Gago,
Guam Ironwood Tree,
Casuarina equisetifolia

Past, Present, Future

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Introduction: This guide serves as an introductory text on plant health care for the Gago or Guam's ironwood (*Casuarina equisetifolia*). It contains some general information about the tree including its history on Guam and its importance to the region. It explains ironwood decline and its underlying causes. Finally, it provides some tree health care recommendations and suggestions for future research.

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BRIEF CONTENTS

SUMMARY

Despite the myriad of utilities and merits of the ironwood tree (*C. equisetifolia* subsp. *equisetifolia*) to the Pacific island of Guam, its future is in doubt because of deteriorating health and survival rate. Ironwood trees (*Casuarina equisetifolia*), like all trees, have a natural finite life span within a given ecosystem; however, Guam's trees are dying at unexpected rates. What is happening on Guam fits the classic definition of tree decline: symptoms are nonspecific such as the thinning of branches; tree health gradually deteriorates leading to tree death over a course of several years; and decline is attributed to a complex environment of infectious and non-infectious agents. However, Guam's trees deviate from the classic model wherein mature trees are more prone to decline.

Decline was first noticed in 2002 by a local farmer. The trees at that site were less than 10 years old and planted in single-row windbreaks of several hundred trees. Less than 5 trees were characterized as wilted with the following symptoms: acropetal progression of chlorosis, tip-burn of lower branchlets giving the tree a singed appearance, and tree death within 6 months. Roughly 15 trees had symptoms of decline, which included internal wood discoloration, thinning of branches, and tree death after several years. Natural Resources personnel with Commander Navy Region Marianas (COMNAVMAR) became aware of trees dying in large numbers at the Naval Station in 2004. At that time approximately one third of all the ironwood trees at the naval station were dead. By 2005, Ironwood Tree Decline (IWTD) was widespread on Guam. In January 2009, a five-day IWTD conference was held with participants from Guam and off-island. Six off-island experts and other participants visited healthy and declined tree sites, collected samples, and reviewed research related to *C. equisetifolia* production worldwide and its growth on Guam. Participants concluded that a complex of biotic and abiotic factors were responsible for the decline and subsequently advanced the theory that an opportunistic conk-producing fungus like *Ganoderma* and/or *Phellinus* in association with wounding could explain the majority of Guam's declining trees.

To assess the level of ironwood tree decline on Guam, photographs of 44 randomly selected trees with varying levels of decline were categorized into small (CBH ≤ 100 cm) or large (CBH >100 cm) based on their circumference at breast height (CBH) and visually catalogued into a five-scale decline severity (DS) rating. On subsequent surveys, trees with different DS ratings were characterized visually for branch thinning and quantitatively for branchlet ("needle") biomass. As DS increased from 0 (healthy tree) to 4 (nearly dead tree), branch thinning progressively increased from 0 to 95.0% and 0 to 92.5% for small and large trees, respectively. There was no significant difference between branchlet biomass for DS 0 and DS 1 nor between DS 2 and DS 3 trees. The greatest branchlet weight loss, at 95.3%, occurred in DS 4 trees. Internal symptoms included various patterns of discolorations in trunks and a white soft-rot in roots. Discoloration was consistently traced into branches through cross-sectioning at the branch-trunk interface. In branches, the presence of discoloration was only 100% consistent in DS 3 and 4 trees. External symptoms start at the top of tree and progress downward; whereas, internal discoloration starts at the tree's base and diminishes acropetally.

To determine the status of the decline problem and to seek possible causes, a survey of 1427 trees was conducted in 2008 and 2009. A highly significant (p=0.0001) linear function

($r^2 = 0.997$) between the presence of basidiocarps and decline severity emerged from the survey. Basidiocarps (“conks”) were either flat (resupinate) or shelflike (conk). Sixty-five percent of the trees at the most severe level of decline (nearly dead) had basidiocarps. Thirty-five "conks" were collected from the survey area under different stages of tree decline. Species from five basidiomycete genera of the class Agaricomycetes, belonging to the orders Polyporales (*Ganoderma, Favolus, Pycnoporus*), Hymenochaetales (*Phellinus*) and Thelephorales (*Sarcodon*) were identified based on macro- and micromorphology and DNA sequencing. The most common species observed was in the genus *Ganoderma.* Diagnostics was based on the prolific production of double walled basidiospores from sporocarps (a characteristic feature of members of the Ganodermataceae). Nuclear ribosomal (ITS) DNA sequencing confirmed Guam's species as a member of the *G. australe* species complex. The second most frequently collected conk belonged to the genus *Phellinus.*

Various modeling techniques were applied to the 1427 tree survey data set. For each sampled tree, the level of decline was measured on an ordinal scale consisting of the five-decline levels ranging from healthy (DS=0) to near dead (DS=4). Several predictors were also measured including tree diameter, fire damage, typhoon damage, presence or absence of termites, presence or absence of basidiocarps, and various geographical or cultural factors. The five decline response levels can be viewed as categories of a multinomial distribution, where the multinomial probability profile depends on the levels of these various predictors. Such data structure is well-suited to a proportional odds model, thereby leading to odds ratios, involving cumulative probabilities which can be estimated and summarized using information from the predictor coefficient. The logistic model used the variable dieback, which is derived from the decline severity variable. Healthy tree (DS=0) was assigned a dieback value of 0, all other decline severity trees DS=1, 2, 3, or 4) were assigned a dieback value of 1. Various modeling techniques were applied to address data set issues: reduced logistic models, spatial relationships of residuals using latitude and longitude coordinates, and correlation structure induced by the fact that trees were sampled in clusters at various sites. Among the findings, factors related to ironwood decline were found to be “conks”, termites, and level of human management.
A conk-producing species in the *Ganoderma australe* complex was identified as the primary wood-rotter. This fungus was commonly found on Guam where IWTD is widespread and rarely on Saipan, a nearby island where the majority of trees are considered healthy. With the addition of GIS map derived variables to those of the original model, it was found that trees are less likely to exhibit ironwood decline symptoms when there is adequate soil moisture holding capacity, as in a forest setting or in a properly managed landscape such as a golf course. Likewise, the amount of declining trees at a given site can be expected to intensify with increases in the occurrence of “conks”, termites, altitude, or tree size. When tree circumference and dieback maps were compared, tree site productivity could not explain the high level of IWTD predicted in central Guam. The increased presence of termites, “conks”, and storm damage with increasing tree size suggests that under ideal tree stand conditions, these variables are part of the normal process of tree senescence.

Bacterial colonization of the xylem is seen in trees with thinning foliage, which is indistinguishable from those attributed to ironwood decline. Three bacteria were consistently isolated: *Ralstonia solanacearum, Klebsiella oxytoca,* and *K. variicola.* We believe *Klebsiella* spp. are responsible for the wetwood symptom associated with Guam's declining trees and that both *R. solanacearum* and *Klebsiella* spp. play a role in tree decline. In the future, the current model will be strengthened, with the addition of *Ralstonia* and *Klebsiella* survey data.

INTRODUCTION

History: *Causarina equisetifolia*, locally known in English as ironwood and in the Chamorro language as "gago," 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 indicate 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). It has been continually propagated on Guam since the 1600's, possibly due to its usefulness and low maintenance requirements. As a result of its tolerance to salt spray and typhoon damage, its ability to support nitrogen-fixing *Frankia*, and endo- and ectomycorrhizae and protioid roots, the tree is able to thrive in the Mariana Islands where typhoon and coral sand beaches and other nutrient-poor soils are commonplace.

Botanical characteristics: The tree is an evergreen angiosperm. Its needle-like jointed branchlets bear the anatomical minute tooth-shaped leaves. As a result of limited leaves and floral structures, the tree has the ability to conserve moisture and tolerate drought. Within the Mariana Islands, the average lifespan of ironwood is estimated to be 35 to 90 years, with a maximum height and circumference at breast height of 13.7 and 2.9 m, respectively. Due to damage from typhoons in the Mariana Islands, exposed trees are often topped with prolific epicormic shoots, resulting in a shorter tree with a wider crown than what is typically seen in Hawaii, an area with few typhoons.

Ecology: Ironwood thickets are a component of Guam's forestland where it is considered a secondary forest species (Liu and Fischer, 2006). Ironwood trees do not compete with native tree species in undisturbed limestone forests (Moore, 1973), although they grow nearly everywhere: beaches, landfills, road shoulders, cleared land, and vacant lots. In the Mariana Islands it grows both in the clay volcanic soils of savanna grasslands and calcareous and loamy sands of coastal strands. In large dense stands, trees produce a thick, slowly decomposing, allelopathic litter layer that eliminates nearly all understory vegetation.

Several prominent forest features of ironwood on Guam were mentioned in a 2002 Guam Forest Bulletin (Donnegan *et al.*, 2004). Ironwood trees were reported to be among the healthiest trees on island with an estimated population of 115,924 for trees greater than 5 inches in diameter at breast height. *C. equisetifolia* was mentioned as a prominent member of the halophytic (sea-salt adapted) vegetation type. This vegetation is found along beaches in the north and south, where it may be composed solely of ironwood or a mixture of other species including *Cocos nucifera*, *Guettarda speciosa*, *Hernandia sonora*, *Pandanus tectorius*, *Scaevola taccada*, *Thespesia populnea*, and *Tournesfortia argentea*. On 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.

MATERIALS AND METHODS

Conference: Participants and attendees included administrators, researchers, students, the general public, and six off-island experts. Fourteen sites were visited during the 5-day conference period where samples in the form of branches, cross-sections (roots, trunks, and branches) and sporocarps were collected and brought to the laboratory at the University of Guam's science building.

Ironwood tree decline (IWTD): Photographs of 44 randomly selected trees with varying levels of decline were categorized into saplings to small trees (DBH $\leq$ 32 cm) or large trees (DBH > 32 cm). These were then visually categorized based on a five-scale decline severity (DS) rating. Percent bare branches (PBB) were determined by analyzing the photographs. Cross-sections of 5 small and 3 large tree trunks and of branch trunk intersections from 34 small and 26 large trees were examined for evidence of discoloration or wood rot. Four to five branches from randomly selected trees were removed (30 cm from branch tip) and growth parameters measured. The branch sections were stripped and branches and branchlets ("needles") weighed. Cones were counted, weighed, and placed in 20-cm diameter Petri dishes on the laboratory bench (temperature 24 - 25 °C and 50 – 55 % relative humidity) to promote seed release.

Nematode extraction: Ten grams of roots were collected from the top 10 centimeters of soil. Eight trees were surveyed: four were in decline and four appeared healthy. Roots were rinsed to remove soil. Roots were cut into sections of a centimeter in length. Ten grams of roots were placed in a flask with 200 ml of water and placed in a shaker at 200 rpm for a total of 57 hours of shaking. The water and roots were passed through a 140-mesh sieve to collect the roots, and a 400-mesh sieve to collect the nematodes. The 400-mesh sieve was flushed and nematodes were collected in 20 ml of water. Two ml of nematode suspension were placed in Petri dishes and identified under an inverted compound microscope. Nematode numbers are per one gram of root tissue.

One hundred ml of soil were collected from the top ten centimeters of soil associated with *Casuarina* roots. The soil samples were processed using a modified Jenkins (1964) centrifugation and flotation technique, using 100 ml subsamples. Twenty ml of the nematode suspension was placed in tubes and a 2.0 ml aliquot was placed in a cover slip-bottom dish and all the nematodes present were identified to the lowest taxon possible. The resulting data were recorded as nematodes per 10 ml of soil.

DeLey's and Blaxter's (2002) system of nematode classification was used. Photographic images were taken of many of the nematode taxa found in this study. An inverted Nikon compound microscope and a Leica DM1000 compound microscope were used for taxon identification. A Motic 2.0 camera and an imaging program were used for the pictures.

Gall wasp damage: The longest branches of a tree attainable by a ladder and/or modified rope system were cut 30 cm from branch tip. Four branches from each of 5 declined trees (DS=0,1,2,3,4) were removed and proportion of "needles" damaged by the gall wasp determined.

Tree survey: In 2008 and 2009, GPS-assisted surveys were conducted along Guam's major thoroughfares, coastal intersecting roads to farmers' fields, agricultural experimental stations, parks, beaches, cliffs, and golf courses. For each sample tree, a set of measurements were taken and selected for analysis. Sites were evaluated for stand origin (natural and planted) and management (slight, moderate and high). Slight management practices were those associated with tree stands (natural or planted) that were allowed to develop unattended. Moderate management practices were those associated with tree stands in parks and cemeteries. High management practices reflect conditions around ironwood trees on golf courses and campuses. 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 (Figure 8). A total of 1398 trees at 38 sites were surveyed for decline from October 2008 to June 2009 (Survey I). From July 2009 to December 2009, a follow-up survey of the original trees was conducted (Survey II). This survey was expanded to include additional characteristics as well as 29 additional trees and 6 sites.

Statistical modeling: Modeling was used to evaluate a set of data from 1427 individual trees, 44 sites, and 16 GIS maps. The primary objective of using statistical models was to find possible factors that could explain tree decline, in other words to find the parameters that have a positive or negative impact on the tree (K. Schlub, 2010). Various modeling techniques were applied to address data set issues. The logic model, which used dieback as the response variable, was found to be the best fit with the data.

Tree sites were examined using the original tree explanatory variables plus those derived from 16 GIS map characteristics (Kennaway, 2010): cemetery buffer, FIA trees with conks (multi-ring buffer), fire risk, fires per year, proximity to golf courses, land cover, management areas, school buffer, soil available water at 150 cm, available water at 25 cm soil depth, soil depth to restrictive layer, soil series, and vegetation. Some maps were dropped from the analysis because of correlations between regressors. A multiplicative change in the odds ratio of unhealthy vs healthy was calculated one regressor at a time by increasing the regressor one unit and holding all remaining regressors constant.

Sporocarp survey: Trees were only surveyed for sporocarps of basidiomycetes "conks", due to infrequent sporocarps of non-basidiomycetes wood rotters. A tree survey was conducted to quantitatively and qualitatively document existing basidiocarps of wood decay fungi on ironwood trees in Guam and Saipan in January and February, 2012. The methodology used to document existing basidiocarps was developed, in part, to be consistent with previous surveys of ironwood on Guam (R. Schlub *et al.,* 2010). Tree surveys were conducted in areas where trees were moderate to large in size, easily accessible and where their health was in question. Three areas on Guam and six on Saipan were surveyed. One hundred three ironwood trees were inspected in three different locations on Guam and 44 trees in six locations on Saipan.

PESTS AND DISEASES

Guam's ironwood tree insects and pathogens are generally considered incidental or opportunistic. Damage by incidental pests are precluded primarily by abiotic disorders. Drought periods especially during the dry season primarily affect plants in poor planting sites where the trees become stressed and consequently become vulnerable to insects and pathogens. Some pathogens may be agents of latent infections; therefore, the infection precedes environmental changes that trigger symptom production.

Scarab beetles: Scarab larvae of the subfamily Cetoniinae, the group to which the beetles *Protaetia pryeri* and *Protaetia orientalis* belong, feed on organic matter in the soil, and some species damage the roots of plants (Borror *et al*., 1989). *P. orientalis* was first noted on Guam in 1972 (Schreiner and Nafus 1986). The discovery of a beetle matching the description of *P. pryeri* was first published in 1990 (Schreiner, 1991). Beetle larvae were found under *C. equisetifolia*, *Pithocellobium dulce* Roxb. and *Leucaena leucocephala* (Lam.) de Wit, and in one instance under turfgrass. Larvae and frass were found under healthy and diseased *Casuarina*. Preliminary results from field research conducted by Campora in 2005 at the naval station and naval magazine in Guam, showed no connection between the invasive beetles *P. pryeri* (Janson) and *P. orientalis* and dying ironwood trees.

Termites: In India, termites feed on underground roots and stems of live *C. equisetifolia.* This type of damage is believed to be occurring in Guam as well. From past entomological surveys and reports, there are at least six species of termites in Guam (Su and Scheffrahn, 1998). 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). The hollowing of trees by termites is often seen in sites with a high decline incidence (**Figure 1**). In some instances, it appears that old conks, serve as a food source and entry point for termites. It is also possible that termites are contributing to the high incidence of xylem residing bacteria and *Ganoderma* in declining trees through transmission and or the creation of points of entry for the pathogens.

Figure 1. A cross-section of a small declined windrow tree (DS=3) infested with termites. Bacterial ooze positive for *Ralstonia solanacearum* was present on the cut surface. No basidiocarps were present.

Gall wasp: Damage to branchlet tips (**Figures 2, 3 and 4**) by an unidentified gall wasp (**Figure 5**) is known to reduce branchlet length and total banchlet mass (Mersha *et al*., 2009). The impact on tree health is probably negligible but may be significant on trees with thinning foliage (**Figure 6**). The wasp reared from branchlet tip galls was identified as belonging to the genus *Selitrichodes* (E*ulophidae: Tetrastichinae)* by John LaSalle, CSIRO, Australia.

Figure 2. Healthy branchlet tip of *C. equisetifolia* (top) and a tip further magnified with gall wasp damage (bottom).

Figure 3. *Casuarina* wasp exit hole on damaged branchlet tip of *C. equisetifolia.*

Figure 4. Witches' broom symptom on ironwood branch caused by infestation of gall wasp (foreground) in comparison to healthy branches (background).

Figure 5. Unidentified *Casuarina* miniature gall wasp belonging to the genus *Selitrichodes* (E*ulophidae: Tetrastichinae)* resting on branchlet of *C. equisetifolia.*

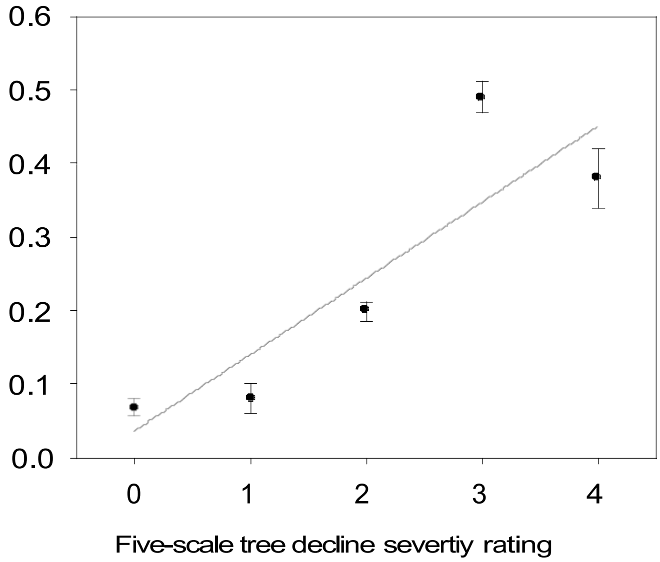


Figure 6. The proportion of ironwood tree branchlet tips damaged by the *Casuarina* gall wasp across the five-scale tree decline severity rating: 0 (healthy) to 4 (nearly dead).

Xylem residing bacteria: *Ralstonia solanacearum*, the cause of bacterial wilt, is among the most common worldwide reported pathogens of *Casuarina*. It is a xylem-resident bacterium mainly entering via roots. Occasionally reported as serious, bacterial wilt has emerged as the most serious disease of *Casuarina* in China (Huang *et al*., 2011) after its discovery in 1964.

Based on culturing from symptomatic tissues, immunostrip data, LAMP data, and other tests, *R. solanacearum* has now been confirmed on Guam. In addition, two companion bacteria (*Klebsiella oxytoca* and *K. variicola*) were found to be associated with the wetwood symptom, which is common in declined trees. Thus, two xylem-resident bacterial genera are associated with IWTD, *Ralstonia* and *Klebsiella*. In Guam, trees that harbor these bacteria do not manifest the same symptoms as those observed in China. In China, the field symptom is rapid tree death (**Figure 7**), which is triggered by severe environmental stress such as that caused by a typhoon or draught. On Guam, bacterial colonization of the xylem results in trees with thinning foliage, which is indistinguishable from symptoms associated with IWTD (**Figure 8**).

Figure 7. Bacterial wilt of *C. equisetifolia* sapling in China (*photo provided by Dr. Chonglu Zhong*).

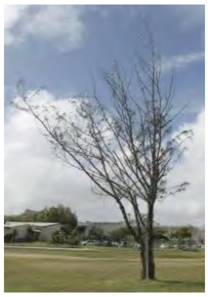
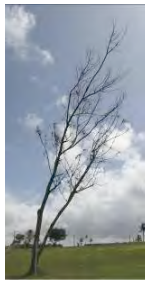
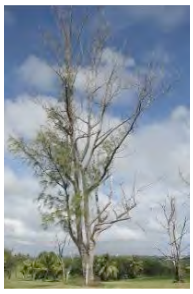
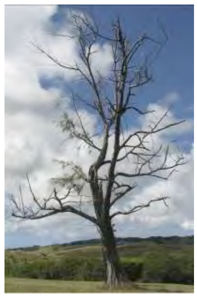
				
DS 0	1	2	3	4
PBB 0	9	49	67	98
				
DS 0	1	2	3	4
PBB 0	13	51	79	96

Figure 8. Representative photographs of small (above) and large (below) solitary trees from locations around Guam depicting five-levels of decline severity (DS) and percentage of bare branches (PBB).

Differences between China and Guam diseases can also be seen in symptoms revealed in cross-sections of the trunks and limbs. In China, xylem vessels of trunk cross-sections contain diffused areas of slightly darker tissue and yield copious amounts of bacterial ooze (**Figure 9**).

Figure 9: Cross-section of a tree in China with bacterial wilt reveals copious amounts of bacterial ooze and tissue discoloration. *From a presentation of Huang Jinshui, He Xueyou, Ke Yuzhu, Cai Shouping, Chen Duanqin, and Tang Chensheng of Fujian Academy of Forestry Sciences at International Casuarina Workshop Haikou, China 21-25 March 2010.*

On Guam, cross-sections of infected trees revealed uncontained areas of dark discoloration "wetwood", with sharply defined borders that radiated from the center of the tree. Droplets of bacterial ooze may or may not appear and are generally restricted to the "wetwood" which has a high moisture content (**Figure 10**).

Figure 10. Cross-sections of infected *C. equisetifolia* tree revealed expanding areas of moist discolored wood (wetwood) that radiated from the center of the tree accompanied by droplets of bacterial ooze composed of *Ralstonia solanacearum* and *Klebsiella* spp.

Nematodes: Not a great deal is known regarding the effects of nematodes on *C. equisetifolia*. However, certain species of nematodes do infect its roots: *Helicotylenchus cavenessi, Radopholus similes, Rotylenchulus reniformis, Tylenchus* sp., *Xiphinema ifacolum;* and Angiospermae: *Cuscuta campestris, Dendrophthoe falcata, Dendrophthoe lanosa.* Nematode infections rarely result in the death of infected hosts, but it is not uncommon for certain root disease fungi to infect nematode-damaged roots, resulting in further damage, including mortality in some cases.

To determine if there is a linkage between the presence of nematodes and ironwood decline, Dr. Marisol Quintanilla extracted nematodes from ironwood roots and associated soils. *Helicotylenchus* sp. was the only herbivore recovered from healthy trees roots. *Tylencholaimellus* sp., *Aphelenchoide*s sp., and one unknown were recovered from trees with dieback. *Helicotylenchus* sp. and *Tylenchus* sp. were consistantly collected from healthy and dieback soil (**Table 1**). It was concluded that *Helicotylenchus* was the only nematode that was isolated with enough consistency to be remotely implicated in ironwood decline.

Table 1. Nematode counts per 10 ml soil samples from healthy ironwood trees and those with dieback.

	HEALTHY					DIEBACK									
SAMPLE	**11-9**	**11-10**	**11-28**	**11-38**	**Average**	**11-8**	**11-31**	**11-29**	**11-35**	**11-33**	**11-15**	**11-41**	**11-42**	**11-25**	**Average**
Acrobeles	0	0	0	10	2.5	0	0	0	10	0	0	0	0	0	1.1
Aphelenchoides	0	10	0	0	2.5	0	0	0	0	0	0	0	0	0	0
Aphelenchus	10	0	0	0	2.5	0	0	0	0	0	0	0	0	0	0
Cephalobida	0	0	0	0	0	0	0	10	0	0	0	0	0	0	1.1
Eucephalobus	0	0	0	0	0	0	0	0	30	0	0	0	0	0	3.3
Helicotylenchus	10	30	0	0	10	30	170	0	10	90	10	10	0	30	38.9
Leptonchus	0	0	10	0	2.5	0	0	0	0	0	0	0	0	0	0
Meloidogyne	0	0	0	0	0	0	0	0	0	20	0	0	0	0	2.2
Mesocriconema	0	10	0	0	2.5	0	0	0	0	0	0	0	0	0	0
Monhystera	0	0	0	0	0	0	0	0	0	10	0	10	0	10	3.3
Paratylenchus	0	0	0	0	0	0	0	0	0	0	0	10	0	0	1.1
Plectus	0	0	0	10	2.5	0	0	0	0	0	0	0	0	0	0
Pratylenchus	0	0	0	0	0	0	10	0	0	0	0	0	0	0	1.1
Prismatolaimus	0	0	0	0	0	0	10	20	0	0	0	0	0	0	3.3
Rhabditidae	0	0	0	0	0	0	0	20	0	0	0	0	0	50	7.8
Rhabditis	0	0	0	0	0	0	0	0	0	10	0	0	0	0	1.1
Tylenchus	0	0	10	20	10	30	10	20	10	0	0	0	0	0	7.8
Wilsonema	0	0	0	0	0	0	0	0	0	20	0	0	0	0	2.2

Fungal wood-rot: There are many fungi involved in wood rot or decay. One group is the basidiomycetes. The fruiting bodies or sporocarps of these fungi are called basidiocarps. The basidiocarps found in Guam and Saipan were either flat (resupinate) (**Figure 11**) or shelflike (conk) (**Figures 12** and **14**). Though usually present, the sporocarp does not have to be present for wood rot to occur. To date, five conk-forming basidiomycete genera have been identified from ironwood on Guam, all in the class Agaricomycetes: *Ganoderma*, *Favolus*, *Pycnoporus*, *Phellinus*, and *Sarcodon* (R. Schlub *et al*., 2011). Distinguishing features for Guam's *Ganoderma* sp. sporocarp include an unvarnished, gray to brown fan-shaped cap, with a white pored undersurface that easily bruises brown when young (**Figure 12**). *Ganoderma* invades woody tissue through an unrestricted mycelial network while sustaining itself on cell and cell wall components (**Figure 13**). Descriptors for Guam's *Phellinus* sp. sporocarp include often formed in overlapping shelves with golden-brown pubescent cap margins when young and a yellow-brown undersurface (**Figure 14**).

Figure 11. Multiple sporocarps of a unidentified resupinate polypore on an ironwood tree (*Casuarina equisetifolia*), on the campus of the University of Guam, Mangilao, Guam.

Figure 12. Sporocarp (conk) of *Ganoderma australe* species complex on *C. equisetifolia.* On Guam, 100% of the trees in decline sites may have conks on their roots or butts.

Figure 13. Cross-section of rotted ironwood tree butt infected with *Ganoderma australe* species complex. Note expanding network of white mycelial strands. This sample was negative for *R. solanacearum.*

Figure 14. Sporocarp (conk) of a *Phellinus* sp. on *C. equisetifolia* trees. This fungus is likely a part of the normal decay process of the ironwood trees in the Mariana Islands and not a contributor to IWTD.

As a result of surveys in January and February 2012, there were mainly two species of basidiomycetes on most affected trees*;* *Ganoderma* sp. (*australe* group) which fruits on tree roots and butts and less commonly on trunk (**Figure 12**) and *Phellinus* sp., which primarily fruits on the butt (**Figure 14**) (R. Schlub *et al.,* 2012). Both are common on Guam (**Figure 15**) and infrequent on Saipan (**Figure 16**). The presence of *Ganoderma* is a consistent indicator of a tree in decline and its occurrence is irrespective of tree size. *Phellinus* is found in association with

Ganoderma or by itself on very large mature trees. On its own, *Phellinus* does not appear to be a contributor to ironwood decline.

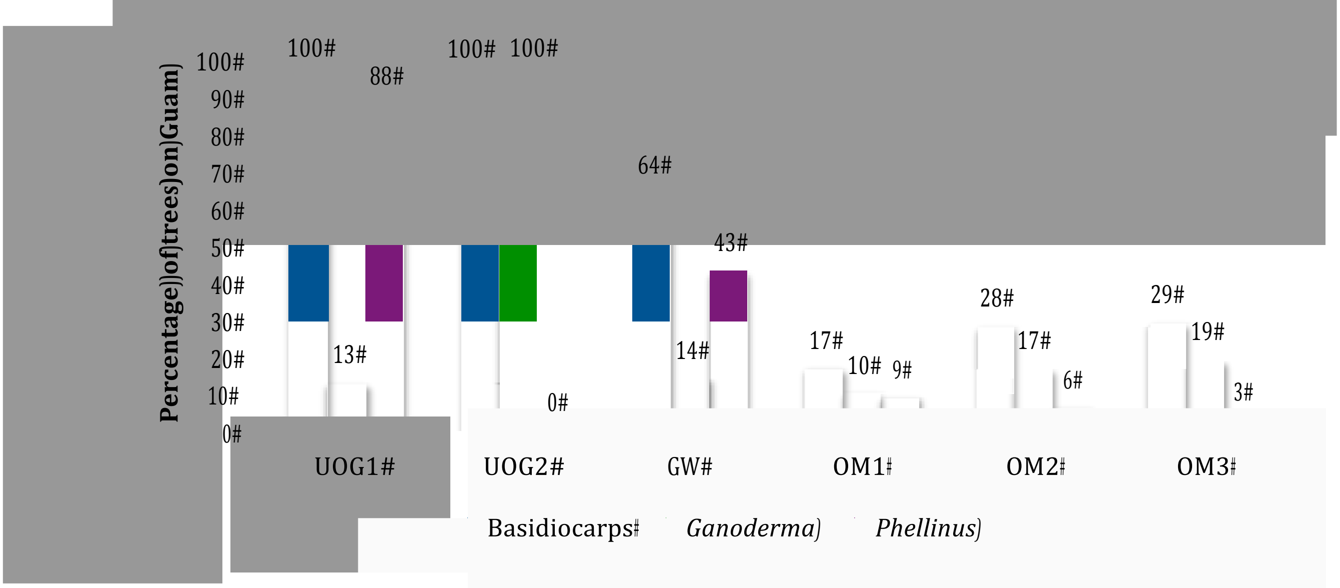


Figure 15. Percentage of trees on Guam with root, butt, or lower trunk basidiocarps, and those trees with identifiable conks of *Ganoderma* (*australe* complex) or *Phellinus*. The survey area and sites include trees flanking sidewalks on the University of Guam campus (UOG 1 & 2), a woodlot at George Washington High School (GW), and windbreaks at Onward Mangilao Golf Course (OM 1, 2, & 3).

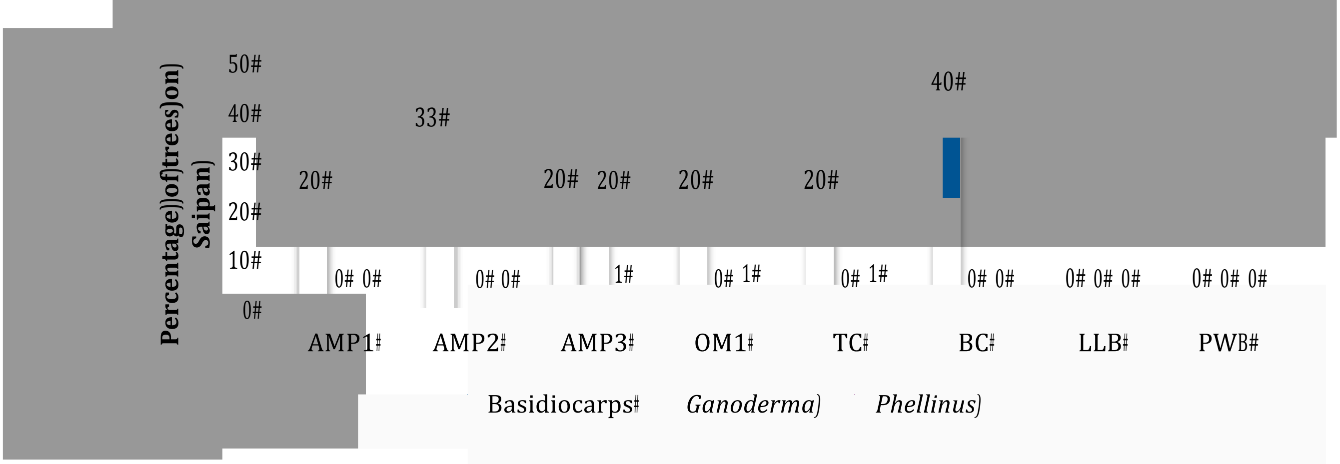


Figure 16. Percentage of trees on Saipan with root, butt, or lower trunk basidiocarps, and those trees with identifiable conks of *Ganoderma* (*australe* complex) or *Phellinus*. The survey area and sites on Saipan include trees in landscaped areas at American Memorial Park (AMP 1, 2, & 3), Fisherman Memorial (FM), Tennis courts (TC), Banzai Cliff (BC), Lau Lau Bay (LLB), and Public Works Beach (PWB).

IRONWOOD DECLINE

Ironwood trees on the island of Guam are in the midst of a decline that was first noticed in 2002 by a local farmer (Mersha *et.al.,* 2009). The trees at that site were less than 10 years old and planted in single-row windbreaks of several hundred trees. Less than 5 trees were characterized as wilted with the following symptoms: acropetal progression of chlorosis, tip-burn of lower branchlets giving the tree a singed appearance, and tree death within 6 months. Roughly 15 trees had symptoms of decline, which included internal wood discoloration, thinning of branches and tree death after several years (**Figure 8**). By 2005, Ironwood Tree Decline (IWTD) was widespread on Guam (Campora, 2005). In January 2009, a five-day IWTD conference was held with participants from Guam and off-island (**Figure 17**). Six off-island experts and other participants visited healthy and declined tree sites (**Figure 18**), collected samples, and reviewed research related to *C. equisetifolia* production worldwide and its growth on Guam (Mersha *et al.*, 2010a, Mersha *et al*., 2010b; K. Schlub, 2010; R. Schlub *et al.*, 2010). Findings of the conference were reported at the 4th International Casuarina Workshop (R. Schlub *et al.,* 2011).

Figure 17. Participants from the 2009 five-day IWTD conference.

Figure 18. Ironwood Decline Conference attendees visit a declined

tree site at Andersen Air Force Base, Yigo, Guam.

Tree survey: Thirty-eight sites (1398 trees) were surveyed for decline from October 2008 to June 2009 (Survey I) (**Figure 19**). From July 2009 to December 2009, a follow-up survey of the original trees was conducted (Survey II) (**Figure 19**). For each tree and site, explanatory variables of decline were measured including tree circumference, fire damage, typhoon damage, presence or absence of termites, presence or absence of "conks", and various geographical or cultural conditions.

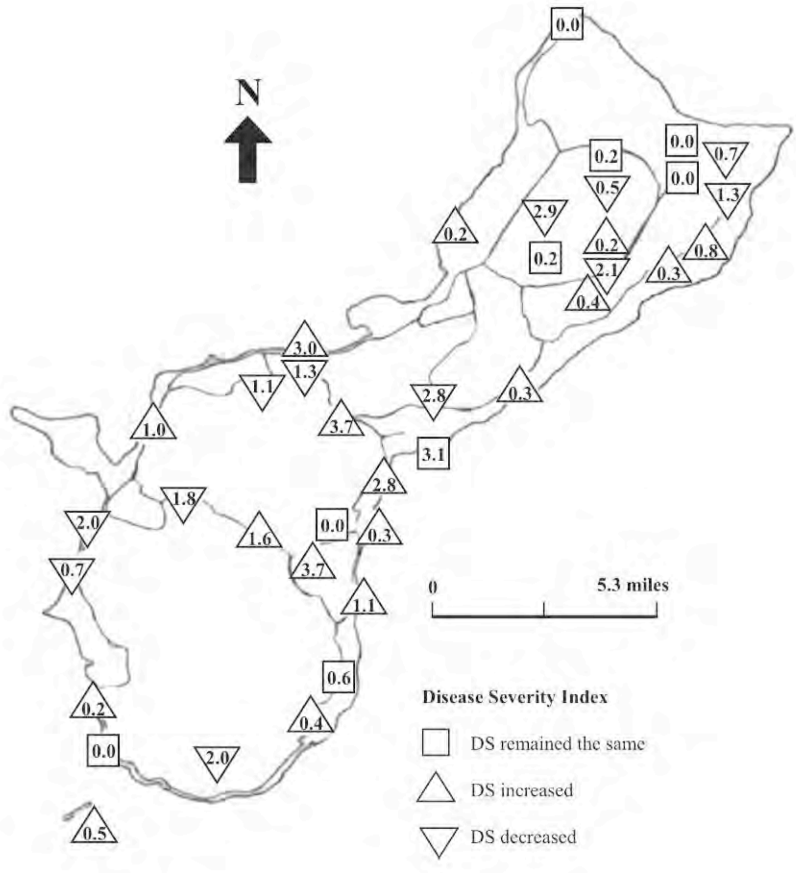


Figure 19. Means of decline severity (DS) found at sites during Survey II (July to December 2009). Values in comparison to Survey I (October 2008 to June 2009) remained nearly the same (square), increased (up-triangle) or decreased (down-triangle).

Symptoms: The presence of discoloration at the branch juncture of large branches of declining trees was consistent for large trees at all DS levels, where it discolored 80 to 100% of the branch cross-sections but was inconsistent for small trees at 1 and 2 DS levels. In healthy small trees, the cuts were clean and non-discolored. In large trees discoloration due to mature heartwood was occasionally observed. There was a clear, consistent gradient of discoloration within the tree trunk of declining trees (**Figure 20**). Linear functions derived from the average proportion of discolored wood at each sampling distance describe well the actual acropetal wood discoloration gradients recorded within small and large trees (**Figure 20**). Wood rotting fungi that produce "conks" are known to cause the internal discoloration and white soft rot commonly found in DS 3 level trees (**Figure 13**). The importance of these fungi in decline is also supported by the fact that the percentage of trees with "conks" increased with IWTD: 2, 18, 35, 47, and 66 % for DS 0, 1, 2, 3, and 4 level trees, respectively.

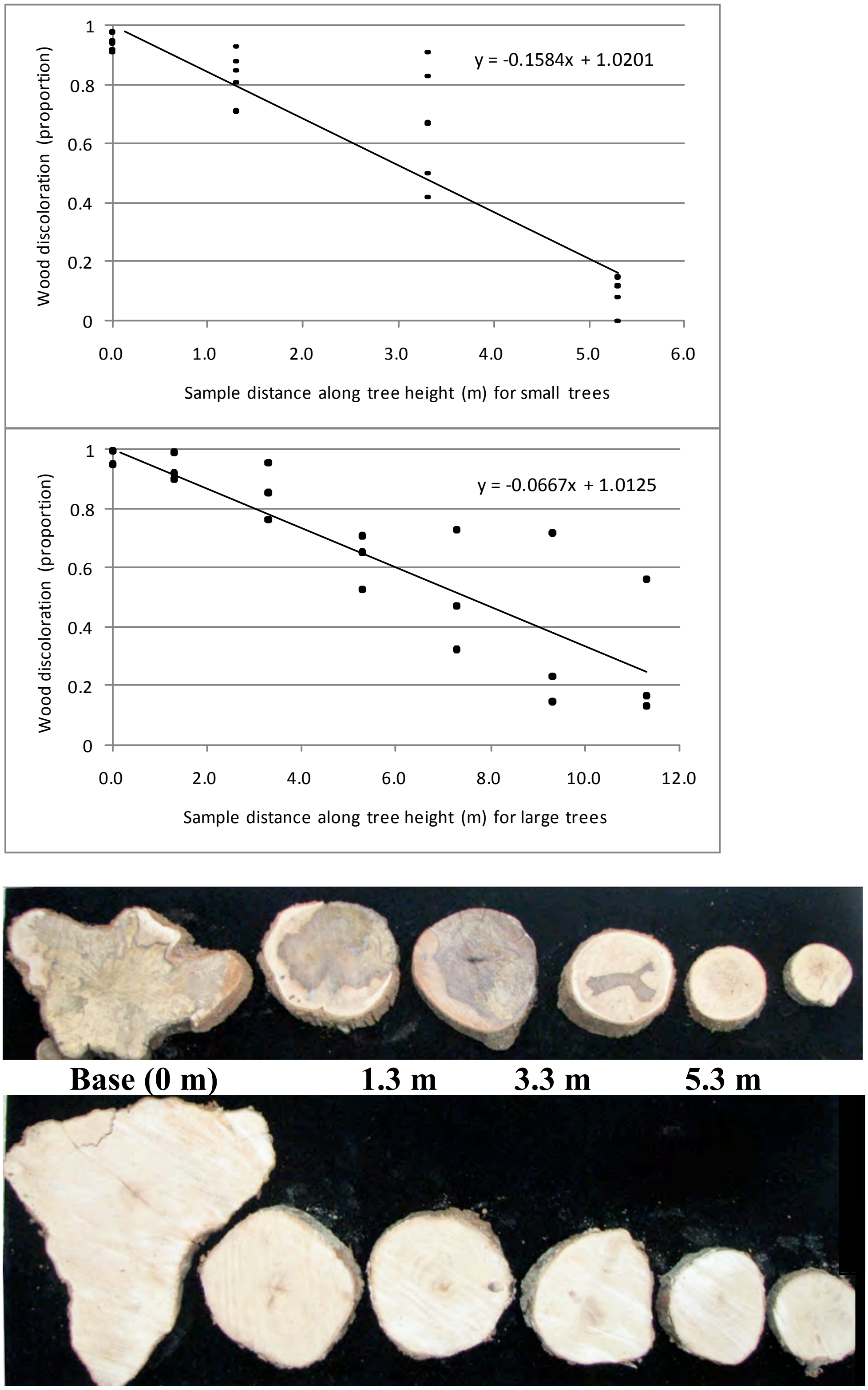


Figure 20. Proportion of wood discoloration in trunk cross-sections fitted to a linear decay function for small (upper) and large (lower) trees and trunk cross-sections from two small trees, one declined (top) and one healthy (bottom).

At DS=1, the outward symptoms of IWTD are indistinguishable from those produced by Guam's xylem-resident bacteria. Internal symptoms (as seen in trunk cross-sections) vary from tree to tree and with decline severity. Small trees (< 50 cm CBH) and those at DS=1 generally have symptoms associated with bacterial infection of the xylem, others have no bacterial ooze and only a small area of centrally-located, contained discoloration. Medium size trees and those at DS=2 usually have bacterial symptoms (**Figure 10**), and less common signs of wood rots caused by *Ganoderma* (**Figure 13**) and termites. Trees in a severe state of decline harbor one or all of the following: bacteria, termites, various resupinate sporocarps (**Figure 11**) and conks of *Ganoderma australe* species complex (**Figure 12**), *Phellinus* (**Figure 14**), and other Agaricoymcetes.

Analysis of individual trees: For each sample tree, measurements were taken and selected for analysis (**Table 2**). The primary objective of using statistical models with the ironwood tree data is to find possible factors that could be related to tree decline (K. Schlub, 2010). Various modeling techniques were applied to address data set issues. The logic model, which used dieback as the response variable, was found to be the best fit with the data. Three explanatory variables were found to be significant and therefore could explain the ironwood's state of health (**Table 2**). Among the three regressors, presence of "conks" had the largest coefficient value at 3.31. The impact of each individual regressor was determined numerically by holding all other regressors constant. The odds favoring decline is 27.3 times greater for a tree with "conks" than without.

Table 2. Grouping and descriptions of ironwood tree variables; those in bold were found to be the most suitable for predictive purposes.

<table>
<tr><th colspan="5">Response Variables</th></tr>
<tr><td>Decline severity</td><td colspan="4">Severity ranking DS=0,1,2,3,4</td></tr>
<tr><td>Tree Dieback</td><td colspan="4">Healthy or unhealthy</td></tr>
<tr><th colspan="5">Explanatory Variables</th></tr>
<tr><td>Structure</td><td>Number of trunks per tree</td><td>Circumference of tree at 1.3 m height</td><td colspan="2">Site density: trees per square meter at site</td></tr>
<tr><td>Stress</td><td>Fire damage: present or not</td><td>"Conks" present or not *</td><td>Storm damage: present or not</td><td>Termites present or not</td></tr>
<tr><td>Geographic</td><td>Latitude</td><td>Longitude</td><td>Altitude</td><td>Site</td></tr>
<tr><td>Miscellaneous</td><td>Level of lawn management none, moderate, or high</td><td colspan="3">Tree origin: natural or planted</td></tr>
</table>

*** "Conks"** refer to any resupinate or shelflike sporocarp of a basidiomycete appearing on a lower trunk (< 0.25 tree height) or roots of an ironwood tree (*Casuarina equisetifolia).*

Analysis of tree sites: Tree sites were examined using the original tree explanatory variables (**Table 2**) plus those derived from 16 GIS map characteristics (Kennaway, 2010): cemetery buffer, FIA trees with conks (multi-ring buffer), fire risk, fires per year, proximity to golf courses, land cover, management areas, school buffer, soil available water at 150 cm, available water at 25 cm soil depth, soil depth to restrictive layer, soil series, and vegetation. Some maps were dropped from the analysis because of correlations between regressors. A multiplicative change in the odds ratio of unhealthy vs healthy was calculated one regressor at a time by increasing the regressor one unit and holding all remaining regressors constant.

There were six positive dieback predictors: increasing circumference, increasing altitude, presence of "conks", presence of termites, planted stand vs natural stand, and urban land location.

There were four negative dieback predictors: increasing water availability at 25 cm soil depth, golf course location, forest location, and decreases in latitude.

In summary, the most beneficial variable identified was soil moisture. Trees in areas with the highest moisture were 3.3 times less likely to be declined. Likewise, the most deleterious variable was the presence of basidiocarps. Trees with "conks" were 27 times more likely to be in a declined state.

Predicting tree size: As a result of multi-linear modeling, several factors were identified that may positively (+) or negatively (-) predict the average size of trees at a site (cm CBH). The size of a tree is restricted by tree stand density, altitude, and soil depth.

Sites with large trees are more likely to be found in urban, forest, national parks, and fire prone areas than in sites at golf courses or in close proximity to a school. It was also found that increased circumference is associated with trees having termites, “conks”, typhoon damage, and multiple trunks. This suggests that large highly-vigorous trees are able to tolerate stresses to which less vigorous trees would have succumbed.

Linking dieback with site productivity: Based on the premise that tree circumference in 2008 and 2009 is an indicator of site productivity, an association between IWTD and circumference was sought. The circumference map supports the concept that nearly the entire island is suitable for the growth of small trees (**Figure 21**). However, as the size of the trees increases the area suitable for sustained growth decreases. When the map for dieback (**Figure 22**) is visually compared to the map for circumference (**Figure 21**), dieback appears poorly linked to site productivity (circumference) and strongly linked to the central area of Guam. This suggests that IWTD is not a natural progression of tree maturation and death. Many factors have been evaluated as possible causes or contributors to ironwood decline. Those that have some perceived relevance by the authors are listed in **Table 3**.

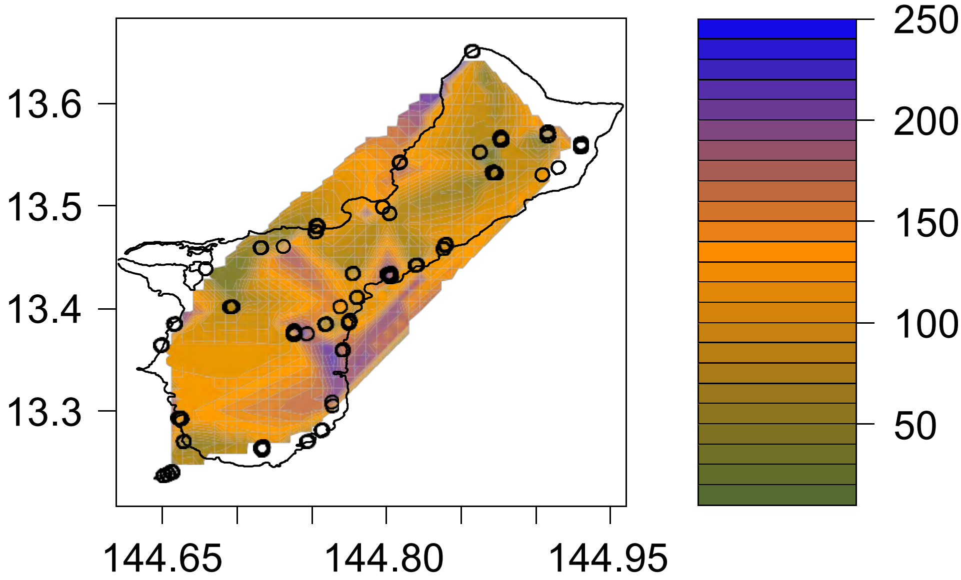


Figure 21. Map of observed tree circumference in cm (CBH) over a longitude-latitude grid of the island of Guam; hence, areas of large trees sites (purple color) have habitats more suitable for ironwood growth irrespective of the presence of IWTD.

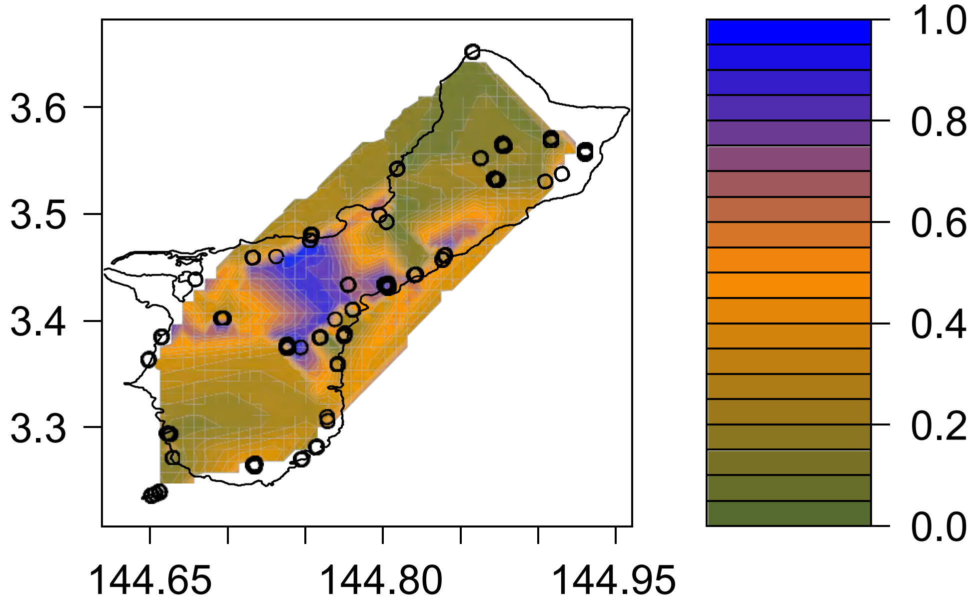


Figure 22. Map of the predicted probability of dieback using a logistic model. Areas in blue indicate regions where dieback is most likely to occur.

Table 3. Likely contributors to ironwood decline and their perceived relevance from low * to high ****.

Biotic factors	Emerging factors	Relevance
Branch dieback	*Pestalotiopsis*	*
Root rot	*Fusarium,*	*
Wood rot	*Ganoderma*	****
Xylem residing bacteria	*Ralstonia solanacearum* *Klebsiella oxytoca, K. variicola*	*** **
Nematodes	*Helicotylenchus*	*
Insects	Termites Gall wasp	** *
Abiotic factors	**Emerging factors**	**Relevance**
Weather	Typhoon damage	*
Management	Poor tree care practices	*
Site environment	Poor site selection	**
Host genetics	Lack of genetic diversity	**

RECOMMENDATIONS

Due to the slow progression and general sporadic nature of IWTD on Guam, it is likely IWTD could be reduced substantially through cultivar selection and cultural practices, which promote healthy growth and preclude favorable conditions for pests (termites) and pathogens (wood-rots, root-rots and bacteria).

Cultivar selection: It was concluded by attendees of the IWTD conference held on Guam in 2009, that the severity of the ironwood decline was likely acerbated by the lack of genetic diversity of Guam's ironwood tree population. Khongsak Pinyopusarerk recommended the evaluation of seedlots used in the 1991-1993 International Provenance trials of *Casuarina equisetifolia* (Pinyopusarerk *et al*., 2004). Though Guam's tree was planted in 21 countries at that time, the actual trial was never conducted on Guam. As a result of funding from the US Forestry Service, a scaled-down version of the international trial was planted at Bernard Watson's farm (N 13.56545; E 144.87790). This trial was planted in late July 2012 in an area of severe IWTD with the hope that in the future superior trees will be identified. The replicated trial (3 blocks) consisted of 11 paired seedlots (similar geography) of 4 trees each from 12 countries including Guam, with 8 ft. tree spacing (**Figures 23** and **24**).

C2	C1	K1	K2	V1	V2	T1	T2	P2	P1
C2	C1	K1	K2	V1	V2	T1	T2	P2	P1
C2	C1	K1	K2	V1	V2	T1	T2	P2	P1
C2	C1	K1	K2	V1	V2	T1	T2	P2	P1
M1	M2	S1	S2	G1	G2	A1	A2	I1	I2
M1	M2	S1	S2	G1	G2	A1	A2	I1	I2
M1	M2	S1	S2	G1	G2	A1	A2	I1	I2
M1	M2	S1	S2	G1	G2	A1	A2	I1	I2
K1	K2	V2	V1	M2	M1	A2	A1	G2	G1
K1	K2	V2	V1	M2	M1	A2	A1	G2	G1
K1	K2	V2	V1	M2	M1	A2	A1	G2	G1
K1	K2	V2	V1	M2	M1	A2	A1	G2	G1
S1	S2	C1	C2	I2	I1	T2	T1	P1	P2
S1	S2	C1	C2	I2	I1	T2	T1	P1	P2
S1	S2	C1	C2	I2	I1	T2	T1	P1	P2
S1	S2	C1	C2	I2	I1	T2	T1	P1	P2
P1	P2	V2	V1	A1	A2	S2	S1	C1	C2
P1	P2	V2	V1	A1	A2	S2	S1	C1	C2
P1	P2	V2	V1	A1	A2	S2	S1	C1	C2
P1	P2	V2	V1	A1	A2	S2	S1	C1	C2
M2	M1	K1	K2	I1	I2	T2	T1	G2	G1
M2	M1	K1	K2	I1	I2	T2	T1	G2	G1
M2	M1	K1	K2	I1	I2	T2	T1	G2	G1
M2	M1	K1	K2	I1	I2	T2	T1	G2	G1

Pairs	Geography	Prov. #
M1	Malaysia	18348
M2	Malaysia	18375
P1	Papua New Guinea	20586
P2	Papua New Guinea	18153
S1	Solomon Islands	18402
S2	Vanuatu	18312
T1	Thailand	18297
T2	Thailand	18299
A1	Australia	19821
A2	Australia	18378
I1	India	18015
I2	India	18119
K1	Kenya	18141
K2	Kenya	18144
C1	China	18267
C2	China	18268
V1	China	18586
V2	Vietnam	18152
G1	Guam, Inarajan	
G2	Guam, Ritidian	

Figure 23. Plot diagram of Guam's *Casuarina equisetifolia* provenance trial, with international trial numbers (Pinyopusarerk *et at*., 2004).

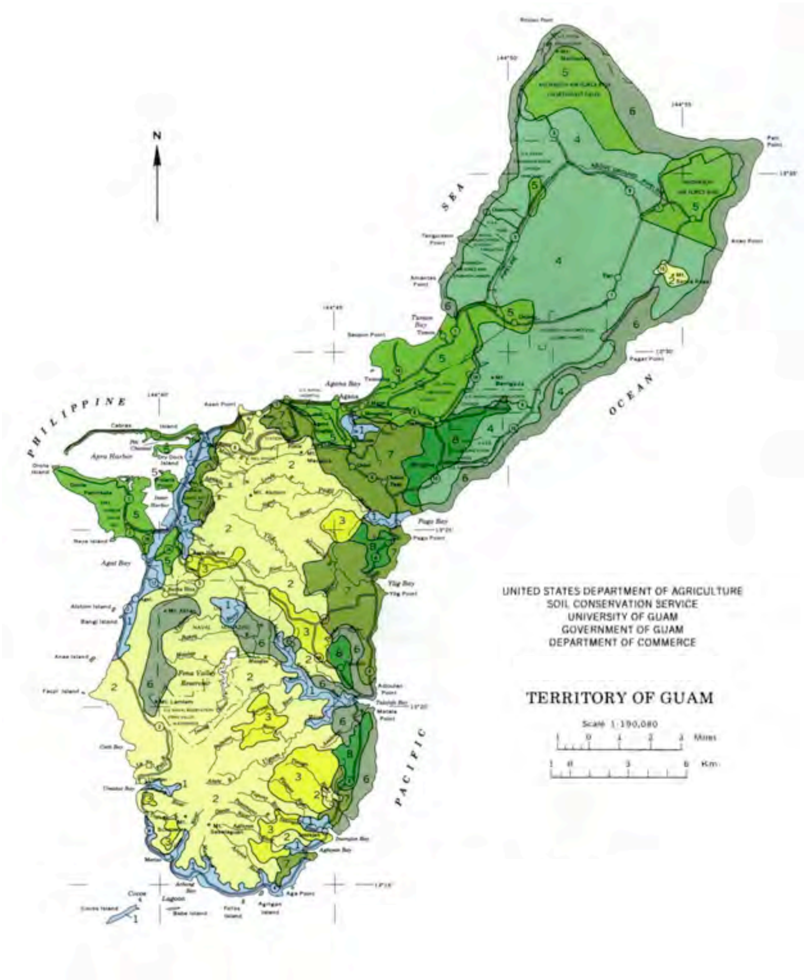
Figure 24. Provenance trial 3.5 months after transplanting.

Site evaluation and soil attributes: Site evaluation and soil care before planting ensures a healthy transplanted plant with increased tolerance to transplant shock as well as a tree that will reach its full maturity. Ironwood is suited for a range of sites and locations. Its growth habit dictates that it be planted 40 feet from houses and 20 feet from each other. In urban, windrow, and agro-forestry situations closer spacing may be necessary.

The island of Guam has three broad landform categories each with their own set of soil parent materials, which are responsible for the formation of 8 major soil units each with unique chemical and physical attributes (**Figure 25**). Chemical attributes of a soil are those related to the activity of ions within the soil solution; measurements include pH and Cation Exchange Capacity (CEC). Though ironwood can grow across Guam's wide range of soil pH, soil nutrients are maximized between pH 6-7. Cation Exchange Capacity is a measure of the soil's ability to hold on to nutrients, which increases with a soil's fertility. Low CEC soils (<11) have a low capacity to hold on to nutrients and are subject to leaching of mobile "anion" nutrients. Landscape treed in low CEC soils are subject to nutrient deficiencies and will benefit with the addition of a slow-release fertilizers with micronutrients.

The physical attributes of a soil are those related to the size and arrangement of its solid particles. Measures of physical properties include soil bulk density, soil texture, soil porosity or percolation. Bulk density is an indicator of soil compaction, which is an indicator of root growth and soil porosity or percolation. The majority of the island of Guam has clay soils with bulk densities of 0.60-1.0 g/cm^3, which are ideal for clayey soil. Unfortunately the soil is shallow often no deeper than 16 cm. The permeability or percolation rate for Guam's soils vary widely from poor (0.1 inches or less / hour) to rapid (5.0 inches or more). Poor soils should be avoided or modified as they promote shallow rooting, poor growth and root rots. Rapid soils are fine for ironwood, provided their roots can reach the water table, which will be critical for their survival in the dry season. Soil in an ideal state for tree growth contains 50% solids (45% mineral material and 5% organic matter) and 25% each of air and water.

Site remediation: Compacted soil in or near a planting pit should be remediated as necessary. The detrimental effects of compacted soil may include inadequacies in infiltration, aeration and water holding capacity. These factors could contribute to decreased root penetrability and thus increased susceptibility to drought and transplant shock. Remediation methods include soil aeration and incorporation of organic matter to improve porosity. Aeration is normally conducted using an air-tool or air-spade. Because Guam's productive layer is thin, vertical mulching also may benefit new planting sites. Vertical mulching consists of using an air-tool or drill to make vertical holes in the soil into which conditioned porous soil is added.



Key	Soil	Horizon depth (cm)	Clay (%)	Bulk density (g / cm³)	pH	CEC
SOILS ON BOTTOM LANDS						
1.1	Inarajan-Inarajan	0–13	50–70	0.90–1.10	5.1–7.3	51
1.2	Shioya	0–25	0–3	1.10–1.25	7.4–8.4	7
SOILS ON VOLCANIC UPLANDS						
2	Akina-Agfayan	0–10	45-80	0.80–0.95	5.1–7.3	23
3	Akina-Togcha-Ylig	0–13	45–70	0.85–1.10	5.1–6.5	36
SOILS ON LIMESTONE UPLANDS						
4	Guam	0–25	35–55	0.60–0.90	6.6–7.8	22
5	Guam-Urban land-Pulantat	0–25	35–80	0.60–1.10	6.6–7.8	27
6	Ritidian-Rock outcrop	0–10	35–40	0.70–0.90	6.6–7.8	——
7	Pulantat	0–16	70–90	0.90–1.10	6.1–7.3	31
8	Pulantat-Kagman-Chacha	0–20	40–80	0.90–1.20	6.1–7.8	26

Figure 25. General Soil Map of Guam (Young *et. al*., 1988)

Tree installation: Plants should be installed in saucer-shaped holes/pits that allow for expansion of the root zone with minimal substrate resistance (**Figure 26**). Soil should be removed with as little disturbance of the soil's profile as possible. Due to Guam's poor subsoil, mixing of topsoil and subsoil should be avoided. When backfilled, the site's profile should match with the original. To enrich the topsoil, amend with organic material. Large rocks on the side or bottom of the pit should be removed with a backhoe or cracked with an air-tool or auger. The planting area should be free of rocks and debris. It is a misconception that adding rocks or gravel in the bottom of the planting hole improves drainage. Care should be taken to avoid planting in holes with steep sides or made with a corer that compresses the sidewalls. In this scenario the roots could encircle among themselves leading to girdling roots. Balled or container trees must be carefully placed in the hole without disturbing the root ball. After installation, the tree should be staked.

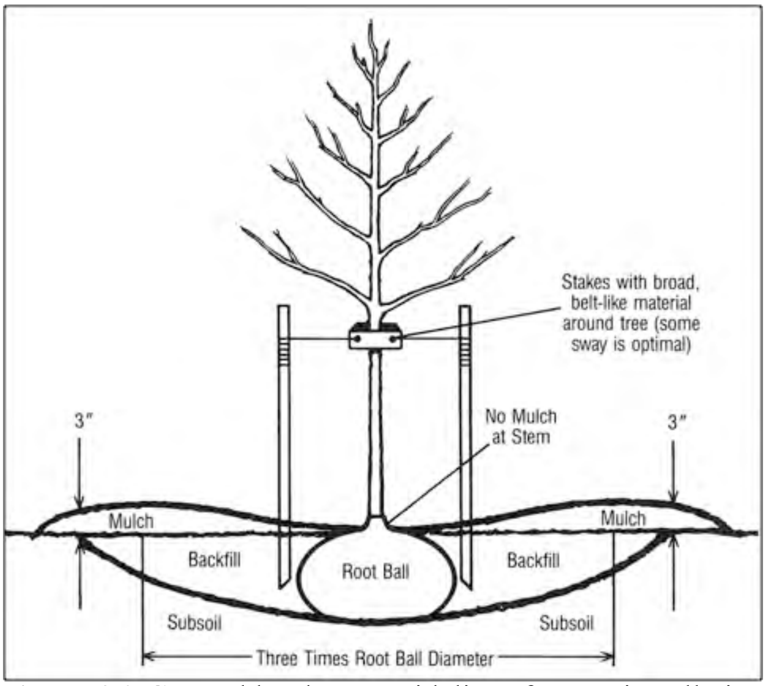


Figure 26. General hardware guidelines for tree installation.

Planting bare root plants: After planting bare rooted trees, gently tap the soil and backfill with water to remove air pockets. Additional staking may be required for bare rooted trees. Bare root plantings, although limited to smaller ironwood plants, allow for earlier adaptation to the new site and faster transplant recovery. However, a drawback in using this technique is that initially roots and the planting pit must be kept sufficiently moist to prevent roots from drying out. It is estimated that in Guam during the dry season, early care should by administered for at least three months and about one month in the wet/rainy season. Early care consists of providing tree transplants a stress free environment, which may include daily watering.

Nutrient management: Guam's soils benefit from nutrient augmentation especially in sandy soil and areas where soil has been disturbed. The soils of northern Guam are calcareous. Trees in these soils will likely benefit from the addition of chelated iron throughout their lifetime. Fertilizer should be used sparingly as the development of nitrogen fixing *Frankia* and beneficial mycorrhizal will be held back with over application. A low nitrogen, slow release fertilizer with micronutrients is ideal. Alternatively, apply a small amount (50 to 100 g) of a low analysis complete fertilizer such as 10-10-10 at transplant.

Mulching: Mulching or placement of organic material around the base of a new plant can be one of the most beneficial cultural practices for young ironwood trees. Mulch is anything used to cover the soil's surface for the purpose of improving plant growth and development. To be suited for plant growth, mulch must allow the exchange of air between the soil and the atmosphere and allow water to infiltrate into the soil profile. The selected mulch (e.g. ironwood needles) should be placed between 1-2 inches deep. Benefits of mulching include: conservation of soil moisture, moderation of soil temperature, improvement of soil quality (organic mulches), suppression of weeds, enhancement of landscape appearance, reduced maintenance, and protection of plants from damage caused by maintenance equipment.

Fertilizing: Fertilizing (also see nutrient management), especially in the early stages of planting, helps root development and may improve drought tolerance, thereby reducing transplant shock.

Watering: Watering or irrigation needs should be a part of the planning process, especially if planting is to occur in the dry season. Any irrigation program implemented should be based on knowledge of the soil percolation rates for the site. Excess moisture could lead to root rot.

Pruning: Pruning for health and training the young tree for structurally optimal strength relies on the judicious removal of plant tissue in a manner, as much as possible, consistent with minimal invasiveness to the plant. Proper pruning practices will enhance the overall health of the plant and should be guided by established standards. Tool sterilization is critical in ensuring sanitation and reducing the potential transfer of pathogens. Wind damaged trees should be correctly pruned as quickly as possible to reduce the amount of deadwood and reduce the surface areas of branches ripped in strong wind. Removal of deadwood reduces the establishment of termites and wood-rotting fungi that contribute to hazardous trees in Guam's urban landscape. Trees broken from typhoons should be felled by excavation instead of sawing where their colonization by a wood rotting fungus could possibly lead to infecting the root systems of neighboring healthy trees.

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Revised on August 6, 2013

Appendix A

Forward: This appendix serves as an update to Robert L. Schlub's August 6, 2013 revised Gago, Guam Ironwood Tree, *Casuarina equisetifolia* Past, Present, Future guide (Guam Ironwood Tree Manual). It contains advances in the research of Ironwood Tree Decline (IWTD), expanded tree survey information, and cultivar evaluations. Finally, it provides suggestions for future research.

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***Casuarina equisetifolia* shoreline protector**

Casuarina equisetifolia subsp. *equisetifolia*, often referred to as ironwood, is indigenous to Southeast Asia, Malaysia, Northern Australia, Oceania and most likely to Guam and the Northern Mariana Islands. It has been introduced into a large number of countries and is now a common feature of the coastal landscape. It is commonly used in agroforestry systems for coastal protection and rehabilitation and soil stabilization reclamation. Since it is salt tolerant and grows in sand, *C. equisetifolia* is used to control erosion along coastlines, estuaries, riverbanks and waterways. It is a very hardy tree and is useful in protecting shorelines from storm surges and tsunamis. The *Casuarina* can withstand a tsunami wave better than the coconut tree, while a young forest of *Casuarina* can provide even more protection. Ironwood is a popular tree species for shelterbelt plantations. The rapid growth of *Casuarina equisetifolia* attracts world climate change practitioners with its potential for climate change mitigation and adaptation in the coastal zones. A special interest in the arena of climate change mitigation lies in this specie's storage of carbon in its rapidly grown biomass.

The tsunami on December 26, 2004 was a major natural disaster, killing some 229,866 men, women and children and causing billions of dollars in damage (United Nations, 2007). Waves crashed into 14 countries and some were 30 meters high. Many *Casuarina* shelterbelts in India, Sri Lanka and Thailand were established to protect coasts from cyclones, tsunamis and other coastal hazards, which proved effective against the 2004 Indian Ocean tsunami as well. Post-tsunami field surveys in Sri Lanka and Thailand showed that older *Casuarina equisetifolia* shelterbelts withstood the tsunami, but failed to provide protection. The tsunami passed through the shelterbelt without resistance from lower-level branches or undergrowth, a condition typical of the species. For a coastal forest of mature casuarina (e.g., 80 cm dbh) the mitigation effect is marginal and only slightly more than *Cocos nucifera*. Very young stands, on the other hand, less than 10-15 cm diameter were uprooted and washed away providing no mitigation. Field observations and laboratory research have established several key parameters that determine the magnitude of tsunami mitigation offered by various types of coastal forests. These parameters include forest width, tree density, age, tree diameter, tree height, and species composition. Forest age (the average age of trees of the dominant size class) is directly correlated with both tree height and diameter. Increases in age, diameter and height generally enhance the mitigation effects of coastal forests. Diameter growth also enhances the breaking strength of trunks and branches. It also raises the resistance of the forest being toppled, up to a point after which resistance falls.

In Sarawak, Indonesia the species is protected because of its importance in controlling coastal erosion. Since 1954, vast plantings of *Casuarina equisetifolia* have been established along the coast fronting the South China Sea. Much of the coast there comprises of bare dunes that formerly were constantly moving inland, destroying arable land. This belt of Casuarina (mainly *C. equisetifolia)* covers more than 1 million hectares and stretches for 3,000 km and varies from 0.5 to 5 km in width. The importance of this tree on the beaches of the world's tropical islands will increase as sea level rise and storms increase in intensity.

The coastline of Bangladesh is mostly exposed to extreme meteorological and hydrological conditions where cyclones and storm surges cause devastating effects including loss of human lives and destruction of properties. Young, dense C. *equisetifolia* is found more effective to reduce storm surge energy than other species previously tested. It was found that shelterbelt C. *equisetifolia* reduced wind speed, increased the size of sand dunes, improved the aesthetic value, increased the protection facilities against cyclones, and enhanced the attractiveness of the beach for tourism. Although casuarina trees have inhibited the native species as undergrowth, the shelterbelt has increased the supply of fuel-wood for local people.

In Hawaii 12 different species of *Casuarina* have been introduced, estimated to occupy 3,800 hectares. Locally known as "ironwood," casuarinas have been planted for erosion control, dune stabilization, windbreaks, fuelwood plantations, beautification, and watershed cover. In the lowlands, the most extensively planted species has been *Casuarina equisetifolia*. In the uplands, *Casuarina glauca* has been most commonly used, primarily for erosion control. In addition, there are plantings of *Casuarina angularis, Casuarina nodiflora*, and an unknown species from Timor. *C. equisetifolia* is listed as one of the exceptional trees of the city and county of Honolulu. The trees are located along Kalakaua Avenue from Kapahulu Avenue to Poni Moi Road, 52 Robinson Lane, and a grove of double row parallel to the Kapiolani Park Bandstand, at Monsarrat Avenue's Waikiki Shell parking lot Makai entrance.

Pros and Cons of *Casuarina equisetifolia*

Casuarina equisetifolia and *Casuarina glauca* are both listed in the National Invasive Species Strategy as plants that should be eradicated or controlled. The Bahamas National Trust has long been on record as supporting the removal of *Casuarina* from island coastlines. Extensive research supports that removal of casuarinas from coastal areas and replanting of the dune ridge with native vegetation will restore the dune and provide an effective barrier against wave action.

Provenance trial: It was concluded by attendees of the IWTD conference held on Guam in 2009, that the severity of the ironwood decline was likely acerbated by the lack of genetic diversity of Guam's ironwood tree population. Khongsak Pinyopusarerk recommended the evaluation of seed lots used in the 1991-1993 International Provenance trials of *Casuarina equisetifolia* (Pinyopusarerk *et al*., 2004). As a result of funding from the US Forestry Service, a scaled-down version of the international trial was planted at Bernard Watson’s farm (N 13.56545; E 144.87790). This trial was planted in late July 2012 in an area of severe IWTD. The replicated trial (3 blocks) consisted of 11 paired seed lots (similar geography) of 4 trees each from 12 countries including Guam, with 8 ft. tree spacing (**Figures 23** and **24**).

After one year, the fastest growing provenances were nearly twice as tall as the average seed lot with nearly 6 times the average biomass. The fastest three geographically paired provenances were those from Solomon-Vanuatu, Malaysia, and China; the slowest were from Australia, Kenya, and Guam (**Figure 27, 28 and 29**). The block effect was significant with the growth of most provenances increasing from block 1 to block 3 (**Figure 27**). This difference was attributed to increasing soil depth from block 1 to block 3.

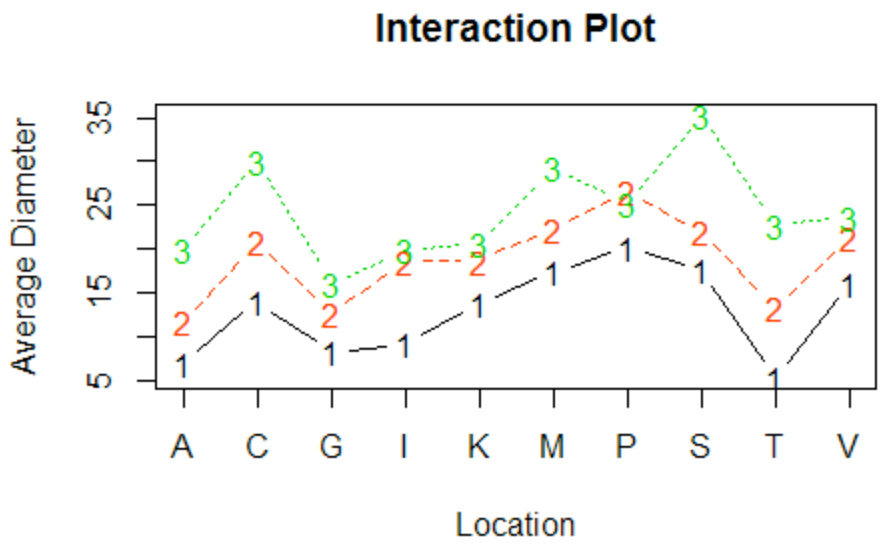


Figure 27. Diameter in millimeters of Guam ironwood provenance trial trees one year after transplant: A (Australia), C (China), G (Guam), I (India), K (Kenya), M (Malaysia), P (Papua New Guinea), S (Solomon Islands & Vanuatu), T (Thailand), V (Vietnam & China).

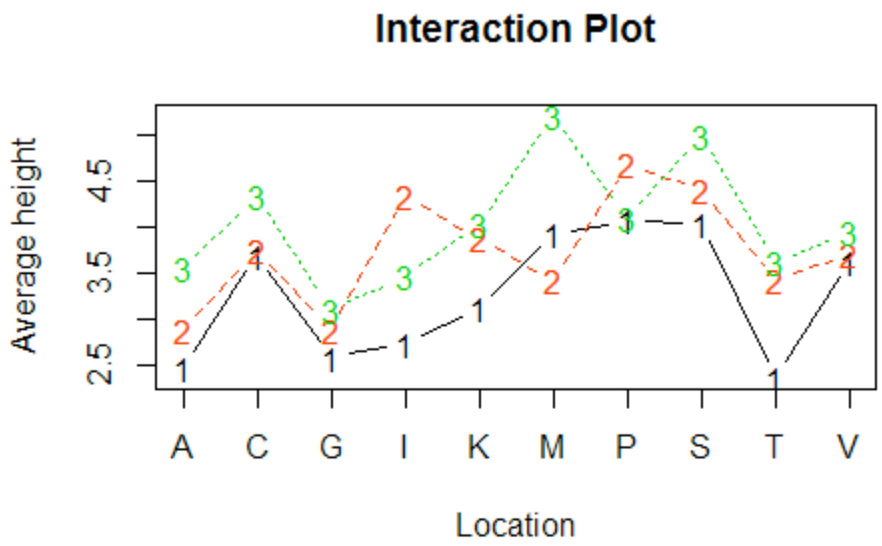


Figure 28. Height in meters of Guam ironwood provenance trial trees one year after transplant: A (Australia), C (China), G (Guam), I (India), K (Kenya), M (Malaysia), P (Papua New Guinea), S (Solomon Islands & Vanuatu), T (Thailand), V (Vietnam & China).

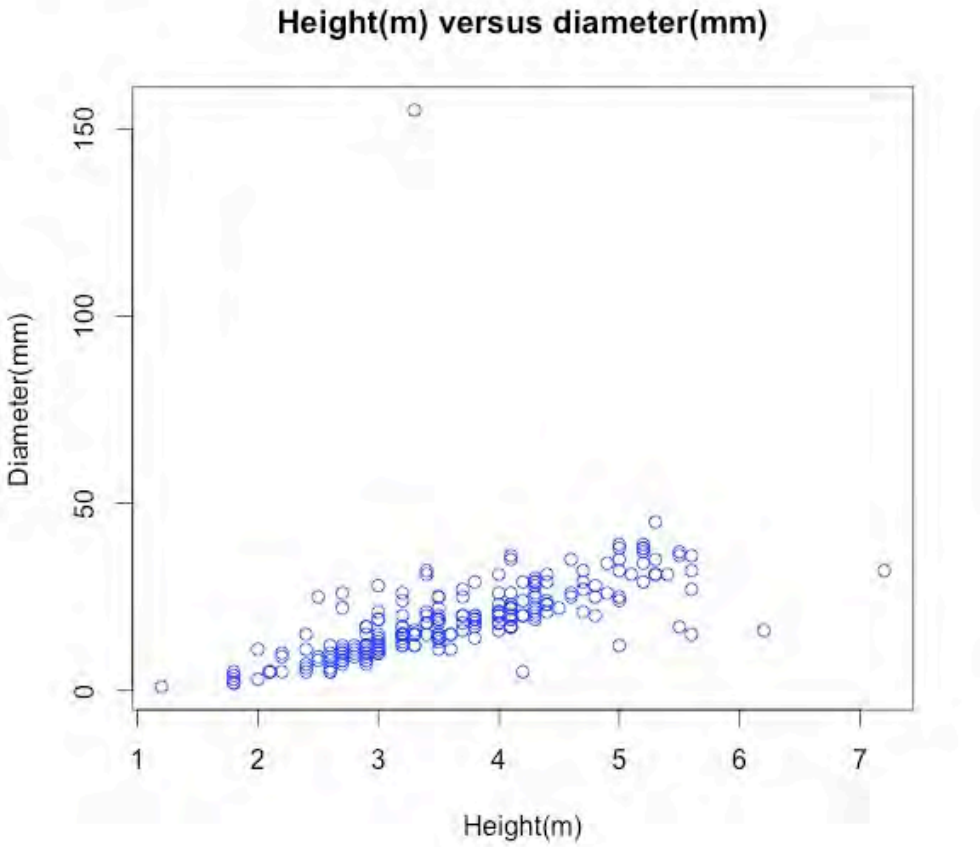


Figure 29. Scatter plot of original data collected on 6/20/2013 consisting of 219 data sets.

By year two the varieties had gained in size by roughly 80% **(Figure 30).** In early September of 2013, Dr. Phil Cannon visited the provenance trial and assisted in selecting trees that were to be thinned from the trial. Subsequently, half of the trees (120 trees) were cut down and taken out of the field, thereby leaving four trees in every paired plot. In late September and mid-October, two typhoons developed near Guam where high winds and heavy rain caused damage to the trial. Tropical storm Pabuk passed near Guam on September 19-20. Nine uprooted trees were removed from the field **(Figure 31),** and 24 bent trees were straightened out and secured with ropes

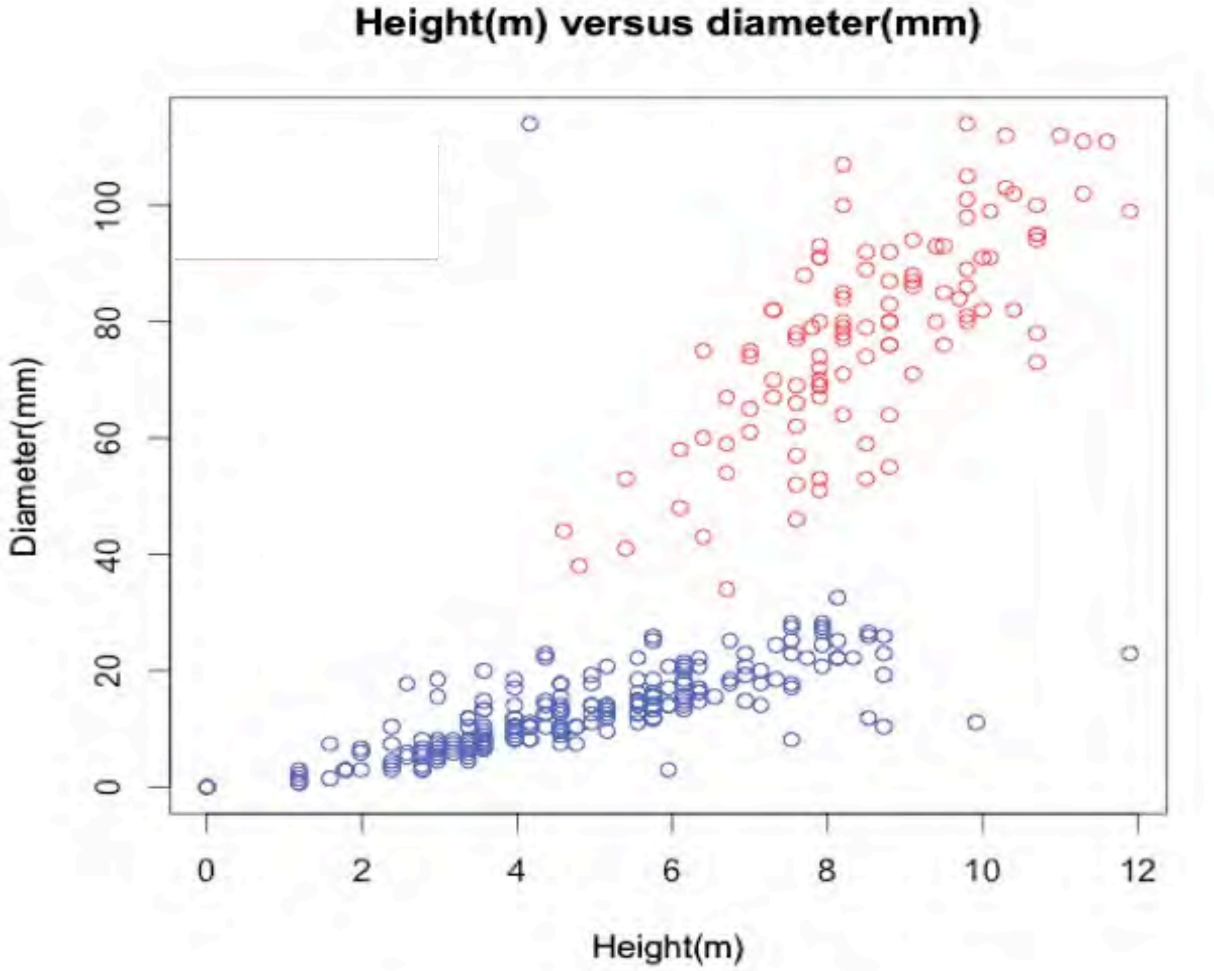
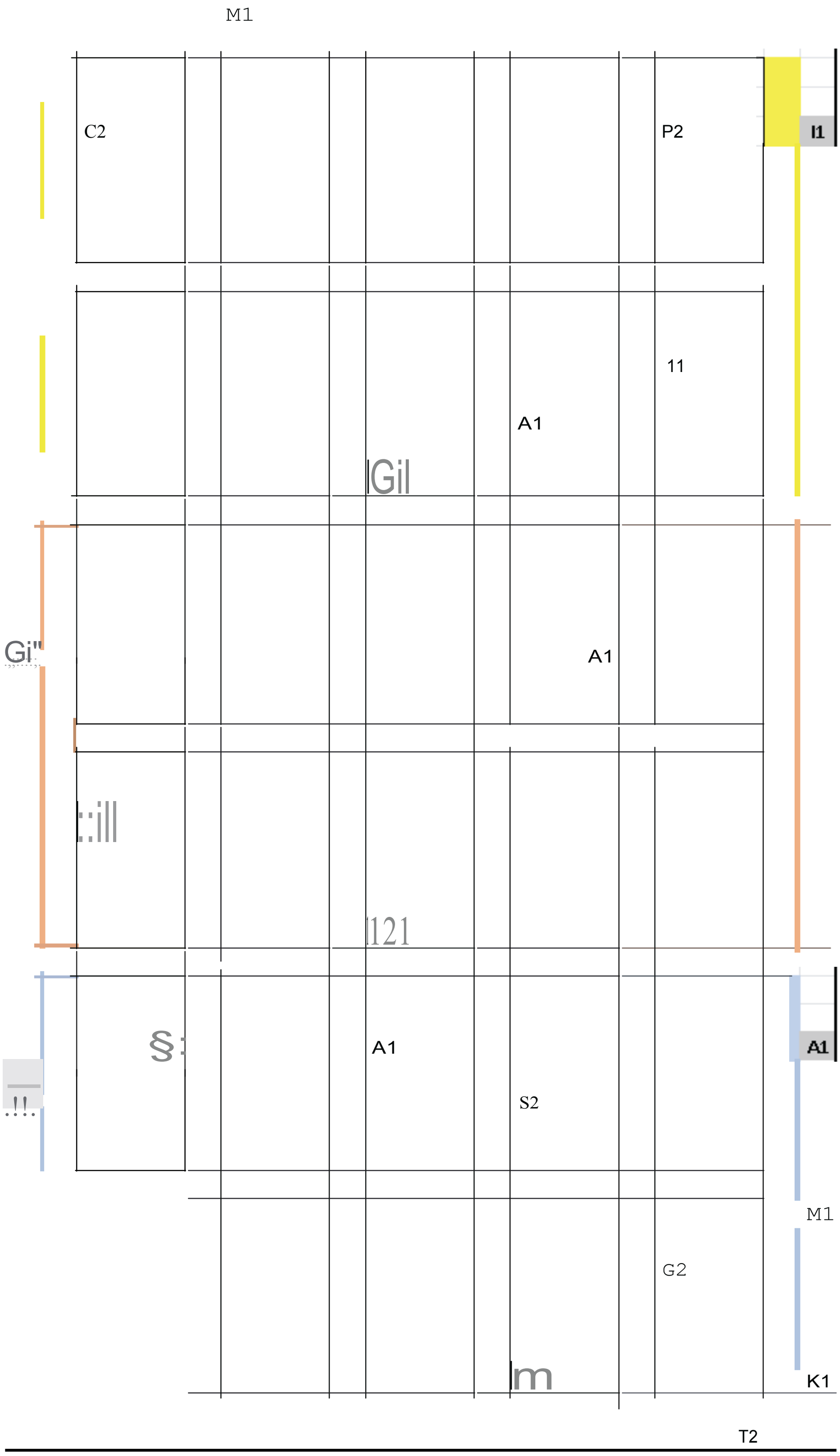


Figure 30. Scatter plot ironwood tree measurements after the first year (blue) and second year (red).

Figure 31. Uprooted tree due to high wind and rain on 9/19/2014.

In the third year, the trial had to be terminated due to tree damage. Typhoon Dolphin's eye passed through the Rota Channel between Guam and Rota Island on May 15, 2015 delivering the typhoon's strongest winds in the eyewall to both locations. Andersen Air Force Base (AAFB) on the northeast side of Guam clocked a peak wind gust of 106 mph just before 7 p.m. local time. One hour later, AAFB was reporting peak sustained winds of 84 mph in the southern eyewall of the typhoon. In addition to the wind, we received 16 inches of rain in two days. In the ironwood provenance trial plot, 44 of 60 trees in the replicated trial and 11 of the 19 trees in the boarder rows were blown-over and uprooted **(Figure 32 and 33)**. One other tree in the replicated trial was snapped in half**.** As much data as possible was collected on remaining trees as well as the felled trees **(Table 4)**. At this time trees were still gaining in height; however the rate of growth had slowed **(Figure 34).** This was particularly true for the trees from Guam and Australia. Their slower grower growth rate and reduced tree height may have contributed to their higher survival level (**Figure 33**).

Figure 32. Trees fallen by Typhoon Dolphin in 2015.



Figme 33. Trees remaining after Typhoon Dolphin struck Guam May 15, 2015.

Table 4: Tree assessment criteria and data collected after Typhoon Dolphin. The notations in green indicate what is considered favorable for a windbreak tree.

<table>
<tr><td>Stem Height (Ht) higher value</td><td>Height of the tree based on tallest stem</td></tr>
<tr><td>Stem Diameter (dbh) higher value</td><td>Trunk/stem diameter at 1.3 m above ground</td></tr>
<tr><td colspan="2">Stem axis persistence: Ability of tree to retain its primary stem axis. Forking is when two or more arising branches emerging from the junction are of near equal diameter.1 = Multiple stems formed at ground level, 2 = Forking in first (lowest) quarter of stem, 3 = Forking in second quarter of stem, 4 = Forking in third quarter of stem, 5 = Forking in fourth quarter of stem, 6 = Persistence complete (no forking) higher value better</td></tr>
<tr><td colspan="2">Stem straightness of trees with 4-6 in axis persistance : 1 = Very crooked, > 2 serious bends, 2 = Slightly crooked, > 2 small bends or < 2 serious bends, 3 = Almost straight, 1–2 small bends , 4 = Completely straight higher value better</td></tr>
<tr><td colspan="2">Tree volume (V): Tree volume = $(G/4)^2 \times H$ higher value better
G = girth (circumference) of tree at breast height in meters (or feet)
H = tree height in meters (or feet)</td></tr>
<tr><td colspan="2">Permanent branches: An indication density of foliage 1=first order branches (originate from stem (trunk)) with maximum number of branch orders or no less then one less, 2,3, and 4 higher value better</td></tr>
<tr><td colspan="2">Branch habit: Is an indication of how dense the “needles” are on the tree. Tree’s maximum orders: first-order branch originates from main stem, second-order branch originates off first –order branch, etc. (do not count deciduous branchlets) higher value better</td></tr>
<tr><td colspan="2">Internodes: Distance between permanent branches taken in lower, middle, upper portions of tree. Branch</td></tr>
<tr><td colspan="2">density rating : 1 = Very high, regularly branched, majority internodes <15 cm (6 in), 2 = High, irregularly branched, internode around 15 cm (6 in), 3 = Low, irregularly branched, internode around 30 cm (12 in) , 4 = Very low, sparsely branched, internode > 30 cm (12 in) lower value better</td></tr>
<tr><td>Branch, max. length, max. diam.</td><td>Length and diameter of longest branch</td></tr>
<tr><td>Branch length rating</td><td>1=long-generally >one-quarter of tree height lower value is better
2=short-generally<one-quarter of tree height</td></tr>
<tr><td colspan="2">Branch thickness rating: ratio of permanent branch and adjacent stem (point where stem is joined with branch)
1 = Very heavy, more than three branches, diameter > 1/3 of adjacent stem.
2 = Heavy, one to three branch, diam. > 1/3 of adjacent stem
3 = Light, branch diameter up to 1/3 of adjacent stem
4 = Very light, branch diameter less than 1/3 of adjacent stem lower value is better</td></tr>
<tr><td colspan="2">Branch angle rate of permanent branches: 1 = Upright, < 60° 2 = Horizontal > 60°</td></tr>
<tr><td colspan="2">Branchlet habit: Is an indication of how thick the “needles’ are on a branch 1=branching common, 2=majority are without branches. lower value is better</td></tr>
<tr><td>Branchlet length</td><td>1= long >15 cm (6 in) 2 = short < 15 (6 in)</td></tr>
<tr><td>Cones</td><td>1= none, 2= yes present of cones is good</td></tr>
<tr><td>Flowers and sex</td><td>1= none, 2= yes (sex), (male, female, both) present of flower is good</td></tr>
<tr><td colspan="2">Root damage, typhoon: 1 = none, 2 = slight (Soil lifted, no roots exposed), 3 = moderate (tops of some roots exposed, not broken, 4 = sever (some exposed and broken, tree would likely survive if left alone), 5 = up-rooted, tree is not likely to survive lower value is better</td></tr>
<tr><td colspan="2">Stem damage, wind: 1= none, 2 = slight (15-45 off vertical), 3 = moderately (45-80 deg off vert.), 4 = severely (greater than 80 deg off vert.), 5 = snapped killing above portion lower value is better</td></tr>
<tr><td colspan="2">Branch damage, wind: 1=no damage or stress injuries, 2=not normal, some bent, none snapped, 3=one or more branches snapped or severely bent, 4= more than one large branched snapped lower value is better</td></tr>
<tr><td colspan="2">Ref. Ravichandran VK, BalaSubramaniam TN & Jeyachandran M. 2003. Development of volume table for Casuarina equisetifolia. The Indian Forestry 26: 7–10.

Pinyopusarerk, K., Kalinganire, A., Williams, E.R., Aken, K.M. 2004. Evaluation of Internation Provenance Trials of Casuarina equiselifolia. ACIAR Technical Reports Canberra, N. 58, 106pp.</td></tr>
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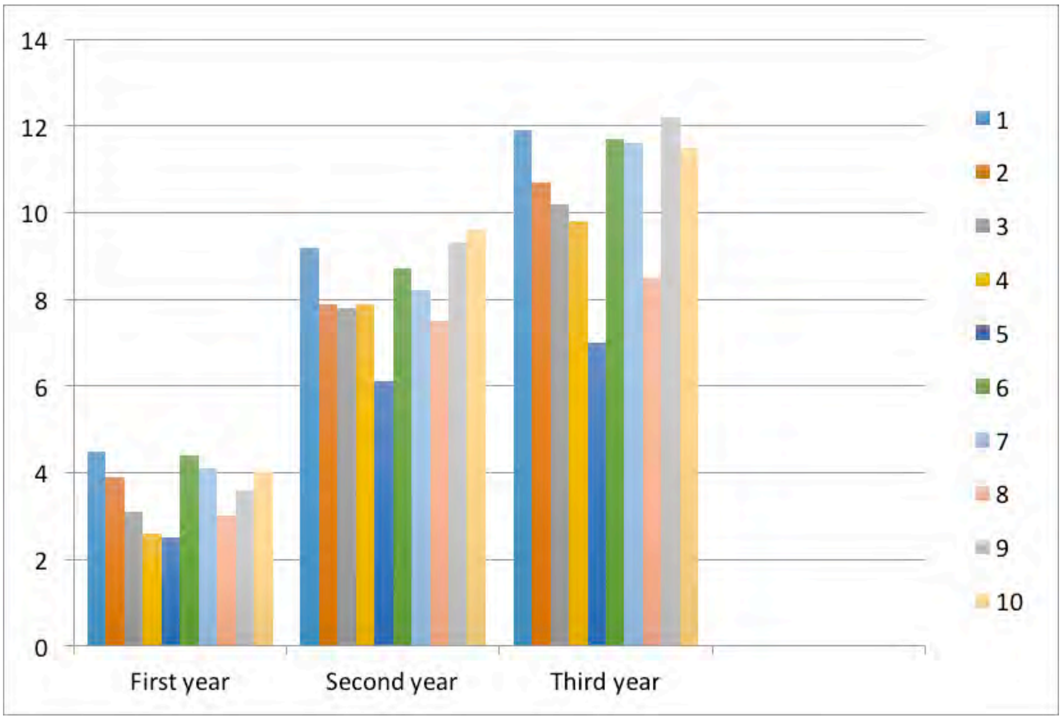

Figure 34. Average height in meters of geographic pairs at end of provenance trial May 2015: 8 (Australia), 10 (China), 5 (Guam), 3 (India), 2 (Kenya), 1 (Malaysia), 6 (Papua New Guinea), 9 (Solomon Islands & Vanuatu), 4 (Thailand), 7 (Vietnam & China).

Contributors to decline

As of 2018, four variables have emerged as useful predictors of ironwood tree decline (IWTD) *Ralstonia solanacearum* (Rs) and *Ganoderma austral* (Ga), percent cross-sectional area with wetwood (WW), and the production of ooze. Rs and Ga are the only two know pathogens of ironwood that have been identified on Guam. Presence of Rs in some healthy trees and the lack of Ga in many severely declining trees, provides antidotal evidence that IWTD is best considered a complex of these two causal agents (**Table 5**).

Table 5. Guam data. Range of growth characteristics of n=77 ironwood trees (*Casuarina equisetifolia*) and percentages of trees at various levels of decline which were positive for *Ralstonia solanacearum* and or *Ganoderma australe.*

Decline Severity *	Tree CBH **	Tree Height***	*Ralstonia solanacearum*	*Ganoderma australe*
DS=0 (n=17)	47 - 218	10.2-21	18%	0%
DS=1 (n=12)	41 -310	9.6-18	42%	8%
DS=2 (n=13)	41-152	4-15	85%	23%
DS=3 (n=15)	44-239	10-13	50%	50%
DS=4 (n=20)	38-249	6-18	84%	37%

*Decline severity based on visual comparisons of trees to a set of photographic standards depicting varying levels of bare branches and thinning foliage as shown in **Figure 1**.
** CBH: Circumference in cm at breast height (1.3 m) was found to be non-significant
***Height in meters was excludes from statistical model due to high collinear with CBH.

Ironwood trees are healthy in Hawaii and Saipan. Groups of trees in various stages of die-back and decline, which is common on Guam, doesn't occur on Saipan or Hawaii. Rs is easily detected in drill shavings from trees on Guam; however, not a single tree tested positive on Saipan over the course of two days of sampling island wide in 2017 nor was bacteria detected in ironwood trees in Hawaii after a morning of sampling trees. On Saipan, the average number of fruiting bodies found at 8 locations was 2.5% whereas on Guam average number at 6 locations was 28%. From casual observations made in Hawaii, the presence of Ga in Hawaii is like that of Saipan.

Termites: In India, termites feed on underground roots and stems of live *C. equisetifolia.* This type of damage is believed to be occurring in Guam as well.

Ironwood trees were surveyed on Guam for termites in 2015-2016. Site and tree information collected included site condition (windrow, landscape, woodlot, etc.) GPS location, tree height and girth, ironwood decline severity rating, presence or absence of basidiocarps of *Ganoderma*, termite colony formation, and relative number of termites present.

The role possibly played by termites in IWTD is still under investigation. Three ironwood tree termite surveys were conducted. The survey conducted in June 2015 consisted of 9 healthy trees, 15 in decline, 2 dead trees and 2 stumps. Of the 24 live trees, 8.3% had basidiocarps of *Ganoderma*, 41.7% tested positive for *R. solanacearum,* and 0% had both. The survey conducted from December 2015 to January 2016 consisted of 6 healthy trees and 13 in decline. Of the 19 live trees, 36.8% had basidiocarps of *Ganoderma*, 36.8% tested positive for *R. solanacearum,* and 15.7% had both. In March 2016, the December survey trees were revisited and samples were collected from 15 trees at 14 sites.

Four species of wood-feeding "higher" termites were found to attack ironwood trees in the areas of collection. Those in the family Termitidae include *Nasutitermes takasagoensis, Microcerotermes crassus,* and a yet to be identified *Microcerotermes species.* Morphological identification showed that the vast majority of ironwood infesting termites were most likely *N. takasagoensis* an arboreal species with a distribution including China. Taiwan, Japan, and Christmas Island (Australia). Two of the samples were morphologically identified as *M. crassus* Snyder, another species known to occur in many countries of South East Asia (including China, India, Malaysia, Myanmar, Thailand, and Vietnam). Nesting habits of this species range from subterranean to arboreal. This species is found around rural dwelling and suburbia and sometimes enters buildings. The unknown *Microcerotermes* species has a 96% best match to *Microcerotermes biroi*. The Rhinotermitidae family only includes *Captotermes gestroi. C. gestroi,* known in Asia as the Philippine milk termite, is endemic to Southeast Asia (China, Taiwan, Indonesia) but has spread to other parts of the world including Madagascar, USA, Brazil, Cuba, Jamaica, Mexico, Puerto Rico, India, Myanmar, Sri Lanka, and French Polynesia. *C. gestroi* is a very damaging termite and a threat to wooden structures wherever it occurs.

The large presence of *Nasutitermes takasagoensis* on ironwood trees across Guam, suggests that this termite was primarily responsible for the termite colony formations that were recorded during the 2008-2009 island wide survey of 1,427 ironwood trees and subsequently included in Karl Schlub's 2010 multinomial model. At the time of the survey it was known that termites were present in 32 of the 44 sites surveyed; but nothing was known about the identification of the termite or termites involved. With the identity of *N. takasagoensis* as the primary colonizer of ironwood trees, it is now known that the presence of termites in an ironwood tree regardless of its condition presents low risk to standing structures.

The risk posed to homes and other structures by the presence of termites in ironwood trees is due to the occasional appearance of *Coptotermes gestrori.* Though *C. gestrori* was only found in 2% of the trees surveyed, these termites are voracious feeders and therefore constitute a threat to nearby wood containing structures. They feed on all sorts of cellulose-containing materials and drill holes in such materials as rubber, plastic, and Styrofoam in their search for food. Though they are reported to attach and consume heartwood of living trees in other locations in the world, this remains to be determined in Guam.

Ralstonia solanacearum **(Rs):** *Ralstonia solanacearum* (Rs) has now been confirmed to occur in Guam's ironwood trees. Rs is a known bacterial pathogen of more than 200 hosts, comprising 53 botanical families. Genetic diversity in global collection of Rs strains has led to the characterization of Rs as a "species complex". Strains of Rs have been reported to cause bacterial wilt of ironwood in several countries where *C. equisetifolia* is propagated including India, China, and Mauritius. In 2012, a survey showed an association of ironwood decline with Rs. Results of this study showed that *Ralstonia* strains isolated from diseased ironwood in Guam were similar to GMI1000, having similar BOX-PCR profiles and belonging to phylotype I and biovar 3. Pathogenicity tests revealed that *Ralstonia* was able to cause wilt in tomato and ironwood seedlings. There were no differences in pathogenicity between Guam *Ralstonia* and control strains, when inoculated into tomato and ironwood from Hawaii. Additionally, there were no observable differences in susceptibility of ironwood from Guam and Hawaii to all strains, suggesting that the association of Guam *Ralstonia* with Guam ironwood is not specific. Phylotype multiplex PCR showed that all Guam Rs strains, along with the Rs reference strain (GMI1000), had bands identical with phylotype I (Asia), and all tested *R. solanacearum* strains contained the 280 bp amplicon, which is specific to the Rs species complex.

Ganoderma **(Ga):** *Ganoderma* is a common heart rotting fungus and normally appears in old trees of many tree species. It has been reported to kill young trees in plantation situations ironwood as well as other trees. The wide spread appearance of Ga in ironwood across the island, seem to point to something very unusual. *Ganoderma*, a genus of more than 300 species of wood-decaying fungi has been reported as wood decay fungi of *C. equisetifolia* throughout its range including Mexico, India, Pacific islands, South Africa, Indonesia and Mataysi. In 2015, DNA of *Ganoderma* sample cultured from ironwood trees from Guam and Saipan was extracted and partial sequences of the internal transcribed spacer region were amplified and edited at the Moscow Forestry Sciences Laboratory (USDA Forest Service-Rocky Mountain Research Station). Sequences were compared to the GenBank® database using the nucleotide BLAST®. Tentative ITS-based taxon identification: Guam, *Ganoderma australe* complex, score 99% to GU213473; Guam, *Ganoderma* sp., score 98% to AY569452; Saipan, *Ganoderma australe* complex, score 99% to FJ 392286.

Wetwood (WW): Wetwood is a type of heartwood in standing trees which has been internally infused with water. In some tree species, wetwood often has a water-soaked translucent appearance. In other species, the typical water-soaked appearance may be absent. In this case, WW has the appearance of either normal heartwood or it has an unusually dark color. WW occurs in both conifers and hardwoods, but its frequency can vary by species (from none to common), age, and the tree's growing conditions (Ward, 1980). Other then causing a reduction in the value of effect lumber, the condition is generally considered benign. Though the causes and mechanisms of wetwood is controversial, most investigators agree that wetwood is the result either of microbial activity (bacterial), injury, or normal aging. Some believe wetwood bacteria become established during early in the life of a tree and only produces the wetwood symptoms when the tree under goes stress.

The best information available on WW in ironwood comes from Mauritius (Orian, 1961). The presence of dark stained wood and ooze is commonly found in declining trees in Guam **(Figure 35).** It consists of droplets or poodles of fluid of various viscosities and colors may result from an infection by *R. solanacearum* or the colonization of tissue by wetwood bacteria **(Figure 36)**.

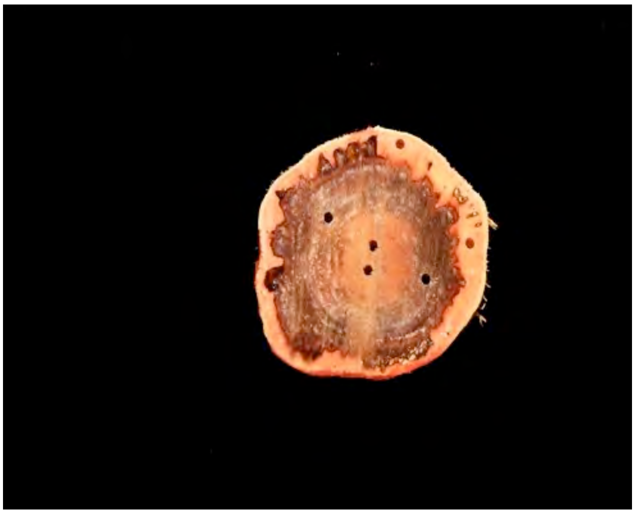

Figure 35. Cross-section of a declining *Casuarina equisetifolia* tree 24 hours after sectioning. Areas of moist discolored "wetwood" radiate from the center. An abundance of creamy, off-white ooze is observed on the cut surface. Drill holes indicate sampled sites; shavings from six holes were combined into a single sample for analysis of bacterial endophytes.

The possible role of wetwood bacteria in decline began to emerge in 2014, these included *K. oxytoca* and *K. variicola.* Six different genera of wetwood bacteria were identified *Kosakonia*, *Enterobacter*, *Pantoea*, *Erwinia, Citrobacter*, and *Klebsiella*. Wetwood symptoms were present in 93% of tree cross-sections, of which 17% had no outward symptoms of IWTD.

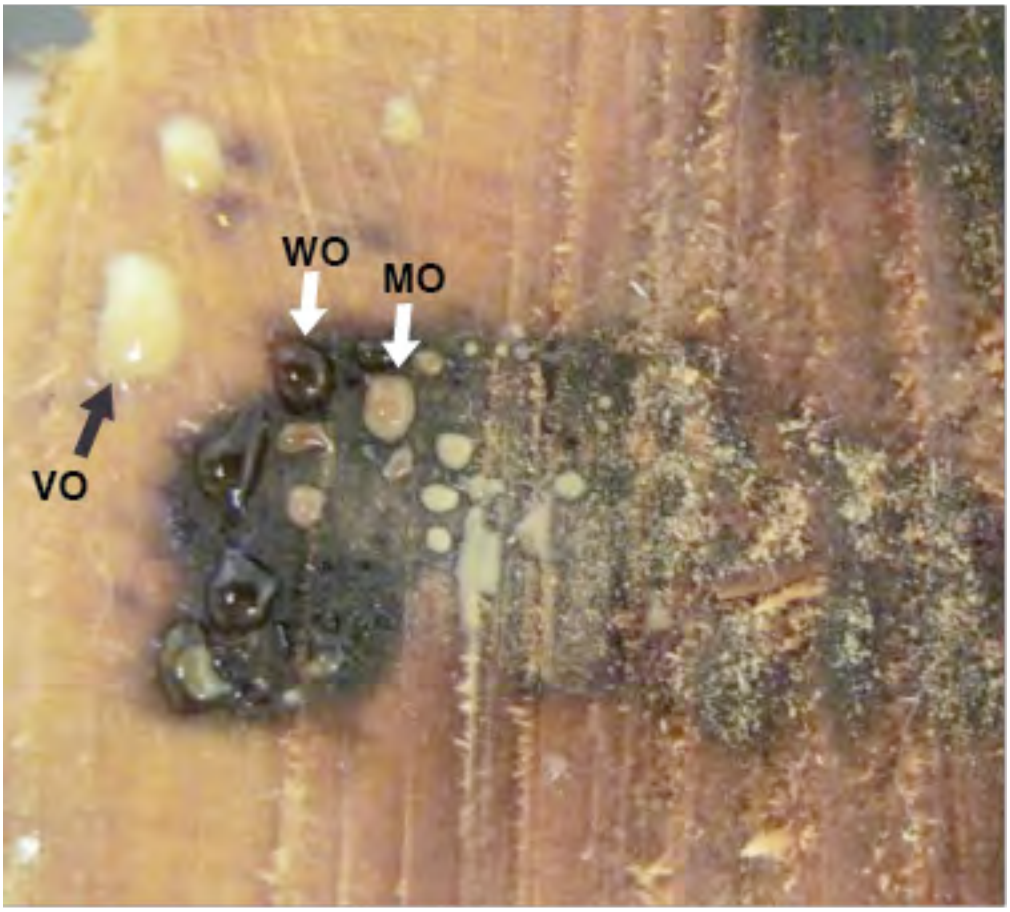

Figure 36. Three types of ooze occur in cross-sections of *C. equisetifolia:* a viscous, white to off-white substance (VO); a watery, amber substance (WO); and a mixture of the viscous and watery ooze (MO). We are not sure as to the causes of each type of ooze. It appears that the white creamy one is due to Rs however, wetwood bacteria are always isolated as well. Watery substance may be the result of the plant's vascular system failing and the leaking of cellular contents brought on by the growth of wetwood bacteria.

Figure 3 (reprint). Cross-section of rotted ironwood tree butt infected with *Ganoderma australe* species complex. Note the presence of white rot, areas of dark stained wetwood and the expanding network of white mycelia strands.

Discussion and Conclusions

The best predictor of decline on Guam is the percentage of a cross-sectional area with wetwood. All trees cut down in Guam that had moderate to serve decline were positive for wetwood but not always positive of Rs or Ga. From tree cross-section, it appears that WW promotes Ga colonization (**Figure 3**). Even though the association between wetwood and decline on Guam is very high, its causality has not been established. We currently know nothing about the occurrence of wetwood in Saipan trees which are very healthy. There are several issues that must be taken into account when dealing with wetwood bacteria, and they include the following: rapid division, there are several number of species, initial population is high, and some grow similarly to Rs on a tetrazolium medium. Caleb Ayin was able to reproduce wilt symptoms in young seedling with Rs but not *Klebsiella.* From the literature termites are known to cause damage to ironwood tree roots in plantations; therefore, we think it is reasonable that they may have spreading Rs or wetwood bacteria around Guam. An Rs culture obtained from China by the University of Hawaii was determined to be phylotype 1 (Asia), the same as Guam and Hawaii.

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Diseases of Plants

Tropical Forest Pathology

Decline of *Casuarina equisetifolia* (ironwood) trees on Guam: Ganoderma and Phellinus

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Ironwood trees (Casuarina equisetifolia), on the island of Guam have been in a state of decline for the past ten years. To determine the status of the decline problem and to seek possible causes, a survey of 1427 trees was conducted. A highly signi�icant (p = 0.0001) linear function (r2 = 0.997) between the presence of conks and decline severity emerged. Sixty-�ive percent of the trees at the most severe level of decline (nearly dead) had conks. Species from �ive basidiomycete genera of the class Agaricomycetes, belonging to the orders Polyporales (Ganoderma, Favolus, Pycnoporus), Hymenochaetales (Phellinus) and Thelephorales (Sarcodon) were identi�ied based on macro- and micromorphology and DNA sequencing. The most common species observed was in the genus Ganoderma. Diagnostics was based on the proli�ic production of double walled basidiospores from sporocarps (a characteristic feature of members of the Ganodermataceae). Nuclear ribosomal (ITS) DNA sequencing con�irmed Guam's species as a member of the G. australe species complex. The second most frequently collected conk belonged to the genus Phellinus. These two known genera of Casuarina wood rotting fungi are most likely playing a prominent role in the decline of Guam's ironwood trees. Due to the high association between levels of management and decline, it is believed that tree wounds from lawn equipment serve as a point of entry for the two fungi.

Diseases of Plants

Tropical Forest Pathology

Decline of Casuarina equisetifolia (ironwood) trees on Guam: Symptomatology and explanatory variables

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Phytopathology 101:S216

Guam's Ironwood trees (Casuarina equisetifolia) are dying at rates that far exceed the norm for the region. The problem �its the classic de�inition of tree decline: symptoms are nonspeci�ic such as the thinning of branches; tree health gradually deteriorates leading to tree death over a course of several years; and decline is attributed to a complex of infectious and non-infectious agents. However, Guam's trees deviate from the classic model where mature trees are more prone to decline. Internal discoloration of the trunk and juncture of large branches was often traced to root and butt rot. By applying various modelling techniques to a set of 1427 individual trees, it was concluded that the presence of basidiocarps, termites, and improper tree care were signi�icant explanatory variables for the decline. A data set created by GIS mapping was also evaluated; however, a reliable model has not yet immerged. At least 5 basidiocarp genera have been identi�ied, of which Ganoderma and Phellinus are most likely contributing to the tree's decline. Termites reported on Guam's ironwood trees include species of Nasutitermes, Microtermes and Coptotermes. Other explanatory factors under study include typhoons Chata'an and Pongsona, a species of wasp belonging to the genus Selitrichodes, nematodes in the genera Helicotylenchus and Aphelenchoides, and the bacterium Ralstonia solanacearum.

Poster Session: New and Emerging Diseases-Fungi and Oomycetes

416-P

Survey of wood decay fungi of Casuarina equisetifolia (ironwood) on the islands of Guam and Saipan.

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As a result of statistical modeling of data from individual trees and tree sites, the occurrence of basidiocarps consistently emerged as the dominant explanatory variable for Guam's declining ironwood trees (Casuarina equisetifolia). A survey was conductedin February 2012 in the Mariana Islands to elucidate which of the know basidiocarpforming genera are most consistently correlated with the decline. Species from five basidiomycete genera of the class Agaricomycetes, belonging to the orders Polyporales (Ganoderma, Favolus, Pycnoporus), Hymenochaetales (Phellinus) and Thelephorales (Sarcodon) were previously identified from Guam based on macro- and micromorphology and DNA sequencing. As a result of the February survey, Ganoderma sp. (G. australe complex) was the basidiocarp found to be most frequently associated with unhealthy trees. Conks of the fungus were commonly found on Guam where they appeared on roots and butts of declining and stumps dead trees. On Saipan where decline does not exist and where the trees are considerably healthier, Ganoderr sp. was rarely found. In contrast, Phellinus sp. was the most widespread fruiting basidiocarp on Guam and Saipan. Though the actual species of Phellinus remains to be determined, it does not appear to represent P. noxious, and is not consistently associated with trees in decline. These and other species associated with ironwood trees in the Mariana Islands will be discussed.

Poster Session: New and Emerging Diseases - Bacteria 422-P

Identification of bacteria associated with decline of ironwood trees (*Casuarina equisetifolia*) in Guam

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Decline of ironwood (Casuarina equisetifolia) in Guam was previously attributed to termite feeding and Ganoderma australe. Recently, we found that bacteria are involved in the disease complex. Ralstonia solanacearum (Rs) and two Klebsiella species were consistently isolated from declining trees that showed no evidence of Ganoderma or termite damage. Discolored wet wood and bacterial ooze gave positive results with Rs-specific Immunostrips (Agdia, Inc. SK 33900/0025) and loop-mediated isothermal amplification. Presumptive Rs cultures isolated from host tissues produced the same positive results. 16S rDNA sequence analysis of presumptive Rs strains showed maximum identity (MI) values of 99% with Rs (strain LMG 2299; K60) and Rs (strain GMI 1000). Klebsiella strains isolated from bacterial ooze and wet wood tissues from the same trees showed 99% MI with two Klebsiella species. Cultures from three trees were identified as K. variicola (strains F2R9 and At-22); cultures from a fourth tree showed 99% MI with K. oxytoca (ATCC 13182). Neither Klebsiella nor Rs were detected in healthy trees. Ironwood and tomato seedlings co-inoculated with Klebsiella and Rs showed distortion, wilt and tissue discoloration. Klebsiella and Rs were reisolated from stems 20 cm above the inoculation point. Identification and pathogenicity tests indicate that the bacterial component of ironwood decline is far more significant than previously suspected.

Poster Session: New and Emerging Diseases - Bacteria 423-P

Casuarina equisetifolia decline in Guam linked to colonization of woody tissues by bacteria.

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Ironwood trees (Casuarina equisetifolia) on the island of Guam are in decline due to a combination of biotic and abiotic factors. Bacteria associated with wet-wood and vascular wilt are emerging as significant biotic factors in addition to those previously established, which include the wood-rotting fungus, Ganoderma australe species complex, and termites. Symptoms include thinning of foliage and dark discoloration of the tree's central core, which are associated with the onset of ironwood tree decline. Ralstonia solanacearum and two other bacterial species were consistently recovered in mixed culture when initial isolations were made from discolored wood tissue and from droplets of bacterial ooze, which often form on stem cross-sections of declined trees.
R. solanacearum and one of the unidentified bacterial species were translocated through xylem vessels of young tomato and C. equisetifolia plants following wound inoculation with the bacterial mixture that oozed from infected wood. Confirmation of R. solanacearum was based on cultural characteristics, Agdia immunostrip SK 33900/0025 and loop-mediated isothermal amplification data. Healthy tissues were negative for both the immunodiagnostic and the LAMP assays. This study presents the first evidence that bacteria are involved in the ironwood decline disease complex.

Poster Session: Ecology and Epidemiology - Analytical and Theoretical Plant Pathology
270-P

Early results of Casuarina equisetifolia provenance trial in Guam and advances in research on its decline.

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In 2009 attendees of a conference held on Guam to address Guam's declining ironwood trees (Casuarina equisetifolia) recommended the evaluations in Guam of seedlots used in a 1991-1993 International Provenance trial. Consequently, in July 2012 a trial was established which contained 3 blocks, consisting of 10 geographically paired seedlots of 4 tree with 8 ft. tree spacing. Trees were from 11 countries, including Guam. All provenances quickly established in Guam. After one year, the fastest growing provenances were nearly twice as tall as the average seedlot with nearly 6 times th average's biomass. The fastest three geographically paired provenances were those from Solomon-Vanuatu, Malaysia, and China; the slowest were from Australia, Kenya, and Guam. The block effect was signi�icant with the growth of mos provenances increasing from block 1 to block 3. This difference was attributed to increasing soil depth from block 1 to block 3. The lack of �it between maps of ironwood circumference and predicted dieback ruled out ironwood tree decline (IWTD) as a mere response of trees to poor site conditions. This and other research and observations support the cause of IWTD as a cascade of biotic events likely starting with root damage and the establishment of bacterial wil (Ralstonia solanacearum) and then quickly followed by establishment of wetwood (Klebsiella spp.), basidiocarps of Ganoderma australe species complex, and termites.

Identi�ication and characterization of bacteria associated with decline of ironwood (Casuarina equisetifolia) in Guam

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Ironwood (Casuarina equisetifolia subsp. equisetifolia) is a nitrogen-�ixing tree of considerable social, economic and environmental importance that commonly occurs in tropical/subtropical zones of Asia, the Paci�ic, Africa, and Central America. Ironwood decline was �irst noticed on Guam in 2002 and is now affecting thousands of trees and impacting the ecosystem. In 2012, a survey showed that Ralstonia solanacearum and Klebsiella spp. were associated with wetwood symptoms of declining trees. R. solanacearum strains isolated from diseased ironwood in Guam were similar to R. solanacearum strain GMI1000, having similar BOX-PCR pro�iles and belonging to phylotype I and biovar 3. Two Klebsiella species (K. variicola and K. oxytoca) wererecovered, with K. variicola being the more prevalent species. Pathogenicity tests revealed that R. solanacearum caused wilt in tomato and ironwood seedlings, whereas neither Klebsiella spp. produced symptoms. There were no differences in virulence between Guam R. solanacearum and control strains following inoculation into tomato and ironwood from Hawaii. Additionally, no observable differences in ironwood susceptibility to Ralstonia strains from Guam or Hawaii, were observed, suggesting that the association of Guam R. solanacearum with Guam ironwood is not speci�ic. Coinoculation studies with both R. solanacearum and Klebsiella variicola and K. oxytoca revealed that Klebsiella sp. did not affect symptoms produced by R. solanacearum alone. In planta studies were feasible only on seedlings and young trees in Hawaii; thus, possible interactions between R. solanacearum and Klebsiealla sp. in adult trees remain to be investigated. A new in-�ield survey of declining ironwood is needed to better understand the role of Klebsiella and Ralstonia in ironwood tree decline in Guam.

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Morphological and Molecular Species Identification of Termites Attacking Ironwood Trees, *Casuarina equisetifolia* (Fagales: Casuarinaceae), in Guam

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Abstract

Ironwood trees (*Casuarina equisetifolia* subsp. *equisetifolia* L.) are ecologically and economically important trees in tropical and subtropical regions of the Indo-Pacific. Ironwood is one of the dominant tree species in Guam, but since 2002, this tree has been declining dramatically. A previous study showed that numerous sick or dead trees were under termite attack. However, the species of termites were not identified. As a first step to investigate causal relationships between termites and ironwood tree death, we assigned termites collected from ironwood trees to species using a combination of morphological characters and DNA barcoding of the 12S, 16S, COI, COII, and ITS2 regions. Based on morphology and comparisons to reference sequences in NCBI GenBank, the most likely species assignments were *Nasutitermes takasagoensis* (Nawa) (Blattodea: Termitidae) found to infest 45 trees, followed by *Coptotermes gestroi* (Wasmann) (Blattodea: Rhinotermitidae) (2 trees), *Microcerotermes crassus* Snyder (Blattodea: Termitidae) (2 trees), and an additional unidentified *Microcerotermes* species (1 tree) with no close sequence match to identified species in NCBI GenBank. However, taxonomic revisions and broader representation of DNA markers of well-curated specimen in public databases are clearly needed, especially for the *N. takasagoensis* species complex.

Keywords: DNA barcoding, ironwood tree decline, wood-feeding pest, Termitidae, Rhinotermitidae

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