, although there were very few settled crawlers. Most scales were located on male cones and the base of the leaf petioles (**Figure 10B**); a few leaflets were densely populated. There was no evidence of predation or parasitism.

Figure 10. A) Cycads at Ija Research and Education Center, Inarajan. **B)** Male cone and leaf petioles infested by the cycad aulacaspis scale. Photos by R. D. Cave.

Monday, March 14 –

I spent most of the day curating the UoG lady beetle collection. With literature and specimens, I was able to sort them in the collection. Three specimens of the species of "cocciduline" that I found during my field work are in the collection. These were taken in Yigo, Ritidian, and Mangilao.

Aubrey Moore and I inspected samples collected in Tinian. We found no predators or parasitoids and no evidence of predation or parasitism.

Tuesday, March 15 –

At UoG, Aubrey Moore and I examined a stand-alone *C. micronesica* next to a building. Populations of CAS were dense on leaves, leaf petioles, and seeds (**Figure 11**). Adults and larvae of *R. lophanthae* were present but not numerous. Extensive predation occurred on the leaves, but there was no evidence of predation on the seeds.

Aubrey Moore and I examined the cycad garden near the Agriculture and Life Sciences Building at UoG. Only *Cycas* species had infestations of CAS; *Cycas angulata* R. Br. and *C. micronesica* were the mostly intensely infested. Infestations on plants of other genera were absent. No predators were seen, but some previous predation was noted. Plant part samples from *C. micronesica* were taken to the laboratory. Nearly 100% parasitism by *A. chionaspidis* was noted.

I met Dr. Ken Puliafico, director of Colorado State's Center for Environmental Management of Military Lands invasive species monitoring project in Guam. His crew collects sampling on Tinian and will look for predatory beetles on the *C. micronesica* plantings there. He described how trees on a cliff above the *ex situ* plantings are heavily infested. Also, the US military planted years ago many *C. micronesica* along roads and in roundabouts; these trees are large and apparently infested. Therefore, infested cycads were already present on Tinian when the *ex situ* plantings were established.

Figure 11. Infestation of the cycad aulacaspis scale on a leaf (**A**) and leaf petioles and seeds (**B**) of *Cycas micronesica* on the campus of the University of Guam, Mangilao. Note the crinkled, deformed leaflets in **A**, caused by the toxic saliva produced by the insect during feeding. Photos by R. D. Cave.

A good discussion at lunch with Toni Mizerek, Ms. Jackie Flores (Head of the USFWS office in Guam), and Aubrey Moore brought up the needs for moving forward on applied biological control of CAS in Guam. It was clear to me that the USFWS and other interested parties want to see in my report what resources (people, money, space) are needed for taking new action against the CAS and what would be short-term and long-term objectives and tasks.

Wednesday, March 16 –

Aubrey Moore and I went to the Yigo Research and Education Center in Dededo to examine plants in a cycad garden established by Thomas Marler. There is a broad diversity of species and genera. Only two plants (one *C. micronesica*, the other *Cycas* sp.) had intense infestations of CAS, but other plants not *C. micronesica* had light to moderate infestations or no CAS. Scales on the *C. micronesica* were on the leaflets only, not on petioles nor crowns.

One plant (not *C. micronesica*) had five species of lady beetles on it: *R. lophanthae*, *T. nitida*, *Nephus roepkei* (Fluiter), "cocciduline", and a species of an undetermined genus. This plant had scales evenly distributed across the leaflet, whose underside was dense with curly trichomes (**Figure 12**). *Cycas micronesica* (with no trichomes on the underneath side of leaflets) had a few adult *R. lophanthae* and several pupae. Adult *A. chionaspidis* were seen on both plants. Samples taken to the laboratory showed very high rates of parasitism by *A. chionaspidis*; many of the adults had already emerged (**Figure 12**). Also on the leaflets were many CAS females producing eggs, fewer mobile and settled crawlers, and a few intact male tests.

Thursday, March 17 – Morning departure from Guam.

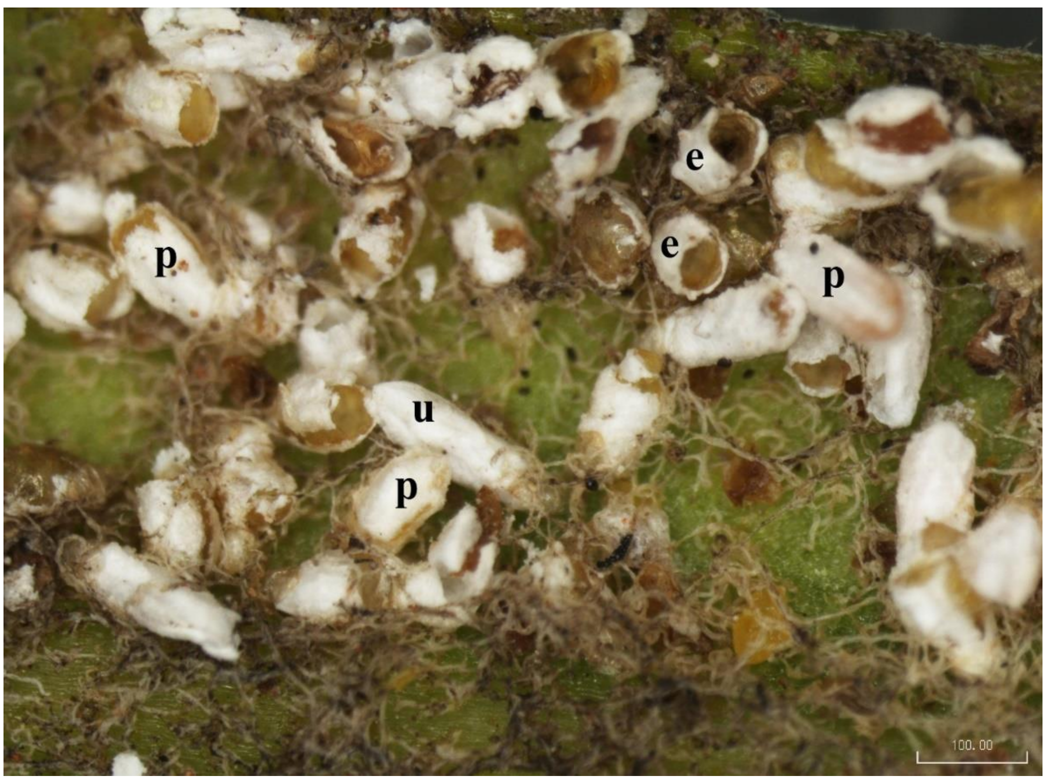

Figure 12. Cycad aulacaspis scales parasitized by Arrhenophagus chionaspidis at Yigo Research and Education Center, Dededo. **e** = empty mummy remaining after emergence of the adult wasp; **p** = parasitized, inflated host scale; **u** = unparasitized male scale. Photo by R. D. Cave.

IV. Status of Current Biological Control of the Cycad Aulacaspis Scale in Guam

Based on my observations in the field in Guam, lady beetles are the most frequently encountered natural enemies and causing the highest rates of mortality of CAS. However, this is not the case at all sites, *e.g.*, Yigo REC and the University of Guam where parasitism rates by *A. chionaspidis* exceeded predation rates. We saw no evidence of the presence of *C. fulvus* at any site. Therefore, it is highly unlikely that the parasitoid is established on the island. This supports the hypothesis of A. Moore that *A. chionaspidis* is the only parasitoid of CAS in Guam.

Six species of lady beetles were collected on cycads with CAS during my field work.

1. *Rhyzobius lophanthae*. This predatory lady beetle has been in Guam since its introduction in 2004 (Moore *et al*. 2005b). During my visits to the localities in the northern part of the island, very high rates of predation were evident on *C. micronesica* that once had leaves densely populated by CAS. However, it was not clear that *R. lophanthae* might have been responsible for this predation because I only observed adults and/or larvae at three localities (Tarague Triangle, UoG, and Yigo REC). It could be that *R. lophanthae* was rare at the time because all the scales on leaves had already been eaten, leaving no prey for the beetles, except for the plant at UoG, which had a thriving population of CAS and numerous *R. lophanthae* adults and larvae. If *R. lophanthae* is the primary predator of CAS in Guam, its effectiveness is probably expressed at a different time of the year. *Rhyzobius lophanthae* preying on CAS is what is termed in biological control as a "new association" because the predator did not evolve with the prey. The lady beetle is native to Australia, whereas CAS is native to Thailand. Although *R. lophanthae* is a voracious predator, its ecology and seasonality may not be finely tuned to that of CAS.
2. "Cocciduline". During my field work, this was the most frequently encountered lady beetle. Twenty-seven specimens were collected (additional specimens were seen but not taken) from infested *C. micronesica* at five localities, all in the northern part of Guam. Interestingly, no larvae were observed, but one pupa was detected. This predator should be studied as it likely is a significant natural enemy of CAS. A taxonomist will have to examine it to determine its identity.
3. *Telsimia nitida*. Three specimens were taken at two localities. Guam is the type locality of this species. It occurs on numerous Pacific islands, including the Hawaiian Islands (Leeper 1976). Armored scale insects are the typical prey of this predator. It may contribute to CAS mortality but appears not to be a significant biological control agent of CAS. Investigation of this species is warranted.
4. *Nephus roepkei*. Two specimens were collected at one locality. This is a widespread species in Southeast Asia and numerous Pacific islands, including the Hawaiian Islands (Leeper 1976). The host range of this predator includes mealybugs (Leeper 1976). It is not likely a significant biological control agent of CAS.
5. *Novius pumila*. One specimen was found. Species of *Novius* prey on scale insects, aphids, and small mites. *Novius pumila* is not likely a significant biological control agent of CAS. Its occurrence on a cycad may have been only incidental.
6. Unknown genus. One specimen was found. It is a minute beetle with two reddish spots on each black elytron. A taxonomist will have to examine it to determine its identity. It is not likely a significant biological control agent of CAS.

V. Assessment of Biological Control Resources in Guam

For biological control of CAS in Guam and Tinian to move forward, two critical resources are required: space and people. Physical laboratory space is needed to rear insects and process samples. Laboratory space does seem to be available at the UoG, but it is also somewhat limited by other activities. The Division of Agriculture & Life Sciences and Center for Island Sustainability appear to have abundant outdoor space for cages to hold plants. A dedicated greenhouse will be required. Also, the Forestry and Soil Resources Division of the Guam Department of Agriculture manages two nurseries with space that they would allow to be used for CAS biological control research and/or rearing natural enemies.

Human resources are a limiting factor. Currently, there are no available personnel for monitoring the 12 Marler plots, collecting samples in the field, and processing the samples in the laboratory. Aubrey Moore is very busy with teaching, extension programming, and the coconut rhino beetle management program. He has no laboratory technicians available for laboratory and field work on CAS. He is in a position supervise a postdoctoral scientist, graduate students, and student interns. The Center for Island Sustainability may be able to provide technical support if funding can be obtained for such purpose. Personnel at the Guam Department of Agriculture are willing to cooperate with issuing import permits.

VI. Action Recommendations

Short-term (1–4 years):

1. Natural enemies are the biological control agents that kill the pest. But to take advantage of these beneficial insects, people need to study them, rear them, introduce them, and disperse them. As mentioned earlier, human resources for work on CAS biological control in Guam are limited. Therefore, the first recommended action is to form a team of qualified individuals willing to dedicate time, knowledge, and experience to the effort of compiling funding from government agencies or funded projects to hire a postdoctoral scientist to lead the laboratory and field activities of the program. The postdoc would also be charged with writing grants, building relationships, performing outreach, monitoring the 12 Marler plots, and supervising a lab/field assistant, graduate students, and student interns. This postdoc could be associated with the University of Guam or possibly be hired as a USFWS biologist.

To support the postdoc, funds will be needed for supplies, travel, and hiring and supervising a lab/field assistant, graduate students, and student interns. There are multiple possible sources of these funds, including USFWS, INRM/Department of Defense, US Department of the Interior (*e.g.*, Office of Internal Affairs), The Nature Conservancy, APHIS PPQ, Guam Department of Agriculture, other granting agencies and foundations, and private donations. And the postdoc will require dedicated laboratory and office space, which may be available at UoG.

To support and advise the postdoc, there should be formed an advisory committee of 4–5 members who would meet with the postdoc 2-3 times per year to provide guidance and recommendations. Membership on this committee should consist of representatives from the UoG, USFWS, Guam Department of Agriculture, and NAVFAC Marianas. I am willing to serve on this advisory committee. Of course, the hiring of a postdoc implies a long-term (5+ years) commitment to a CAS biological control program.

2. A two-year, quantitative survey of the resident lady beetle species in Guam is needed. The information gathered in the survey will reveal which species are mostly closely associated with CAS, how predation rates vary over time, and when and where they are influential as drivers in the population dynamics of CAS. Citizen Science might be a useful tool to gather data, and the data can be analyzed with GPS technology. The survey information will be critical when considering the introduction of other CAS natural enemies, especially other predatory beetles.

3. Surveys should be conducted in Tinian and Rota to determine the lady beetle species that are resident on the islands and the presence of *A. chionaspidis*. The survey will inform which natural enemies might need to be introduced. *Rhyzobius lophanthae* is already on Rota (Marler and Marler 2018), but its effect on CAS populations there is unknown.

4. *Arrhenophagus chionaspidis* is capable of producing high parasitism rates in Guam, as evidenced at UoG and Yigo REC. The parasitoid was commonly found in China, Vietnam, and Thailand during exploration for CAS natural enemies in 2006, 2007, and 2009 (Cave *et al*. 2013). The parasitoid first appeared parasitizing CAS in Florida in 2012, and within a few years it became widespread in the state (R. D. Cave personal observation). It may be coincidental, but CAS infestations in Florida are now less intense.

Arrhenophagus chionaspidis should be prevalent in all of Guam, but apparently this is not the case. Its distribution on the island (and introduction in Rota and Tinian) could be ameliorated by collecting adult wasps where they are abundant (UoG and Yigo REC) and placing them in organdy bags enclosing well-infested leaves on carefully selected individuals of *C. micronesica* or *C. revoluta*. These organdy bags will exclude predators that would eat parasitized scales, and they will maintain the wasps in proximity to their hosts.

5. The larvae and adults of *Cybocephalus nipponicus* Endrödy-Younga (Coleoptera: Cybocephalidae) (**Figure 13**) are predators of armored scale insects. From July to December, it occurs abundantly on king sago palms infested with CAS throughout Florida. Adults are long-lived and consume hundreds of scales during adulthood (**Table 1**). Females may lay over 300 eggs during their lifetime. The predator is widely distributed in Southeast Asia, where it occurs in very large numbers on scale-infested cycads, some very densely, in southern China and Vietnam (R. D. Cave personal observations). The beetle also occurs in Texas and Hawaii (Smith and Cave 2006a). The wasp *Aphanogmus albicoxalis* Evans and Dessart (Hymenoptera: Ceraphronidae) parasitizes the prepupae and pupae of *C. nipponicus* (Evans *et al*. 2005) in Florida, which might affect the predator's ability to significantly suppress CAS population.

Figure 13. Adults and larva of *Cybocephalus nipponicus*. The adult female is all black, whereas the adult male is bicolored. Photos by D. Caldwell.

Cybocephalus nipponicus does not occur in Guam. Serious consideration should be given to its introduction in Guam and other Pacific islands. Due to its specificity to armored scale insects, it is not a threat to native fauna. It is sympatric with *R. lophanthae* in Texas and Hawaii, thus the two species can co-exist, but this needs to be studied experimentally. During exploration in Asia, *A. chionaspidis* and *C. nipponicus* were seen together on plants at multiple localities (Cave *et al*. 2013), giving evidence that these two biological control agents may be able to complement each other's actions.

6. One more attempt at the introduction of the parasitoid *C. fulvus* may be a worthy effort with new techniques. Three attempts at establishing *C. fulvus* in Guam have been made, but there are several possible reasons why establishment did not occur. The wasp should be cultured on securely enclosed *Cycas* plants that have a clean colony of CAS. To increase the potential for establishment, organdy bags can be used to enclose well-infested leaves on carefully selected individuals of *C. micronesica* or *C. revoluta*. These organdy bags will exclude predators that would eat parasitized scales, and they will maintain the wasps in proximity to their hosts.

Coccobius fulvus was released in Barbados with material from Florida, established, and reportedly controls CAS (van Lenteren and Colmenarez 2020). In Florida, however, control of CAS by this natural enemy was deemed unsatisfactory (Cave 2006). Average parasitism by *C. fulvus* on a leaf averages 10–40%, with greater parasitism occurring on leaves with high scale densities. Moreover, female *C. fulvus* seem not to access CAS in cryptic sites on the plant (R. D. Cave personal observation).

7. The species of *Aphytis* that attacks CAS in Hawaii is interesting. This parasitoid causes high parasitism rates that apparently suppress CAS populations in Hawaii (M. G. Wright personal communication, 2022), but this phenomenon should be more closely examined. *Aphytis* sp. is much smaller than *R. lophanthae*; it is hoped that it will attack CAS in refuges too small for the lady beetle to access and will do a better job at protecting cycad seedlings. This can be examined in the field in Hawaii and in the laboratory.

The genus *Aphytis* was thoroughly reviewed by Rosen and DeBach (1979), who based their taxonomy on morphology. The species of *Aphytis* parasitizing CAS has been tentatively identified as *Aphytis lingnanensis*. This parasitic wasp attacks several species of armored scale insects (Noyes 2019) and has been moved around the world for biological control of armored scales insects on citrus. It is very similar to *Aphytis holoxanthus* DeBach and *Aphytis melinus* DeBach, which also parasitize various armored scale insect species and have been dispersed worldwide for biological pest control. Specimens of so-called *A. lingnanensis* have been collected on different hosts in different localities and cannot be morphologically distinguished but do not hybridize. Rosen and DeBach (1979) admitted:

> "Further biosystematics investigation will undoubtedly reveal additional biologically distinct forms which are at present referred to *lingnanensis*. Although very confusing to the taxonomist, discovery of such forms may prove to be of great importance for biological control."

Nowadays, molecular techniques are readily used to distinguish species. The identification of the *Aphytis* species parasitizing CAS in Hawaii should be confirmed with modern genetic analysis.

There was an attempt in 2012 to introduce the *Aphytis* species from Hawaii in Guam but culturing it in the laboratory failed. New techniques can be used to establish a clean colony of CAS in the laboratory to rear *Aphytis* sp., once its species determination has been clarified. Alternatively, wasps collected in Hawaii could be released directly in the field. Whether from the laboratory or Hawaii, the wasps should be placed in organdy bags enclosing well-infested leaves on carefully selected individuals of *C. micronesica* or *C. revoluta*.

<u>Long-term (4–8 years)</u>:

8. The lady beetle *Phaenochilus kashaya* Giorgi and Vandenberg (**Figure 14**) was discovered in Thailand in 2007 (Cave *et al*., 2009a) and found again there in 2009. The cycads in the area where the beetle was found were very sparsely infested with CAS or had no scales at all (R. D. Cave personal observation). Biological studies of the beetle in quarantine in Florida revealed it is a voracious and quite fecund predator (Manrique *et al*. 2012). Instar IV eats 3–5 times more scales per day than instars I–III at 25°C. The predation rate of *P. kashaya* larvae is substantially greater than that of *R. lophanthae* larvae (**Table 1**). Adult *P. kashaya* eat 12 times more scales per day than adult *R. lophanthae*, and they can live up to six months (males live up to four months) at 25° C. Because *P. kashaya* adults eat more scales per day and live considerably longer than *R. lophanthae* adults, *P. kashaya* can kill about 3.5 times more scales than *R. lophanthae* (**Table 1**). At 20° C, a female *P. kashaya* can lay 339 eggs during her adult life, while a female *R. lophanthae* consuming CAS at 24° C lays only about 50 eggs (Thorson 2009).

Figure 14. *Phaenochilus kashaya* adult and larva. Photos by R. D. Cave.

Table 1. Average longevity (L, in days) and daily consumption (DC) and total consumption (TC) of life stages of *Cybocephalus nipponicus* (at 25° C, data from Smith and Cave 2006b), *Phaenochilus kashaya* (at 25° C, data from Manrique *et al*. 2012), and *Rhyzobius lophanthae* (at 24° C, data from Thorson 2009). ND = no data.

	C. nipponicus			***P. kashaya***			***R. lophanthae***		
Stage	**L**	**DC**	**TC**	**L**	**DC**	**TC**	**L**	**DC**	**TC**
Instars I–III	ND	ND	ND	19	4–8	130	14	1–5	29
Instar IV	ND	ND	ND	12	21	246	6	5	58
Larva	14	ND	ND	31	4–21	380	20	1–5	87
Adult female	110	4	440	158	29	915	104	3	281
Adult male	89	4	356	130	29	753	103	3	194

A petition to release *P. kashaya* from quarantine in Florida was denied by USDA APHIS PPQ for various reasons. However, six of nine petition reviewers from Canada, Mexico, and the United States did recommend that it be released, and a representative of Agriculture and Agri-Food Canada indicated there was a consensus that the predator be released in Guam and Rota (P. G. Mason *in litt*. to R. Tichenor, 2014).

Because CAS and *P. kashaya* are native to the same region of Asia, they likely co-evolved, and the predator is finely tuned to the ecology and seasonality of the armored scale insect. Serious consideration should be given to introducing *P. kashaya* to Guam, Rota, and Tinian. However, important questions must be discussed before action is taken. Four questions are:

1. *Is there a real need for another predatory lady beetle feeding on CAS populations in Guam? Rhyzobius lophanthae* is broadly present in Guam, and if not already present in Rota and Tinian, it should be introduced there. Marler *et al*. (2013) presented data from 2006 to 2012 that indicate *R. lophanthae* appears to suppress CAS populations, but outbreaks of the scale do occur. From my observations, predation of CAS was very high in and around the Guam NWR. However, it is unknown if this is just a seasonal observation (*i.e*., relative predation might be less in August–October). Nor was it clear which predator, *R. lophanthae* or the "cocciduline", was responsible for the highest proportion of predation. It is thus imperative to know now if satisfactory control of CAS is occurring where *R. lophanthae* and the "cocciduline" are present, and therefore an additional lady beetle may not be required.

2. *What are the potential antagonistic effects on the populations of resident lady beetles and other possible non-target effects by introducing P. kashaya in Guam and elsewhere?* Biological studies in the laboratory can answer this question to some extent. If *R. lophanthae* and other lady beetles, such as the "cocciduline", are key biological control agents of CAS in Guam, the risks and consequences of *P. kashaya* adversely influencing their populations should be carefully assessed. Non-target testing in quarantine in Florida revealed that *P. kashaya* can feed on non-armored scale insect prey, including larvae of *C. nipponicus* and *R. lophanthae* (**Appendix A**).

3. *Will P. kashaya feed extensively on CAS on seedlings and small individuals of C. micronesica?* This can be determined in a greenhouse study.

4. *Who will collect the P. kashaya stock material in Thailand, who will culture it in quarantine, who will perform the studies in quarantine, and who will mass-rear the beetle, and what will be the source of substantial funding for the endeavor?*

If there are no predators or parasitoids of CAS in Tinian, *P. kashaya* should certainly be introduced to the island to assess its performance outside of its native land.

9. There may be other good natural enemies of CAS in Thailand, which are yet to be discovered. Additional exploration in Thailand, and maybe Cambodia, may be rewarding. As with the collection of *P. kashaya* stock material in Thailand, there are the questions of who will do the exploration (certainly would involve the hired postdoc) and what will be the source of substantial funding for the endeavor.

10. Many questions unrelated to biological control were discussed during meetings and field site visits. The following is a listing of some of these discussion points that would be good topics for research by scientists with the appropriate expertise.

a. Within a small population of *C. micronesica*, some trees have CAS and others do not. Also, some infested trees seem not to suffer from the pest attack, whereas others are very unhealthy or do not survive. Intraspecific genetic diversity of *C. micronesica* may be responsible for tolerance or resistance to infestation by CAS.

b. We observed several quite unhealthy individuals of *C. micronesica* with no or few CAS. Several trees had old, dry leaves drooping from the trunk. There may be a plant pathogen afflicting these trees, which might make them more susceptible to infestation by CAS.

c. Armored scale insects injure their host plants by removing sap and injecting toxic saliva during feeding (McClure 1990). It is not known how many CAS on a plant will cause the death of cycad, either by intense feeding or weakening the host to make it susceptible to lethal plant pathogens.

VII. Closing Remarks

It must be clearly understood that no biological control agent(s) can eradicate CAS from Guam or any other Pacific island. All that can be hoped from biological control is that the pest be suppressed to levels at which it is no longer considered an ecological pest. In the case of CAS threatening Guam's *C. micronesica* trees, that is a big ask. Biological control agents of CAS may be able to do this, but only in an environment that favors them. The cycad aulacaspis scale has now been in Guam for nearly 20 years. During that time, valid attempts have been made to bring it under biological control, but no established natural enemies have adequately suppressed the armored scale insect. Other biological control options may or may not be successful, but they will not be easy to implement. No solution to the CAS problem or the amelioration of the condition of the *C. micronesica* population in Guam and elsewhere will be easy. If it were easy, it would have happened by now. But to give any option the best chance possible, there must be a focused scientific effort backed by adequate and continuous funding and public awareness and support that can be cultivated by effective Extension programming. People, money, and time. Applied biological control is a team effort.

VIII. Acknowledgments

I am grateful to Dr. Toni Mizerek for facilitating my visit to Guam, and to the US Fish and Wildlife Service for providing the funding for my travel. I thank Dr. Aubrey Moore for hosting me, taking me to field sites, arranging my seminar, and allowing me to use his imaging and microscope equipment. The use of a University of Guam vehicle for travel between the hotel and the university was great appreciated.

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Professor and Center Director
May 5, 2022

APPENDIX A

Host range testing of *Phaenochilus kashaya* in quarantine at the Biological Control Research and Containment Laboratory, Indian River Research and Education Center, University of Florida. Excerpted from the “Petition for the Release from Quarantine of *Phaenochilus kashaya* Giorgi and Vandenberg for Biological Control of the Cycad Aulacaspis Scale, *Aulacaspis yasumatsui* Takagi Petition for the Release from Quarantine of *Phaenochilus kashaya* Giorgi and Vandenberg for Biological Control of the Cycad Aulacaspis Scale, *Aulacaspis yasumatsui* Takagi” submitted to APHIS PPQ by Ronald D. Cave in 2014.

3.8 Known host range

The host range of adult *P. kashaya* was studied in the Gainesville and Ft. Pierce quarantine facilities. Thirty-six potential prey species (Tables 7 and 8) were offered to adult *P. kashaya* under laboratory conditions. All *P. kashaya* used in these trials were starved for 24 hours prior to testing. The number of prey offered, either individually or in small groups, varied among species. Experiments were conducted inside environmentally controlled chambers (25 ± 2°C, 60-80% RH, 14L: 10D photoperiod) using two arena types: 1) plastic Petri dishes (9 cm diameter) containing a moistened cotton wick or ball and sealed with Parafilm; or 2) potted host plants enclosed by a clear acrylic cylinder cage (45 cm height × 15 cm diameter). Trials with the predators and parasitoid as offered prey were conducted only in Petri dishes. Each species listed in Tables 7 and 8 was exposed to one adult *P. kashaya* during a 24-hour period under no-choice conditions (no CAS provided). Mummies of CAS parasitized by *C. fulvus* were extracted from underneath their covering armor and exposed to one *P. kashaya* adult. Choice tests used only the Petri dish arena with 5-10 female CAS presented with the offered prey for 24 hours. Control groups comprised the potential prey species in the same arena types without *P. kashaya* present.

No mortality or missing individuals were detected in the control groups. Therefore, dead and missing prey individuals in the arenas with *P. kashaya* were assumed to have been killed or consumed by the lady beetle. *Phaenochilus kashaya* adults fed on 10 of the 30 herbivorous insect species tested (Table 7). Three of four species of armored scales (Diaspididae) were eaten, but none of the soft scales (Coccidae) or red date scale (Phoenicococcidae) was consumed. Among the three species of mealybugs (Pseudococcidae), only one species, the Madeira mealybug, was eaten, but this species was offered as very small nymphs (first and second instars) and adults, and only the nymphs were eaten, not the adults. Only 5% of the sweetpotato whitefly nymphs offered was eaten, but no nymphs of the giant whitefly, a larger insect, were consumed. Although there was no predation on the Asian citrus psyllid, there was some apparent predation on the melaleuca psyllid (two out of eight nymphs exposed). The *P. kashaya* adults were not observed feeding on the melaleuca psyllid, but two psyllid nymphs could not be found in the sealed Petri dish, and the conclusion was that the nymphs had been consumed by the beetle. However, in choice tests in which CAS was offered along with one melaleuca psyllid per replicate (four replicates), no predation on the psyllid was evident (Table 9). Among the three species of aphids, only one species, the green peach aphid, was eaten. However, similar to the predation on the Madeira mealybug, only the very small nymphs of the aphid, no adults, were consumed. In no-choice tests, adults of *P. kashaya* were exposed to eggs of three species of butterflies: the painted lady, the buckeye butterfly, and the Julia butterfly. In two of 10 replicates with painted lady eggs, feeding by the predator was evident. In one of 10 replicates with buckeye butterfly eggs, feeding by the predator was evident. There was no evidence of feeding on Julia butterfly eggs in any of the 10 replicates. However, in choice tests with eggs of the three butterfly species presented along with CAS, no feeding on eggs by *P. kashaya* was observed (Table 9).

Some intraguild predation was observed when *P. kashaya* adults were offered the larvae of the predatory beetles *R. lophanthae* and *C. nipponicus*, the eggs of the green lacewing (*Chrysoperla rufilabris* Burmeister), and mummies of *C. fulvus* (Table 8). The young larvae of *R. lophanthae* and *C. nipponicus* and mummies of *C. fulvus* were fully exposed larvae and mummies in the trials. The early instars of *R. lophanthae* and *C. nipponicus* and the larvae and pupae of *C. fulvus* in CAS mummies normally reside below the armor of CAS, and this armor may provide some protection from *P. kashaya* predation. Predation by *P. kashaya* adults on green lacewing eggs not on stalks was very high in both no-choice and choice tests (Table 8). Larvae of *P. kashaya* also fed on green lacewing eggs, consuming 16 of 25 eggs not on stalks and 13 of 25 eggs on stalks.

Predation by *P. kashaya* on the predatory beetle larvae may be a behavioral mechanism to reduce interspecific competition under limited spatial conditions when primary prey (CAS) is relatively scarce. On the other hand, being such voracious predators, the *P. kashaya* adults may prey on the *R. lophanthae* and *C. nipponicus* larvae only after all the CAS have been consumed; this was observed in the choice tests. However, both these scenarios may not play out in the field. A *P. kashaya*-*C. nipponicus* compatibility experiment on whole plants was conducted inside a rearing room (26 ± 2°C, photoperiod of 14:10 L:D, 60-80% RH). Adults (equal sex ratio) of *P. kashaya* and *C. nipponicus* were simultaneously placed together in two cages (60 × 60 × 60 cm): cage 1 was inoculated with eight *P. kashaya* and eight *C. nipponicus*; cage 2 was inoculated with 16 *P. kashaya* and 16 *C. nipponicus*. Each cage contained two potted *C. revoluta* infested with abundant CAS and a cotton wick with honey: water (1:1) solution; plants were watered as needed. Every 10 days, another scale-infested cycad plant was added to each cage. The experiment was terminated at 1½ months, and the number of adults of each predator species in each cage was counted. Cage 1 had 20 *P. kashaya* and 40 *C. nipponicus* adults; cage 2 had 41 *P. kashaya* and 55 *C. nipponicus* adults. According to these results, both predatory beetles are able to co-exist and population densities increased when caged together.

Predation by *P. kashaya* on mummies of CAS parasitized by *C. fulvus* is not too surprising since this prey smells like CAS, which may have elicited feeding behavior by the beetle. This may also be a reason why larvae of *C. nipponicus* and *R. lophanthae* were preyed upon, because after crawling among and feeding on CAS, they smelled like CAS. *Cybocephalus nipponicus*, *R. lophanthae*, and *C. fulvus* are non-native insects in Florida, and though they are documented natural enemies of CAS, none of them appear to be providing adequate control of the pest.

Table 7. Predation by adult *Phaenochilus kashaya* offered herbivorous arthropods in no-choice tests. Species consumed are in **bold**.

Species offered	Arena type	# prey offer	# consumed
HEMIPTERA			
Diaspididae			
1. ***Aonidiella orientalis*** (oriental scale) ♀♀	caged host plant	32	5
2. *Fiorinia theae* (tea scale) ♀♀	Petri dish	52	0
3. ***Pseudaulacaspis cockerelli*** (false oleander scale) ♀♀	Petri dish	50	50
4. ***Pinnaspis strachani*** (lesser snowscale) ♀♀	Petri dish	65	32
Coccidae			
5. *Ceroplastes ceriferus* (Indian wax scale) ♀♀	Petri dish	12	0
6. *Saissetia coffeae* (hemispherical scale) ♀♀	caged host plant	17	0
Phoenicococcidae			
7. *Phoenicococcus marlatti* (red date scale) ♀♀	Petri dish	25	0
Pseudococcidae			
8. *Maconellicoccus hirsutus* (pink hibiscus mealybug) ♀♀	Petri dish	67	0
9. *Pseudococcus longispinus* (longtail mealybug) ♀♀	Petri dish	23	0
10. ***Phenacoccus madeirensis***			
(Madeira mealybug) nymphs	Petri dish	30	21
♀♀	Petri dish	5	0
Aleyrodidae			
11. ***Bemisia tabaci*** (sweetpotato whitefly) nymphs	Petri dish	37	2
12. *Aleurodicus dugesii* (giant whitefly) nymphs	Petri dish	14	0
Psyllidae			
13. *Diaphorina citri* (Asian citrus psyllid) nymphs	caged host plant	37	0
14. ***Boreioglycaspis melaleucae*** (melaleuca psyllid) nymphs	Petri dish	8	2

Table 7 continued.

Aphididae			
15. *Aphis craccivora*	caged host plant	27	0
(cowpea aphid) ♀♀			
16. *Toxoptera citricida*	caged host plant	14	0
(brown citrus aphid) ♀♀			
17. ***Myzus persicae***			
(green peach aphid) nymphs	Petri dish	75	60
♀♀	Petri dish	10	0
Blissidae			
18. *Blissus insularis*	Petri dish	10	0
(southern chinch bug) adult			
THYSANOPTERA			
Phlaeothripidae			
19. *Pseudophilothrips ichini*	Petri dish	10	0
(Brazilian peppertree thrips) nymphs			
20. *Selenothrips rubrocinctus*			
(red-banded thrips) nymphs	Petri dish	8	0
adults	Petri dish	4	0
LEPIDOPTERA			
Gracillariidae			
21. *Phyllocnistis citrella*	Petri dish	41	0
(citrus leafminer) larvae in mines			
Pyralidae			
22. *Cactoblastis cactorum*	Petri dish	10	0
(cactus moth) larvae			
Noctuidae			
23. *Trichoplusia ni*	Petri dish	15	0
(cabbage looper) eggs			
Nymphalidae			
24. ***Vanessa carduii***	Petri dish	10	2
(painted lady) eggs			
25. ***Junonia coenia***	Petri dish	10	1
(buckeye butterfly) eggs			
26. *Dryas julia*	Petri dish	10	0
(Julia butterfly) eggs			
COLEOPTERA			
Curculionidae			
27. *Oxyops vitiosa*	Petri dish	10	0
(melaleuca weevil) larvae			

Table 7 continued.

COLEOPTERA			
Chrysomelidae			
28. *Microtheca ochroloma* (yellowmargined leaf beetle) larvae	Petri dish	20	0
29. *Gratiana boliviana*			
(TSA tortoise beetle) eggs	Petri dish	5	0
larvae	Petri dish	5	0
ACARI			
Tetranychidae			
30. *Tetranychus urticae* (two-spotted spider mite) adult	Petri dish	90	0

Table 8. Predation by adults of *Phaenochilus kashaya* on immature stages of five predators (species 1-5) and one parasitoid (species 6) in no-choice and choice tests in Petri dishes.

	No-choice tests		**Choice tests**	
Species offered	**# exposed**	**# consumed**	**# exposed**	**% consumed**
COLEOPTERA				
Cybocephalidae				
1. *Cybocephalus nipponicus*				
early instars	5	5	4	1
Coccinellidae				
2. *Rhyzobius lophanthae*				
early instars	4	3	6	4
late instars	not tested		5	2
3. *Cryptolaemus montrouzieri*				
early instars	7	0	not tested	
4. *Cycloneda sanguinea*				
early instars	4	0	not tested	
NEUROPTERA				
Chrysopidae				
5. *Chrysoperla rufilabris*				
eggs	100	95	75	70
early instars	5	0	5	0
HYMENOPTERA				
Aphelinidae				
6. *Coccobius fulvus*				
larvae/pupae in mummies	4	4	6	1

Table 9. Predation by adult *Phaenochilus kashaya* in choice tests pairing female *Aulacaspis yasumatsui* with alternate herbivorous species offered in Petri dishes.

Species offered	# prey offered	# consumed
HEMIPTERA		
Boreioglycaspis melaleucae		
(melaleuca psyllid) nymphs	4	0
LEPIDOPTERA		
Vanessa carduii		
(painted lady) eggs	78	0
Junonia coenia		
(buckeye butterfly) eggs	57	0
Dryas julia		
(Julia butterfly) eggs	9	0

Predation by *P. kashaya* on mummies of CAS parasitized by *C. fulvus* is not too surprising since this prey smells like CAS, which may have elicited feeding behavior by the beetle. This may also be a reason why larvae of *C. nipponicus* and *R. lophanthae* were preyed upon, because after crawling among and feeding on CAS, they smelled like CAS. *Cybocephalus nipponicus*, *R. lophanthae*, and *C. fulvus* are non-native insects in Florida, and though they are documented natural enemies of CAS, none of them appear to be providing adequate control of the pest.

The high rate of predation on green lacewing eggs is somewhat surprising since consumption of lepidopteran eggs was very limited. Extensive examination of numerous CAS-infested cycads in the field has never detected green lacewing eggs or larvae on the plants (R. D. Cave, personal observation). Any green lacewing eggs laid on cycads would be incidental since the lacewing females lay their eggs on any substrate (Canard *et al*. 1984). Green lacewing larvae feed on a variety of small, soft-bodied arthropods (Tauber *et al*. 2000). They are considered excellent aphid killers (Hydorn and Whitcomb 1979; Canard *et al*. 1984; Dean and Schuster 1995; Chen and Liu 2001; Knutson and Tedders 2002), but they are also known as biological control agents of whiteflies (Legaspi *et al*. 1994; Dean and Schuster 1995), moth eggs (Lingren *et al*. 1968; Hydorn and Whitcomb 1979), leafhoppers (Daanel and Yokota 1997), and mites (Canard *et al*. 1984). For this reason, green lacewings are commercially available to growers for pest management of these arthropods. Except for saprophagous mites, none of these arthropods are associated with cycads (R. D. Cave, personal observation). Green lacewing larvae are not predators of armored scales because the immature predator cannot penetrate the scale's armor with its mandibles to suck out the nutrients from the female scale's body. Given the information described herein, *P. kashaya* is not expected to interfere with green lacewings serving as biological control agents of plant pests. The prey preferences of the two natural enemies are different, although *P. kashaya* does eat small mealybugs, aphids, and whitefly nymphs and if it does feed on these insects in the field, then it might enhance the biological control of these plant pests. Moreover, green lacewings do not restrict their oviposition to sites where *P. kashaya* is expected to occur, and green lacewings are geographically widespread, even occurring in areas in the northern temperate region not likely to be populated by *P. kashaya*.

Predation on green lacewings by coccinellids in intraguild predation studies in the laboratory has been documented in two studies. Lucas *et al*. (1998) observed that fourth instar and adult *Coleomegilla maculata* (De Geer) frequently attacked groups of green lacewing (*C. rufilabris*) eggs. Larval and adult *C. maculata* consumed 24-84% of the lacewing eggs in a group, but this occurred in less than half of the tests. *Coleomegilla maculata* is a generalist predator with a geographic range from southern Canada to northern South America (Gordon 1985). Green lacewing larvae also preyed on the lady beetle eggs and larvae. At the species level, Lucas *et al*. (1998) consider the intraguild predation between these two species as mutually equalized. Phoofolo and Obrycki (1998) showed that *C. maculata* and *Harmonia axyridis* Pallas are able to complete their larval and pupal development when the eggs of *Chrysoperla carnea* Stephens serve as prey. Weight of adult females of both lady beetles was less than when pea aphids, *Acyrthosiphon pisum* (Harris), were offered as prey. Phoofolo and Obrycki (1998) noted that exclusive consumption of green lacewing eggs may have some negative consequences, particularly on reproduction, since fecundity is directly related to female body size (Stewart *et al*. 1991). Larvae of *Coccinella septempunctata* L., a non-native species in North America, were unable to complete their development with a strict diet of green lacewing eggs (Phoofolo and Obrycki 1998); this lady beetle is an aphid-feeding specialist. Phoofolo and Obrycki (1998) commented that levels of intraguild predation between green lacewings and aphidophagous lady beetles might not reflect those that may occur in the field because in the laboratory experiments the predators were confined, thus magnifying interaction. This concept also applies to the green lacewing-*P. kashaya* interaction. Coupled with the distinct habitat and prey preferences of the two species, the intraguild predation between these two species in the field is likely to be negligible.