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Ecology of Guam's *Casuarina equisetifolia* and research into its decline

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Abstract

On Guam, the estimated lifespan of *Casuarina equisetifolia* subsp. *equisetifolia* is 35 to 90 years and trees may reach a height of 24 m and a diameter at breast height of 92 cm. The majority of its trees are monecious. In 2002, it was noticed that many trees were in a slow decline and when felled, droplets often formed on their fresh stumps. In 2009, this condition was coined ironwood tree decline (IWTD). The droplets which consisted of various mixtures of plant exudates and bacterial ooze were found to contain *Ralstonia solanacearum* and various wetwood bacteria. Reported in 2015 and confirmed in 2020, Guam's *R. solanacearum* strains are of the phylotype 1 (Asia). Cross-sections of IWTD trees from Guam are very similar in appearance with those of bacterial wilt from China. In 2012, a project was initiated to diversify the gene pool and identify resistance of *C. equisetifolia* in Guam trees. Using the 1991-1993 international provenance trials of *C. equisetifolia*, 11 geographically paired seedlots were planted at a farm where IWTD was present. Over the succeeding years, none of these trees have developed IWTD nor tested positive for *R. solanacearum*. Over the years, the following pests were investigated and ruled out as contributors to IWTD: *Protaetia orientalis*, *Protaetia pryeri*, *Selitrichodes casuarinae*, *Helicotylenchus* sp. and *Phellinus* sp. Between 2010-2019, data analyses identified three prominent variables as predictors of IWTD: bacterial wilt pathogen *R. solanacearum*, butt and root rot fungus *Ganoderma australe* and termites. In 2019, *Nasutitermes takasagoensis* complex was found to be the dominant termite species infesting Guam's *C. equisetifolia*.

Keywords: *Casuarina equisetifolia*, *Ralstonia solanacearum*, *Ganoderma australe*, wetwood, ironwood tree decline on Guam, bacterial wilt China, bacterial wilt India

1 Introduction

Much of the information presented in this article is contained in a general review of Guam's *C. equisetifolia* (Schlub, 2019).

1.1 History

Casuarina equisetifolia, subsp. *equisetifolia*, is tightly integrated into the local culture and the Guam's environment, where it is locally known as ironwood (in English) and "gago" (in the native Chamorro language). It has been continually propagated on Guam since the 1600s. It is a hardy, pioneer, salt-resistant tree that occurs on the island's main soil types: limestone, volcanic, and coral sand. It is propagated for windbreaks, erosion control, and urban landscapes. Because *C. equisetifolia* is the dominant tree species on many of the sandy beaches of the Mariana Islands, it has become an important perching tree for the white-collared kingfisher (*Halcyon chloris*), the Mariana fruit-dove (*Ptilinopus roseicapilla*), and the white fairy tern (*Gygis alba*), which commonly lays eggs in ironwood trees. Since the 1980s and prior to appear of tree decline in the 2000s, the Guam Department of Agriculture provided approximately 250,000 seedlings to farmers, the public, and government agencies for various tree planting projects.

1.2 Ecology

On Guam, estimated lifespan of *C. equisetifolia* is 35 to 90 years, and it may reach a height of 24 m and DBH of 92 cm. Its population on Guam is estimated to be 80% monecious, 3% male, and 10% female. Ironwood thickets are a component of Guam's forestland, where *C. equisetifolia* is considered a secondary forest species. In the Mariana Islands, it grows in the clay volcanic soils of savanna grasslands and in the calcareous and loamy sands of coastal strands. On Guam, *C. equisetifolia* is only one of eight tree species larger than 28 cm in diameter at breast height (DBH). In addition, *C. equisetifolia* is a prominent member of the halophytic (sea-salt adapted) vegetation type. This vegetation type is found along beaches in northern and southern Guam, where it may be composed solely of *C. equisetifolia* or a mixture of other species, including *Cocos nucifera, Guettarda speciosa, Hernandia sonora, Pandanus tectorius, Scaevola taccada, Thespesia populnea*, and *Tournefortia argentea*.

1.3 Ironwood tree decline (IWTD)

Symptomatic *C. equisetifolia* began appearing in tree stands across Guam in 2002. In one farm location, five 10-year-old trees planted as part of a windbreak exhibited symptoms of rapid yellowing (chlorosis) and mortality. At the same time, trees at this location and elsewhere on Guam were exhibiting symptoms of thinning foliage and a lethal progressive dieback. Age of affected trees ranged from 10 years to several decades. A study was commissioned in 2004, after Natural Resources personnel with Commander Navy Region Marianas (COMNAVMAR) observed high mortality among trees at the Naval Station. The study failed to identify a cause for the mortality but did rule out two invasive beetles, *Protaetia pryeri* (Janson) and *Protaetia orientalis* (Gory and Percheron). By 2005, one third of all trees at the Naval Station were dead. In 2008-2009, the condition of foliage thinning and dieback on *C. equisetifolia* was referred to as ironwood tree decline (IWTD) (Schlub et al., 2011). At that time a visual tree scoring system was developed based on five ordinal categories of branch fullness and dieback: 0= symptomless, 1=slight damage, 2=distinctly damaged, 3=heavily damaged, and 4=nearly dead) (Schlub et al., 2011). As of today, trees continue to die, although the rate of loss appears less than that in 2005.

2 Materials, methods and results

2.1 International provenance trial

To identify potential resistance to IWTD, 11 geographically paired seedlots obtained from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Tree Seed Centre were planted on Guam in 2012. The majority of these seedlots were used in the 1991-1993 international provenance trials of *C. equisetifolia*. The fastest growing pairs were those from Solomon (CSIRO 18402), Vanuatu (CSIRO 18312) and Papua New Guinea (CSIRO 20586 and CSIRO 18153), while the slowest pairs were from Australia (CSIRO 19821 and CSIRO 18378), Thailand (CSIRO 18297 and CSIRO 18299), and Guam. Though these trees were planted in the immediate area where IWTD occurs, today these trees remain healthy and free of infection by *R. solanacearum* (bacterial wilt pathogen) or *G. australe* (wood-rot fungus).

2.2 Gall-inducing wasp

After discussions among attendees of Guam's IWTD conference in 2009 (Schlub et al., 2011) and subsequent surveys, the gall-inducing wasp (*Selitrichodes casuarinae*) was discovered. Subsequently, this gall-inducing was found to be widespread across Guam (Fisher et al., 2014). On healthy trees, its impact is likely negligible, but may be significant on trees in decline.

2.3 Nematodes

In 2011, over a 5-day period, nematodes were extracted from *C. equisetifolia* roots and associated soils from five sites with healthy trees, and nine sites with declining trees. Soil samples from sites with decline contained a higher number of nematodes and nematode species than samples from sites with healthy trees. *Tylenchus* and *Helicotylenchus* were the most common genera isolated, with both genera found on 50% of samples from healthy sites, and 44% and 78%, respectively, of samples sites with declining trees. The highest average nematode counts from 10 g samples from both sites (healthy and declining trees) were those of *Helicotylenchus*, with 10 (healthy tree sites) and 39 (declining tree sites). It was concluded that *Helicotylenchus* sp. was likely the only nematode isolated with a potential to negatively impact *C. equisetifolia.*

2.4 Termites

In 2015, termites from 48 infested *C. equisetifolia* trees across Guam were collected. *Nasutitermes takasagoensis* or a closely related species from the *Nasutitermes takasagoensis* complex was found to have infested 45 trees. *Coptotermes gestroi* and *Microcerotermes crassus* were found to infested two trees (Park et al., 2019).

2.5 Conk forming basidiomycetes

Tree surveys during 2008-2009 found that 65% of *C. equisetifolia* were nearly dead (DS=4) and conks (fruiting bodies/basidiocarps of wood-decay fungi) were visible on most of these trees. In 2010, five conk-forming 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 micro-morphology and DNA sequencing (e.g. ITS rDNA). A short survey for conk-forming basidiomycete was conducted in 2012 in healthy and IWTD sites on Guam and the nearby island of Saipan where IWTD does not occur.

Conks found growing on live trees belonged primarily to two species: *Ganoderma* sp. (*austral* group), which fruits on the tree roots, butt, and less commonly bole, and *Phellinus* spp., which primarily fruited on the butt. Both species were commonly found on Guam and infrequently found on Saipan. *Phellinus* does not appear to be a primary contributor to IWTD by itself. In contrast, *Ganoderma* appears more likely as a factor that contributes to IWTD on Guam, because it is a consistent indicator of IWTD (or a tree in decline) and its occurrence is irrespective of tree size. The *G. australe* complex identification was confirmed from samples collected at three sites on Guam and one site on Saipan in 2013. ITS sequences of a sample (*G. austral* complex) from Guam showed a 99% identity with sequences of *Ganoderma* sp. from China (GenBank GU213473), and a sample from Saipan showed a 99% identity with other sequences of *Ganoderma* sp. from China (GenBank FJ392286). Appropriate taxonomy within the *G. australe* complex remains unclear.

2.6 Detection and isolation of *R. solanacearum* on Guam

Within minutes of felling a tree in decline, droplets often formed on the cut surface. The droplets, hereafter referred to as ooze, consist of various mixtures of plant exudates and bacterial ooze. In 2011, it was discovered that ooze and tissue samples from decline trees tested positive using *R. solanacearum* specific Immunostrips (Agdia, Inc.) (Ayin et al., 2015; Ayin et al., 2019).

Three forms of ooze were observed: white to off-white viscous ooze (VO), watery amber ooze (WO), and a mixture of the two (MO). Drops of VO commonly appear in sapwood and sapwood-transition zones, may occur in unstained tissue, and frequently tested positive for *R. solanacearum*. Drops of WO commonly appear in the sapwood-transition and heartwood zones, always appear in stained tissue, and frequently tests negative for *R. solanacearum*. Drops of VO and MO were not randomly distributed, but appeared to coincide with growth rings (Fig. 1).

Though *R. solanacearum* could be detected from wood chips and drill shaving from roots, stems and branches of trees, attempts to isolate these bacteria from these same drill shavings failed. The only means

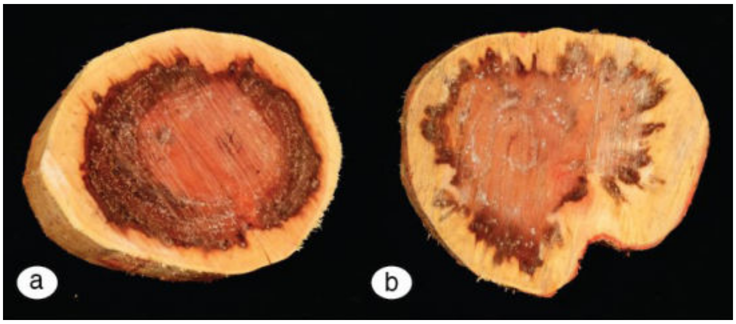

Fig. 1. Cross-sections of *Casuarina equisetifolia* trees on Guam with decline symptoms: (a) decline level DS=1 (slight damage), 63% wetwood symptomatic tissue present in sapwood and sapwood-heartwood transition zones, extensive bacterial ooze formed within minutes of felling, the majority of which is viscous, positive for *R. solanacearum*, positive for *Klebsiella oxytoca*, and negative for *Ganoderma australe*; (b) decline level DS=3 (heavily damaged), 58% wetwood symptomatic tissue present in sapwood and sapwood-heartwood transition zones, extensive bacterial ooze, of which the majority is viscous, positive for *R. solanacearum*, and negative for *Ganoderm australe*.

by which *R. solanacearum* could be isolated was by streaking ooze that formed on slices of stems, roots, or large branches from infected trees onto selective medium. To enhance the production of ooze, slices were placed on saturated paper-towel in a moisture chamber for 24 hrs. Once formed, the ooze was streaked on Engelbrecht's semi-selective medium (SMSA) (Ayin et al., 2015; Ayin et al., 2019). Colonies were re-streaked on to SMSA, which was followed by streaking onto modified Kelman's tetrozolium chloride medium (TZC) before subculturing on TZC.

2.7 Statistical links to decline

Various statistical methods have been applied to variables in search of predictors for IWTD. These variables were related to tree growth (e.g. diameter), abiotic factors (e.g. management practices and site density), or biotic organisms (e.g. termites and bacteria). In 2008-2009, 1,427 trees were surveyed for decline severity (DS) using an ordinal scale consisting of five categories (0=no damage, 1=slight damage, 2=moderately damaged, 3=heavily damaged, and 4=nearly dead). In addition 13 predictive variables were measured: latitude, longitude, altitude, number of tree stems, tree stand density, tree site location, typhoon damage, fire damage, CBH, intensity of management practices (none, moderate, or high), whether the tree was naturally planted or not, and whether a tree had conks or termites (Schlub et al., 2011). Through the application of multinomial modeling, three variables were determined to be significant (Schlub, 2010) (Table1).

In 2012, variables derived from 16 GIS map characteristics were added to the survey data. These GIS-derived variables included: cemetery 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, vegetation, and the 2002 USDA Forest Service Inventory Analysis (FIA) map of trees with conks. The soil series was dropped from the analysis because of correlations with regressors. Nine GIS-derived variables were determined to be significant (Table 1).

After establishing in 2013 that *R. solanacearum* (bacterial pathogen) and wetwood bacteria were present in declining trees (Ayin et al., 2015), trees were surveyed to determine the relationship of bacteria and *G. australe* (wood-rot fungus) with IWTD. Using data collected in 2015 from a set of 77 whole trees, a proportional odds logistic regression model was fit with the following covariates: DBH, height, and presence/absence of *R. solanacearum* and *G. australe*. In addition, nine covariates (percent wetwood area, ooze initiation, ooze quantity, ooze type, and presence/absence of *Klebsiella* colony types, *K. oxytoca*, *R. solanacearum*, and *G. australe*) were applied univariately to data from a 30-tree subset of the original 77. From the two studies, four explanatory variables or covariates were determined to be significant (Ayin et al., 2019)

Table 1 Explanatory variables that were found to be significant positive (+) or negative (-) predictors of ironwood tree decline (*Casuarina equisetifolia*) (IWTD) from three statistical studies, variables are listed in order of significance.

Study*	Variable	Significance
2010	When conks were present	+
	When termites were present	+
	With increases in landscape management practices	+
2012	When conks were present	+
	When trees were intentionally planted	+
	When located where soil water is available at 25 cm	-
	With increases in altitude of location	+
	When located on a golf course	-
	When located in a forested areas	-
	With increases in tree circumference	+
	When termites were present	+
	When located in an urban landscape area	+
2019	When *Ralstonia solanacearum* was detected	+
	When *Ganoderma australe* was present	+
	With increases in percent wetwood in tree cross-sections	+
	When ooze bacterial ooze forms within 24 hrs of tree felling	+

*Analyses (Schlub 2010; 2019 and Ayin et al., 2019).

(Table 1).

2.8 Comparison between IWTD in Guam and bacterial wilt in China and India

Though IWTD in Guam and bacterial wilt in China and India have the pathogen *R. solanacearum* in common, IWTD differs with respect to symptomatology and abiotic and biotic contributors (Table 2).

3 Discussion

Based on our current information, it appears that IWTD is unique to Guam and it has no single cause. Though *R. solanacearum* occurs in Guam, China and India, it only accounts for 65% of the trees with IWTD symptoms in Guam, whereas in China and India is accounts for 100% of trees with bacterial wilt symptoms. Another stark difference between Guam and China and India is that the symptoms are nondistinctive and gradual with IWTD in Guam, whereas in China and India thedisease symptoms are distinct and sudden.

The identification of several significant explanatory variables strengthens the concept that IWTD is not solely caused by *R. solanacearum*, but it is instead the result of a disease complex comprising multiple biotic and abiotic factors in which biotic factors play a dominant role. The presence of *R. solanacearum* and *Klebsiella* colony types in symptomless trees suggests that at least some trees could possibly remain symptomless when infected with *R. solanacearum* and wetwood bacteria, provided its sapwood is not compromised through the formation of wetwood or ooze (Ayin et al., 2019).

4 Conclusion

On Guam, where IWTD is prevalent 20% of the outwardly asymptomatic trees test positive for *R. solanacearum* and 50% have some degree of wetwood. Only a few trees exhibit no internal discoloration or bacterial ooze, and test negative for *R. solanacearum* (Fig. 9).

Table 2. Comparison between ironwood tree (*Casuarina equisetifolia*) decline (IWTD) in Guam and bacterial wilt caused by *Ralstonia solanacearum* in China and India.

	Guam	**China**	**India**
Age at onset	10 years and older	Several months to 10 years (Fig. 4)	Sapling to one year
Foliage symptoms	Gradual thinning and die-back of branches (Fig. 3a)	Rapid wilt and death of branchlets (Fig. 4, 5)	Rapid yellowing of lowest branchlets and progressing upward
Onset to tree death	Months to years	Weeks to months but heavy rain can extend the period	Weeks
Appearance of freshly cut stomp or stem cross-sectional disc/slice	Those in severe decline usually produce ooze (Fig. 1), others may (Fig. 2) but often do not (Fig. 3b), wetwood may occur in the sapwood, sapwood heartwood transition zone (Fig. 1) or heartwood (Fig. 2), asymptomatic trees may (Fig. 2) or may not (Fig. 3b) have wetwood	Usually produce ooze (Fig. 6, 7) and may have evidence of wetwood in the sapwood or the sapwood-heartwood transition zones (Fig 6.)	Often show ooze and discoloration
Occurrence	Roughly 5% of the tree population, dense tree cohorts often <0.5 ha or as a few scattered trees across several hectares	Roughly 5,000 hectares mainly in Guangdong province, occurring dense tree cohorts close to agricultural lands (Fig. 4)	Dense plantations
Maximum Incidence	Approximately 85%	>90% within 1-2 months following a typhoon	40%
Phylogenetic Analysis	phylotype 1 (Ayin et al., 2015)	phylotypes 1, biovar 3 or 4 and race 1 (Jiang et al., 2017)	Phyloptype I, biovar 3 & 4, race 1 (Ramesh et al. 2014; Singh et al., 2018)
Contributing factors	In sites with decline approx. 65% of the symptomatic trees are infected with *Ralstonia solanacearum*, other factors linked to IWTD included site conditions, management practices, bacterial wetwood, the termite *Nasutitermes takasagoensis*, and the butt and root rot fungus *Ganoderma australe*	100% of the symptomatic trees are infected with *R. solanacearum*, appears sporadically at first and gradually reaches >90%, sudden death often follows typhoon (Fig. 4,5), occurs in plantations, disease level increases with repeated plantings in infected sites, disease is greatest near crops infected with bacterial wilt	Usually occurs under wet conditions, most of the plantation are adjacent to tomato fields, biocontrol organisms such as *Trichoderma* and *Micromonospora* controlled the disease and reversed the symptoms if applied during early stages of infection, revived trees showed no discoloration of stem or oozing when tested at the age of 18 to 24 months (Fig. 8)

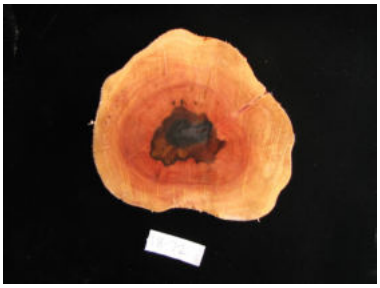

Fig. 2. Cross-section of a *Casuarina equisetifolia* tree on Guam with no outward symptoms of decline: DS=0 (symptomless), 14% wetwood symptomatic tissue present in heartwood, slight viscous and watery bacterial ooze, negative for *Ralstonia solanacearum* and *Ganoderma australe*

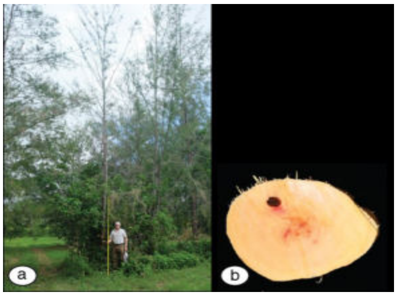

Fig. 3. *Casuarina equisetifolia* tree on Guam with decline symptoms: (a) level DS=2 (distinctly damaged) (b) tree 'a' cross-section, 0% wetwood symptomatic tissue, no bacterial ooze, positive for *Ralstonia solanacearum*, and negative for *Ganodera australe*.

Fig. 4 Mortality of various aged clonal *Casuarina equisetifolia* trees in China, following a strong typhoon in 2015.

Fig. 5. Mortality of bacterial wilt-infected *Casuarina equisetifolia* trees in a clonal trial in Wuchuan, China, 2 months after a strong typhoon in 2015.

Fig. 6. Cross-section of bacterial wilt-infected *Casuarina equisetifolia* tree in Xuwen, China in 2019: severe bacterial wilt, 38% wetwood symptomatic tissue present in sapwood-heartwood transition zone, extensive viscous bacterial ooze is formed within minutes of felling, *Ralstonia solanacearum* positive.

Fig. 7. Cross-section of bacterial wilt infected *Casuarina equisetifolia* tree in Xuwen, China in 2019: severe wilt, 0 % wetwood symptomatic tissue, slight viscous ooze bacterial exudate is formed within minutes of felling, *Ralstonia solanacearum* positive.

Fig. 8. Cross-section of an 18-month-old tree in India that recovered from bacterial wilt with no signs of wood discoloration or bacterial ooze, after showing symptoms at 6 months and following treatment with *Micromonospora maritima.*

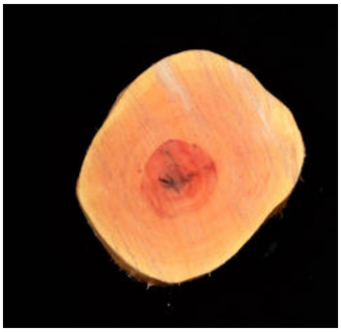

Fig. 9. Cross-section of a *Casuarina equisetifolia* tree on Guam with no outward symptoms of decline: IWTD level DS=0, symptomatic tissue 3%, no wetwood or bacterial ooze, negative for *Ralstonia solanacearum,* negative for *Klebsiella oxytoca,* and negative for *Ganoderma australe.*

These healthy trees tend to occur in natural stands, at low altitude or areas not prone to drought. Due to the slow progression of IWTD and its general sporadic nature, it is likely that IWTD could be reduced through the following measures: (1) increasing the genetic diversity and species diversity of *C. equisetifolia* in Guam, (2) removal of *R. solanacearum / G. australe* infected trees, (3) prevention of root-grafts, and (4) the application of cultural practices that promote healthy growth.

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