# Appendix 1: Diagnostic Report on signs, symptoms and fungi associated with Casuarina equisetifolia.

## *Casuarina* sample information

**Sample 11-1** (N13.31943 E144.52473)
[I am not sure if this number is correct, as the image I have for this sample is not of fine roots – I do not know if the fine roots and the wood were given different numbers. The coordinates are correct.]

Root sample collected 1/29/11 and examined 1/30/11. In my notes I have this as the second tree that we looked at at Yigo on the first day. This tree was in the left windrow if you stood facing the row of *Casuarina* trees that are severely declined and which had conks on them (at one edge of the papaya field). This was the one at the end of the row, and was the first one from which we collected roots. This was the tree which was later pushed over, and I believe that this was the one originally marked 6a and 6b, and which was later given the number 11-44. (There was some confusion over samples 6a/b and sample 7 – sample 7 was the one that was near this tree, and from which we cut a wedge showing severely decayed wood. That sample was later renamed 11-7). The roots of this tree were covered in many inches of needles, and many roots were present in this needle duff. The tree had some termites active on one side.

These roots were 1-3 mm in diameter; there were few new, white roots present (these may have been pulled off with sampling). Some roots with dark zone lines suggesting fungal infection. The whitish roots present had some superficial fungi present on the surface, some of which were dark and ectotrophic, and some of which had clamp connections. No fungal structures in the root interiors. Older roots that look non-functional had thick mycelial mats on the surface (I believe I took photos of these roots with the dissecting microscope.). No nodules present.

The image I have for this sample is of the wedge which had been cut from the tree on 1/29/11:


2/5/11
Tissue from the dark, watersoaked area was tested for the presence of *Phytophthora* using an Agdia immunostrip (lot 00004), and results were negative.

Bacterial isolation results.
Plated 2/6/11; evaluated 2/8/11

Two potential *Pseudomonas*/*Ralstonia* type colonies on NA.

**Sample 11-2** (N13.31943 E 144.52473)

This was a branch sample from the same tree as above. There was watersoaking in the heartwood. On 2/4/11, a scalpel was used to scrape the dark, watersoaked areas for a *Ralstonia* test using Agdia immunostrip (lot 00012). It was positive.

Bacterial plate results. Plated 2/5/11, evaluated 2/8/11

Negative for *Pseudomonas*/*Ralstonia* type colonies on NA.

Sample 11-2


11-3

**Sample 11-3**

Collected at Yigo 1/29/11. Originally labeled 3rd Yigo 12:01 pm.

**Sample 11-4**

No information on this sample.

**Sample 11-5**

No information on this sample.

**Sample 11-6**

No information on this sample.

**Sample 11-7**

This was originally labeled sample 7b, from Yigo, collected 1/29/11. It was a trunk sample.

11-7

Isolation results

Isolations were made the same as for sample 11-44, and evaluated 2/4/11.

PDA+C: R1

1 min = Mucorales

2 min = Mucorales (same as the 1 min piece) and *Penicillium*

3 min = same as 2 min

PDA +C R2

1 min = very little growth of a white, sterile fungus

2 min = wood piece is missing, but there are a lot of whitish mycelia

3 min = *Penicillium*

PDA+C R3

1 min = sterile fungus; white above, yellowish beneath. Bob said this is frequently recovered.
2 min = same as above
3 min = wood piece is missing; white sterile mycelia

NA R1

1 min = no growth
2 min = no growth
3 min = no growth

NA R2

1 min = bacteria on plate surface. Was streaked on 1/31/11 to NA, and testedo2/8/11 with Agdia immunostrip for *Ralstonia* (lot 00012). Results negative.
2 min = white appressed fungal growth
3 min = bacteria only; on plate surface where wood meets agar

NA R3

1 min = yellowish-white sterile mycelial growth
2 min = same
3 min = same

**Sample 11-8**

This was a bag of soil with only a few root fragments. Collected for class purposes from a declining *Casuarina*. Used for baiting. *Casuarina* needles and twig pieces were floated over the soil using tap water. Baits placed 1/31/11 and harvested on Friday, 2/4/11 by rinsing in a beaker of water, blotting, and placing tissue pieces on WA and fresh PAR medium. Plates evaluated .on 2/8/11. Both WA and PAR plates negative for any fungal growth.

**Sample 11-9**

Soil sample collected for class from around a healthy *Casuarina* tree, and treated as above.. *Phytophthora* present on PAR and WA. Sporangia present by 2/8/11.

**Sample 11-10.**

Collected for class purposes from around a tree that was not *Casuarina*, and treated as for 11-8 and 11-9. Fungal growth present on both WA and PAR, but not *Phytophthora*.

**Samples 11-11 and 11-12** (two root and soil samples from the same tree)

Yigo, collected 2/2/11; originally labeled tree 3. This was from a different windrow than the other two, and was adjacent to the aquaculture facility. The tree sampled was the tall one in the image to the right. Roots were well developed and there was no obvious root rot and no basidiocarps preset. A major branch (about 5 inches in diameter) when cut had no internal discoloration, but the heartwood of the main trunk was brown and showing evidence of decay (pictured). The amount of discoloration did not seem sufficient to cause the amount of canopy thinning. Soil here was of better quality than with trees 1 and 2, as there was more of it.

**Sample 11-11**

Root and soil sample. Fine roots mostly completely dead. Some pinhead-sized holes in roots which suggest insect feeding damage. Larger diameter roots (about 3 mm) are dying, with dead cortex. Most of the largest roots (pencil-diameter) look perfectly fine. No nodules present.

**Sample 11-12**

Root and soil sample. Some necrosis of fine roots; larger ones look OK. No evidence of insect feeding. Some large clusters of roots (Frankia?)

**Sample 11-13**

Mostly soil sample, with some fine, detached roots. Can't tell what they are from. Did not sample for processing.

**Sample 11-14**

No information on this sample.

11-14

**Sample 11-15**

This was a root sample from the tree originally labeled as Tree 4 (Yigo, collected 2/2/11) and is the same tree as was sampled on Sunday (1/29/11) from the end of the windrow on the windward side of the papaya field (this tree was originally labeled as sample 6, now labeled 11-1). This tree was multi-stemmed

and had a dark canker at the base of the main trunk. There was watersoaking in one main stem. Roots did not look all that bad. The wedge cut from the buttress root did not show any discoloration, but it was taken from the side opposite of the cankered area.

**Sample 11-16**

This is another root sample from the same tree as 11-15.

**Sample number missing** (I didn't get the new number).

This was originally labeled Tree 5 from Yigo, sampled on 2/2/11 and was a few trees down from Tree 4. The tree was mostly dead. There were a lot of dead roots, some with exterior fungal growth present, but no basidiocarps. There was watersoaking of the main stem.

**Sample 11-18**

This was a wood sample from the same tree as 11-15 and 11-16.

**Sample 11-19**

No data on this one. Large root pieces from this sample with internal darkening were tested with an Agdia immunostrip for *Ralstonia solanacearum* (lot 00012), and results were

11-19

11-20

positive.

**Sample 11-20**

Bacterial plate results. Plated 2/6/11' evaluated 2/8/11.

R1 – One potential *Pseudomonas*/*Ralstonia* type colony on NA

R2 Two. *Pseudomonas*/*Ralstonia* type colonies on NA

R3 Negative for *Pseudomonas*/*Ralstonia* type colonies on NA. All one colony type, and look to be clean. Yellowish small colonies. Transferred.

**Sample 11-21**

11-21

Bacterial plate results. Plated 2/5/11 and evaluated 2/8/11.

R1 - Negative for for *Pseudomonas*/*Ralstonia* type colonies on NA

R2 – Lots of growth; one potential. *Pseudomonas*/*Ralstonia* type colony on NA

R3 – negative for *Pseudomonas*/*Ralstonia* type colonies on NA

11-22

**Sample 11-22.**

Yigo, collected 2/2/11; originally labeled Tree 2. This was adjacent to tree 1 in the row. It had very thin and sparse canopy (the tree to the right in the image). Most roots of ½ - 1 inch in diameter were dead. There was major discoloration in cut branches and at the base of the trunk 3 feet from the root collar. No conks present.

**Sample 11-23**

No information on this sample.

**Sample 11-24**

Bacterial plate results. Plated 2/6/11, evaluated 2/8/11.

R1 – Negative for *Pseudomonas*/*Ralstonia* type colonies on NA

R2 – One questionable *Pseudomonas*/*Ralstonia* type colony

R3. Two. *Pseudomonas*/*Ralstonia* type colonies on NA

11-24. Superficial fungal growth not originally present.

**Sample 11-25**

Yigo, collected 2/2/11. This sample and the following one were from a windrow which was (I think) two trees deep (two rows of trees, one in front of the other), right next to the entrance to the experiment station. Originally labeled tree 1. This tree had pretty good foliage, with not much (if any) symptoms of decline. Branch about 3 inches in diameter had no internal discoloration when cut from the trunk, but the main stem did, about 3 feet up from the root collar. Not many obviously diseased roots, but some about ¾ inch in diameter were discolored. Watersoaking of areas within the heartwood present. No evidence of conks or other external fungal colonization. I believe this is the tree in the left in the photo. This plant was chosen because it was near the declining tree, but it looked pretty good. The soil here was very shallow; most roots were in bedrock.

**Sample 11-26**

This was another root sample from the same tree as described in 11-25

**Sample 11-27**

Bacterial plate results. Plated 2/6/11; evaluated 2/8/11.

R1 – No growth

R2 – One potential *Pseudomonas*/*Ralstonia* type colony on NA

R3 – No *Pseudomonas*/*Ralstonia* type colonies on NA

11-27

**Sample 11-28**

No information on this sample.

**Samples 11-29** (N13.38567 E 144.65802)

This was a root and bark sample, originally labeled Tree 4, collected near the War in the Pacific Park at Agat on 2/3/11. This was one of a row of trees along the road on the east side of the road (across the street from the park). This tree had been sampled in a previous survey (603 UOG 08/18/09). *Ganoderma* was fruiting in the buttress roots in association with physical injury; we sampled by cutting into a root flare where a conk was, and found white rot. There was a small amount of dark watersoaking in the cut surface. A detached stick beneath the tree had a resupinate polypore fungus on it. Tree has 4 stems. Large open wounds on the interior side of two of the main stems. Had been fire (possibly) at the base of the trunk. Lots of branch dieback present, very thin canopy. Low growing ground cover under the tree, and lots of needles on the ground. DS-2..

11-29 (tree in center, to left of Dave, Joe

**Sample 11-30**

This was a root flare sample from the same tree as 11-29

11-30

11-30

**Sample 11-31** (N13.48067 E144.75316)
Collected 2/3/11 in Pasaeo Park. Originally labeled Tree 1. This was a large tree with a wound at the trunk base on the east side, with dieback of branches above the wound. There were holes in the ground around the roots on the west side. Broken branches, probably due to storm damage, were present. No basidiocarps. In grassy area with no leaf litter. Subject to winds on two sides. Lots of ants in the old wound on the east side. Tree rated as DS-1.


11-31

**Sample 11-32**
White resupinate fungal growth on dead branch snag on tree 11-31..


11-32


11-31

**Sample 11-33** (N13.38090 E144.75356)

Tree 2 from Pasaeo Park. One of a group of three that look pretty good from a distance, but there is some branch dieback and broken branches up top. There is an old wound on a buttress root at the root flare. Piece of bark had been removed at some time in the past. Mower damage to exposed roots. No evidence of decay at the base of the trunk. No basidiocarps, no insects. DS-1.

11-33

**Sample 11-34.**

Originally labeled "2a A Yigo, second Good." Sampled 1/29/11 and examined 1/30/11. Root sample. Some darkened roots had mycelia emerging from them. Did not have a chance for a more thorough exam.

11-34

**Sample 11-35** (N13.43920 E144.67792)

Originally labeled Tree 3, collected from Polaris Point on 2/3/11. This was one of a bunch of trees in a waste area next to asphalt. Soil is about an inch deep, and calcareous immediately beneath the surface. DS-3. Lots of fallen needles and duff, but also vegetation around the base of the trees. This was on the west side of a group of trees at the edge of the stand, with smaller trees (pea family) growing beyond the *Casuarina*. No basidiocarps on tree. Short needles, lots of dead branches present. Old wound at the base of the trunk, with dirty yellowish flat growth (fungal?) in wound. The trunk had interior discoloration of wood, and white rot present.. Took a wedge out of the base of the trunk in the old wound site, where the white rot/fungal growth is.

11-35

**Sample 11-36**

This is from the same tree as 11-35 and consists of a branch and fungal growth on trunk.

11-36

11-36

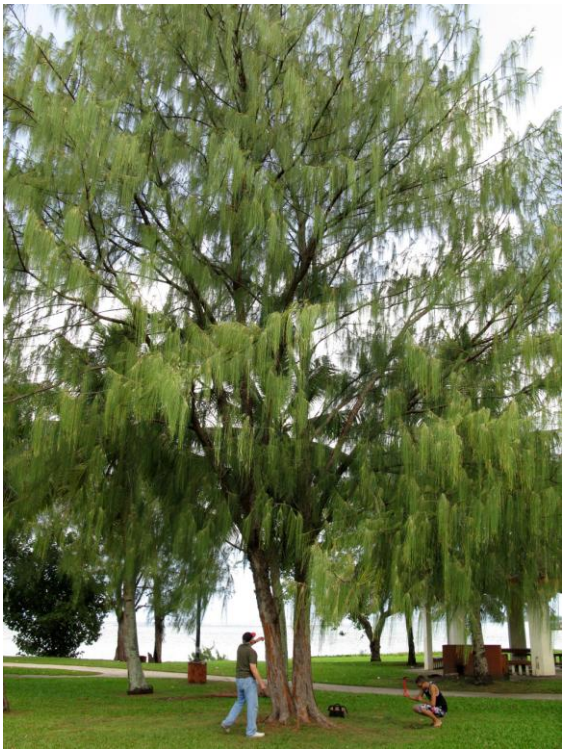
**Sample 11-37**

This is the same tree as 11-35, but was taken from the tree base.

11-37

**Sample 11-38**

This was originally labeled Tree 5 and was collected on Nimitz beach on 2/3/11.There was a bit of off-color look to the needles, but otherwise it looks OK. DS-0. A few branches broken off from storm damage. In grassy area about 24 feet from a similar tree with obvious decline. Tree is 2 trunks growing together. Real soil here. Base of trunk looks good. Sampled roots and soil only. No basidiocarps present.



11-38

**Sample 11-39**

This is a root and soil sample from a tree on Cocos Island, collected on 2/3/11. This was a large tree with conks on the root flares. DS-1. Scattered and lower branch dieback. Canopy a bit thin. Big pruning wound on the trunk from where a large branch had been, on seaward side (we are on the leeward side of the island) The site is a few 100 yards from the main landing site/pavilion, about 15 feet from an old shelter. The tree is in a neglected site on sandy soil with lots of needle duff. Small divot in tree root flare is where an old conk was.

11-39

**Sample 11-40**

This was an old basidiocarp from tree 11-39.

11-40

**Sample 11-41** (N 13.24108 E144.65652)

This was sample 2 from Cocos Island, and it consisted of roots and soil. This tree has conks on it. The tree is very tall and is an a natural part of the forest, with lots of needle duff on the ground. *Ganoderma* is present on wounds in the trunk; multiple trunk wounds present. This tree is within 6 feet of a fallen and long dead *Casuarina* tree. The top of the tree sampled had been killed out – snapped from a storm, and the rest of the canopy is thinning out. . DS-2/3.

11-41

11-41 is the larger tree in background

**Samples 11-42**

This was a root and soil sample from tree 3 from Cocos Island. The conk (*Phellinus*?) was found on a dead standing stem next to this tree, and was not part of this tree, which did not have any evident basidiocarps. This tree is about 30 feet from tree 2, towards the windward side. The tree is in an unmanaged area. Upper canopy is thin, with lots of dead branches. A major branch has been broken out. DS-2/3.

11-42

**Sample 11-43**

This is a basidiocarp from tree described above (tree adjacent to 11-42).

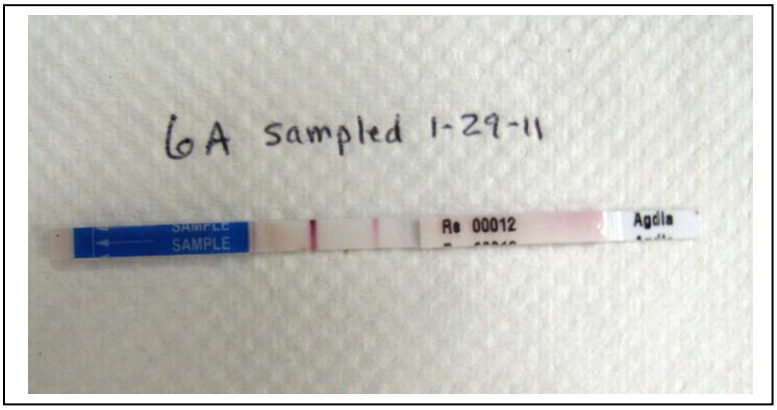
11-43

11-43 on ground at right

**Sample 11-44**

This was originally labeled 6, collected at Yigo on 1/29/30. (I believe this is from the same tree as the roots labeled 11-1).

This was a branch piece which had been taken as two pieces, one of which was oozing. One piece was put into a shallow pan of water to encourage oozing, and a slice off the other was flamed and put into a moist chamber (which probably was too wet, as the whole thing later was completely watersoaked, and no fungi were recovered). The piece in the pan of water the following day (1/30/11) was showing what looked like bacterial ooze, and this was scraped with a scalpel and tested for *Ralstonia solanacearum* with an Agdia immunostrip (lot 00012). The response appeared positive (pictured). A second test conducted the next day on the same piece of wood was also positive. Sawdust collected with a drill from the same piece also tested positive.




Pieces of wood from the second branch piece (6b) were plated to APDA, WA and NA.

<u>Isolation results for 11-44 (plates evaluated 2/4/11)</u>

Bob had plated after bleaching pieces of wood 1, 2, or 3 minutes. The bark had been removed before pieces were taken.

PDA +C  This was labeled R1 (meaning of the R designations on last page).

1 min = *Pestalotiopsis, Aspergillus, Trichoderma* (I would not consider any of these significant)

2 min = some sort of Mucorales; *Fusarium* growing beneath on the wood and not on the plates

3 min = Mucorales, *Pestalotiopsis* fruiting on wood, *Trichoderma*

PDA + C;  R2

1 min = *Penicillium, Fusarium* (I would not consider any of these significant)

2 min = Mucorales; *Pestalotiopsis*

3 min = White sterile mycelium, with no clamp connections

PDA + C; R3

1 min = bacterial growth only. Streaked to NA, which produced *Pseudomonas*-type colonies. Fluoresced yellow green under long-wave UV light. Not *Ralstonia*.

2 min = *Pestalotiopsis* (identified 2/6/11)

3 min = bacteria only. Streaked to NA, which produced *Pseudomonas*-type colonies. By 2/6/11, *Pestalotiopsis* was present. Took photo of spores under microscope.

WA

All fungal growth is sterile.

NA; R1 and R2

Mostly bacteria, with some secondary fungal growth growing out from all 3 pieces.

NA; R3

Bacterial growth only.

**Sample 11-45**

No information on this sample.

**Sample 11-46** (N13.43475 E 144.80270)

This sample had originally been labeled sample 1 and had been collected on UOG campus on 1/30/11. It was rated DS-2. It was from a lawn situation, with mown grass and no tall weeds. The base of the trunk was clean, with no needle duff. Had to dig to get roots.

The roots were examined the same day. Roots were colonized by a basidiomycete (clamp connections present). Some fine roots were blackened, but a *Phytophthora* test on them (Agdia immunostrip, lot # 0004) was negative.

**Sample 11-47** (N13.143482 E144.80257)

This was a root sample (originally sample 2 of the UOG samples) collected on 1/30/11 from the UOG campus rated DS-1. Some roots had small clusters of roundish roots (looking like a grape cluster) – early *Frankia*? Another species of *Frankia*? Some fine roots were necrotic, and there were roots of about 2 mm diameter in which the cortex was dead. Can see dark fungal hyphae in the cortex, but not enough detail to tell anything about them. Some groups of what I think are *Frankia*-colonized roots (many short roots arising perpendicular to the main axis of a root, to give a bottle-brush appearance).

**Sample 11-48** (N13.43474 E 144.80261)

This was a third UOG sample collected on 1/30/11, rated DS-0. Many clusters of "bottle brush" roots and "grape clusters" the size of acorns. A few dead roots present, but most have intact cortex and stele, with no significant hyphae present.

R1 = first area in from the bark; normal xylem, showing no discoloration

R2 = the leading edge of abnormal wood discoloration; toward the center of the stem usually.

R3 = middle of the discolored area

Note: I did not include any information on the *Xylella* test results, which I interpret as all negative (I suspect the one that came up faintly positive was actually negative), nor have I included the wood plating results that I did (with flamed pieces of wood plated to PDA), as these plates had not grown sufficiently to ID by the time I left.

## Appendix 2: Nematodes associated with Casuarina equisetifolia on Guam.

# NEMATODES ASSOCIATED WITH CASUARINA ROOTS AND SOIL

## MATERIALS AND METHODS

### Root Sampling and Nematode Extracting

Ten grams of roots were collected from the top 10 centimeters of soil. Eight trees selected and four were in decline and four appeared healthy. Roots were rinsed to remove soil. Roots were cut into sections of 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 was passed through a 140-mesh sieve to separate the roots, and the water was collected in a 400-mesh sieve. Twenty ml of nematode solution was placed tubes. Two ml were placed in petry dishes and identified under a inverted compound microscope. The resulting nematode numbers are per one gram of root tissue.

### Soil Sampling and Nematode Extraction

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 subsample. Twenty ml of the nematode solution 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 numbers are in number of nematodes per 10 ml of soil.

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

## RESULTS

### Nematodes Extracted from Roots-

Table 1. Number of Nematodes in one gram of Casuarina roots collected at Yigo Agriculture Exp. Station (AES) in Guam January and February 2011 and identified in Dr. Schlub's lab on February 15 and 16 of 2011.

| Clinic ID | Tree health | Taxon | Quantity | Trophic group |
|---|---|---|---|---|
| 11-62 | DS=3 | None | 0 | |
| 11-60 | DS=2 | None | 0 | |
| 11-63 | DS=2 | Tylencholaimellus | 1 | |
| 11-63 | DS=2 | Panagrolaimus | 1 | bacterievore |
| 11-64 | DS=3 | Aphelenchoides | 3 | herbivore |
| 11-64 | DS=3 | Unknown | 1 | herbivore |
| 11-64 | DS=3 | Rhabditidae | 1 | bacterievore |
| 11-58 | DS=0 | None | 0 | |

| | | | | |
|---|---|---|---|---|
| 11-61 | DS=0 | Helicotylenchus | 1 | herbivore |
| 11-59 | DS=0 | Unknown | 1 | |
| 11-59 | DS=0 | Panagrolaimus | 1 | bacterievore |
| 11-65 | DS=0 | Helicotylenchus | 1 | herbivore |
| 11-65 | DS=0 | Unknown | 2 | |
| 11-65 | DS=0 | Cephalobus | 1 | bacterievore |

Decline Severity (DS) DS = 0 for healthy trees, 0% branchlet thinning; DS=1 slight decline, 16% reduction in branchlets ; DS= moderate decline, 47% reduction in branchlets; DS=3 severe decline, 69% reduction in branchlets; DS=4 extreme decline, 94% reduction in branchlets.

Yigo AES is in the northern part of the island is characterized as Lithic Ustorthents, clayey, gibbsitic, nonacid, isohyperthermic with pH of 6.4–7.5. It is very shallow soil laying on a limestone bed.

**Nematodes Extracted from Soil**

Table 2. Number of Nematodes in 10 ml of soil associated with Casuarina roots collected in Guam in January and February 2011 and identified in Dr. Schlub's lab on February 15 and 16 of 2011.

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| Clinic ID | Location | Tree health | Taxon | Quantity | Trophic group |
|---|---|---|---|---|---|
| 11-11 | Yigo, AES | DS =1 | Prismatolaimus | 1 | Bacterievore |
| 11-11 | | Roots were well | Helicotylenchus | 1 | Herbivore |
| 11-11 | | developed and | Tripyla | 1 | |
| 11-11 | | appeared health | Panagrolaimus | 2 | Bacterievore |
| 11-11 | | | Aphelenchoides | 1 | Herbivore |
| 11-11 | | | Unknown | 1 | |
| 11-22 | Yigo, AES | DS=3 | Helicotylenchus | 17 | Herbivore |
| 11-22 | | Most of 1/2-1 | Plectus | 1 | Bacterievore |
| 11-22 | | inch roots were | Rhabditis | 3 | Bacterievore |
| 11-22 | | dead | Panagrolaimus | 1 | Bacterievore |

Table 3: Soil samples collected and prepared in January and February of 2011 and analyzed in April of 2011. Samples were stored at 5 degrees centigrade in a cold incubator in Dr. Schlub's Lab. Columns headings are nematode ID with number of nematodes found below.

| Sample | Acrobeles | Aphelenchoides | Aphelenchus | Cephalobidae | Cephalobus |
|---|---|---|---|---|---|
| 11-8 | | | | | |
| 11-28 | | | | | |
| 11-31 | | | | | |
| 11-29 | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 11-35 | 10 | | | | |
| 11-38 | 10 | | | | |
| 11-41 | | | | | |
| 11-15 | | | | | |
| 11-42 | | | | | |
| 11-25 | | | | | |
| 11-39 | | | 10 | | |
| 11-33 | | | | | |
| 11-9 | | | 10 | | |
| 11-10 | | 10 | | | |
| 11-13 | | | | | 10 |
| | Eucephalobus | Helicotylenchus | Leptonchus | Meloidogyne | Mesocriconema |
| 11-8 | | 30 | | | |
| 11-28 | | | 10 | | |
| 11-31 | | 170 | | | |
| 11-29 | | | | | |
| 11-35 | 30 | 10 | | | |
| 11-38 | | | | | |
| 11-41 | | 10 | | | |
| 11-15 | | 10 | | | |
| 11-42 | | | | | |
| 11-25 | | 30 | | | |
| 11-39 | 10 | 120 | | 40 | |
| 11-33 | | 90 | | 20 | |
| 11-9 | | 10 | | | |
| 11-10 | | 30 | | | 10 |
| 11-13 | | 20 | | | |
| | Dorylaimidae | Monhystera | Paratylenchus | Plectus | Pratylenchus |
| 11-8 | | | | | |
| 11-28 | | | | | |
| 11-31 | | | | | 10 |
| 11-29 | | | | | |
| 11-35 | | | | | |
| 11-38 | | | | 10 | |
| 11-41 | | 10 | 10 | | |
| 11-15 | | | | | |
| 11-42 | | | | | |
| 11-25 | | 10 | | | |
| 11-39 | 10 | | | | 30 |
| 11-33 | | 10 | | | |
| 11-9 | | | | | |
| 11-10 | | | | | |
| 11-13 | | | | | 10 |
| | Prismatolaimus | Rhabditidae | Rhabditis | Tylenchus | Wilsonema |
| 11-8 | | | | 30 | |
| 11-28 | | | | 10 | |
| 11-31 | 10 | | | 10 | |
| 11-29 | 20 | 20 | | 20 | |
| 11-35 | | | | 10 | |
| 11-38 | | | | 20 | |

| | | | | | |
|---|---|---|---|---|---|
| 11-41 | | | | | |
| 11-15 | | | | | |
| 11-42 | | | | | |
| 11-25 | | 50 | | | |
| 11-39 | 10 | | 60 | 10 | 10 |
| 11-33 | | | 10 | | 20 |
| 11-9 | | | | | |
| 11-10 | | | | | |
| 11-13 | | | | | |

**REFERENCES**


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# Appendix 3: Database of samples collected during year three

| Clinic Identifier year- | | Date: | Nature of sample: | Other Identifier: | Host: | Village: | GPS E: | GPS N: |
|---|---|---|---|---|---|---|---|---|
| 11 | 1 | 1/29/2011 | trunk | 3 in red 1st 10:55 am | ironwood | yigo AES | 144.52473 | 13.31943 |
| 11 | 1 | 1/29/2011 | roots | 11:00 AM | ironwood | yigo AES | 144.52473 | 13.31943 |
| 11 | 2 | 1/29/2011 | branch | 2 in red | ironwood | Yigo AES | 144.52473 | 13.31943 |
| 11 | 3 | 1/29/2011 | branch | 5 in red 3rd in yigo | ironwood | Yigo AES | | |
| 11 | 4 | 1/29/2011 | needles | 2nd | ironwood | Yigo AES | 144.52423 | 13.31845 |
| 11 | 5 | 1/29/2011 | needles & bark | 8 in red | ironwood | Yigo AES | 144.52423 | 13.31943 |
| 11 | 6 | 1/29/2011 | branch | 4 in red 1st 10:55 am | ironwood | Yigo AES | 144.52946 | 13.31948 |
| 11 | 7 | 1/29/2011 | trunk | 7 | ironwood | Yigo | | |
| 11 | 8 | 1/31/2011 | soil & roots - decline | | ironwood | Mangilao UOG | 144.79968 | 13.43081 |
| 11 | 9 | 1/31/2011 | soil - healty | | ironwood | Mangilao UOG | 144.80035 | 13.43079 |
| 11 | 9 | 1/31/2011 | roots - healthy | | ironwood | Mangilao UOG | 144.80035 | 13.43079 |
| 11 | 10 | 1/31/2011 | soil & roots - healthy | | ironwood | Mangilao UOG | 144.79967 | 13.43032 |
| 11 | 11 | 2/2/2011 | soil & roots | 3 | ironwood | Yigo AES | 144.8725 | 13.53304 |
| 11 | 12 | 2/2/2011 | soil | 3 1:45 pm | ironwood | Yigo AES | 144.8725 | 13.53304 |
| 11 | 13 | 2/2/2011 | soil and roots | 3 1:45pm | ironwood | Yigo AES | 144.8725 | 13.53304 |
| 11 | 14 | 2/2/2011 | branch | 3 1:45 pm | ironwood | Yigo AES | 144.8725 | 13.53304 |
| 11 | 15 | 2/2/2011 | soil and roots | 4 10:45 pm | ironwood | Yigo AES | 194.52473 | 13.31943 |
| 11 | 16 | 2/2/2011 | roots | 4 2:10 pm end row | ironwood | Yigo AES | 194.52473 | 13.31943 |
| 11 | 17 | 2/2/2011 | root large | 2:10 pm 4 | ironwood | Yigo AES | 194.52473 | 13.31943 |
| 11 | 18 | 2/2/2011 | branch | 2:10 pm 4 | ironwood | Yigo AES | 194.52473 | 13.31943 |
| 11 | 19 | 2/2/2011 | roots | 2:20 pm 5 | ironwood | Yigo AES | 144.8745 | 13.53229 |
| 11 | 20 | 2/2/2011 | branch | 2:20 pm 5 | ironwood | Yigo AES | 144.8745 | 13.53229 |
| 11 | 21 | 2/2/2011 | trunk | 2 1:50 pm | ironwood | Yigo AES | 144.8725 | 13.53304 |
| 11 | 22 | 2/2/2011 | soil and roots | 2 red 9:10 am | ironwood | Yigo AES | 144.87141 | 13.53351 |
| 11 | 23 | 2/2/2011 | roots | 2 1:10:00 PM | ironwood | Yigo AES | 144.87141 | 13.53351 |
| 11 | 24 | 2/2/2011 | trunk | 2 1:20 pm | ironwood | Yigo AES | 144.87141 | 13.53351 |
| 11 | 25 | 2/2/2011 | soil and roots | 1 9:00 am | ironwood | Yigo AES | 144.87137 | 13.53353 |
| 11 | 26 | 2/2/2011 | roots | 1 1:20:00 PM | ironwood | Yigo AES | 144.87137 | 13.53353 |
| 11 | 27 | 2/2/2011 | trunk | 1 1:15 pm | ironwood | Yigo AES | 144.87137 | 13.53353 |
| 11 | 28 | 2/2/2011 | soil and roots | 9:50 AM | ironwood | Yigo Ritidian | 144.85749 | 13.65234 |

| Clinic Identifier year- | | Date: | Nature of sample: | Other Identifier: | Host: | Village: | GPS E: | GPS N: |
|---|---|---|---|---|---|---|---|---|
| 11 | 29 | 2/3/2011 | soil, roots and conk | 9:50 AM | ironwood | Agat | 144.65802 | 13.38567 |
| 11 | 30 | 2/3/2011 | root | 9:50 AM | ironwood | Agat | 144.65802 | 13.38567 |
| 11 | 31 | 2/3/2011 | root and soil | 8:10 AM | ironwood | Paseo | 144.75314 | 13.48067 |
| 11 | 32 | 2/3/2011 | branch, fungus | 8:20 AM | ironwood | Paseo | 144.75316 | 13.48067 |
| 11 | 33 | 2/3/2011 | soil and roots | 8:25 AM | ironwood | Paseo | 144.75356 | 13.4809 |
| 11 | 34 | 2/3/2011 | roots - healthy | | ironwood | Yigo | none | none |
| 11 | 35 | 2/3/2011 | soil, roots and bark | 9:00 AM | ironwood | Polaris | 144.67792 | 13.4392 |
| 11 | 36 | 2/3/2011 | branch | 9:00 AM | ironwood | Polaris | 144.67792 | 13.4392 |
| 11 | 36 | 2/3/2011 | trunk, fungus | 9:00 AM | ironwood | Polaris | 144.67792 | 13.4392 |
| 11 | 37 | 2/3/2011 | trunk | | ironwood | Polaris | 144.67792 | 13.4392 |
| 11 | 38 | 2/3/2011 | soil and roots | 10:15am | ironwood | Nimitz Beach | 144.64952 | 13.3653 |
| 11 | 39 | 2/3/2011 | soil and roots | 12:40 PM | ironwood | Cocos | 144.65585 | 13.24085 |
| 11 | 40 | 2/3/2011 | conk and soft rot | | ironwood | Cocos | 144.65585 | 13.24085 |
| 11 | 41 | 2/3/2011 | soil, roots and conk | 1:10 PM | ironwood | Cocos | 144.65652 | 13.24108 |
| 11 | 42 | 2/3/2011 | soil and roots | 1:20 PM | ironwood | Cocos | 144.65646 | 13.2411 |
| 11 | 43 | 2/3/2011 | conk | 1:15 PM | ironwood | Cocos | 144.65646 | 13.2411 |
| 11 | 44 | 1/29/2011 | trunk discolored | 6a, 6b | ironwood | Yigo | none | none |
| 11 | 45 | 2/3/2011 | branch with pustual | | ironwood | Polaris | 144.67792 | 13.4392 |
| 11 | 46 | 1/30/2011 | roots | 11:36 AM | ironwood DS=2 | UOG | 144.8027 | 13.43475 |
| 11 | 47 | 1/30/2011 | roots | 11:42 AM | ironwood DS=1 | UOG | 144.8057 | 13.143482 |
| 11 | 48 | 1/30/2011 | root (tree ds = 0) | 11:48 AM | ironwood DS=0 | UOG | 144.80261 | 13.43474 |
| 11 | 49 | 1/30/2008 | bacterium | GG1-B2 #2 Yigo | ironwood | | | |
| 11 | 50 | 2008 | bacterium | #2 | ironwood | | | |
| 11 | 51 | 6/17/2008 | bacterium | LBA-B | ironwood | | | |
| 11 | 52 | 12/25/2008 | bacterium | | ironwood | | | |
| 11 | 53 | 6/12/2008 | bacterium | LBA-C | ironwood | | | |
| 11 | 54 | 2/9/2011 | whole plant | Sample from Phoebe | zukinie | Mangilao | | |
| 11 | 55 | 2/9/2011 | whole plant | Sample from Phoebe | dacon | Mangilao | | |
| 11 | 56 | 1/29/2011 | fine roots | | ironwood DS=1 | Mangilao UOG | | |

| Clinic Identifier year- | | Date: | Nature of sample: | Other Identifier: | Host: | Village: | GPS E: | GPS N: |
|---|---|---|---|---|---|---|---|---|
| 11 | 57 | 1/29/2011 | fine roots | | | | | |
| 11 | 58 | 2/15/2011 | roots | | ironwood DS=0 | Yigo AES | 144.87447 | 13.53182 |
| 11 | 59 | 2/15/2011 | roots | | ironwood DS=0 | Yigo AES | 144.87323 | 13.53104 |
| 11 | 60 | 2/15/2011 | roots | | ironwood DS=2 | Yigo AES | 144.87372 | 13.53205 |
| 11 | 61 | 2/15/2011 | roots | | ironwood DS=0 | Yigo AES | 144.87415 | 13.53065 |
| 11 | 62 | 2/15/2011 | roots | | ironwood DS=3 | Yigo AES | 144.8735 | 13.53211 |
| 11 | 63 | 2/15/2011 | roots | | ironwood DS=2 | Yigo AES | 144.87361 | 13.5326 |
| 11 | 64 | 2/15/2011 | roots | | ironwood DS=3 | Yigo AES | 144.87444 | 13.53214 |
| 11 | 65 | 2/15/2011 | roots | | ironwood DS=0 | Yigo AES | 144.87326 | 13.533104 |
| 11 | 66 | 1/29/2011 | photos tree and branch | | ironwood stressed | Ygio, AES | | |

## Appendix 4: Two Abstracts on Ironwood Tree Decline presented at the 2011 American Phytopathological Society meeting.

### APS Citations

Mersha, Z., Aime, M. C., Cannon, P., Nandwani, D., Nelson, S., Spaine, P.C., and Schlub, R.L. 2011. Decline of *Casuarina equisetifolia* (ironwood) trees on Guam: Ganoderma and Phellinus. Phytopathology 101:S216.

Schlub, R.L., Moore, A., Marx, B., Schlub, K., Kennaway, L., Quintanilla, M., Putnam, M., Mersha, Z., 2011. Decline of *Casuarina equisetifolia* (ironwood) trees on Guam: Symptomatology and explanatory variables. Phytopathology 101:S216

### Abstracts

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

R. L. Schlub[1], A. Moore[1], B. D. Marx[2], K. A. Schlub[2], L. F. Kennaway[3], M. A. Quintanilla[4], M. L. Putnam[5], and Z. Mersha[6],

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Ironwood trees (*Casuarina equisetifolia*), like all trees, have a natural finite life span within a given ecosystem; however, Guam's trees are dying at rates that far exceed the norm. 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 where mature trees are more prone to decline. Internal discoloration of the trunk and juncture of large branches was consistent for moderate and severely declined trees and attributed 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 significant 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 identified, 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*.

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

**Z. Mersha**[1], M. C. Aime[2], P. Cannon[3], D. Nandwani[4], S. Nelson[5], P. C. Spaine[6], J. A. Smith[7] and R. L. Schlub[8]

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Ironwood trees (*Casuarina equisetifolia* ssp. *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 1400 trees was conducted in 2008 and 2009. A highly significant ($p=0.0001$) linear correlation ($r^2 = 0.997$) between the presence of conks and decline severity emerged from the survey. Sixty- five percent of the trees at the most severe level of decline (nearly dead) had conks. Thirty- five conks were collected from the survey area under different stages of 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*. The prolific production of double walled basidiospores (a characteristic feature of members of the Ganodermataceae) from the sporocarps were consistently diagnostic of the genus *Ganoderma* and nuclear ribosomal (ITS) DNA sequencing confirmed this species as a member of the *G. australe* species complex. The second most prominently fruiting 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 associations 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.