Pre and Post January 2009 Guam Ironwood (*Casuarina equisetifolia*) Tree Decline Conference

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ABSTRACT

Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in the midst of a decline that was first noticed in 2002 by a local farmer, Mr. Bernard Watson. His trees were less than 10 years old at the time and planted in windbreaks. Some trees had symptoms of wilt and others had decline symptoms. Wilt symptoms were characterized by chlorosis and tip-burn of lower branchlets, which progressed acropetally, killing the tree within 6 months. The dead foliage often remained on the tree giving the tree a singed appearance. In contrast to wilt, symptoms of decline began as a thinning of upper branches and may last for years. By 2005, Ironwood Tree Decline (IWTD) was widespread on Guam.

In January 2009, six invited off-island scientists together with participants from Guam took part in a 5-day IWTD conference. As a result of the conference and subsequent research, it was concluded that a complex of biotic and abiotic factors are most likely responsible for the decline. The fungus genus *Ganoderma*, emerged as the most likely biotic factor based on the high prevalence of conks in some of the sites. Other biotic agents isolated and considered were *Pestalotia*, *Botryosphaeria*, *Fusarium* and several yet unidentified fungi and bacteria. Insects that may play a role in IWTD are termites and a newly discovered gall-forming eulophid wasp which forms galls in branchlet tips. Among the abiotic factors are the two major typhoons in 2002 (Chata'an and Pongsona), the intervening severe drought, and human impact.

Post-conference analysis of cross-sections of felled declining trees revealed large areas of discoloration radiating from their centers and decreasing in size as distance from the tree base increased. Island-wide surveys, using a five-scale visual estimation method, indicated higher decline prevalence in planted tree stands than in natural established tree stands.

Key words: *Casuarina equisetifolia*, Ironwood Tree Decline Conference, Guam, biotic, abiotic

INTRODUCTION

The ironwood tree (*Casuarina equisetifolia*) is known to be indigenous to Australia, the Malayan Islands, the east side of the Bay of Bengal, and occurs on many islands of the Pacific, extending eastward to the Marquesa Islands and northward to the Mariana Islands (Safford, 1905). Pollen records indicated that ironwood has grown on Guam for thousands of years (Athens and Ward, 2004) and is likely native to Guam (Fosberg *et al.*, 1979; Stone, 1970). Being indigenous to the region and virtually pest and disease free, the ironwood tree is one of the dominant agroforestry species in the Pacific and is tightly integrated into the environment and its many ecosystems. Since *Casuarina* is the dominant tree species on many of the sandy beaches of the Mariana Islands, it has become an important perching tree for the white-collared kingfisher *(Halcyon chloris)* and the Mariana fruit-dove, *Ptilinopus roseicapilla* (Marshall, 1949). The white tern, *Gygis alba*, commonly lays eggs in ironwood trees. Only a few trees in the Pacific Islands can surpass ironwood in restoring organic matter and usefulness as a fuel wood, mulch, windbreaker, and nitrogen fixer.

As a result of the major role it plays in the flora composition of Guam, recent surveys have separately listed *Casuarina* thickets as a component of Guam's forestland (Liu and Fischer, 2006). It does not compete with native tree species in undisturbed limestone forests (Moore, 1973). However, it does grow nearly everywhere else: beaches, land-fills, wetlands, road shoulders, cleared land, and vacant lots. It is common on Guam's southern volcanic soils where it is commonly associated with savannah vegetation and is probably restricted in extent by fires (Young, 1988). A healthy population of white tern (*Gygis alba*) on Guam is located on Cocos Island, a small 100-acre island heavily covered with ironwood trees located 2.5 km from Guam's most southern point (GDAWR, 2005). In the north, ironwood trees, that grow on cliff faces, serve as one of several support trees for Guam's only Mariana fruit bat colony.

The ironwood tree, locally known in the Chamorro language as "gago", has been continually propagated on Guam since the 1600s for its good construction and firewood qualities, rapid growth, and ability to grow on marginal lands. It is conspicuously mentioned in prominent forest vegetation surveys in modern times. Due to its ability to withstand salt spray and poor soil, it is ideal for reducing soil erosion on hills and beaches, for providing protection against the trade winds and typhoons, for land reclamation, and for island beautification. In a 2002 forestry inventory, with an estimated 115,924 ironwood trees larger than 5 inches in diameter at breast height, ironwood was reported to be the healthiest tree species on the island (Donnegan, *et al.* 2004). Furthermore, the same report indicated the tree as one of 8 species of trees growing larger than 11 inches in diameter.

Since the 1980s and prior to widespread prevalence of decline (Campora, 2005), Guam Department of Agriculture provided approximately 250,000 seedlings to farmers, the general public and government agencies for various tree planting projects. At the time decline was first noticed in 2002, symptoms were very mild and largely unreported as was likely the case in Guam's 2002 forest resources report (Donnegan, *et al.* 2004). Today, tens of thousands of ironwood trees are dying on Guam due to Ironwood Tree Decline (IWTD).

By 2005, many sites associated with urban development (windbreaks, beaches, parks, housing complexes, and golf courses) had large numbers of declining or dead trees. After preliminary field and lab diagnostic results at the University of Guam failed to provide any conclusive answers to either the cause of the wilt or decline, external funds and expertise were sought. With funding from the Western Sustainable Agriculture Research and Education

(WSARE) program and the Western Integrated Pest Management (WIPM) Center, research began with the planning of the 2009 Guam IWTD Conference at the University of Guam. This paper gives a historical account of decline on Guam, reports on the findings of a 5-day IWTD conference held in January 2009, and results from the subsequent research.

MATERIALS AND METHODS

January 2009 Guam Ironwood Tree Decline (IWTD) Conference

Conference participants and attendees included administrators, researchers, students, the general public, and six off-island experts. The off-island experts, all of whom co-authored this research paper, were from the fields of plant/forest pathology, tree breeding, and extension. Fourteen sites were visited during the 5-day conference period where samples in the form of branches, cross-sections (roots, trunks and branches) and conks were collected and brought to the laboratory at the University of Guam's science building. Conference presentations included botanical descriptions of the tree, typhoon history of Guam, tree photography, Guam's soil, forest pathology, entomology, and information on the Department of Agriculture's ironwood seedling distribution programs.

Visual and Quantitative Characterization of IWTD

Prior to the January 2009 Guam IWTD Conference, 44 randomly selected isolated trees with varying levels of decline were visually assessed and given a percent decline value and photographed. Trees were measured at breast height (CBH) then categorized as small (CBH $\leq$ 100 cm) or large (CBH > 100 cm). Enlarged color photographs of these trees were printed, evaluated, and visually catalogued by researchers into a five-scale decline severity (DS) rating

(Fig. 1). Photographs were further examined and percentage of bare branches of each tree was estimated (Fig. 2).

Branch Growth and IWTD

Four to five branches from randomly selected trees were removed and growth parameters measured. The longest branches of a tree, attainable by a ladder and/or modified rope system, were cut 30 cm from branch tip. Under laboratory conditions, the 30-cm branch sections were stripped, and branchlets and cones separated. Cones were counted, weighed and allowed to shatter their seeds in open 20-cm diameter Petri dishes on the laboratory bench (temperature 24-25°C and 50-55% relative humidity). Seeds from 15 trees (comprising of three trees from each of the five-scale decline severity levels) were collected on a weekly basis and stored in vials at 4°C. Germination of seeds from each tree was evaluated by sowing forty seeds onto the surface of a planting medium contained in a plastic pot (9.0 cm diameter and 8.3 cm depth). Each pot was filled with a different planting medium: 1:1 proportion of Guam beach sand to Sunshine mix (Sun Gro Horticulture, Canada Ltd.); 1:1 proportion of Guam beach sand to Sunshine mix plus 18.2 g of *Casuarina/Frankia* nodules from 20 seedlings, pulled from the sandy coastal beach area; and Guam clay soil collected from the vicinity of healthy ironwood trees.

Discoloration and IWTD

Cross-sections of branches from healthy and declined trees were examined for presence of discoloration and decay. The longest easily accessible branch was pruned from the tree trunk and two 5-cm slices, one from the juncture of the branch with the main stem and the other 2 m away from the juncture, were cut using a machine chainsaw (Shindaiwa Inc., Japan). To enhance the examination process, some of the wood pieces were cut again on a table saw. The faces of each branch slice were tallied for presence of discoloration and decay. Patterns of discoloration and decay were noted on each slice.

In a separate experiment, small and large trees from the University of Guam (UOG) campus and Mr. Bernard Watson's ironwood windbreaks were felled and cross-sectioned. Five small trees, with and without decline, as well as three large trees with decline, were sampled. Trees were felled by cutting at ground level. Acropetally along the trunk, cross-sections of 5-cm thickness were removed first from the base (0 m), next at 1.3 m and then at 2.0 m intervals. Trunk cross-sections were photographed and discoloration quantified.

Island-wide Survey of IWTD

A GPS assisted survey was conducted between October 2008 and June 2009 along Guam's major thoroughfares as well as a number of coastal intersecting roads to farmers' fields, agricultural experimental stations, parks, beaches, cliffs, and golf courses. The survey covered 38 sites which were evaluated for stand (natural and planted) and management (slight, moderate and high). Slight management practices were those associated with tree stands (natural or planted) that are allowed to develop unattended. Moderate management practices were those associated with tree stands in parks and cemeteries, and high management practices reflect conditions around ironwood trees on golf courses and campus areas. The GPS receiver (GPSmap 76CSx, Garmin International Inc.) was read 1 m above ground level held against the north-side of the tree. Each tree was given a decline rating by two researchers using the five-scale IWTD severity rating (Fig. 1).

Statistical Data Analyses

For most treatment comparisons a one- or two-way ANOVA was computed and mean separation was performed according to Tukey's test at $P = 0.05$ level. Relationships were verified using linear and non-linear regression analyses. The following exponential function was used for the non-linear regression analysis of the proportion of wood discoloration in tree trunk cross-sections:

$$y(x) = a * \exp(-b * x) \qquad \text{eqn. 1}$$

Where $y(x)$ refers to proportion discoloration at a given distance *x*, parameters *a* and *b* refer to proportion discoloration at the origin $(x = 0)$ and steepness of the discoloration gradient, respectively.

RESULTS

Branchlet Thinning and IWTD

Photographs of ten trees, from among the 44, were selected as pictorial depictions for the five-scale IWTD severity rating system (DS) for small (n = 17) and large (n = 27) trees (Fig. 1). Percent bare branches and percent decline increased progressively as DS increased from 0 to 4 (Fig. 2). The difference between the two estimation methods was negligible. For small trees at DS 1, 2, 3, and 4 the percent (%) values were 12.5/8.5, 42.5/48.8, 66.0/67.0, and 95.0/99.8 for the quantification of branchlet thinning and percent decline, respectively (Fig. 2a). The respective values for the large trees were 20.0/12.5, 52.0/51.0, 72.8/78.6, and 92.5/95.7 (Fig. 2b).

There is an inverse relationship between tree growth (in terms of branchlet biomass and bare branch weight) and DS for small (Fig. 3a) and large (Fig. 3b) trees. This linear relationship fitted better with branchlet weight (r^2 ranging between 0.35 – 0.44) than with bare branch weight (r^2 ranging between 0.05 – 0.15). Though not presented here, data from cone number and cone weight showed a similar inverse relationship. The negative slope terms -4.77 (± 0.68) and -5.99 (± 0.77) of the branchlet weight (in g) for the small and large trees, respectively, indicates the rate at which branchlet fresh weight decreases as decline severity increases by a unit. A statistical analysis of branchlet fresh weight for the small and large trees, indicated no significant difference between DS 0 and 1, and DS 2 and 3 (Fig. 3c). Comparison of the decline severity scales at 0 (healthy) and 4 (nearly dead) for small and large trees resulted in respective branchlet weight losses of 95.3 and 88.7 (Fig. 3c).

In a satellite experiment, 30-cm branches (sampled as described in Branch Growth and IWTD section), were removed from declining trees and branchlet tips with and without the eulophid wasp galls were counted. Proportion of the wasp damaged branchlets were 0.07, 0.08, 0.20, 0.49 and 0.38 for DS 0, 1, 2, 3 and 4, respectively (Fig. 4). The average length of branchlets (with and without galls) that formed in bundles were 13.06, 14.04, 13.70, 10.62 and 6.67 cm for DS 0, 1, 2, 3 and 4, respectively.

Percent germination of seeds was not significantly affected by the extent of decline severity ($P = 0.138$). Percent seed germination of 31.3, 30.4, 29.5, 44.7 and 24.6% were recorded for trees at DS 0, 1, 2, 3 and 4, respectively. However, germination was significantly ($P = 0.041$) affected by planting medium; Sunshine mix (37.5%) and Sunshine mix plus *Frankia* nodules (36.1%) were better than the clay soil brought from the root area of ironwood trees (22.8%).

Internal Discoloration and IWTD

Branches and trunk sections from small and large healthy trees did not show any discoloration, except for a single case of a large tree with limited discoloration. This case was not attributed to IWTD as it was not characteristic of that seen in declined trees. Discoloration that was attributed to IWTD increased with severity, ranging proportionally from 0.46 to 1.00 (Table 1). Although discoloration was found consistently and exclusively on all the branches at the juncture with the main stem (trunk) of large trees, cross-sections on side branches 2 m away from the juncture were not always discolored. For small trees, however, discolorations at the juncture was consistent for DS 3 and 4 but not for DS 1 and 2 trees.

Wood discoloration extended from the tree base acropetally to a height of at least 5.3 m for small trees (Fig. 5a, c) and as high as 11.3 m for large trees (Fig. 5b). The proportion of discolored area of trunk cross-sections showed a clear gradient acropetally which was well described by the exponential function (eqn. 1). This non-linear regression model was highly significant ($P = 0.0001$) with R^2 values of 0.85 and 0.73 for the small and large trees, respectively. In this model, the rate of gradient was 0.28 for small trees and 0.10 for large trees (Fig. 5). The 0.10 value for large trees is partially due to the fact that only large trees of DS 4 were available for measurement.

There were variations in patterns and types of discoloration encountered. Some discoloration appeared to originate from the edge and others from the center of cross-sections. The discoloration ranged from slight to intense and most of the section from the base and the roots showed a white soft root-rot symptom which in some cases became spongy. Advancing edges of these discolorations varied from smooth to serrated. At points where conks are attached, usually the white soft rot association was observed (Fig. 6).

Island-wide IWTD Survey

Decline across the island appears to be spatially random (Fig. 7). The latitude based category showed a higher concentration of the decline at the central region of the island compared to the southern and northern ends. Tree decline severity, however, is likely geoculturally linked as decline is less in natural stands (mean = 0.08) and more severe in planted stands (mean = 1.60) such as in golf courses, housing subdivisions, school yards and commercial lots (Table 2). Categorizing surveyed sites based on intensity of management also showed a clear tendency of increased decline severity with increased management (Table 2). Farm windbreaks in northern Guam are severely affected, forcing in some instances, complete removal of trees.

Throughout the course of the surveys, when lower tree branches were visually accessible, flower types were noted and the tree was categorized. The majority of trees (80.3%) were best described as monecious: male and female flowers on the same individual tree. Guam's monecious trees contained female flowers produced on short peduncles, female flowers with male flowers on their peduncles, and male flowers produced on slender cylindrical spikes at branchlet tips and/or in the axillary position. A few trees (13.2%) are best described as dioecious: male and female flowers on separate trees. A tree was categorized as female if prolific cone production was present on its middle or lower portions. Male trees (2.9%), bore flowers on slender cylindrical spikes at branchlet tips and/or at the axillary position. None of the trees were absolutely dioecious as there were always a few cones (<12) on male trees and a few female flowers with stamens attached to their peduncles on the female trees. Trees with no inflorescences account for 6.5% and were labeled as sterile. Since flowers are not produced year round, a more in-depth study is needed to assess the sex of Guam's trees.

DISCUSSION

Damage on a given tree species or a forest stand in time (temporal) or space (spatial) dimensions can be quantified using various methods such as percentiles of crown transparency (defoliation), scale categories of decline severity and/or foliar color and size gradients. McLaughin *et al.* (1992) used tree crown silhouettes and a five-class decline rating in their forest health surveys in Ontario. Similar works of tree and/or forest health assessments were reported by Silverborg and Boss (1968), Augustin *et al.* (2007), and Kneib and Fahrmeir (2007). Our current five-scale decline severity rating system was generally effective in measuring decline at various survey sites on the island of Guam.

Evaluation of decline based on a standard visual scale, as reported here, however, has its shortcomings. Foremost, it is a subjective judgment and lacks clear demarcations of the lower three levels, DS 0, 1 and 2. Confounding the issue further was the fact that trees were generally intermingled and hence estimation of decline on an individual tree basis was difficult. Part of the problem was resolved by flexible judgment of the decline severity, by taking into consideration the prevailing circumstances. For instance, it was noted that healthy trees growing in an open setting have a thicker set of branches and branchlets than those growing elsewhere. To adjust for this, the amount of fullness for healthy trees in non-open settings had to be adjusted downward; that is from 1 to 0, based on the five-scale decline severity rating. Although there were many instances whereby a tree with substantial lower canopy bareness rated as 0 or 1, owing to the natural dynamics in a crowded tree stand, our overall assessments of isolated trees (averaged values of small and large trees) resulted in percentage of bare branches of 0.3, 16.3, 47.8, 74.4 and 99.0 for DS 0, 1, 2, 3, and 4, respectively. The relationship between tree thinning and decline was also supported by the pooled data (small and large trees) of branchlet biomass which resulted in percent weight loss of 0.0, 44.0, 43.1, 55.7 and 91.7 at DS 0, 1, 2, 3 and 4, respectively. Outputs from the current research in terms of growth comparisons may not be directly translated to other ecosystems as there have been reports of variations in growth and morphological traits of *C. equisetifolia* from different provenances. For example, in a provenance trial in Thailand, Pinyopusarerk *et al.* (1996) indicated that three provenances from Malaysia and Thailand formed distinct groups and grew faster than those from Australia, Fiji, Guam, and the Philippines.

Decline does not appear to impact seed germination, as the germination difference across the decline levels was not significant. Though addition of *Casuarina/Frankia* to soil was reported to improve growth of field grown *Casuarina* (Sougoufara *et al.*, 1989), in our study it had no effect on germination.

Microscopically, wood is composed of three main substances, cellulose, hemicellulose and lignin. From the roughly 10 declined trees in which roots were examined, most had evidence of a soft white rot, presumably caused by a wood rotting fungus capable of decomposing lignin and cellulose. Depending on the tree species, such fungal genera as *Phellinus*, *Echinodontium*, *Ganoderma*, *Cryptoporus*, *Sterum*, *Trichaptum*, *Trameters*, *Armillaria*, and *Heterobasidion* could cause white rots. Trees, on the other hand, develop built-in systems that keep out most wood-destroying microorganisms and/or walling-off, confining, or compartmentalizing the few microorganisms that are able to surmount the chemical protective barrier and infect the wood (Shigo, 1967). Symptoms ranging from simple discolorations to distinct patterns of compartmentalization were associated with the current decline problem on Guam. Cross-section cuts at the tree base and at the juncture of one of the declining branches exclusively portrayed discoloration which usually flared from the middle towards the outer wood cells. Despite the fact that most thinning-out symptoms commenced from the tip of the tree, internal discoloration gradients were formed acropetally. The maximum height of internal discoloration recorded for small trees was 5.3 m and for large trees was 11.3 m. Except for a few small discolored or cankered spots, healthy trees were clean in contrast to declining trees. Exponential function very well described the progression of discoloration across the tree height and, thus, can be used for forecasting extents of discoloration on those ironwood trees grown under similar circumstances.

When trees are dying in large numbers, it is usually assumed that they are dying as a result of a plant pathogen or other biotic agent (Mueller-Dombois, 1992). On Guam, from the surveys conducted in the past 17 months, it is not uncommon to see declining trees next to healthy ones (Fig. 8). Association of conks on some of the trees under decline, as well as the seemingly contagious nature of decline development, implicates biological organism/s in causing the decline on Guam. From the literature, *Ganoderma appalanatum* is known to cause root and butt rot on ironwood but is of minor importance (Kohler *et al.*, 1997); however, it has never been reported from Guam. Molecular characterization of conk samples collected by conference participants were identified as *Ganoderma gibbosum* and *G. japonicum* (Smith J., Spaine, P. and Nelson, S., personal communication). Currently, there is an ongoing effort to characterize the most frequent conk types of ironwood on Guam. This collaborative work is underway by conference participants, with the help of Dr. M. Catherine Aime from Louisiana State University. Other pathogens being considered as components of IWTD on Guam are *Botryosphaeria*, *Fusarium*, *Pestalotia*, and yet unidentified fungal and bacterial cultures. In an effort to detect graft transmission of any possible biotic agent, grafting of decline scion to healthy root stock was attempted but failed. However, grafting of a healthy scion to healthy root stock was successful. Based on a discussion of symptomatology during the conference, it was concluded that brown root rot caused by *Phellinus noxius*, wilt or blister bark caused by *Subramanianospora vesiculosa* and bacterial wilt caused by *Pseudomonas* are not likely pathogens of IWTD on Guam.

The role played by insects in IWTD is believed to be less than that played by pathogens. Though two of Guam's invasive beetles, *Protaetia pryeri* (Janson) and *Protaetia orientalis* (Gory and Percheron) have been shown to be associated with ironwood trees, they do not appear to be correlated with IWTD (Campora, 2005). Most of the severely declining trees (DS 2 to 4) on Guam were heavily infested with termites. From past entomological surveys and reports, colonies of *Nasutitermes* sp. and *Microtermes* sp. were found feeding on dead ironwood trees (Moore, A., personal communication). The Philippine milk termite *Coptotermes gestroi* was responsible for killing ironwood trees transplanted onto a new golf course (Yudin, L. S., personal communication). A species of wasp reared from branchlet tip galls was identified as belonging to the genus Selitrichodes (*Eulophidae*: *Tetrastichinae*) by John LaSalle, CSIRO, Australia. Damage to branchlet tips by this wasp is correlated with decline severity in restricted locations on the island; however, future studies are needed to confirm whether the wasp is responsible for any significant impact on health of ironwood trees on Guam.

Preliminary survey results, as presented in this research paper, highlighted that the ironwood tree decline problem is associated partly with the level of human intervention. Data collection on variables like edaphic characteristics, typhoon history, grassland fires, and water table are still underway and will be presented in future publications of the ongoing research. The two major typhoons, Chata'an (July, 2002) and Pongsona (December, 2002), accompanied by the intervening drought were mentioned as possible contributing factors to IWTD on Guam (Guard, C. and Lander, M., personal communications).

CONCLUSIONS

Many of Guam's ironwood trees are dying at levels previously never recorded. Ironwood trees, like all trees, have a natural finite life span within various ecosystems and under various abiotic and biotic pressures. Based on a report for the US Navy (Campora, 2005) and as a result of discussions and findings from the January 2009 Guam Ironwood Tree Decline conference, it was concluded that Guam's trees are not only dying from natural causes but are also in a state of decline attributed to unknown biotic and abiotic factors. It is believed that researchers on neighboring islands, using tools designed in this study, will be able to monitor their tree stands for evidence of IWTD. As a result of this research, tree coring and branch sampling are recommended for adoption as non-invasive means of detecting internal wood discoloration and decay, thereby allowing future studies to focus on determining the rates of the gradients at each of the five-scale decline severity levels. Future research should also explore the soil environment as the possible source/s of decline.

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Table 1. Relationship between the five-scale tree decline severity rating and prevalence of discoloration in terms of the average proportion of two cross-section slices sampled at the juncture with the main stem and 2 meters away.

Five-scale tree decline severity rating (DS)	Number of trees pruned		Proportion of cross-section slices showing wood discoloration	
	Small	Large	Small	Large
0	5	5	0.00	0.05
1	5	5	0.50	0.80
2	7	5	0.46	0.80
3	8	5	1.00	0.95
4	9	6	0.75	1.00

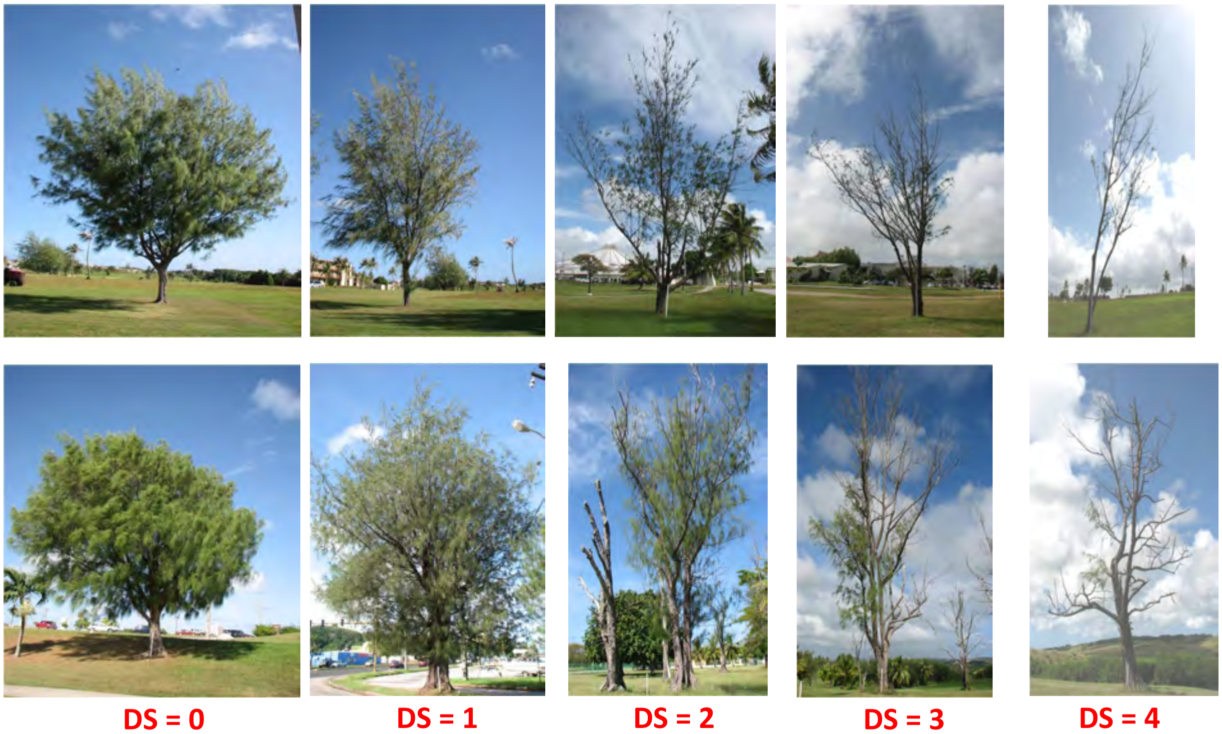

Fig. 1. Representative tree photographs depicting a five-scale visual estimation of ironwood (*Casuarina equisetifolia*) tree decline severity (DS) rating which ranged from 0 (healthy) to 4 (nearly dead) on small **(top)** and large **(bottom)** trees on Guam.

Fig. 2. Percentage of bare branches derived from photograph examination and percentage of decline from the on-site visual estimation for **(a)** small and **(b)** large ironwood trees at each of the five-scale tree decline severity rating ranging from 0 (healthy) to 4 (nearly dead). NB: Decline severity scale 0 for small trees was not shown on the graphs because of zero values.

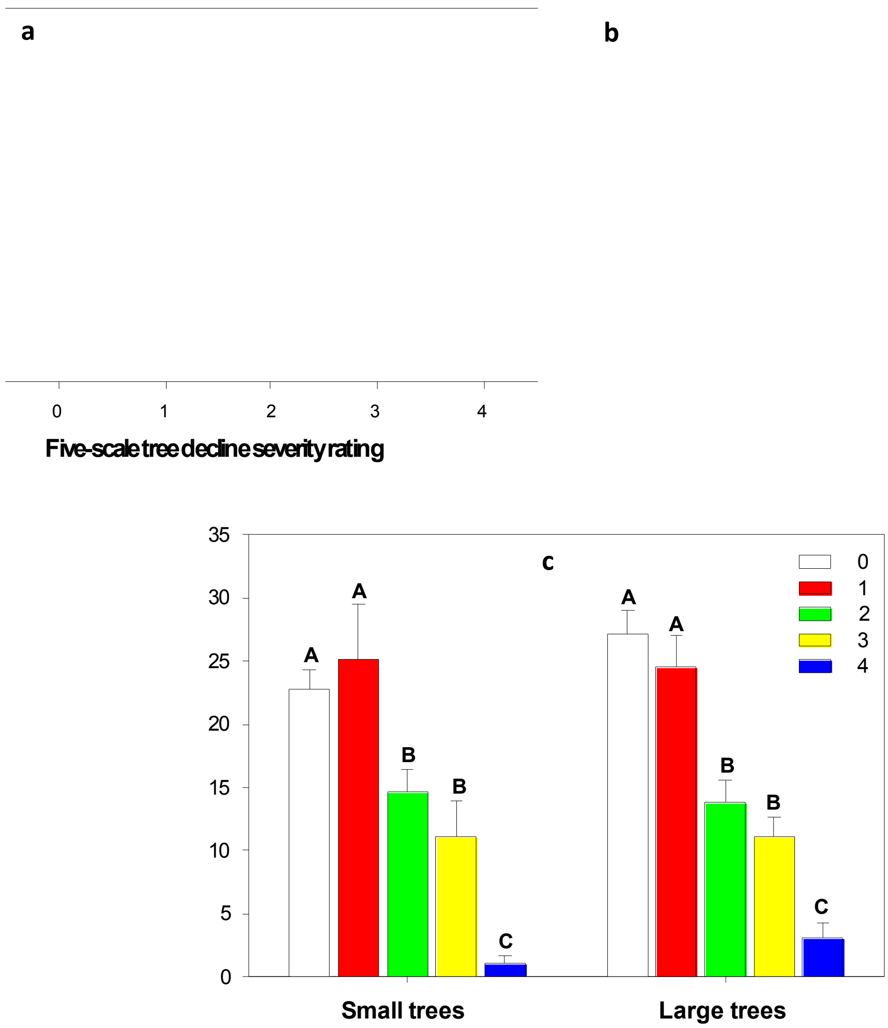

Fig. 3. Relationship between fresh weight of branchlets and their respective 30-cm branch section for **(a)** small and **(b)** large ironwood trees and **(c)** mean separation of branchlet weight across the five-scale tree decline severity rating: 0 (healthy) to 4 (nearly dead). NB: means with the same capital letters on top of each bar group for the small and large trees indicate non-significant difference at P = 0.05.

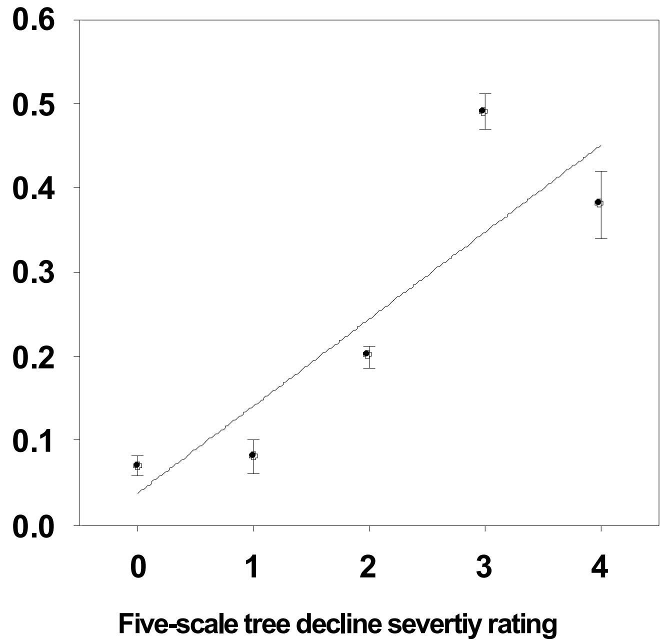

Fig. 4. The proportion of ironwood tree branchlet tips damaged by the gall-forming eulophid wasp across the five-scale tree decline severity rating: 0 (healthy) to 4 (nearly dead).

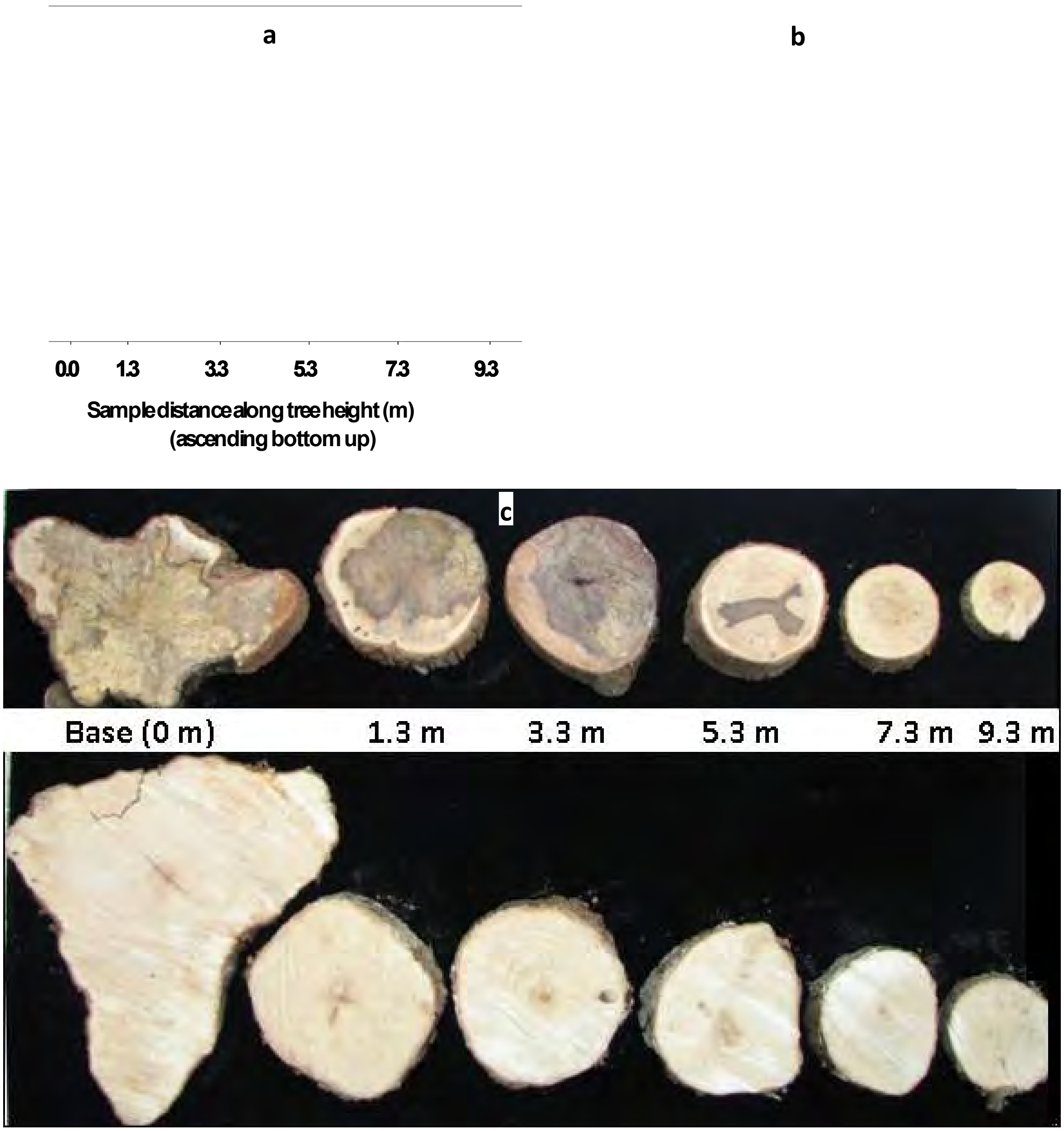

Fig. 5. Non-linear regression analysis of proportion of wood discoloration in trunk cross-sections sampled acropetally for **(a)** small and **(b)** large ironwood trees (*Casuarina equisetifolia*), and **(c)** pictures of cross-sections from small declining **(top)** and healthy **(bottom)** tree.

Fig. 6. Patterns and appearances of discoloration and decay from cross-sectional slices of trunks **(top)** and roots **(bottom)** of declining ironwood trees (*Casuarina equisetifolia*) on Guam.

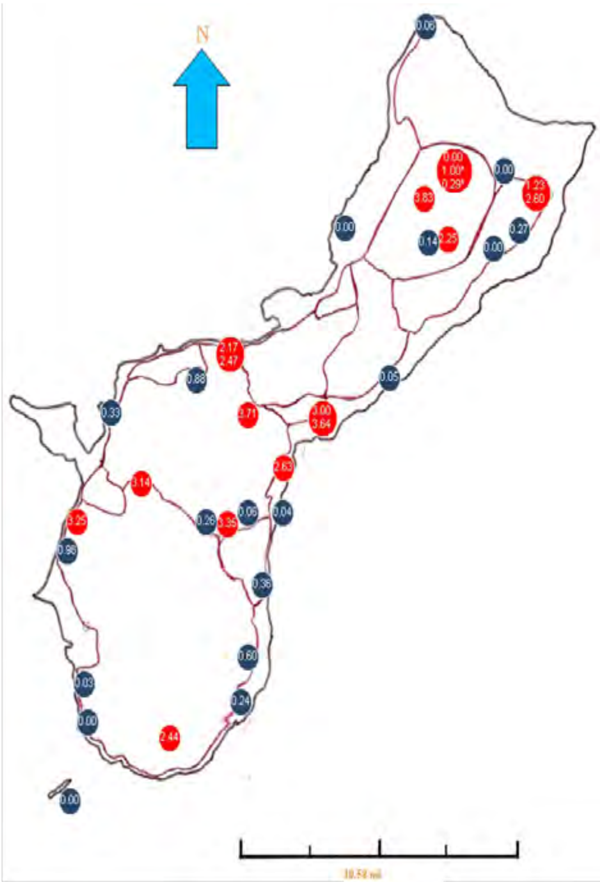

Fig. 7. Mean values of ironwood tree decline severity of each surveyed site on Guam based on a five-scale decline severity rating assessment from October 2008 to June 2009: 0 (healthy) to 4 (nearly dead).

Table 2. Summary of mean values for the five-scale decline severity rating and the prevalence of conks on ironwood trees at various locations across Guam categorized based on latitude, stand and levels of management.

Criteria	No. of Sites	Decline severity (mean ± SE)	Conk prevalence (mean ± SE)
Latitude			
Northern	15	0.80±0.31	0.08±0.03
Central	13	1.95±0.41	0.35±0.13
Southern	10	0.83±0.36	0.06±0.03
Stand			
Natural	10	0.08±0.05	0.01±0.00
Planted	28	1.60±0.26	0.19±0.05
Management			
Slight/low (0)	8	0.16±0.11	0.00±0.00
Moderate (1)	9	1.44±0.51	0.22±0.13
High (2)	21	2.15±0.30	0.21±0.05

NB: **Latitude:** Northern (from N13°30′ upward to the northern tip), Central (N13°30′ ≤ x ≤ N13°22′) or Southern (from N13°22′ downward to the southern tip); **Management:** 0 (very negligible to non-disturbed), 1 (slightly disturbed) and 2 (highly disturbed or intensively managed).

Fig. 8. A windbreak of ironwood trees with healthy and severely declined trees at the Yigo Agricultural Experimental Station, Guam.