Temporal Variations in Leaf Miner, Butterfly, and Stem Borer Infestations of *Cycas micronesica* in Relation to *Aulacaspis yasumatsui* Incidence

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Abstract. Four phytophagous insects are among the major threats to *Cycas micronesica* K.D. Hill on Guam, and the temporal comparisons of infestation levels may inform horticultural and conservation decisions. Incidence of *Aulacaspis yasumatsui* Takagi, *Erechthias* Meyrick sp., *Chilades pandava* Horsfield, and *Dihammus marianarum* Aurivillius infestations on *Cycas micronesica* plants were recorded every 6 months from 2004 to 2013 to determine if the incidence of *A. yasumatsui* was related to patterns of the other three insects. *Dihammus marianarum* infestations reached ephemeral maximum and minimum levels ≈ 2 years after *A. yasumatsui* infestations reached maximum and minimum levels. *Erechthias* sp. infestations disappeared in early 2006 when *Cycas micronesica* leaves were being killed by acute *A. yasumatsui* infestations. *Erechthias* sp. infestations increased thereafter as *A. yasumatsui* incidence declined in response to biological control. *Chilades pandava* infestations appeared to increase and decrease inversely with *A. yasumatsui* infestations throughout the years. *Aulacaspis yasumatsui* may be indirectly affecting *D. marianarum* damage by direct control of changes in overall tree health. *Aulacaspis yasumatsui* may be indirectly influencing *Erechthias* sp. through direct control over leaf longevity. The *C. pandava* and *A. yasumatsui* populations appear to exhibit direct competition with inverse patterns of incidence.

Cycas micronesica is a tropical cycad species that is endemic among several western Pacific islands (Hill, 1994). Paleoenviromental investigations document its prevalence in Guam for more than 9000 years (Athens and Ward, 2004). This tree has enjoyed a dominant position in horticulture and agroforestry settings of the region for centuries (Barratt, 2003; Edwards, 1918; Safford, 1905).

Dihammus marianarum (Cerambycidae) is a native stem borer (Marler and Muniappan, 2006). Larvae of this beetle bore tunnels in the cortex of *Cycas micronesica* stems (Fig. 1A). In the absence of a stressor that initially decreases plant health, the beetle does not attack the trees. When tree health declines, subsequent signs of borer attack are unambiguous with mucilage and frass building up on stem surfaces (Fig. 1B) and around the base of the stem on the soil surface. No other local plant species are known hosts for this stem borer.

Several 21st century insect invasions have threatened Guam's *Cycas micronesica* and

removed the horticultural appeal of the cycad species. The cycad leaf miner *Erechthias* sp. (Tineidae) was first reported in 2003 when it was restricted to the southern habitats of the island (Marler and Muniappan, 2006). Caterpillars of this microlepidopteran insect tunnel in leaflets exclusively on hardened, mature *Cycas* leaves (Fig. 2A–B). The armored scale *Aulacaspis yasumatsui* (Diaspididae) invaded Guam in 2003 (Marler, 2012; Marler and Muniappan, 2006), and its predator *Rhyzobius lophanthae* Blaisdell was purposefully introduced in 2005 (Moore et al., 2005). Damage to host plants occurs during a lengthy sessile stage during which waxy covering protects the insects. The butterfly *Chilades pandava* (Lycaenidae) was found in northern Guam in 2005 (Moore et al., 2005). The caterpillar stage of this butterfly requires soft, expanding *Cycas* tissue as food. The three herbivore insects established readily and then spread throughout the island, and the predator was transported by local biologists to new localities of armored scale outbreaks until it had established throughout the island. Additionally, exotic termites that attack *Cycas micronesica* stem tissue are located in some northern habitats (Marler et al., 2011).

The threats to the plant inflicted by these insect pests have been augmented by feral ungulate damage, and this nexus of threats has led to greater than 90% plant mortality in less than one decade (Marler and Lawrence,

2012). The previously healthy *Cycas micronesica* populations and their sustainable relationship with the native stem borer have been acutely disrupted by the recent pest invasions. Infestation levels of the armored scale and damage by the leaf miner, the butterfly, and the stem borer on *Cycas micronesica* trees were recorded from 2004 until 2013 to determine the patterns of infestation among the pests. Results may be useful for informing horticultural or conservation management decisions.

Materials and Methods

Four linear transects were established in northern Guam in Oct. 2004 in a study site where the presence of the stem borer and leaf miner had been documented. The armored scale immigrated into the study site in Jan. or Feb. 2005. *Rhyzobius lophanthae* was released in the study site in Feb. 2005 to counter the initial armored scale outbreak. The butterfly was first discovered in the habitat in July 2005.

The established transects were 2 m wide and contained 100 individuals per transect. Because the rate of tree mortality could not be predicted in 2004 when the study was initiated, each transect was positioned such that the length could be extended at each successive visit as a means of achieving 100 individuals per transect. Initially each transect was ≈ 100 m in length, but they ranged from 600 to 850 m in length by 2013.

For each date of data collection, a binary yes or no was recorded to indicate current presence of damage for each of the four herbivore insect species on each tree. For the leaf miner and butterfly, this consisted of inspecting every leaf. For the armored scale, the surface of every exposed organ required inspection. For the stem borer, the entire circumference of the stem was inspected up to 2 m in height. For unknown reasons, this pest infests stems up to ≈ 1 m in height, regardless of tree height (Marler and Muniappan, 2006). Therefore, the January and July visits each year documented the relationships among the four insects as their competition for the *Cycas micronesica* trees was established.

Results

The armored scale was not present on the trees as the study was initiated but infested 100% of the trees by 2006 (Fig. 1C). As the predator biological control became established, the armored scale infestations declined. Biological control effectiveness exhibited ephemeral disruptions for unknown reasons, which led to armored scale irruptions in early 2009 and mid-2010.

The stem borer infested less than 5% of the trees at the beginning of the study (Fig. 1C). Stem borer incidence began to increase as the scale population reached its peak and reached $\approx 90\%$ infestation of trees by 2008. Thereafter, stem borer infestations declined until 2010 and then increased during a second irruption in 2011.

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Fig. 1. *Dihammus marianarum* is a stem borer that attacks *Cycas micronesica*. (A) The larvae stage causes the damage from tunneling in stem cortex tissue. (B) Mucilage exudation and surface frass are unambiguous for the signs of *D. marianarum* infestations. (C) Proportion of *Cycas micronesica* trees infested with *Aulacaspis yasumatsui* (□) or *D. marianarum* (○) from 2004 to 2013. Mean $\pm$ SE.

The leaf miner infested 100% of the trees at the beginning of the study but could not be found on any of the trees by early 2006 when the scale had defoliated all of the original leaves (Fig. 2C). As the scale population was brought under biological control by the predator, leaf miner incidence increased and then was sustained in greater than 80% of the trees.

The butterfly was not found in the study site in 2004, but steadily increased in incidence thereafter until reaching the greatest infestation levels in 2008 (Fig. 3C). Incidence remained relatively high until 2010. Butterfly caterpillar damage was present in 40% to 60% of the trees from mid-2010 until early 2013.

Discussion

As indigenous or endemic species respond spatially and temporally to populations of alien insects, they may be affected negatively or positively in complex ways (Gandhi and Herms, 2010). Furthermore,

abiotic factors may influence the manner in which multiple phytophagous insects compete (Staley et al., 2011). If the presence of one insect species or an abiotic factor increases or decreases damage by a second insect species, this knowledge may improve management decisions.

The initial outbreak of armored scale in 2005–06 was followed by an epidemic outbreak of the stem borer roughly 2 years after the scale outbreak reached its peak. Interestingly, the duration of this lag time was confirmed by a second armored scale irruption and then a second maximum level of stem borer damage. Furthermore, the two lowest levels of stem borer infestation occurred roughly 2 years after the two lowest levels of armored scale incidence. The patterns indicate that this armored scale may be indirectly controlling stem borer incidence by way of a direct influence on general tree health.

Stem borer species are known to attack weakened trees (Hlášny and Turčáni, 2013).

For example, after Hurricane Andrew, outbreaks of stem borer damage occurred in Florida, which further decreased the health of trees damaged during the hurricane (Armentano et al., 1995; Platt et al., 2002). Case studies showing how damage by an insect pest leads to increased stem or bark borer damage are uncommon. However, a well-studied example is how defoliation of *Quercus* L. species by the gypsy moth *Lymantria dispar* L. facilitated colonization by twined chestnut borer (*Agrilus bilineatus* Weber), probably by compromising tree defenses to this phloem-feeding wood borer (Dunbar and Stephens, 1975; Muzika et al., 2000; Wargo, 1977).

The armored scale also appeared to exert control over the leaf miner infestations by pre-empting larval food. For unknown reasons, the leaf miner does not oviposition in young leaves. Therefore, when leaves are lost or senesce before reaching an age sufficient for ovipositioning by the leaf miner, no

Fig. 2. *Erechthias* sp. is a microlepidopteran leaf miner that attacks *Cycas micronesica* leaves. (A) The larvae mines coalesce into sections where the leaflets are cleared. (B) The moth ovipositions preferentially on older leaves. (C) Proportion of *Cycas micronesica* trees infested with *Aulacaspis yasumatsui* (□) or *Erechthias* sp. (○) from 2004 to 2013. Mean $\pm$ SE.

appropriate food is available for the caterpillar stage. During the few acute irruptions of the armored scale, most of the leaves were killed before they reached sufficient age. This was most apparent after the initial scale irruption, which preceded adequate establishment of the *R. lophanthae* predator. Every tree in the initial survey revealed leaf mines, but no tree in the early 2006 survey exhibited leaf mines. The armored scale may be indirectly influencing leaf miner damage through direct control over leaf longevity.

Butterfly caterpillar damage and armored scale incidence seemed to exhibit the most direct relationship. As one pest increased in severity, the other pest simultaneously decreased in severity. In this setting, the butterfly

caterpillar and armored scale populations appear to exhibit direct competition with inverse patterns of incidence.

Improved horticultural and conservation decisions for managing cycad plants will require a better understanding of how identified threats interact (Denno et al., 1995; Kaplan and Denno, 2007). To illustrate, Gómez et al. (2012) studied simultaneous attack of *Tsuga canadensis* (L.) Carrière by a scale insect and a gall-forming insect to reveal that feeding by one insect altered the tissue quality for and damage by the other insect. Moreover, the novel interactions that result from biological invasions need to be understood for improved pest management. In the Guam case, an increase in stem borer damage can now be

anticipated long before it actually occurs simply by monitoring severity of the armored scale damage. Additionally, we have been unsuccessful in our multiple attempts to establish two parasitoid species for scale control, but we continue to pursue this goal (Marler and Terry, 2013). If we achieve successful augmented parasitoid biological control, these results indicate an increase in butterfly caterpillar damage may follow.

There are no other geographic locations where a *Cycas* species is being threatened by these same four insect species that are attacking Guam's *Cycas* trees. However, similar studies are warranted in various locations where this butterfly and armored scale simultaneously attack *Cycas* trees. This is true for

Fig. 3. *Chilades pandava* is a specialist butterfly that requires cycad tissue for larvae food. (A) Female adult butterfly ovipositioning on *Cycas* leaflets. (B) A *Cycas revoluta* plant with a recent leaf flush in which all leaflets were consumed by *Chilades pandava* larvae. (C) Proportion of *Cycas micronesica* trees infested with *Aulacaspis yasumatsui* (□) or *Chilades pandava* (○) from 2004 to 2013. Mean $\pm$ SE.

various locations in Asia where the butterfly, the armored scale, and a *Cycas* species coexist in a native habitat. Taiwan could provide an informative additional case study, where the native *Cycas taitungensis* C.F. Shen, K.D. Hill, C.H. Tsou & C.J. Chen and the butterfly coexist (Hsu, 2002; Wu et al., 2010), but a recent *A. yasumatsui* invasion is threatening the cycad population (Haynes, 2010). Botanic gardens that specialize in *Cycas* conservation with multiple resident insect pest species also offer the potential for informative case studies (e.g., Marler et al., 2012). In these settings, multiple *Cycas* species could be compared in addition to the pest comparisons. Studying each new case may help predict the potential outcomes of future novel interactions that result from further invasions.

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