Observed plant recovery of the endangered *Cycas micronesica* populations on the island of Guam: plant resistance or biocontrol

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Abstract

The island of Guam's only gymnosperm and historically one of the island's most abundant trees, *Cycas micronesica*, has been devastated by high mortality due primarily to the armoured scale *Aulacaspis yasumatsui.* This cycad-specific scale pest invaded Guam in 2003 and, in only a few years, this pest caused over 90% mortality to maturing trees and 100% mortality to seedlings. In 2015, *C. micronesica* was listed under the United States Endangered Species Act. Continuous surveillance of tree mortality throughout the island showed extreme decline in health until recent surveys have demonstrated that there has been improved tree health with little evidence of leaf herbivory on some remaining trees. Suggested explanations for this observed reduction in scale herbivory include some form of resistance in the surviving trees or a biocontrol agent (or several agents) that previously existed or has been introduced unintentionally and is controlling the scale to a greater degree. A combination of resistance and biocontrol are possibly both involved. We discuss in depth these possibilities and then propose experimental approaches that will help resolve this question.

Keywords

Aulacaspis yasumatsui, biological control, *Cycas micronesica*, plant resistance, *Rhyzobius lophanthae*

Overview and background

Over the last few decades, the cycad-specific scale pest, *Aulacaspis yasumatsui* Takagi (Cycad Aulacaspis Scale, CAS), which is native to Southeast Asia, has spread and is infesting cycads throughout many parts of the world (Cave et al. 2022). In some cases, CAS population growth rate is rampant and plants die from continuous scale feeding on most plant tissues. On the Mariana Islands, mortality of Guam's native cycad and only gymnosperm, *Cycas micronesica* K.D.Hill, was exponential following the invasion by this scale pest in 2003. CAS rapidly spread on cycads throughout the island and, within six years, over 92% of the trees were dead and no seedlings were surviving (Marler and Lawrence 2012, Marler and Krishnapillai 2020). Fig. 1 shows a healthy tree prior to being infested by CAS. Mortality of trees continued and led to the plant's being given Endangered status by the IUCN Red List in 2006 and then later listed under the United States Endangered Species Act in 2015. Other cycad herbivores have added to the decline in health, but CAS remains the major threat to date (Deloso et al. 2020).

Prior to the CAS invasion, *C. micronesica* was a dominant forest tree throughout Guam's different native habitats (Donnegan et al. 2004), giving life support to forest health by contributing useable nitrogen (N) to other plants by means of its N-fixing cyanobacteria located in coralloid roots. The widespread mortality of the cycad has led to other forest fitness declines. Throughout the more than 20 years since CAS invaded, numerous recommendations have been made with repeated calls for investment of funds for researching different potential biological control agents for this pest (Marler and Lindström 2014, Marler et al. 2024). Establishing an effective biological control programme has been seen as the only long term viable option for managing this pest in Guam's forests (Marler et al. 2024). These recommendations included surveys for CAS biological control agents in its native habitat of Thailand where there are no major outbreaks of this pest on native *Cycas* species and then establishment of these potential control agent populations for release into Guam's forests. Early attempts to test potential predators did have some success (Moore et al. 2005) with the release and establishment of the predator ladybird beetle, *Rhyzobius lophanthae* Blaisdell; however, the parasitic wasp, *Coccobius fulvus* Compere & Annecke, failed to establish. Even with this early success with the predatory beetle, CAS continued to ravage the cycad populations and extinction of this iconic tree appeared imminent. Further recommendations included the introduction of a scale-specific parasitoid that would supplement the ladybird beetle and a list of potential agents was provided (Cave et al. 2013, Tang and Cave 2016, Cave et al. 2022). Despite these and other recommendations for biocontrol (Marler et al. 2021), management and funding for these conservation projects have not been realised (Cave et al. 2022).

Improvement in the health of in situ *C. micronesica* trees in Guam during the last few years has been observed, with many trees showing no signs of herbivory, ushering in a remarkable change compared with previous years of continued devastation by CAS and other pests (Marler and Terry 2023, Lindström et al. 2023). In the early years after the CAS infestation was noted, entire above-ground parts of the tree (leaves, trunks, reproductive organs) were covered with scale adults and crawlers causing leaf collapse (Fig. 2), so the current observation that trees have flushes of healthy leaves is a startling, but promising sign of recovery. This improvement occurs in all parts of the island where this cycad grows in different soils, at different elevations and in open and closed canopy habitats.

Potential causes of recovery

We consider three major drivers as to the cause of this fortuitous development. First, the efficacy of pre-existing biological control organisms may have improved such that the contemporary CAS population is being controlled at levels that did not occur during the previous years. Second, a chance arrival and establishment of a new biological control organism may have occurred on Guam in the recent past and this new CAS enemy has not been identified to date. Third, the persisting living trees may have a degree of genetic resistance to CAS herbivory that the rest of the 2003 population did not have and the 96% mortality between 2003 and 2020 (Marler and Krishnapillai 2020) has selectively removed the less resistant genotypes from the population. These three broad drivers of population dynamics may not be mutually exclusive, but developing a greater understanding of their roles in the persistence of *C. micronesica* will improve future species recovery efforts.

Objectives and implementation

Herein we discuss evidence that may provide information for this conversation, then discuss approaches to determine which of the drivers, or combination of drivers, is most likely causal.

The evidence

We believe that a recent improvement in biological control efficacy of CAS is the most likely cause of the recent improvements in plant health and the details of this improved biocontrol have not been adequately studied. However, we also consider how the current trees' survival was accomplished during the early years, and it may be through some form of resistance (antixenosis, antibiosis, or tolerance, nutritional status) (Emden 2002). As a result, the causes are not understood without more dedicated research. We will discuss evidence that suggests where plant resistance or biocontrol may or may not play a role in plant recovery.

- First, if the 2024 *C. micronesica* trees that appear to have improved in health status comprised the small percentage of 2003 trees endowed with the phene of greater constitutive genetic resistance to CAS in terms of antixenosis (plant surface, texture or volatiles etc., that make the plant unattractive) or antibiosis (negative effects on life cycle of the herbivore; for example, through chemical defences), these individual trees would likely have shown greater health and less CAS herbivory throughout the entire time span since 2003. This was not the case. After the CAS population was ubiquitous amongst all Guam habitats by 2006-2007, there were no *C. micronesica* trees that exhibited consistently greater health than the remaining sympatric trees (Marler and Lawrence 2012, Marler and Krishnapillai 2020). On the contrary, every tree within a habitat exhibited similar levels of CAS infestation and declines in health during every post-invasion year for more than a decade. If constitutive or induced genetic resistance is responsible for the recent increases in health status of the persisting trees, we believe that genetic resistance would have expressed itself during the past 20 years and it would not begin to express itself after almost two decades of scale herbivory. Resistance through tolerance or nutritional status cannot be ruled out, however. Plants that were able to thrive throughout the last 20 years may have had better starch storage or other abilities to recover from the herbivory through the years of continuous attacks by CAS.

- Second, some of the 2024 trees supported by a crown of healthy-looking leaves with minimal CAS herbivory have produced basal leaves from suckers or pups. These leaves near the soil surface invariably become heavily infested with CAS. An individual plant with genetic antibiosis chemical resistance to CAS would not likely exhibit one level of resistance at the 3-4 m stratum and a different level of resistance at the 0-1 m stratum. These observations indicate that differential levels of biological control as defined by vertical stratification (see section on regeneration below) (Marler 2013, Marler et al. 2013) are causal of the strata heterogeneity in CAS herbivory within individual trees. However, these observations do not rule out antixenosis resistance if leaves or plant parts of different maturities have different features (toughness, chemistry) that affect establishment by CAS crawlers.
- Third, some of the 2024 trees contain multiple branches and commonly one can find trees with heavy CAS infestation in one area of the canopy and minimal infestation in a second area of the same canopy. The most parsimonious explanation for this spatial heterogeneity of CAS infestation level within the same tree's canopy is spatial heterogeneity of biological control, as every part of one tree's canopy would likely contain the same level of genetic resistance to CAS. The question why some recovered trees have partial canopy heavily infested may be due to spatial conditions where biocontrol is restricted. As above, we cannot rule out that some differences in the location of the leaves (e.g. sun/shade) may affect leaf chemistry or physical traits that may play a role in CAS establishment.
- Fourth, regeneration and recruitment have been severely reduced by the chronic CAS herbivory, and very few ovules that are fertilised during the pollination phase have grown to maturity due to direct CAS herbivory of the developing seeds. In some of the habitats where mature seed production has happened in recent years, the germinating seedlings readily become heavily infested with lethal levels of CAS shortly after emergence of the first leaf. If Guam's persisting mature trees are a result of their greater genetic antibiosis chemical resistance to CAS, then the pollen parent and ovulate parent of every contemporary seedling is endowed with this genetic resistance. Yet 100% of the newly-emerging seedlings continue to be rapidly killed by immediate CAS herbivory. There is the possiblity that antibiosis or antixenosis resistance, via chemical or physical properties, differs with maturity of leaves or plant stage as found in some other plant groups (such as Souza et al. (2021)). In cycads, for example, a chemical produced by cyanobacteria or the strain of cyanobacteria in the coralloid roots of mature plants

may affect CAS, as has been demonstrated for some plants with symbiotic N-fixing rhizobia (Godschalx et al. 2015, Iriart et al. 2024). However, based on the results of a study that examined biocontrol of CAS, the stratification/height of seedling leaves was a factor, with biocontrol active only in higher structures of the plant (Marler et al. 2013).

Proposed studies

There are several straight-forward, easily conducted studies that could provide information about the knowledge deficits. The following are a few recommended studies that would immediately provide this information

- First, a detailed survey of parasitoid and predator impacts on CAS population dynamics could be conducted throughout Guam. A CAS second instar or adult that has been consumed by a predator exhibits unique traits that are easily observed. The remains of the CAS armour reveal the damage caused by the predator's mandibles which were used to break open the covering. Additionally, predation of the crawler stage is possible (Hill et al. 1993, El-Sawi and Momen 2006), and observational studies could confirm this kind of predation. Finally, parasitoid exit holes in CAS armour and mummified remains are also unique and unambiguously identified. The small circular hole through which the parasitoid adult emerged appears in sharp contrast to the hole caused by the predator's behaviour. Widespread surveys of CAS to tabulate these forms of evidence for predation and parasitism would enable an understanding of the extent of biological control amongst Guam's CAS population, even if the organisms responsible for the biocontrol are not known.
- Second, the release of the parasitoid, *Arrhenophagus chionaspidis* Aurivillius, began in 2008, but evidence suggests that it had a fortuitous arrival prior to the release (Cave et al. 2022). Evidence of parasitism by this species was reported to be throughout Guam in 2013 (Cave et al. 2022), and surveys in 2017 -2018 indicated that this is the only CAS parasitoid of CAS in Guam. Surveys should be devoted to assessing the current distribution and prevalence of this agent. Unfortunately, this parasitoid does not parasitise female CAS, as suggested by females still laying eggs (Cave et al. 2022). Therefore, a dedicated search for any parasitoid exit holes in female CAS armours on Guam would indicate the presence of a second unidentified parasitoid on the island.
- Third, rearing parasitoids from field-collected CAS is a simple endeavour and could be conducted from *C. micronesica* leaflets collected throughout Guam. Counting adult parasitoids that emerge from caged *C. micronesica* leaf tissue infested with CAS would provide information for this conversation conundrum even if funds were never made available to identify the resulting parasitoids that emerge in the cages.
- Fourth, a repeat of the vertical stratification study conducted in 2008 (Marler et al. 2013) would add greatly to the contemporary need for new information. Contemporary seeds, from surviving trees that are free of CAS, could be collected,

germinated in a container nursery, and then the resulting seedlings would be used to repeat the stratification methods. We predict the elevated seedlings will exhibit less CAS infestation than the ground-level seedlings in congruence with what was shown in earlier years of the invasion. This would demonstrate that these seedlings do not have genetic antibiosis or antixenosis resistance. Again, this would point to some stratification behaviour by a biological control agent.

- Fifth, *Cycas* leaves on plants can be caged with fine-mesh screening to keep out biocontrol agents, even the smallest parasitoids and then leaves would be experimentally infested with CAS in the absence of predator or parasitoid control. Leaves in open cages would also be infested. If biocontrol is the reason that 2024 *C. micronesica* trees are beginning to recover in health, these close-caged cycad leaves will increase in CAS density, while open-caged leaves on the same trees will continue to exhibit reduced CAS density. This is the classic exclusion method for assessing the effectiveness of biological control agents. Contrarily, if genetic antixenosis or antibiosis resistance of the surviving *C. micronesica* plants is causal of the healthier trees today compared to previous years, then the caged exclusion of biological control agents would not influence the level of CAS infestation inside the cages versus outside the cages.
- Sixth, if antixenosis or antibiosis is indicated by the above experiments, then further studies on the leaf chemistry and physical features should be conducted.

Impact

All five of these experimental approaches we suggest do not represent difficult endeavours. The studies could be accomplished in a very short amount of time and the results would produce unambiguous data to help parse the causes of the recent recovery of these iconic cycad trees. Previous conservation funding, research and managment of this plant has not placed a major effort focused on biocontrol. However, the evidence of our research from earlier studies presented here clearly point to biocontrol being a major driver of the recovery, with the possibility of some tolerance by trees that survived the early devastation by CAS. Regardless of the outcome of the proposed studies, the answers would provide the evidence to empower decision-makers how to approach future conservation measures. If results of these studies reveal only biocontrol as the cause of the current recovery, then efforts should further promote the use of biocontrol agents in Guam and in other cycad locations where this pest has been introduced. In the event that biocontrol by one or more agents is effective, new leaf flushes (Fig. 3) on the surviving trees should continue to be protected and aid in overall tree recovery.

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Conflicts of interest

The authors have declared that no competing interests exist.

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Figure 1.

A healthy male *Cycas micronesica* plant with a maturing pollen cone.

Figure 2.

A male *Cycas micronesica* plant heavily infested with aulacaspis scale pest showing the collapse of leaves from different leaf flushes and the scale-covered pollen cone.

Figure 3.

New leaf growth of previously infested *Cycas* plants may be free of CAS if adequate biological control is established.