IUCN/SSC Cycad Specialist Group – Subgroup on Invasive Pests
Cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae): Review of biocontrol agents and the process of their introduction into infected wild populations of cycads

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Introduction

Cycads are recognized as the most endangered group of plants on earth by the International Union for the Conservation of Nature (IUCN). For each endangered group the IUCN aims to produce an Action Plan to guide conservation efforts. The first and only IUCN Action Plan for cycads was published in 2003 (see Donaldson 2003). Unfortunately, that plan omitted one of the most important threats that these plants face, the introduction of invasive insect pests into wild populations. In 2003 the armored scale cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi (Hemiptera: Diaspididae), hereafter referred to as CAS, was inadvertently introduced into Guam, an island in the western Pacific. The impact on Guam's native cycad, *Cycas micronesica,* was devastating, with some 88% of the mature trees, representing hundreds of thousands of plants dying within the first five years of this biological invasion (Marler and Krishnapillai 2020). In an initial assessment this cycad was projected to go extinct in the wild by 2019, however, the introduction of two biocontrol agents, the beetle *Rhyzobius lophanthae* and the wasp *Arrhenophagus chionaspidis*, both natural enemies of CAS, appears to have lowered the damaging effects of this pest to a level that has allowed some recovery of health in surviving plants and a deceleration of the decline in population numbers (Marler et al. 2012, Lindström et al. 2023). CAS was first detected on the Island of Taiwan in 2000 and soon reached the wild populations of *C. taitungensis*, located in the Taitung Cycas Forest Reserves and Taitung Hongyeh Village Cycas Nature Reserve. By 2010 in the Hongyeh Reserve mortality in this cycad had reached 62% (Liao et al. 2018). Survey of this population in 2019 revealed continuing poor health of surviving plants and the absence of any seed production or seedling recruitment (Lindström pers. obs.). Biocontrol agents of CAS were surveyed in this population, revealing the presence of predaceous beetles *Cybocephalus nipponicus* and an undescribed species of *Cybocephalus* (Bailey & Lai 2006) and the parasitic wasp *Arrhenophagus chionaspidis* (Chao et al. 2010). It is not known whether foreign biocontrol agents were introduced into these reserves to control CAS.

Recently CAS has invaded wild populations of *Cycas revoluta* in Japan, emphasizing the urgency for developing better biocontrol methods. The threat of Diaspididae on wild cycad populations is not limited to *Cycas*, as an undescribed *Aspidiotus* armored scale has been associated with severe dieback within *Macrozamia communis* habitats (McDougall et al 2021).

This review is an update to a Report and Recommendations on CAS published earlier by the Cycad Specialist Group (see Tang et al. 2005). In that report various control options for CAS were discussed. The discovery of further biocontrol agents not discussed in that report and new developments call for the need of an update. This Review is meant to provide further guidance on biocontrol, currently the only effective means for controlling established CAS infestations.

Objective & Tasks

This review discusses:

1) Currently known biocontrol agents and the effectiveness of each in controlling CAS
2) The scientific process of finding and identifying biocontrol agents
3) The scientific process of testing the effectiveness of biocontrol agents
4) The political process of certifying a biological control agent for release
5) Options for facilitating the biocontrol process

Known Biocontrol agents of CAS

Classical biological control, hereafter referred to as biocontrol, is the use of predators and parasites, usually sourced from regions where the pest is native, as a way to control a targeted pest. This method of pest control dates back to the 1800s prior to the invention of modern pesticides. The natural enemies of armored scales such as CAS include parasitic wasps, often in the families Aphelinidae and Encrytidae, predaceous beetles and entomopathogenic fungi. One fifth of all successful biocontrol projects have targeted armored scales and about half of all successful biocontrol projects have employed aphelinid and encrytid wasps (Miller and Davidson 2005). A review of biocontrol efforts in Florida involving the introduction of 59 arthropods and one nematode showed that this approach has had minimal documented effects on non-target organisms (Frank and McCoy 2007), which suggests that if conducted with proper research and safeguards, biocontrol can have few to no noticeable side effects. A review of CAS biocontrol agents was published by Tang & Cave (2016) and an update is presented here based on Cave (2022):

Listed below are known biological control agents of CAS, regions where they have been used and level of success. The effectiveness of these agents varies from region to region and is probably influenced by climate and complex interactions among biocontrol agents and with other existing organisms.

1) *Cybocephalus nipponicus* (Coleoptera: Cybocephalidae*):* First identified from surveys in Thailand by Richard Baranowski (University of Florida), with Banpot Napompeth (National Biological Control Research Center, Thailand), this natural predator of CAS was released in Florida, but has not provided adequate control on plants of *Cycas revoluta*.
2) *Rhyzobius lophanthae* (Coleoptera: Coccinellidae): Introduced to Florida and Hawaii prior to the outbreak of CAS, this beetle does not provide effective control of CAS in Florida, but appears to provide effective control in Hawaii and possibly Guam. In Taiwan it does not provide effective control of CAS in the wild population of *C. taitungensis*. In Texas, in conjunction with an aphelinid wasp, *Aphytis lingnanensis,* it provides effective control on *C. revoluta* (Flores and Carlson 2009). Introduced into Guam in 2005 it provides some protection for mature plants, but not to seedlings near ground level (Marler et al. 2013). *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 (Cave 2022). This lady beetle is native to Australia, whereas CAS is native to mainland SE Asia. Although *R. lophanthae* is a voracious predator, its ecology and seasonality may not be finely tuned to that of CAS.
3) *Arrhenophagus chionaspidis* (Hymenoptera: Encrytidae): Ronald Cave (University of Florida) and Ru Nguyen (Florida Department of Agriculture) observed this parasitic wasp attacking CAS in China, Thailand, and Vietnam. It was previously established in Florida to control San Jose scale, but was not detected attacking CAS until 2009. It now appears widespread throughout the southern half of the state. Initially considered to provide minimal control since it attacks only the male scales, its effectiveness is being reassessed as CAS infestations in Florida are now less intense. In Taiwan 37% of CAS in the wild population of *C. taitungensis* were found to be parasitized by this wasp (Chao et al. 2010).
4) *Coccobius fulvus* (Hymenoptera: Aphelinidae): First identified from surveys in Thailand by Richard Baranowski (University of Florida), with Banpot Napompeth (National Biological Control Research Center, Thailand), this wasp parasitoid of CAS was released in Florida. Although high rates of parasitism have been observed, it has not provided satisfactory control of CAS. This wasp was introduced into Guam in 2005 but did not become established.

5) Other aphelinid wasps: In Hawaii, *Aphytis* sp. possibly *lingnanensis* causes high parasitism rates in Hawaii that apparently suppressed CAS populations (Cave et al. 2013). This wasp appears to be part of a species complex that may require genetic analysis to sort out, as specimens collected in different localities and hosts, which cannot be morphologically distinguished, do not hybridize.
6) *Isaria fumosorosea* (entomopathogenic fungus now called *Cordyceps javanica*): In laboratory trials, this fungus achieved over 70% infection rates of CAS (Castillo et al. 2011). Limited field trials were inconclusive and it is uncertain how commercial products of this organism might be employed and whether or not it would also attack biocontrol organisms used to control CAS. Studies by Avery et al. (2008, 2019, 2020) and Sánchez Barahona et al. (2018) demonstrated the compatibility of this fungus with parasitoids and predators.
7) *Telsimia nitida* (Coleoptera: Coccinellidae): Guam is the type locality of this species. It occurs on numerous Pacific islands, including the Hawaiian Islands (Leeper 1976). Three specimens were taken at two localities in Guam feeding on CAS. 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 due to its relatively broad host range (many species of armored scales). Investigation of this species is warranted.
8) *Phaenochilus kashaya* (Coleoptera: Coccinellidae): Ronald Cave (University of Florida) and Ru Nguyen (Florida Department of Agriculture) found this beetle feeding on CAS on wild *Cycas siamensis* populations in Thailand (Giorgi and Vandenberg 2012). Laboratory trials (Manrique et al. 2012) have demonstrated that the larvae and adults of this beetle are voracious predators of CAS, with each individual beetle estimated to consume over 5000 CAS over its lifespan. Although the release of this predatory beetle has been denied in Florida twice, this is a promising new biocontrol agent that needs to be considered further.
9) *Scymnus* (*Pullus*) sp. (Coleoptera: Coccinellidae): Surveys of natural enemies of CAS on Guam in March 2022 discovered a species of small lady beetle that was frequently found on plants displaying substantial levels of predation on CAS (Cave 2022). Many adults and one pupa were encountered. The beetle is unlikely a native species of Guam or the Mariana Islands. Rather, it possibly is an accidental introduction from Japan or mainland Asia; it might be an undescribed species. This predator should be studied as a potentially significant natural enemy of CAS. The species needs to be determined.

<u>The Process of finding and identifying biocontrol agents</u>

At the beginning of an outbreak the first step for a biocontrol effort is to determine what biocontrol agents are already present. For example, the beetle *Cybocephalus nipponicus* is widely established in Asia and may already be present in infested areas in Japan. Such a survey will also determine which of the known biocontrol agents listed above are not present and thus may be selected for possible introduction. This kind of survey work can be conducted by a post-doctoral entomologist or a trained entomology student under the supervision of an experienced specialist in biocontrol agents. Besides direct observation and capture of CAS predators in the field, infested *Cycas* leaves can be housed inside a cage in a laboratory to see what parasites or predators can be reared out to maturity. Mature life stages are the most readily identified with morphological techniques, however genetic analysis may be employed to more rapidly identify potential biocontrol organisms that may be present.

If new biocontrol agents are found during such a survey, besides the identification process, a lengthy evaluation process may ensue before they can be introduced to other islands or infested areas (see below). Since the CAS outbreak in Japan is a crisis that needs a remedy within a two-year window before major collapse of the population will occur, focus should be placed on known biocontrol agents of CAS.

The hunt for more effective biocontrol agents is necessary, as there is yet no agent or combinations of agents that can provide complete control of CAS, although *Phaenochilus kashaya* discussed above may have this potential. This will be a more strategic endeavor that may take many years. This kind of survey can be carried out in the native range of a pest, where predators may exist and have specifically evolved to prey on the pest in question. This is how the lady beetle *Phaenochilus kashaya* (discussed earlier) was discovered in Thailand. The native range of CAS extends east-west from Vietnam to Thailand and possibly to India and north to southern China. Suitable control agents may possibly be found on other species of the genus *Aulacaspis*, which contains over 100 described species that extend east to the Philippines and as far south as to northern and eastern Australia (Takagi 2013). For each new CAS invasion, the exploratory efforts for finding new biocontrol organisms should focus on portions of the native range of CAS with a climate that most closely resemble the newly invaded region.

<u>The process of testing the effectiveness and safety of biocontrol agents</u>

In most developed as well as several developing countries, there are laws regulating the introduction of non-native organisms into the environment. In the case of exotic biocontrol agents, these must be tested for their host specificity, possible harm to non-target plants and animals, and effectiveness in controlling the target pest. This kind of work is usually carried out in specialized containment facilities, where the control agents can be secured and prevented from escape, while they are being studied and evaluated for possible release. In general, this process will take a minimum of many months to 3-5 years. For parasitoids and predators, insect species closely related to the target pest and beneficial organisms such as other parasitoids and predators from the target area of introduction and nearby regions must be tested to clearly determine what the exotic biocontrol agent will eat and what it will not eat. Studies on temperature-dependent development, cold tolerance, and reproductive biology must also be investigated.

Some biocontrol agents with known efficacy are commercially available and can be quickly obtained for study or release. *Cybocephalus nipponicus*, for example, is available from five commercial sources in the USA (*Cybocephalus nipponicus* - Bugwoodwiki viewed 2023).

<u>The political process of certifying a biological control agent for release</u>

The modern process of discovery and investigating the biology, specificity, and efficacy of a potential biocontrol agent is a scientific process. That is to say, scientific methods of trials and experiments are employed to draw conclusions. Once this process is completed and if scientific evaluations are favorable, a formal request or petition is submitted to a country's authorities for approval of release of the exotic biocontrol agent into the environment. The permitting process varies from country to country. In democratic countries a committee of various stakeholders may jointly make this decision. For example, Cave (2022) describes the permit process for the lady beetle *Phaenochilus kashaya,* which holds promise as an effective biocontrol agent for CAS. The deciding committee of the North American Plant Protection Organization consisted of stakeholders from the United States, Canada, and Mexico, as these countries are contiguous and impacted by the release of the organism. Similar international plant protection organization cover other regions of the world (Japan is a member of the Asia and Pacific Plant Protection Commission). The stakeholders may represent various groups that might be impacted by the release of the organism. For example, an exotic lady beetle may become a competitor of a native coccinellid beetle and drive the native to extinction. Some members of the group will view this potential impact as unacceptable. In the case of CAS some members may be less interested in protecting cycads from CAS and more concerned about such potential unintended consequences such as predation or parasitism of native fauna. The final decision for release is thus a political process among various stakeholders, government authorities, public opinions, and conservation interests. *Cycas revoluta* has deep cultural roots in Japan, with a long history as an ornamental plant, food source and subject of art (Thieret 1958, Osborne and Tomiyama 1995). It is not inaccurate to say it is a cultural icon in Japan. Therefore, in Japan it will be necessary to convince authorities that saving such a plant is more important than potential negative impacts on native scale insects, which would be the main potential non-target organisms. It is up to citizens in Japan who value this plant to make their voices heard and facilitate the biocontrol process that may help save their wild *C. revoluta* populations.

<u>Options for facilitating the biocontrol process</u>

The process to discover, study, release, and evaluate biocontrol agents for CAS is long. For example, efforts to find and evaluate biocontrol agents in Florida lasted over a decade and further work remains (Tang & Cave 2016, Cave 2022). Observations have shown that when CAS first enters vulnerable populations of cultivated or wild plants most will be killed within a two-year period (Howard et al. 1999, Marler and Krishnapillai 2020). The biocontrol process could easily take too long to be of assistance in saving wild populations of *C. revoluta*. What are some options for the Cycad Specialist Group, concerned individuals and citizen groups in Japan for facilitating and speeding the process of studying and releasing biocontrol agents for *C. revoluta*?

1) The IUCN SSC Invasive Species Specialist Group: "promotes and facilitates the exchange of invasive species information and knowledge across the globe and ensures the linkage between knowledge, practice and policy so that decision making is informed. The two core activity areas of the ISSG are policy and technical advice, and information exchange through our online resources and tools and through networking". They are a possible source of knowledge about how to facilitate the introduction of biocontrol agents in Japan. The Chair of this group, Piero Genovesi, has identified several people in Japan who may be of assistance on this topic: Hana Matsuzaki, from the Minister of Environment and working on invasive species policies, Furio Tohru Ikeda, leading expert

on IAS vertebrates in Japan, and Fumio Yamada, also working on invasive species in Japan.

2) Establish communications and form a working relationship with biocontrol specialists and authorities in Japan and connect them with scientists with experience in CAS biocontrol.
3) Provide funding to biocontrol researchers and their students to work on CAS on *Cycas revoluta* in Japan. This will likely speed the research aspects of the biocontrol effort.
4) Promote news coverage of the CAS invasions and citizen efforts to control it: media include newspapers, television, webpages, Youtube, Facebook, Twitter.
5) Lobby politicians and government officials: besides bureaucratic inertia there is the issue of many problems and interests vying for the attention of government officials. Any concerned citizen or citizen groups must compete with these other interests for the attention of politicians. Political authorities are ultimately the ones who can make the decisions to facilitate the release of biocontrol agents. Points 1-4 essentially serve to educate decision-makers and advocate for action.

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