Integrated Perspective on Tree Decline of Ironwood (*Casuarina equisetifolia*) on Guam

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ABSTRACT

Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in decline. Research on the cause of ironwood tree decline (IWTD) began in earnest in January 2009 when six invited off-island scientists, together with participants from Guam, took part in a 5-day IWTD conference (Mersha et al., 2009). The findings of that conference and subsequent research were published in the proceedings of the 4th International *Casuarina* Workshop, Haikou, China, in 2010 (Schlub et al., 2011). Therein, it was reported that a complex of biotic and abiotic factors were responsible for the decline. The theory that opportunistic conk-producing fungi such as species of *Ganoderma* and *Phellinus* as an explanation for the majority of Guam's declining trees was advanced. Much of what was identified as contributing IWTD factors has now been confirmed and will be clarified in this Western International Forest Disease Work Conference (WIFDWC) Proceedings. 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. Bacterial colonization of the xylem is seen in trees with thinning foliage, which is indistinguishable from that attributed to ironwood decline. At least three bacteria are consistently present in declining trees: *Ralstonia solanacearum, Klebsiella oxtoca* and *K. variicola*. We believe the *Klebsiella* spp. are responsible for the wetwood symptom associated with Guam's declining trees and that both *R. solancearum* and *Klebsietta* spp. play a role in IWTD. When Geographic Information System (GIS) map derived variables were added to the original model, it was found that trees are less impacted by ironwood decline when there is adequate soil moisture holding capacity, when trees are 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 and termites, height above sea level and tree size. The current model will be strengthened, with the addition of *R. solanacearum* and *Klebsiella* spp. survey data. 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.

INTRODUCTION

History: *Causarina equisetifolia*, which is native to Guam and locally known in the English language as ironwood and in the Chamorro language as "gago," has been continually propagated on Guam since the 1600s. As a result of its nitrogen-fixing ability and tolerance to salt spray and typhoon damage, the tree is able to thrive in the Mariana Islands where typhoons and coral sand beaches and other nutrient-poor soils are commonplace.

Botanical Characteristics: The tree is an evergreen and 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 an average 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, which results in a shorter tree with a wider crown than what is typically seen in Hawaii.

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 it grows nearly everywhere: beaches, landfills, road shoulders, cleared land, and vacant lots. In the Mariana Islands it grows both in savanna grasslands in clay volcanic soils and in coastal strands in calcareous sands and loamy sand soils. In large dense strands trees produce a thick, slowly decomposed, 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 mixtures of other species including *Cocos nucifera*, *Guettarda speciosa*, *Hernandia sonora*, *Pandanus tectorius*, *Scaevola taccada*, *Thespesia populnea*, and *Tournesfortia argentea*.

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 will primarily affect plants in poor planting sites where the trees become stressed and consequently become vulnerable to these insects and pathogens. Some pathogens may be agents of latent infections; therefore, the infection precedes environmental changes that trigger symptom production.

In India, termites feed on underground roots and stems of live *Casuarina equisetifolia*. This type of damage is believed to be occurring in Guam as well. From past entomological surveys and reports, colonies of *Nasutitermes* sp. and *Microtermes* sp. were found feeding on dead ironwood trees (Moore, A., personal communication). The Philippine milk termite *Coptotermes gestroi* was responsible for killing ironwood trees transplanted onto a new golf course (Yudin, L.S., personal communication).

Damage to branchlet tips by an unidentified gall wasp 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. The wasp reared from branchlet tip galls was identified as belonging to the genus *Selitrichodes* (E*ulophidae: Tetrastichinae)* by John LaSalle, CSIRO, Australia.

Ralstonia solanacearum the cause of bacterial wilt is among the most commonly worldwide reported pathogens of *Casuarina*. It is a xylem-resident bacterium mainly entering via roots Though only 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 field observations, culturing from symptomatic tissues, immunostrip data, LAMP data, and other tests, it was concluded that on Guam *R. solanacearum* is part of the disease complex referred to as ironwood tree decline (IWTD). Inaddition, *R. solanacearum* is accompanied by two companion bacteria. In March 2013, it was confirmed that ooze from cross-sections of declining tree stems contained *R. solanacearum* and two species of *Klebsiella*: *K oxytoca* and *K. variicola*. Both bacteria are translocated through xylem vessels of *C. equisetifolia* seedlings following wound inoculation.

On Guam, trees that harbor *R. solanacearum* and the companion bacteria, do not manifest the same symptoms as those observed in China. In China, the field symptom is rapid tree death (Figure 1), which is triggered by severe environmental stress such as a typhoon. On Guam, bacterial colonization of the xylem results in trees with thinning foliage, which is indistinguishable from symptoms associated with IWTD (Figure 2). Differences between China and Guam diseases can also be seen in symptoms revealed in cross-sections of trunks and limbs. In China, xylem vessels of trunk cross-sections contain diffused areas of slightly darker tissue and copious amounts of bacterial ooze (Figure 3). 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

Figure 1. Bacterial wilt of *C. equisetifolia* sapling in China (*photo provided by Dr. Chonglu Zhong*).

Figure 2. Comparison of a health *C. equisetifolia* tree, DS=0, (left) with a tree at the first level of Decline Severity, DS=1, (right). Note thinning foliage and slight tip-dieback of fine branches.

restricted to the "wetwood" which has a high moisture content (Figure 4).We believe the *Klebsiella* spp. are responsible for the wetwood symptom associated with Guam's declining trees and themselves plays a role in Guam's ironwood decline.

There are many fungi involved in wood rot or decay, those contributing to ironwood decline are the basidiomycete. The fruiting body or sporocarp of these fungi are called basidocarps. The basidiocarps found in Guam and Saipan were either flat (resupinate) or shelflike (conk) (Figures 5 and 6). 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* (Schlub et al., 2011). The death of large branches and later roots may occur without the presence of basidiocarps.

As a result of a survey in February 2012, the basidiomycete found to be the most frequently associated with unhealthy trees and wood-rot was that of *G. australe* species complex (Figure 5) (Schlub, et al., 2012). It invades woody tissue through an unrestricted mycelial network while sustaining themselves on cell and cell wall components (Figure 7). Its conks appeared on roots and butts of declining trees and stumps of dead trees. On Saipan where decline does not exist and where the trees are considerably healthier, *G. australe* species complex was rarely found, and then only in association with a few unhealthy appearing trees.

IRONWOOD TREE DECLINE

Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in the midst of a decline that was widespread by 2005. Several trees stands on Guam began showing decline symptoms in 2002 and 2003 following typhoons Chata'an in July and Pongsona in December of 2002. Observations by Bart Lawrence, long time resident and forester, believes decline may have begun in isolated areas following typhoon Gay in November 1992.

Figure 3: 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,* et al. *at International Casuarina Workshop Haikou, China 21-25 March 2010*.

Figure 4. Cross-section 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 an *Klebsiella* spp.

Ironwood Tree Decline (IWTD) starts with thinning of foliage and slight dieback of fine branches at DS=1 (Figure 3) and processes to the final stage (DS=4) characterized by dieback of large branches and 95% defoliation. At DS=1, the outward symptom of IWTD is indistinguishable from those produced by Guam's two xylem-resident bacteria. Internal symptoms (as seen in trunk cross-sections) vary from tree to tree and with decline severity. Small trees (< 50 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 4), and less common signs of wood rots caused by *Ganoderma* (Figure 7) and termites. Trees in a severe state of decline harbor one or all of the following: bacteria, termites, various resupinate sporocarps and conks of *Ganoderma australe* species complex (Figure 5), *Phellinus* (Figure 6) and other Agaricoymcetes.

Statistical Modeling: Modeling was used to evaluate a set of data from 1427 individual trees, 44 sites, and 16 GIS maps.

Analysis of Individual Trees: For each sample tree, a set of measurements were taken and selected for analysis (Table 1). The primary objective of using statistical models with the ironwood tree data is to find possible factors that could be related to tree decline, in other words to find the parameters that have a positive or negative impact on the tree (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.

Figure 5. 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 6. 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 Marina islands and not a contributor to IWTD or stand losses.

Three explanatory variables were found to be significant and therefore could explain the ironwood's state of health (Table 1). 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" (basidiocraps) than a tree without.

Figure 7. Cross-section of rotted ironwood tree butt infected with *Ganoderma australe* species complex. Note expanding network of white mycelial strands.

Table 1. Grouping and descriptions of ironwood tree variables, those in bold were found to be the most suitable for predictive purposes.

<table>
<tr><td colspan="6">Response Variables</td></tr>
<tr><td>Decline severity</td><td colspan="5">Severity ranking DS=0, 1, 2, 3, and 4</td></tr>
<tr><td>Tree dieback</td><td colspan="5">Healthy or unhealthy</td></tr>
<tr><td colspan="6">Explanatory Variables</td></tr>
<tr><td>Structure</td><td>Number of trunks per tree</td><td colspan="2">Circumference of tree at 1.3 m height</td><td colspan="2">Site density: trees per square meter of site</td></tr>
<tr><td>Stress</td><td>Fire damage: present or not</td><td>"Conks": present or not *</td><td colspan="2">Storm damage: present or not</td><td>Termites: present or not</td></tr>
<tr><td>Geographic</td><td>Latitude</td><td>Longitude</td><td colspan="2">Altitude</td><td>Site</td></tr>
<tr><td>Miscellaneous</td><td>Level of lawn management: none, moderate, high</td><td colspan="4">Tree origin: natural or planted</td></tr>
</table>

* "Conks" refers to any resuspinate or shelflike sporocarp of a basidiomycete appearing on 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 1) 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 6 positive IWTD predictors: increases in circumference, increases in altitude, presence of conks, presence of termites, planted stand vs natural stand, and urban land location.

There were 4 negative IWTD 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 conks. 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 in circumference at 1.3 m). 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 observed circumference was sought. The circumference map supports the concept that nearly the entire island is suitable for the growth of small trees (Figure 8). However, as the size of the trees increases the area suitable for sustained growth decreases. When the map for dieback (Figure 9) was visually compared to the map for circumference, IWTD 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.

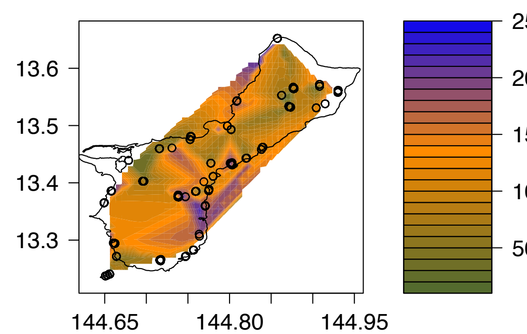


Figure 8: Map of observed tree circumference in cm at breast height (CBH) over a longitude-latitude grid of the island of Guam; hence, areas of large trees sites (purple color) have habitats more suitable for ironwood growth irrespective of the presence of IWTD.

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 2

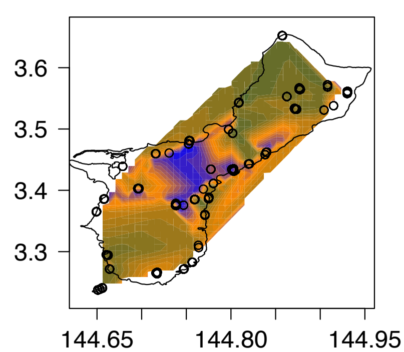


Figure 9: Map of the predicted probability of dieback using a logistic model. Areas in blue indicate regions where dieback is most likely to occur.

Table 2. 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		
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 of IWTD and its general sporadic nature, decline on Guam 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). Currently, experiments are underway to identify new provenances of *C. equisetifolia* that might grow well under pressure from Guam's pests, diseases and typhoons. There are 23 provenances in this test which represent *C. equisetifolia* sources from around the globe.

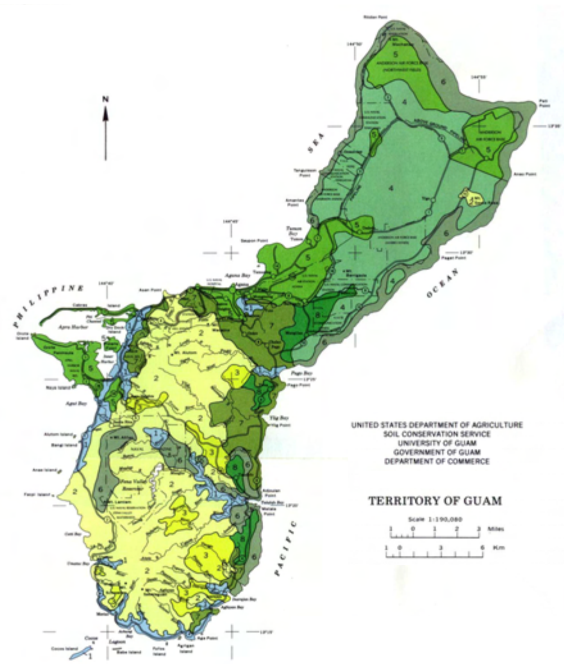
Experimental plans are also underway to determine if there are particular spacing patterns or pruning practices that can enable ironwood tree to be more wind resistant. Good site selection and good establishment techniques are among the recommended practices for avoiding ironwood decline along with all activities that could lead to the damage of the cambium in the boles of the stem. Grass trimming near trees and ground fires are estimated to have been particularly detrimental to ironwood trees in the past couple of decades.

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 10). Chemical attributes of a soil are those related to the activity of ions within the soil solution; measurements include pH and CEC. Though ironwood can grow across Guam's wide range of soil pH, soil nutrients are maximized between pH 6-7. Cation Exchange Capacity (CEC) is a measure of the soil's ability to hold unto nutrients, which increases with a soil's fertility.

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/cm3, which are ideal for clayey soil. Unfortunately the soil is 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 reached 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.

Figure 10. General Soil Map of Guam (Young et.al.,1988)



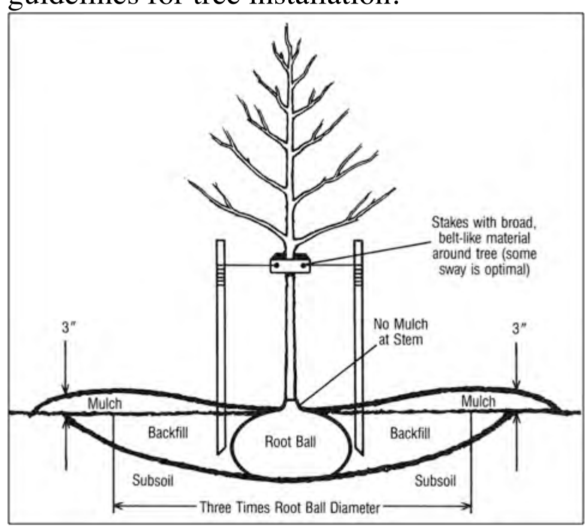
Key	Soil	Horizon depth (cm)	Clay (%)	Bulk density (g / cm^3)	pH	CEC
SOILS ON BOTTOM LANDS						
1.1	Inarajan-Inarajan	0–13	50–70	0.90–1.10	5.1–7.3	51
1.2	Shioya	0–25	0–3	1.10–1.25	7.4–8.4	7
SOILS ON VOLCANIC UPLANDS						
2	Akina-Agfayan	0–10	45-80	0.80–0.95	5.1–7.3	23
3	Akina-Togcha-Ylig	0–13	45–70	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

Site Remediation: Compacted soil in or near a planting pit should be remediated as necessary. The detrimental effects of planting in compacted soil may include decreased water holding capacity, poor infiltration rates and decreased aeration. These factors could contribute to decreased root penetrability and thus increased susceptibility to drought and transplant shock. Remediation methods include 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 add benefit to new planting sites. Vertical mulching, a process of drilling or blasting with an air-tool vertical holes in the soil into which conditioned porous soil is added.

Tree Installation: Plants should be installed in saucer- shaped hole / pit that allow for expansion of the root zone with minimal substrate resistance (Figure 11). 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 matching the original. To enrich the topsoil, amended 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 hole 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 because in this scenario the roots could encircle amongst 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.

Planting Bare Root Plants: After planting bare rooted trees, gently tap the soil and backfilling with water to remove air pockets. Additional staking may be required of 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.

Figure 11: General installation/hardware guidelines for tree installation.



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 life time. Fertilizer needs to be use 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 micro nutrients is ideal. Alternatively, apply a small amount (50 to 100 g) of a low analysis complete fertilizer (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. A 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 (eg 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 neighbouring healthy trees.

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