The latest version of this document and source files are available from https://github.com/aubreymoore/CAS/

Biological Control of the Cycad Aulacaspis Scale, *Aulacaspis* yasumatsui

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Ecological impact of CAS invasions varies greatly with location, largely due to differences in characteristics of host plant populations, climate, and presence of natural enemies. When CAS arrived in Florida (1995) and Hawaii (1998), it became an economic pest of ornamental cycads which could be protected using a combination of pesticide applications and biological control. However, when CAS arrived in Guam (2003), it started out as an economic pest of ornamental cycads Cycas revoluta and Cycas micronesica but rapidly spread to the wild Cycas micronesica population, causing an uncontrolled island-wide outbreak. This chapter will be focused on the ecological impact to C. micronesica and the forest ecosystem on Guam.

4.1 *C. micronesica* taxonomy and pre-invasion status

Cycas micronesica was described as a species in 1994 (Hill, 1994). Prior to description, it was identified as C. rumphii or C. circinalis. This tree-sized cycad is endemic to Micronesia and it is the only endemic gymnosperm on these islands. It grows in the wild in a wide variety of habitats on Guam, the Northern Mariana Islands, the Yap Islands, and the Palau Islands. Individuals are either male or female.

Based on an island-wide census of Guam's trees performed by the U. S. Forest Service in in 2002, a year before arrival of CAS, *C. micronesica* was identified as the most abundant tree in Guam's forests Donnegon et al., 2004. It thrived because it had evolved tolerance to local abiotic threats such as typhoons and drought and for this reason it was often used as a low maintenance ornamental plant. Indigenous people of the Mariana Islands, the Chamorros, named this plant *fadang* and used it as a source of starch. But there is no evidence that the plant was ever planted as a crop.

Haynes and Marler, 2005 described Guam's *C.micronesica* population from a historic perspective:

Our height increment data indicate that many of Guam's coastal cycad plants are hundreds of years old. These plants have survived the Spanish-American War and two world wars; they have survived innumerable tropical cyclones; they have survived the invasion of intentionally introduced feral deer and pig populations and the accidental introduction of various insect species. Some of these individual plants "watched" Ferdinand Magellan and his fleet sail along Guam's coast on 6 March 1521. And they endured the Spanish-Chamorro Wars that decimated the indigenous human population. Truly, the remaining plants comprise a long-lived botanical and cultural treasure, one that is in danger of disappearing forever. We simply cannot elect to continue to watch this tragedy without attempting to intervene. The impending cascade of detrimental effects is looming too large to justify apathy.

4.2 First detection and invasive pathway for arrival of *A. yasumatsui* on Guam

Arrival of CAS on Guam was predicted: on February 13 2000 T. E. Marler published an article in the Gardening section of the Pacific Daily News entitled *Looking out for scale insects* (Haynes and Marler, 2005). Alarmed by establishment of CAS in Hawaii, Marler warned of pending arrival on Guam and pleaded for a ban cycad imports to the island.

CAS was first detected in the Tumon Beach hotel area of Guam near the end of 2003 on *C. micronesica* and *C. revoluta* growing as ornamental plants at two hotels. In those days, almost every hotel on Guam had cycad displays near their entrances.

The invasion pathway along which CAS travelled to Guam is unknown. It is likely that this pest arrived via importation of infested cycads from Hawaii, Florida or elsewhere. However, there are is no evidence of this: there are no records of legal cycad importation to Guam in the two years prior to detection of CAS on the island (R. Campbell, Guam Plant Inspection Facility, personal communication to AM).

An intriguing alternative is that CAS arrived on Guam as crawlers. For several years prior to arrival, there was an active infestation of CAS on *C. revoluta* growing in an outdoor garden at the Honolulu International Airport located within a few hundred meters of where passengers boarded a daily 7.5 hour flight to Guam. Possibly, crawlers were carried on clothing of passengers visiting this garden or airborne scale crawlers may have been blown into cargo holds, wheel wells, or other spaces on Guam-bound aircraft. The location of initial CAS detection sites in Tumon Bay are only about 1 km downwind of the Guam International Airport.

4.3 Impacts of CAS on the Guam population of *C. micronesica*

The Guam CAS outbreak, which was severe and spread rapidly throughout the island (Table 4.3, Figure 1), was well documented by Marler and others. Within 12 years, *C. micronesica* went from being the most abundant tree in Guam's forests to being listed under the U.S. Endangered Species Act. It should be noted that since arrival of CAS, several other recently arrived invasive insect species and even some native species have begun attacking and damaging Guam's cycads (Marler and Muniappan, 2006, Moore, Miller, Marler, and Yudin, 2013, (Deloso et al., 2020)). However, CAS is considered to be the prime driver of population decline because it is the only herbivore causing damage severe enough to result in mortality.

	Table 1: Timeline for CAS Pacific island invasions.
2000	T. E. Marler predicts of arrival of CAS on Guam in a Pacific
	Daily News article (Haynes & Marler, 2005) in response to
	detection of this pest in Florida during 1996 (Howard et al.,
	1999) and Hawaii during 1998 (Heu et al., 2003)
2000	CAS first detected in Taiwan infesting wild <i>C. taitungensis</i> ,
	the endemic cycad of Taiwan
2002	An initial US Forest Service survey indicates that C .
	micronesica is Guam's most abundant tree (with stem
	diameter greater than 5 inches) and estimates a population
	size of 1,571,556 (Donnegon et al., 2004)
2003	CAS first detected on Guam infesting ornamental Cycas
	revoluta and C. micronesica at hotels in Tumon Bay
2006	C. micronesica added to the IUCN Red List of Threatened
	Species
2007	CAS first detected on Rota
2007	CAS first detected in Palau
2008	C. micronesica seedlings from Guam established in a
	germplasm collection on Tinian because this island was
	assumed to be free of A. yasumatsui
2015	C. micronesica listed as a threatened species under the US
	Endangered Species Act (United States Government, 2015)
2019	Scale insects found infesting <i>C. micronesica</i> in the Tinian
	germplasm conservation plots (Presumed to be CAS, but
	identification is still pending) (Andersen Air Force Base, 2021).
2020	Data from annual surveys of 120 permanent plots established
	in 12 habitats dispersed throughout Guam in 2005 show that
	the $C.$ micronesica suvival is 4% of the initial population and
	no natural reproduction is occurring (Marler & Krishnapillai,
	2020)

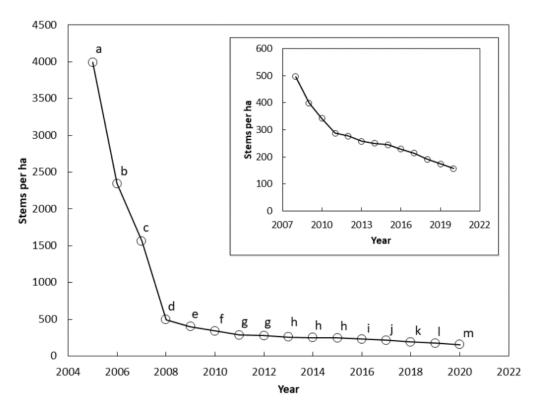


Figure 1: [From Marler and Krishnapillai, 2020] The number of *Cycas micronesica* stems per ha (all size categories) among 12 Guam habitats from 2005 until 2020. The inset shows results from 2008 until 2020 with smaller vertical axis range. Ordinates of markers with the same letter are not significantly different.

Rapid decline of the Guam *C. micronesica* population was documented by Marler and Krishnapillai, 2020 (Figure 1). Data are annual cycad stem counts in 120 permanent plots established after infestation of Guam's wild cycads, but prior to any mortality. It can be seen in the figure that cycad stem count declined to only 12.5% of the original within the first 3 years of the survey (2005-2008) and continued to decline to only 4% of the original count in succeeding years (2009-2020). In addition to high plant mortality surviving cycads stopped reproducing in the Guam plots: the last seedling (0-10 cm tall) was seen in 2014. Between 2014 and 2020 the annual mortality rate for mature plants was relatively stable at about 14 per ha.

4.4 Impacts of CAS on C. micronesica individuals

Immediate impacts of CAS on health of *C. micronesica* individuals is very obvious. On Guam, unprotected plants typically die within 18 months (REF) after being infested. Several overlapping generations of CAS totally encrust all leaves within a few months of infestation. Plant mortality is caused by a combination of nutrient removal by CAS sap feeding plus blockage of photosynthesis. Scales covers of dead insects are tightly affixed to the plant and these do not easily wash off. So photosynthesis may be blocked even after scale insects are killed. Muniappan et al., 2012 suggest that toxic effects of CAS saliva may also be involved in cycad mortality.

Secondary impacts of CAS on health of C. micronesica individuals are not obvious.

4.4.1 Mature plants which have survived CAS infestation have reduced reproductive capacity

Perhaps the most important secondary impact is much lower reproductive capability in plants recovering from CAS-infestation.

Marler and Cruz, 2019 reported that seeds from CAS-infested plants are deficient in nonstructural carbohydrates and germination rates are much lower: 43% of seeds from healthy plants germinated versus 7% of seeds from infested plants.

In addition, Marler and Terry, 2021 reported that mature male plants which survived the initial CAS invasion have significantly smaller cones than prior to infestation. Average cone volume was 24% of pre-infestation levels in 2007 and this increased to 57% in 2021.

4.4.2 Mature plants which have survived CAS infestation are more susceptible to typhoon damage

C. micronesica and other endemic plants on Guam have evolved tolerence for typhoon strength winds (greater than 118 km/h) because tropical cyclones frequently visit this island. (Marler, 2013) reported on results of nondestructive winching stress tests performed on C. micronesica stems to simulate effects of typhoon strength winds. Stems of plants which had not been infested by CAS were significantly stiffer than those which had been infested by CAS for either two or five years. Marler hypothesized from this finding that CAS-infested plants were much more susceptible to stem failure during typhoons.

Evidence supporting this hypothesis came two years after the original article was published, from a damage survey after Typhoon Dolphin passed over Guam on May 15, 2015. Marler et al., 2016 compare the level of damage from Typhoon Dolphin with that of a previous cyclone, Supertyphoon Paka:

Supertyphoon Paka caused severe damage to Guam's forest resources in 1997 when the *C. micronesica* population was healthy and not threatened by any known invasive insect herbivores. Less than 2% of the healthy *C. micronesica* population exhibited windsnap damage during peak winds of 298 km/h. In contrast, Typhoon Dolphin's peak winds of only 170 km/h. caused windsnap of 6% (mean of our eight sampling sites) of Guam's unhealthy *C. micronesica* population after only 10 years of *A. yasumatsui* infestations.

Windsnap is synonymous with stem failure. It is interesting to note that, because energy is a function of the square of wind speed, maximum winds generated by Paka were about 3 times more powerful than those of Dolphin.

4.5 CAS-induced cascading impacts on Guam's forest ecosystems

An ecological cascade effect is a series of secondary extinctions that are triggered by the primary extinction of a key species in an ecosystem. *C. micronesica* is considered to be a key species in Guam forest ecosystems because it was the most abundant tree prior to arrival of CAS. Loss of this plant is likely to threaten survival other organisms.

4.5.1 Herbivores which depend on C. micronesica for food

Marianus fruit bat, *Pteropus mariannus* Haynes and Marler, 2005 reported:

The fleshy, aromatic covering of fadang seeds is a preferred food item for the endangered Mariana fruit bat, *Pteropus marianus marianus*. Fadang is so resistant to most types of disturbance that its seeds are sometimes the only bat food item available in the forest following the destructive winds of a passing cyclone. Fewer than 100 Mariana fruit bats remain on Guam and it is unknown what effect the loss of fadang will have on these endangered bats.

In 2020, the US Fish and Wildlife Service estimated that only 45 fruit bats remain on Guam in a single roost site on Andersen Air Force Base.

Dihammus marianarum and Anatrachyntis sp. Marler and Lawrence, 2013 reported:

At least two arthropods depend on the *C. micronesica* population; the endemic stem borer *Dihammus marianarum* exploits cycad stem tissue for larval food, and the cone borer *Anatra-chyntis* sp requires microstrobili (male cones) for larval food.

Dihammus marianarum (=Acalolepta marianarum) is an endemic longhorn beetle (Cerambicidae), which became a secondary pest of *C. micronesica* after plants were infested with CAS. This beetle has many larval host plants (Marler & Muniappan, 2006) so *C. micronesica* is probably not critical for its survival.

On the other hand the cosmopterigid moth, *Anatrachyntis* sp, has been identified as a probable pollinator of *C. micronesica* and it has been suggested that this insect may be an obligate symbiont, meaning that it cannot survive without the cycad.

An abundance of larvae are found in every male cone following pollen shedding. Pupation takes place in a silken cocoon on the surface of the cone (Marler & Muniappan, 2006). Terry et al., 2009 used sticky traps to sample insects and pollen in the vicinity of *Cycas micronsica* cones. They reported:

On female cone sticky traps, 30% of the pollen grains were associated with Anatrachyntis moths or moth scales and less than 5% with other insects; however, over 60% of the pollen was not associated with any insect, suggesting some pollen is wind dispersed.

Based on these observations, they hypothesized that *C. micronesica* is pollinated both by wind and insects, with *Anatrachyntis* sp being an important insect pollinator.

No lepidopteran had previously been identified as a cycad pollinator. However, nine years later Hua et al., 2018 reported that a moth in the same genus, *Anatrychintis badi*, was discovered feeding in male cones of a native Florida cycad, *Zamia integrifolia*, and suggested that this species may also be an insect pollinator.

Details of the hypothesized symbosis between the unidentified *Anatra-chyntis* species and *C. micronesica* on Guam are not fully understood. The relationship between partners in this symbiosis differs markedly from than between flowering plants and their insect pollinators:

- 1. The reward for pollination service is provided to the caterpillar stage by the male plants which provide food in the form of sacrificial tissues within the male cone. The male cone may also provide a site free from competitors and natural enemies. The presumption here is that the caterpillars are protected by cycad toxins to which they have evolved tolerance.
- 2. There is no known reward provided to adults moths carrying pollen to female cones for pollination to take place. It is not known how *Anatrahynitis* moths are attracted to female cones of *C. micronesica*.

The biology of *Atrachyntis* on Guam is largely unknown apart from its association with *C. micronesica*. It is possible that this species is highly specialized and that its survival is totally dependent on availability of male cones. Alternatively, it is possible that this moth is not an obligate symbiont, but has broad host range, making it less dependent on *C. micronesica*.

4.5.2 Ecosystem services provided by *C. micronesica*

Nitrogen fixation and impacts on forest soils *C. micronesica* is the only dominant native tree on Guam which nitrogen-fixing sympionts. Therefor, the native biota of Guam's habitats has developed with this living resource provisioning the forest ecosystem with nitrogen (Marler & Terry, 2011). In the case of cycads, the nitrogen fixing symbionts are cyanobacteria growing in specialized structures called coralloid roots. Marler and Krishnapillai, 2018 showed that *C. micronesica* plants impact the health of forest soils in ways additional to nitrogen fixation.

4.5.3 Prognosis for Guam's Forests

Currently, mature *C. micronesica* in Guam's forests are dying and these are not being replaced by seeds or juvenile plants. It is obvious that without a change, this endemic plant, the most abundant tree in Guam's forests only two decades ago, is headed towards local extinction.

Pending extirpation of Guam's native cycads is not the first or last ecological disaster caused by invasive species in Guam's forests (Moore, 2018). The brown tree snake, *Boiga irregularis*, first detected in 1953 caused extinction or extirpation of all Guam's forest birds, also removing the ecosystem services they provided such as seed disperal, insectivory, and pollination. The coconut rhinoceros beetle, *Oryctes rhinoceros*, first detected in 2007 is killing large numbers of coconut palms, *Cocus nucifera*, and the introduced palma brava, *Heterospathe elata*. These palm species were identified as Guam's second and third most abundant trees in the 2002 forestry survey. (Donnegon et al., 2004).

Restoration of Guam's forests to their pristine state is no longer possible. However, there is an urgent need to control CAS and other key invasive species so that some recovery can take place, without further loss of biodiversity.

5 Natural enemies of CAS - Cave

6 Classical biological control

- 6.1 Florida Cave
- 6.2 Hawaii Wright
- 6.3 Guam Moore

6.3.1 Rhyzobius lophanthae

About 100 adults of *Rhyzobius lophanthae* were field collected on Maui and imported to Guam during November 2004. This coccinelid was originally introduced to California from Australia in 1892 and to Hawaii from California in 1894. It was observed feeding voraciously on CAS shortly after arrival of this new pest in Hawaii.

R. lophanthae was previously introduced to Guam on two separate occasions under various synonyms: R. satelles Blackburn, Lindorus lophanthae (Blaisdell), and R. pulchellus Montrouzier (Nafus and Schreiner, 1989). In 1925 and 1926 Rhyzobius satelles was imported to Guam from California to control the coconut scale, Aspidiotus destructor Signoret. However, attempts at field establishment failed.

Nafus and Schreiner, 1989 also reported:

In 1971, Rhyzobius satelles Blackburn (as R. pulchellus Montrouzier) was introduced to Guam from New Caledonia to aid in the control of coconut scales and citrus scales. A single specimen of R. satelles was recovered in 1978, indicating establishment. The beetle, however, is very uncommon; an intensive survey of coconut insects in 1984 yielded no specimens.

The beetles from Maui were reared on scale-infested *C. micronesica* cuttings placed in a large screened camping tent set up in a laboratory. Adult offspring were collected for field release by aspirating them from the walls of the tent into plastic vials. Field releases were initiated on February 16 2005 at the Guam National Wildlife Refuge at Ritidian Point. The beetles established readily. By July 7 2005 high densities on adults were observed on cycads anywhere within a 1 km radius of the release site. Establishment and dispersion of the beetles were monitored using yellow sticky traps deployed between June 2005 and May 2006. Unexpectedly, we were also able to monitor CAS crawlers and adult males using these traps (Fig. 2) (Moore, 2017). Following establishment of *R. lophanthae* at Ritidian Point, laboratory-reared and field-collected beetles were released at about 30 other sites throughout Guam.

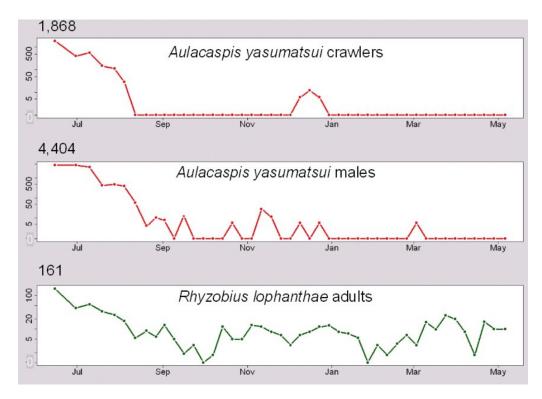


Figure 2: Insects trapped on yellow sticky cards at Ritidian Point, Guam following field release of Rhyzobius lophanthae in February, 2005. X axis runs from July 2005 through May 2006; Y-axis, in log scale, is number of insects trapped per square meter per day. [Figure from Moore, Miller, and Marler, 2013]

By about 2010, *R. lophanthae* larvae or adults could be found on almost every CAS-infested cycad on Guam, preventing CAS from killing mature cycads. By 2010, about 90% of wild cycads had been killed on Guam (REF). Unfortunately, the *C. micronesica* population is not recovering because almost all seeds and seedlings are being killed by CAS and other causes (REF). Marler et al., 2013 showed that *R. lophanthae* predation of CAS is significantly reduced close to the ground and suggest that this may partially account for failure of the beetle to protect CAS seedlings. They also suggested:

The causes of reduced scale predation by $R.\ lophanthae$ near the ground are unknown, but a parasitoid biological control agent may not exhibit these same limitations. Furthermore, because a parasitoid would be much smaller than $R.\ lophanthae$, it would likely be better able to access scale infestations within cracks and crevices on $C.\ micronesica$ and $C.\ revoluta$ trees.

6.3.2 Coccobius fulvus

- According to Reddy's Hatch project report (USDA-REEIS, 2007) *C. fulvus* (China strain) was imported from FL and released at 3 sites on Guam (by Muni in 2005?) checking on this
- According to Reddy's Hatch project report *C. fulvus* (Thailand strain) was imported from FL (not sure if any were released) checking on this
- Moore imported field collected *C. fulvus* from Cave in FL on 2 or 3 occasions; lab rearing failed; some were directly field released at Ritidian Point (will check hardcopy records)

6.3.3 Aphytis lignanensis

Moore attempted to import this species from field collections in HI but Wright and Kaufman were unable to establish to colonize them because they were outcompeted by *Rhizobius lophanthae* (will check hard copy records)

6.3.4 Arrhenophagus

In 2013 Moore discovered *Arrhenophagus* attacking CAS on Guam and it has since become very common. It was assumed that this was a fortuitous introduction. However, Reddy's 2007 Hatch report indicates import of A. chionaspidis from Florida to Guam. Checking to see if any of these were released on Guam.

6.4 Elsewhere - Cave

7 Prospects for future action - Cave, Wright, and Moore

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