

5-1-2010

**Soil modification and potential allelopathy: an investigation into how the invasive *Casuarina equisetifolia* L. (Australian pine) modify their environment**

Charlotte P. Buehler

Follow this and additional works at: <https://scholarsjunction.msstate.edu/td>

---

**Recommended Citation**

Buehler, Charlotte P., "Soil modification and potential allelopathy: an investigation into how the invasive *Casuarina equisetifolia* L. (Australian pine) modify their environment" (2010). *Theses and Dissertations*. 3982.

<https://scholarsjunction.msstate.edu/td/3982>

This Graduate Thesis - Open Access is brought to you for free and open access by the Theses and Dissertations at Scholars Junction. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of Scholars Junction. For more information, please contact [scholcomm@msstate.libanswers.com](mailto:scholcomm@msstate.libanswers.com).

SOIL MODIFICATION AND POTENTIAL ALLELOPATHY: AN INVESTIGATION  
INTO HOW THE INVASIVE *CASUARINA EQUISETIFOLIA* L. (AUSTRALIAN  
PINE) MODIFY THEIR ENVIRONMENT

By

Charlotte P. Buehler

A Thesis  
Submitted to the Faculty of  
Mississippi State University  
in Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
in Geosciences  
in the Department of Geosciences

Mississippi State University

May 2010

Copyright 2010

By

Charlotte P. Buehler

SOIL MODIFICATION AND POTENTIAL ALLELOPATHY: AN INVESTIGATION  
INTO HOW THE INVASIVE *CASUARINA EQUISETIFOLIA* L. (AUSTRALIAN  
PINE) MODIFY THEIR ENVIRONMENT

By

Charlotte P. Buehler

Approved:

---

John C. Rodgers III  
Associate Professor of Geography  
Department of Geosciences  
(Director of Thesis)

---

Karen McNeal  
Assistant Professor of Geology  
Department of Geosciences  
(Committee member)

---

Gary Ervin  
Associate Professor of Biological Sciences  
Department of Biological Sciences  
(Committee member)

---

Chris Dewey  
Associate Professor of Geosciences  
and Graduate Coordinator,  
Department of Geosciences

---

Darrel Schmitz  
Professor and Department Head,  
Department of Geosciences

---

Gary L. Myers  
Dean of the College of Arts & Sciences

Name: Charlotte P. Buehler

Date of Degree: May 1, 2010

Institution: Mississippi State University

Major Field: Geosciences

Major Professor: Dr. John Rodgers

Title of Study: SOIL MODIFICATION AND POTENTIAL ALLELOPATHY:  
AN INVESTIGATION INTO HOW THE INVASIVE  
*CASUARINA EQUISETIFOLIA* L. (AUSTRALIAN PINE)  
MODIFY THEIR ENVIRONMENT

Pages in Study: 103

Candidate for Degree of Master of Science

*Casuarina equisetifolia* L. is a noxious plant species known to be invasive in the West Indies. Not much is known about its impact on its host environment. This study's objective was to quantitatively compare physical and chemical soil differences between sites dominated by and devoid of Casuarinas. This study also conducted growth chamber experiments to determine potential for allelopathy. It was demonstrated that sites dominated by Casuarinas differed significantly in K, P, organic matter, and leaf litter depth. Application of Casuarinas leachate showed reduction in germination of radish and bean seeds by at least 32% and 70%, respectively. Once germinated no growth suppression of seedlings were observed with applications of Casuarinas leachate. Investigation with liquid chromatography mass spectrometry (LC-MS) revealed the potential presence of the allelochemical chalepin. This research demonstrates that Casuarinas have the potential to modify their host environment and therefore perpetuate their existence as a noxious invasive species.

## DEDICATION

To my parents for encouraging me to spread my wings, and to my grandparents-- especially Grandpa Hawk-- for their loving support.

## ACKNOWLEDGEMENTS

Acknowledgements are extended to Tom Rothfus and the Gerace Research Centre who provided me with funding for this research project and to the Department of Geosciences at Mississippi State University for their support. Thank you to Dr. Ervin who graciously allowed me use of his laboratory and Bill Holmes and Andrew McDaniel at Mississippi State Chemistry Lab for their assistance in chemical analysis. Thank you to Dr. Mercer for his statistical guidance and to Mrs. Mylroie, Eric Ezell, and the TIG students of 2009 for their assistance in the field. Lastly, thank you to my committee members Dr. Ervin and Dr. McNeal for their guidance and to my thesis advisor Dr. Rodgers who-- in every step of the way-- provided his support, encouragement, and benevolence.

## TABLE OF CONTENTS

	Page
DEDICATION .....	ii
ACKNOWLEDGEMENTS .....	iii
LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
CHAPTER	
I. INTRODUCTION .....	1
II. ANALYSIS OF SOIL PROPERTIES FROM SITES DOMINATED BY CASUARINAS ANS SITES DEVIOD OF CASUARINAS .....	7
Overview .....	7
Introduction.....	8
Background.....	12
Casuarinas on San Salvador Island.....	12
Methods .....	15
Study Area: San Salvador Island, The Bahamas .....	16
Site Descriptions.....	17
Casuarinas sites.....	19
Sites devoid of Casuarinas.....	21
Soil Field Sampling .....	22
Soil Lab Analysis.....	24
Statistical Analysis.....	25
Results.....	26
Discussion.....	38
Conclusion .....	48
III. ALLELOPATHY ANALYSIS OF <i>CASUARINA EQUISETIFOLIA</i> .....	51



Overview.....	51
Introduction.....	52
Background.....	55
Allelopathy.....	55
Study Organism .....	58
Methods .....	60
Study Area .....	60
San Salvador Island, The Bahamas.....	61
Field Collection.....	62
Laboratory Methods.....	62
Seed Germination .....	65
Seedling Growth .....	66
Chloroform extraction/ GC-MS analysis and SPE/LC-MS Analysis.....	67
Results.....	68
Seed germination/seedling growth.....	68
LC-MS Analysis .....	80
Discussion.....	83
Conclusion .....	89
 IV. CONCLUSION.....	 91
REFERENCES .....	95

## LIST OF TABLES

TABLE		Page
1	Comparison of soil nutrient abundances between sites dominated by Casuarinas and sites devoid of Casuarinas. Significant differences in soil variable abundances are denoted by an asterisk (*)......	27
2	LC-MS analysis of compounds detected in Casuarinas leachate and Quercus leachate. Six compounds found in Casuarinas leachate were not present in Quercus leachate. The leachates shared one compound in common, however, it was 100x more intense in Casuarinas leachate.....	82

## LIST OF FIGURES

FIGURE	Page
1	<i>Casuarina equisetifolia</i> located on Bonefish Bay beach, San Salvador Island, The Bahamas. Casuarinas ability to thrive in a variety of soil types allow these trees to live in close proximity to the ocean and tolerate seaspray (Elfers 1988).....14
2	Map of San Salvador Island, The Bahamas depicting study site locations. ....20
3	Casuarinas forest with dense leaf litter at Field Station (FS) site (left). Graham’s Harbour (GH) site devoid of Casuarinas approximately 100 m away from FS (left).....22
4	Sample site area comprised of five 25- m transects spaced 5-m apart. Five study plot locations were contained in each transect.....23
5	Soil acidity comparison of sites dominated by Casuarinas (Cas) vs. sites devoid of Casuarinas (Non). The median is indicated by the center line within the boxplot. The measure of interquartile variability is represented by length of the boxplot with 25% of the data occurring on either side of the median line. The length of the whiskers represent skewness with outliers represented by a circle above or below the whiskers.....28
6	Leaf litter comparison between sites dominated by Casuarinas and sites devoid of Casuarinas (Non).....29
7	Abundant Casuarinas leaf littler (at Field Station site) compared to leaf litter abundance from a site devoid of Casuarinas (Graham’s Harbour). Both sites are adjacent to one another (100 m).....29
8	Soil moisture comparisons between Casuarinas dominated sites (Cas) and sites devoid of Casuarinas (Non).....30
9	Comparison of phosphorous concentration in sites dominated by Casuarinas (Cas) and sites devoid of Casuarinas (Non).....31

10	Comparison of potassium concentration between sites dominated by Casuarinas (Cas) and sites devoid of Casuarinas (Non).....	32
11	Comparison of calcium between sites dominated by Casuarinas and sites devoid of Casuarinas (Non).....	33
12	Comparison of magnesium concentration between Casuarinas (Cas) dominated sites and sites devoid of Casuarinas (Non).....	34
13	Comparison of percent organic matter between Casuarinas dominated sites (Cas) and sites devoid Casuarinas (Non).....	35
14	Comparison of nitrate-nitrogen concentration in Casuarinas dominated sites (Cas) and sites devoid Casuarinas (Non).....	36
15	CEC comparison between Casuarinas dominated sites (Cas) and sites devoid of Casuarinas (Non).....	37
16	Comparison of K:Mg ratio between Casuarinas dominated sites and sites devoid of Casuarinas (Non).....	38
17	Tropical soil fungi (white) present in Casuarinas leaf litter at a depth of approximately 5cm. Specific fungi species were not identified.....	44
18	Map of San Salvador Island, The Bahamas depicting study site locations.....	63
19	Bean seeds applied with Casuarinas treatment (A) after 7 days compared to bean seeds applied with control (B). Radish seeds applied with Casuarinas treatment (C) after 3 days compared to radish seeds (D) applied with control.....	69
20	Percent germination of bean seeds when applied with Casuarinas treatment and control treatment during six trials.....	70
21	Percent germination of radish seeds applied with Casuarinas treatment and control during six trials.....	71
22	Percent germination of radish seeds applied with Quercus treatment compared to seeds applied with control treatment with six trials.....	72
23	Percent germination of bean seeds when applied with Quercus treatment and control with six trials.....	73
24	Comparison of growth between radish seedlings applied with control treatment and seedlings applied with Casuarinas treatment. No significant difference ( $p > 0.05$ ) of seedling growth existed	

	between control treatments and Casuarinas treatment for each time interval. ....	75
25	Average radish seedling growth applied with Quercus treatment at 24 hr intervals (T1, T2, T3) for 72 hrs. Only a slight difference of seedling growth existed between control and treatment per time interval ( $p > 0.05$ ). Seedling growth in T1 was significantly greater than T3 ( $p < 0.05$ ). ....	76
26	Comparison of growth between bean seedlings applied with control and seedling applied with Casuarinas leachate. T1 and T2 show more growth of seedlings applied with control compared to seedlings applied with Casuarinas treatment. T3 demonstrates more growth. ....	77
27	Comparison of growth between bean seedlings applied with control treatment and seedlings applied with Quercus treatment. T1 and T2 show more growth of seedlings applied with control treatment compared to seedlings applied with Quercus treatment. ....	79
28	Fresh and dry mean mass per radish seedling. Dry mass of Casuarinas treatment seedlings was not significantly different from the dry mass of the control treatment seedlings ( $p = 0.328$ ) or Quercus treatment seedlings ( $p = 0.336$ ). Wet mass of the control treatment seedlings was also not significantly different from the wet mass of the seedlings applied with Casuarinas treatment ( $p = 0.335$ ) or Quercus treatment ( $p = 0.298$ ). ....	80

## CHAPTER I

### INTRODUCTION

The invasion of non-native species has become recognized as one of the major threats to biodiversity and ecosystem stability (Mack et al. 2000) and research on what exactly makes a species capable of invasion and what communities are vulnerable to invasions have recently become a major concern (Gordon 1998). Non-native species cost the United States at least 120 billion dollars/yr in control and eradication methods and contribute to reduced agricultural productivity (Pimentel et al. 2005). Gordon (1998) states that the presence of invasive species can create disturbances that result in permanent changes to ecosystem processes. For example, invasive species alter geomorphological processes like erosion rates and water channels, biogeochemical and hydrological cycling such as nutrient mineralization and soil moisture content, and can change the frequency, intensity, and duration of disturbance regimes. At a community level invasive species alter stand structures by adding new life forms, modify resource competition for light, water, nutrients, and space, alter soil dynamics involving soil microbes, or add biochemicals into the soil (Vitousek et al. 1990; Gordon 1998).

Some of the most recognized examples of invasive species modifying their environments include *Myrica faya* (myrica) which colonizes young volcanic soil and alters nutrient availability and community stand structure (Vitousek 1990). *Bromus*

*tectorum* (downy brome or cheat grass) has been found to change fire patterns in the western rangelands and, as a result, increase frequency and intensity of natural fire regimes (Invasive, 2006). *Tamarix* spp., with their rapid regeneration rate, are recognized to change nutrient cycling within the soil as well as contribute to geomorphological changes along stream banks (Invasive, 2006). *Mimosa pigra* causes sediment accumulation and disruption of waterways in Thailand (Lonsdale et al. 1989). *Eichornia crassipes* and *Pistia stratiotes* have shown to increase siltation rates by slowing water flows (Schmitz et al. 1993). Callaway and Ridenour (2004) have found *Centaurea diffusa* inhibits native species ability to acquire phosphorous in the soil and suggest *C. diffusa* also suppresses growth of native species by means of releasing biochemicals into the soil (allelopathy). These studies illustrate that invasive species can modify their environments, potentially facilitating their spread and leading to the exclusion of native species-- unquestionably-- more research is needed to understand these complex mechanisms.

Many hypotheses exist for how and why invasive species come to dominate communities. Gordon (1998) suggests that in order for an invasive species to be successful they must possess certain characteristics which include: the ability to occupy open niches, an ability to outcompete native species in the native ecosystem, the potential to alter the invaded site by modifying resource availability, disturbance regimes, or both, and have effective reproduction and dispersal mechanisms. More specifically the enemy release hypothesis (ERH) has been introduced to describe how invasive species come to establish in new communities (Hierro and Callaway 2003; Blumenthal 2006; and Catford

2009). This hypothesis states exotic plants upon introduction to a new region experience a decrease in regulation by herbivores and other natural enemies and, as a result, rapidly increase in distribution and abundance.

Indeed, a combination of mechanisms in conjunction with the enemy release hypothesis are thought to play a role in how some invasive plants come to dominate communities (Hierro and Callaway 2003; Catford 2009). One of these mechanisms known as allelopathy, introduced by Callaway and Ridenour (2004) as the novel weapons hypothesis, maintains that biochemicals can be released from invading plants which have negative effects on native neighboring plants-- allowing the invader a competitive advantage. Allelopathy is often described as the chemical reaction between plants or between plants and microbes and, as described by Weir et al. (2004), allelopathy literally translates from the Latin root *allelon* to mean 'of each other' while *pathos* translates to 'feel or suffer.' Invasive species which exhibit the potential for allelopathy are known to induce changes to an ecosystem which can have devastating, long term impacts on plant communities (Weir et al. 2004).

Research on allelopathy has recently been resurrected as new studies have evidence that some biochemicals or secondary metabolites, which when applied in laboratory environments to seed and seedlings, suppress seed and seedling germination and growth plants (Hierro and Callaway 2003; Callaway and Ridenour 2004; Weir 2004; Blanco 2007). According to Weir (2004), allelopathy has not had widespread acceptance into the modern scientific community primarily because of its difficulty to prove. Obtaining accurate results from field experiments and corroborating those results with



greenhouse or laboratory bioassay experiments is complex and, consequently, has proved as a hindrance to the field (Weir et al. 2004).

Determining if invasive species employ allelopathy can be challenging as there are several implications. According to Heirro and Callaway (2003), skepticism exists in methodological approaches. For example, laboratory conditions are not similar to natural field conditions and even in natural conditions allelochemicals can be difficult to detect as they can persist in low concentrations in the soil and be continuously affected by biogeochemical interactions with soil microorganisms (Blanco 2007).

The difficulty in researching and establishing if certain invasive species employ allelopathy has, undoubtedly, only stimulated this field study and generated even more inquires into the realm of invasive species biology. One of the most studied species that is thought to be allelopathic is *Centaurea maculosa*, which has been shown to suppress seed germination in plants especially *Arabidopsis thaliana* (Bias et al. 2002; Heirro and Callaway 2003). *Tribulus terrestris* has been shown to inhibit the germination and radicle elongation of target species as well as increase soil concentrations of nitrogen, phosphorous and potassium and increase soil moisture content (El-Ghareeb 1991; Davis et al. 2000). Evidence supports the idea that higher resource availability increases the susceptibility to invasion of plant communities (El-Ghareeb 1991). The invasive *Kochia scoparia* has also been shown to suppress seedling growth of the native grass *Bouteloua gracilis* (Karachi and Peiper 1987).

In the West Indies and south Florida, *Casuarina equisetifolia* (Elfers 1988; Swearingen 1997; Hammerton 2001) is especially problematic in that it is known to

modify dune structures, potentially change physical and chemical soil properties of areas which it dominates (Gochenaour 1975; Elfers 1988; Jiqin et al. 1991, Batish 1998; Parrotta 1993) and is suggested to be allelopathic (Suresh et al. 1987; Batish 1998; Swearingen 1997; Hammerton 2001). Very few studies exist on how the Casuarinas in the West Indies modify soil, affect native vegetation growth, and overall induce change to its environment. However, Casuarinas' presence in sub-tropical areas such as India and China have been studied. Jiqin et al. (1991) suggest Casuarinas have the potential to decrease soil pH, Suresh and Rai (1987) suggest Casuarinas inhibit germination and root growth of some crops such as *Vigna mungo*, *Cajanus cajan*, and *Glycine max*, and Batish (1998) suggests reduced forest floor vegetation under Casuarinas forest plantations. Although research on Casuarinas' effect on its environment has been suggested in various studies (Suresh and Rai 1987; Jiqin et al. 1991; Batish 1998), no research has been conducted on Casuarinas in the West Indies where it is considered to be a particularly noxious species (Hammerton 2001, Rodgers 2005).

The main objectives for this research include analyzing the physical and chemical soil properties in sites dominated by Casuarinas and sites devoid of Casuarinas to determine how and to what degree certain soil nutrients are depleted or enhanced by the presence of this invasive species. The second objective is to examine if Casuarinas have the potential for allelopathy. Though an application of a leachate made from leaves of Casuarinas, this research will examine how seed germination and seedling growth of common cultivars of bean (*Phaseolus vulgaris*) and radish (*Raphanus sativus*) are perhaps altered.

This research is significant because understanding the roles of invasive species, like the Casuarinas, may provide new case studies for understanding how invasive species contribute to environmental modification. Not only do invasive species cost billions of dollars each year to maintain (Pimentel et al. 2005) but invasive species can cause irreversible damage to ecosystems. Understanding more about Casuarinas' effects on soil and its potential to employ allelopathy will qualitatively assess how Casuarinas modify invaded areas in the West Indies, which may lead to better management and eradication strategies. Studying invasive non-native species is pertinent to the study of Geosciences as these investigations are spatial in nature—entwining invasive plant dispersal with their affect on the environment. This research will not only contribute to a better understanding of *Casuarinas equisetifolia*, but it will shed new light on the mechanisms by which Casuarinas modify the landscape in the West Indies. Understanding the environmental harm associated with invasive species like Casuarinas will lead to increased awareness and better wiser land management practices.

CHAPTER II  
ANALYSIS OF SOIL PROPERTIES FROM SITES DOMINATED BY CASUARINAS  
AND SITES DEVOID OF CASUARINAS

Overview

*Casuarina equisetifolia* is considered a noxious invasive species particularly problematic in the West Indies. Although studies suggest Casuarinas can alter dune morphology (Sealey 2003) and establish ecologically sterile monocultures (Swearingen 1997; Batish 1998; Hammerton 2001), not much is known about how it specifically modifies physical and chemical soil properties. Soil collected from sites dominated by Casuarinas and sites devoid of Casuarinas on San Salvador Island, The Bahamas was analyzed and revealed several physical and chemical differences. Sites dominated by Casuarinas have soils with significantly less potassium, phosphorous, and organic matter, higher pH, and greater leaf litter abundance. No significant differences in the soil cations calcium and magnesium and no differences in nitrate-nitrogen and soil moisture were detected. The results of this soil analysis suggest that Casuarinas have the ability to modify their environment and thereby possibly perpetuate their own existence.

## Introduction

It is recognized that invasive species can alter geomorphological processes like erosion rates and water channels as well as biogeochemical and hydrological cycling such as nutrient mineralization and soil moisture content (Gordon 1998). The frequency, intensity, and duration of disturbance regimes can also be changed by the presence of invasive species. At a community level, invasive species alter stand structures by adding new life forms, modify resource competition for light, water, nutrients, and space, and alter soil dynamics (Vitousek et al. 1996; Gordon 1998). It is for these reasons that the invasion of non-native species has been widely discussed in academic literature (Bais 2003; Fitter 2003; Hierro and Callaway 2003; Callaway and Ridenour 2004; Inderjit et al. 2008) and why understanding how the invasive Casuarinas alters the environment that ultimately results in the facilitation of their own establishment and/or the decline of their native competitors.

Many invasive species have been studied to assess their impact on their host environment. By physically modifying its host environment through rapid growth *Mimosa pigra* (Giant sensitive tree), will colonize any area of standing water and will form mono-specific communities. *Mimosa pigra* is also known to colonize stream banks where it contributes to increased sediment accumulation (Lonsdale et al. 1989). *Tamarix* spp. (salt cedar) has been studied because of its ability to use enormous amounts of water whereby drying up riparian zones and inhibiting neighboring native plants from obtaining moisture (Invasive, 2006). Although prescribed burns were at one time thought to control this invasive plant, it was established that fires actually encourage *Tamarix* root

nodules to sprout whereby facilitating its propagation (Invasive, 2006). Pollen-Bankhead et al. (2009) studied the impact Tamarisk and Russian-olive have on streambank stability. Results indicated that the effects of root reinforcements provided by Tamarix and Russian-olive have a significant impact on bank stability and even bank-failure frequency. As Pollen-Bankead et al. (2009) suggest, banks are dominated by sands in which roots play a key role in cohesion. So conversely, when Tamarisk and Russian-olive are removed along a riparian corridors the removal can potentially lead to a loss of sediment and gradual bank widening thereby altering stream channel morphology.

Many invasive species can also change water column chemistry. The aquatic species *Eichhornia crassipes*, *Hydrilla verticillata*, and *Pistia stratiotes* are known to decrease dissolved oxygen, pH, and phosphorous contents while increasing dissolved carbon dioxide, and turbidity (Schmitz et al. 1993). Because of these changes, the growth of native aquatic vegetation and phytoplankton abundances have declined in areas dominated by *E. crassipes*, *H. verticillata*, and *P. stratiotes* (Schmitz et al. 1993). The legumes *Abrus precatorius*, *Acacia auriculiformis*, and *Pueraria montana* are known as nitrogen fixers and are thought to contribute to higher concentrations of nitrogen and phosphorous (Vitousek 1986). The production of mass quantities of leaf litter is also thought to affect soil chemistry as seen by *Eichhornia crassipes* and *Melaleuca quinquenervia* which produce abundant leaf litter and are thought to cause anoxia, increased phosphorous release, and neighboring plant mortality. *Sapium sebiferum* is also detrimental to soil environments in that it is suggested to increase nitrogen, phosphorous, magnesium, nitrogen, and calcium concentrations compared to soils found

under native vegetation (Cameron and Spencer 1989). These modifications have shown to alter soil nutrient distributions and enhance productivity in areas where *S. sebiferum* has colonized. A study on the invasive *Imperata cylindrica* (cogongrass) found that it maintains a significantly faster decomposition rate compared to native grasses which can lead to increased nutrient cycling and allow *I. cylindrica* to persist in invaded areas (Holly 2008).

Altering natural disturbance regimes is another way invasive species modify their environment. A study on the invasive *Bromus tectorum* (downy brome) found that it can alter fire patterns in the western rangelands because as the grass dries it forms tiny seed-filled pods which contribute to an increased fuel source. Its presence can actually increase the probability of rangeland fire to every 3-5 years instead of every 60-100 years (Invasive, 2006). It has also been shown that once *B. tectorum* establishes it regenerates faster than native perennial grasses after fires and only with extensive intervention using chemical controls can *B. tectorum* be eradicated (Invasive, 2006).

The invasive *Casuarina equisetifolia* are suggested to alter both physical and chemical soil properties (Gochenaur 1975; Suresh and Rai 1987; Jiqin et al. 1991; Swearingen 1997; Batish 1998; Hammerton 2000). Geomorphological processes such as shoreline erosion and dune destabilization are altered by presence of Casuarinas. The shallow root system of the species does not facilitate trapping and holding sand which eliminates their ability to stabilize sand dunes contributing to beach-width reductions (Deaton 1994; Sealey 2003). The dense mat of leaf litter (5-10 cm thick) produced by Casuarinas is also thought to suppress recruitment of native species as well as contribute

to increased nutrient cycling (Fernald and Barnett 1981; Duever et al. 1986; Vitousek 1986). Gochenaur (1975) studied Casuarinas forest and coconut grove plant communities in New Providence Island, The Bahamas and data showed markedly different soil conditions between species. Areas with Casuarinas had more acidic soil (pH = 7.3-7.5) than Coconut grove forests (pH = 8.4-8.6) and a greater soil moisture content ( $35.8 \pm 8.5$  g H<sub>2</sub>O/100g dry soil) compared to coconut groves forests ( $1.4 \pm 0.4$  g H<sub>2</sub>O/100g dry soil) (Gochenaur 1975). Organic matter content was greater in Casuarinas sites ( $8.3 \pm 2.6\%$ ) than Coconut sites ( $1.1 \pm 0.2\%$ ). Casuarinas sites also had more total soluble salts ( $1.06 \pm 0.11\%$ ) than coconut sites ( $0.36 \pm 0.04\%$ ). Mesophilous fungi also dominated Casuarinas sites (mean = 32,800 fungi) opposed to coconut grove (mean = 9,900 fungi) even though both communities were established within the same original soil type.

Various studies suggest invasive species have the potential to alter physical and chemical soil properties. Furthermore, the invasive Casuarinas have been suggested to demonstrate some of these environmental modifying characteristics. The purpose of this research is to quantitatively assess how the presence of Casuarinas modify physical and chemical properties of the soil on San Salvador Island, The Bahamas. Specifically, the hypotheses are that differences in soil properties exist in sites dominated by the Casuarinas compared sites devoid of Casuarinas in leaf litter abundance, organic matter content, soil pH, soil moisture content, cation exchange capability, potassium:magnesium ratio, and concentrations of potassium, magnesium, phosphorous, calcium, and nitrate-nitrogen. This research is important in that it may help identify how the invasive Casuarinas can alter their environment whereby outcompeting native vegetation



and provide evidence which illustrates to what degree modification of the soil occurs on San Salvador Island, The Bahamas.

## Background

### *Casuarinas on San Salvador Island*

Also known as Shea (She)-Oak, Beefwood, and Horsetail Tree, Casuarinas is in the magnoliopsida class (flowering plants) although its appearance resembles coniferous trees. According to Elfers (1988) eighty-two species of *Casuarinaceae* exist, however, the most common in The Bahamas are *Casuarina equisetifolia* (L.) and *Casuarina glauca* (Sieb.). Casuarina can grow up to 40 m tall and have evergreen needle leaves (Figure 1). Pollinated by the wind, Casuarinas produce multiple flowers throughout the year and disperse thousands of seeds (Elfers 1988).

Casuarinas also have the ability colonize in various habitats from beaches and dunes to rocky cliffs and mangroves due to their ability to fix nitrogen which allows them the ability thrive in a wide range of soil types (Swearingen 1997). Parrotta (1993) suggests Casuarinas require warm frost-free temperatures year round and tolerate various precipitation amounts, though not prolonged periods of flooding.

According to Parrotta (1993) Casuarinas are present as non-native species on most of the world's beaches including Florida, Hawaii, and Africa. Most commonly Casuarinas are used as a decorative shade tree in coastal areas because it can tolerate nutrient poor soil and salt spray. Frequently they are used as a vegetative border and

shaped into hedgerows for privacy or the wood can be used as firewood (Hammerton 2001).

Native to tropical areas of the Indian Ocean and the South China Sea, Casuarinas were intentionally introduced to the West Indies in the late 1800's as an ornamental tree because of its ability to thrive in saline beach environments (Elfers 1988; Hammerton 2001). Casuarinas were also thought to stabilize eroding beaches, however it has now been proved as a faulty assumption (Sealey 2006). Regardless of how or why Casuarinas were introduced it has rapidly spread as a non-native species across the Bahamian Archipelago.

Casuarinas are problematic for a variety of reasons: they can form dense thickets that crowd out native vegetation, produce an abundant amount of leaf litter which is thought to inhibit rain water infiltration, and is also hypothesized to release allelopathic chemicals. Swearingen (1997) maintains that land occupied by Casuarinas becomes ecologically sterile in that little or no germination and growth exist of other plant species.



Figure 1 *Casuarina equisetifolia* located on Bonefish Bay beach, San Salvador Island, The Bahamas. Casuarinas ability to thrive in a variety of soil types allow these trees to live in close proximity to the ocean and tolerate seaspray (Elfers 1988).

As a result of their rapid colonization, Casuarinas as a non-native species in The Bahamas has created various problems (Swearingen 1997). They form dense monocultures which crowd out native vegetation and deter native birds because it is suggested that stands inhibit native vegetation from growing and thereby reduce food sources (Swearingen 1997). Casuarinas also affect coastal dune formation (Elfers 1988). Unlike native dune vegetation which has root and stem morphologies that trap and hold down sand, Casuarinas have shallow root mats which *contribute* to erosion and in strong winds are known to blow over contributing even further to dune destabilization (Swearingen 1997; Sealey 2003).

Native vegetation is thought to be inhibited from growing in Casuarinas dominated areas because leaf litter is so dense and inhibits rainwater from infiltrating (Hammerton 2001). Another hypothesis maintains is that Casuarinas leaves contain allelopathic chemicals which, when released into the soil via leaf litter, are thought to inhibit native seed germination and growth (Batish 1998; Hammerston 2001). Indeed, it is surprising how few studies exist which quantify how Casuarinas modify their environments given that preceding studies deem it is such a nuisance to the West Indies.

## Methods

Research for this investigation took place December 2008 and June 2009 on San Salvador Island, The Bahamas with the goal to address specific questions including how soil properties differ between sites containing Casuarinas and sites absent of Casuarinas in physical characteristics such as organic matter, leaf litter depth, and soil moisture and

chemical characteristics such as pH, cation exchange capacity, and abundance of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , N, P, and  $\text{K}^+$ . The approaches used to examine these soil variables included collecting and analyzing soil from San Salvador Island, The Bahamas and identifying which particular soil variables were significantly different among soil collected from Casuarinas sites and sites devoid of Casuarinas.

*Study Area: San Salvador Island, The Bahamas*

The Commonwealth of the Bahamas is a chain of twenty-nine islands and numerous cays in the Caribbean located in the North Atlantic Ocean southeast of Florida, USA and northeast of Cuba. The northwest-southeast trending archipelago extends 1400 km from the Florida peninsula to the tectonically active Caribbean Plate (Carew and Mylroie 1995). The combined land area of The Bahamas is 10,010 km<sup>2</sup>.

Lithified coral sediments, oolitic sediments, grapestone, pellet mud, and clay and silt make up the carbonate Bahamas Platform (Sealey 2006). The trade winds contribute to the warm waters and tropical marine climate of The Bahamas, however, as a consequence of its geographic location, The Bahamas can receive up to a quarter of its rainfall from tropical storms and hurricanes (Sealey 2006). Temperatures with normal trade winds in the Spring usually lie between 19-29 °C and Autumn temperatures lie between 24-30 °C (Sealey 2006).

San Salvador Island's geology, as described by Carew and Mylroie (1995), is a complex array of eolianite limestones deposited in the middle to late Quaternary. Soil on San Salvador Island, as well as on other islands in The Bahamas, is alkaline with the

texture ranging from sandy to stony with loam (a mixture of sand and clay) being absent because the soils are so poorly developed (Sealey 2006). The soil is considered azonal, or immature, though a red clay soil and organic soil can be recognized and classified in some areas (Sealey 2006).

The vegetation on the island grows in three main zones: the inland zone, the nearshore zone, and the coastal zone (Gerace et al. 1995). The inland zone contains dense thickets with mangrove communities that surround inland hypersaline lakes. The nearshore zone is the transition area between dune grasses and shrub communities of the coastal zone and the more dense thickets of the inland zone (Gerace et al. 1995). Coastal vegetation grows in nutrient poor-carbonate rich sand with vegetation inland growing in older more organic soil.

San Salvador Island is estimated to receive 100 cm of rain annually with evaporation rates exceeding precipitation rates (Sealey 2006). The beginning of the rainy season lasts from June to December with October being San Salvador Island's wettest month (San Salvador 1999). Northern islands in the archipelago can receive up to 75-100 more centimeters of rain per year than San Salvador Island (Gerace 2009).

### *Site Descriptions*

Bahamian soil is invariably alkaline and consequently the dominate soil group is pedocal soil with nutrients such as potassium and nitrogen invariably lacking in abundance (Sealey 2006). Though San Salvador Island is dominated by limestone bedrock and only one or two thin soil protocols exist, three types of soil can be found:

1) red clay soil, 2) sedimentary soil, and 3) organic soil (Sealey 2006). The red clay soil was originally derived from the Sahara desert 125,000 years ago and deposited on the surface of the limestone (Sealey 2006). Mature and immature aluminous laterites compromise the red clay soil and are found mainly on the ridglands (Sealey 2006). Sedimentary soils include the presence of sandy soil based from Holocene marine calcareous sand (Sealey 2006). The sand layer is less than 30 cm and usually a greyish-brown in color (Sealey 2006). The organic soil is commonly found on rockland contains humus and is often a depth of less than 15 cm (Sealey 2006). This soil type is the most common in the Bahamas and accounts for at least 90% of the surface area (Sealey 2006).

Casuarinas distribution on San Salvador Island is not uniform in that they primarily dominate the western and northern areas of the island (Rodgers 2005). Riding Rock Marina/Cockburn Town contains the largest clusters one magnitude greater than anywhere else on the island (Rodgers 2005). The second highest abundance of Casuarinas on San Salvador is located on the northern portion of the island comprising the Field Station, Coast Guard Station, and United Estates regions (Rodgers 2005). The locations with the highest density included Riding Rock Marina and the Field Station which suggest Casuarinas distribution is associated with human settlement and land disturbance (Rodgers 2005).

Three study sites were selected that represent areas dominated by Casuarinas and three sites were selected that represent areas devoid of Casuarinas (Figure 2). In order to maintain site isotropism, site locations were designated in which a site with Casuarinas was adjacent to or in close proximity ( $\leq 100$  m) of a site absent of Casuarinas

### *Casuarinas sites*

Field Station- The Gerace Research Centre, located on the northern perimeter of the island, contains a dense (0.68 individuals/m<sup>2</sup>) 30 m x 40 m stand (> 20 individuals) approximately 50 years old (Figure 3) (Rodgers 2005). The understory of the Field Station site has a paucity of vegetation, though about 5% of the area contains *Metopium toxiferum* (Poisonwood), *Smilax laurifolia*, (green brier), *Cocothrinax* (silverthatch), and other unidentified Myricaceous and Ficus shrubs. This site is approximately 30 m from the ocean with an elevation of 6m and minimal topographic variability. Soil is dominated by sand to gravel size sediment.

Marina 1- Another dense (0.31 individuals/m<sup>2</sup>) Casuarinas stand (> 20 individuals) approximately 30m x 50m (Rodgers 2005) in area is located near Riding Rock Marina and south of the airport on the western perimeter of the island. This site is approximately 15 m west from the ocean, 1.5 m above sea level, and has minimal topographic variability. About 90% of the vegetation cover comprises Casuarinas with the other 10% comprising *Agave americana* (agave), *Myrica linnaeus* sp. (bayberry), *Metopium toxiferum* (poisonwood), *Smilax laurifolia*, (green brier), and other unidentified shrubs. The soil consisted of sand to gravel size sediment.



## Casuarinas and Non-Casuarinas Study Sites San Salvador Island, The Bahamas

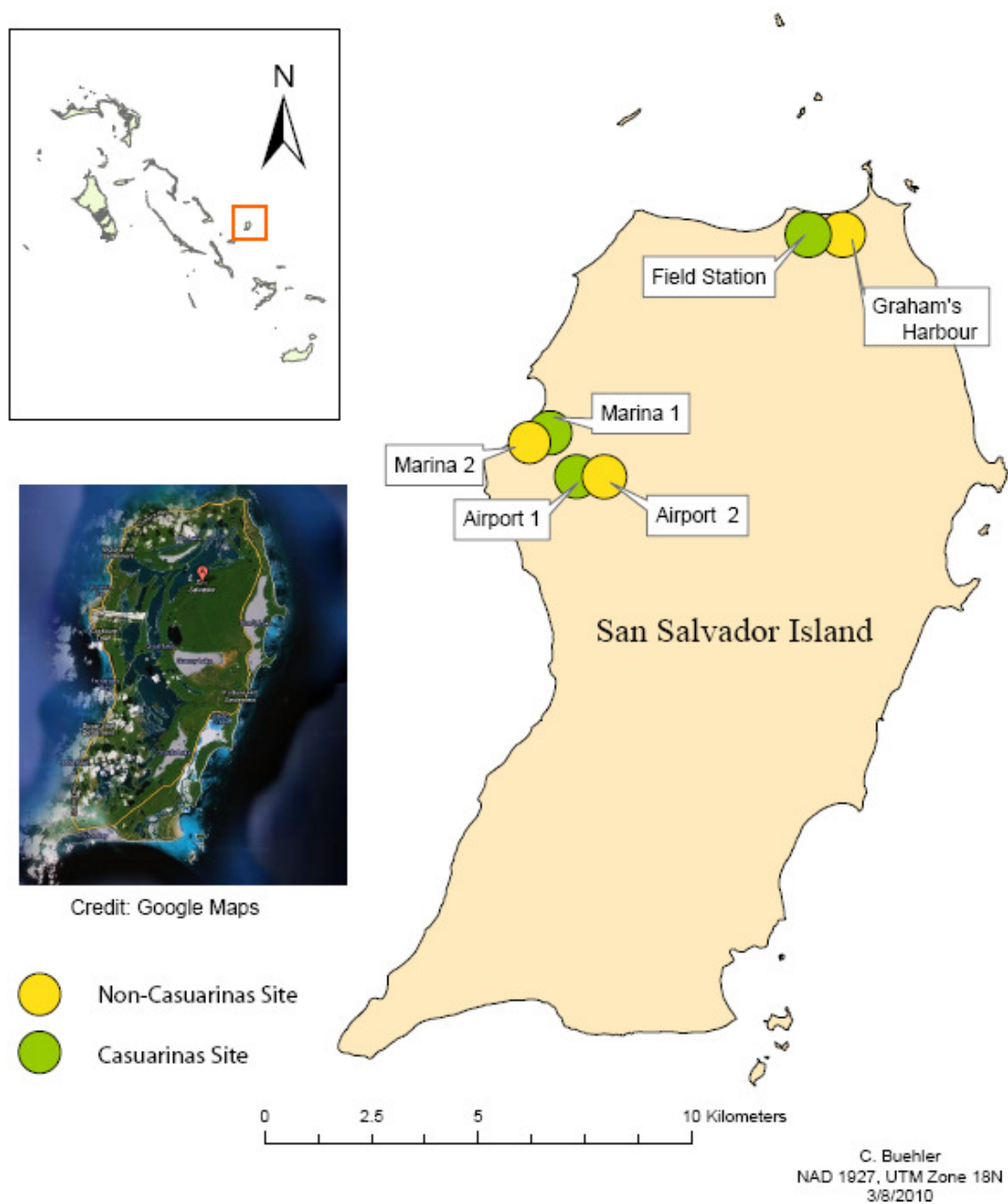


Figure 2 Map of San Salvador Island, The Bahamas depicting study site locations.

Airport 1- Located 100 m from San Salvador Island International Airport, this dense stand (stand  $\geq 20$  individuals) of *Casuarinas* is approximately 40m x 50 m in area. Located 2.5 m above sea level this area had minimal topographic variability. Ninety percent of the vegetation cover was comprised of *Casuarinas* with the other 10% comprising *Myrica linnaeus* sp. (bayberry) *Metopium toxiferum* (poisonwood), *Smilax laurifolia*, (green brier) and other unidentified shrubs. This site location also contained sand to gravel size sediment.

#### *Sites devoid of Casuarinas*

Grahams Harbour- Located near the Gerace Research Centre this 80 m x 50 m plot is dominated by dense shrubbery with little organic material covering the soil (Figure 3). Sixty percent of the vegetation cover is comprised of *Coccoloba uvifera* (seagrape), with 40% comprising *Coccothrinax argentata* (silver thatch palms), *Myrica linnaeus* sp. (bayberry), *Ficus* spp. (fig), and other unidentified shrubs. This site is situated partially on the crest of an eolian dune with an elevation of 3 m above sea level. Soil consisted sand to cobble size sediments

Marina 2- This site located at Riding Rock Marina was adjacent to Marina 1 and isolated on three sides by dense brush. Approximately 40 m x 40m in area, this site contains 50% vegetation cover consisting of “coastal coppice” *Metopium toxiferum* (poisonwood), and 50% *Smilax laurifolia* (green brier), silverthatch or *Coccothrinax* shrub. The topography

had no visible relief and soil texture consisted of gravel to sand size sediment. Elevation was 2 m above sea level.

Airport 2- Dominated 90% by vegetation type called “Black Coppice” (Smith 1993) which includes *Metopium toxiferum* (poisonwood) and *Smilax laurifolia* (green brier) this site is 40m x 40m and located 100 m from the airport. Very little variability in relief exists and elevation is 3 m above sea level. The soil consisted of gravel to sand size sediment with some organic matter incorporated.



Figure 3 Casuarinas forest with dense leaf litter at Field Station (FS) site (left). Graham’s Harbour (GH) site devoid of Casuarinas approximately 100 m away from FS (right).

### *Soil Field Sampling*

Soil for soil analyses was collected on San Salvador Island, The Bahamas June 2009. Soil samples were obtained from three sites containing Casuarinas: Field Station (FS), Marina 1 (M1), and Airport 1 (A1), and three sites devoid of Casuarinas: Graham’s

Harbour (GH), Marina 2 (M2), and Airport 2 (A2). In order to maintain soil isotropism, site locations were designated in which a site with Casuarinas was adjacent to or in close proximity ( $\leq 100$  m) of a site absent of Casuarinas.

Sites M1, GH, M2, and A2 contained five transects with 5 plots on each transect spaced 5 m apart. Spacing between each transect was also 5 m, forming a grid with 25 plots within a 400 m<sup>2</sup> area (Figure 4).

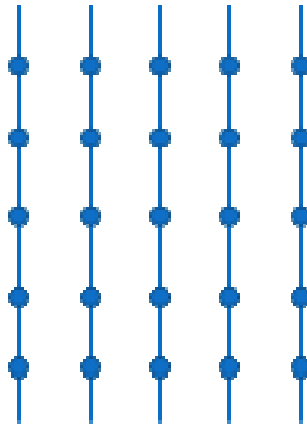


Figure 4 Sample site area comprised of five 25- m transects spaced 5-m apart. Five study plot locations were contained in each transect.

Due to study site area, site A1 contained six transects with 4 plots on each transect spaced 5 m apart. This formed a grid with 24 plots within an area of 375 m<sup>2</sup>. Casuarinas site FS

contained five transects with 6 plots on each transect spaced 5 m apart. At each site, GPS coordinates were recorded along with soil moisture measurements, pH, and leaf litter depth. A 0.25 m<sup>2</sup> sampling frame was used in which the average depth of leaf litter was quantified using a ruler. Also sampled within the sampling frame included soil pH which was measured with a handheld pH meter and soil moisture which was measured with a HH2 Delta-T Moisture Meter (Cambridge, England).

Soil samples were also collected from the top 10 cm of soil systematically at 5 m intervals along each transect with each transect contributing to one soil bag for a total five soil sample bags per study location. Soil collection bags were obtained from A & L Laboratories, Inc., Memphis, TN and held approximately 453 g (1lb) soil, the amount necessary for a soil analysis. A total of 30 soil samples were collected for all six study locations on San Salvador Island, The Bahamas. Conditions set by the United States Department of Agriculture and The Bahamas Ministry of Agriculture required a special permit for soil collection and the cost of soil analysis and shipping limited the number of samples collected. All thirty soil samples were packaged together and shipped from Nassau, The Bahamas to A & L Laboratories, Inc., Memphis, TN for analysis four days after field work was conducted.

#### *Soil Lab Analysis*

Soil collected on San Salvador Island, The Bahamas during June 2009 was analyzed by ETC Environmental Testing & Consulting, Inc., Memphis, TN. Potassium, Magnesium, Phosphorous and Calcium were all analyzed using the Mehlich-3 test

method. Mehlich-3 (M3) is advantageous because it offers the possibility of using one test for all nutrients, opposed to the Brey-P1 or Olsen (sodium bicarbonate) tests for phosphorous and the ammonium acetate test for potassium, magnesium, and calcium (Mallarino 1999). Mallarino (1999) suggests M3 results are similar to other analyses, however, M3 has shown to provide better results than other tests on high pH soils. Nitrate content was analyzed using a Konelab Aqua 20 discrete chemical analyzer, wavelength 660nm (ETC 2009).

Organic matter content was analyzed by the Walkley Black method which utilized a wet-chemical oxidation technique to determine organic matter (Blaisdell et al. 2003; ETC 2009). Because collected soil samples were calcareous in nature, carbonates were removed using a strong-acid digestion in which the sample was heated to 90°C for 90 minutes. This processes allowed for a more precise dichromate digestion. The determinations of organic matter were converted using the van Bemmelen correction factor which assumes soil organic matter is 58% carbon (Blaisdell et al. 2003).

### *Statistical Analysis*

Statistically significant ( $p < 0.05$ ) differences in soil properties (using three sites for replication) were tested by permutation tests utilizing CRAN (Comprehensive R Archive Network) because it provides a variety of statistical techniques and as well as produces publication-quality plots (Hornik 2010) Permutations tests are considered a natural generalization of the Wilcoxon-Mann-Whitney test, in which given two samples measuring the same variable data can be compared for possible differences in magnitude

(Wilks 2006). Permutation tests, compared to analysis of variance (ANOVA), do not have an assumption of normality so permutation tests can be beneficial for many kinds of ecological data (Taylor 1961; Gaston and McArdle 1994). Instead of normalizing data using transformation matrixes or liner models, permutation tests utilize the re-ordering (permutations) of observations and a  $p$  value is calculated as the proportion of values obtained under the permutation that are greater than or equal to the observed value (Anderson and Braak 2003). The soil data in this study were sampled with replacement using 2,000 iterations. Data were considered significant if  $p < 0.05$ .

## Results

Analysis of soil data indicates sites dominated by Casuarinas have distinct differences in abundances of some soil variables compared to sites deviod of Casuarinas (Table 1).

Table 1 Comparison of soil nutrient abundances between sites dominated by Casuarinas and sites devoid of Casuarinas. Significant differences in soil variable abundances are denoted by an asterisk (\*).

<i>Variable</i>	<i>Mean of Casuarinas sites</i>	<i>Mean of Non-Casuarinas sites</i>	<i>p value (<math>\alpha = 0.05</math>)</i>
<i>Leaf litter depth (cm)</i>	7.37±2.89	1.84±1.56	0.000*
<i>Soil moisture (<math>m^3.m^{-3}</math>)</i>	0.195±0.08	0.202±0.06	0.635
<i>pH</i>	6.61±0.74	6.81±1.56	0.042*
<i>P (kg/ha)</i>	7.86± 2.14	11.07± 3.01	0.002*
<i>K<sup>+</sup> (kg/ha)</i>	112.57± 36.42	204.93± 73.89	0.000*
<i>Ca<sup>2+</sup> (kg/ha)</i>	40917.14± 3145.31	40806.67± 8396.85	0.967
<i>Mg<sup>2+</sup> (kg/ha)</i>	730.43± 236.25	716.67± 187.59	0.855
<i>OM (%)</i>	4.41±1.31	5.72± 1.06	0.007*
<i>NO<sub>3</sub><sup>-</sup>- N (kg/ha)</i>	19.21±10.17	21.13± 6.40	0.525
<i>CEC (meq/100g)</i>	83.71±6.54	83.69± 16.00	0.996
<i>K:Mg ratio</i>	0.05±0.02	0.09± 0.02	0.000*

Sites dominated by Casuarinas had slightly ( $p = 0.042$ ) more acidic soil ( $pH = 6.609 \pm 0.74$ ) than sites devoid of Casuarinas ( $pH = 6.813 \pm 0.47$ ) (Figure 5).



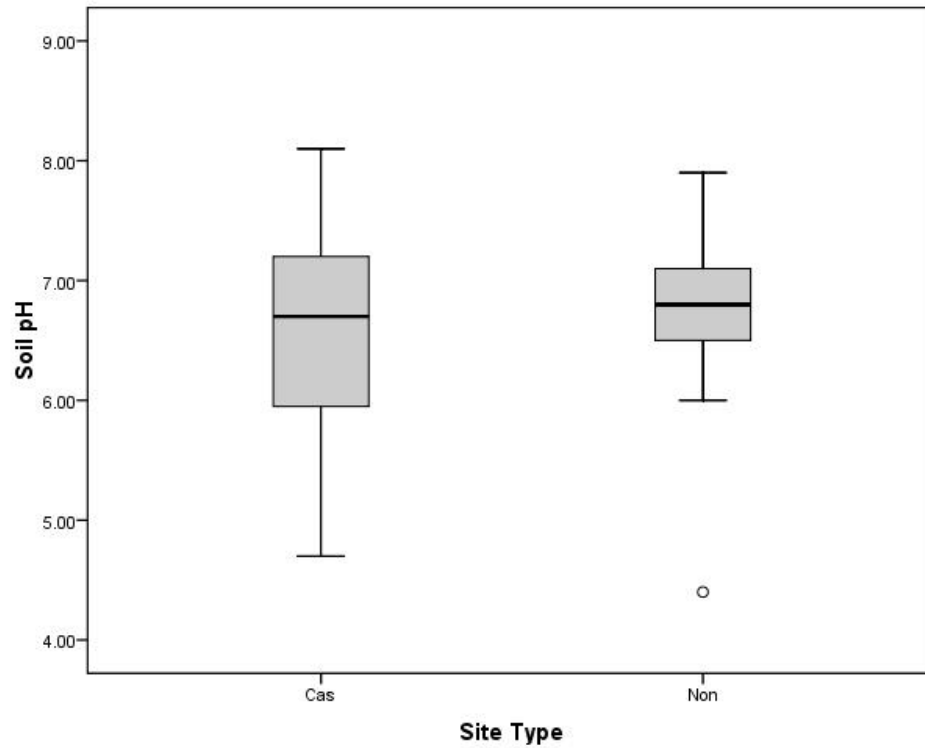


Figure 5 Soil acidity comparison of sites dominated by Casuarinas (Cas) vs. sites devoid of Casuarinas (Non). The median is indicated by the center line within the boxplot. The measure of interquartile variability is represented by length of the boxplot with 25% of the data occurring on either side of the median line. The length of the whiskers represent skewness with outliers represented by a circle above or below the whiskers.

Leaf litter in sites dominated by Casuarinas was significantly greater than in sites devoid of Casuarinas ( $p < 0.01$ ) ( Figure 6; Figure 7).

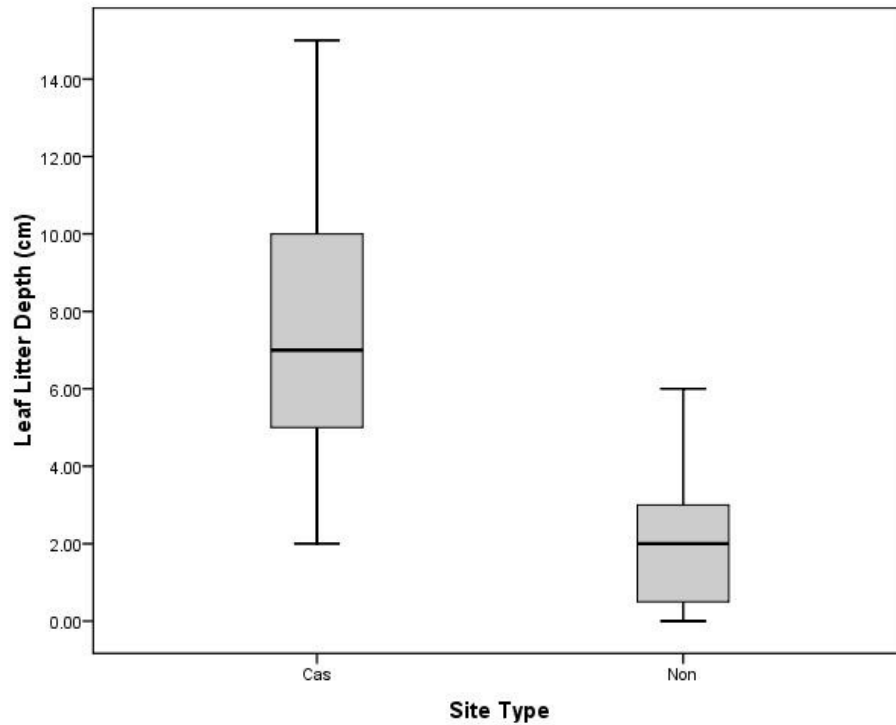


Figure 6 Leaf litter comparison between sites dominated by Casuarinas and sites devoid of Casuarinas (Non).



Figure 7 Abundant Casuarinas leaf litter (at Field Station site) compared to leaf litter abundance from a site devoid of Casuarinas (Graham's Harbour). Both sites are adjacent to one another (100 m).

Volumetric soil moisture content was not significantly different ( $P= 0.6355$ ) between sites dominated by Casuarinas ( $0.195 \pm 0.08 \text{ m}^3\text{m}^{-3}$ ) and sites devoid of Casuarinas ( $0.202 \pm 0.06 \text{ m}^3\text{m}^{-3}$ ) (Figure 8).

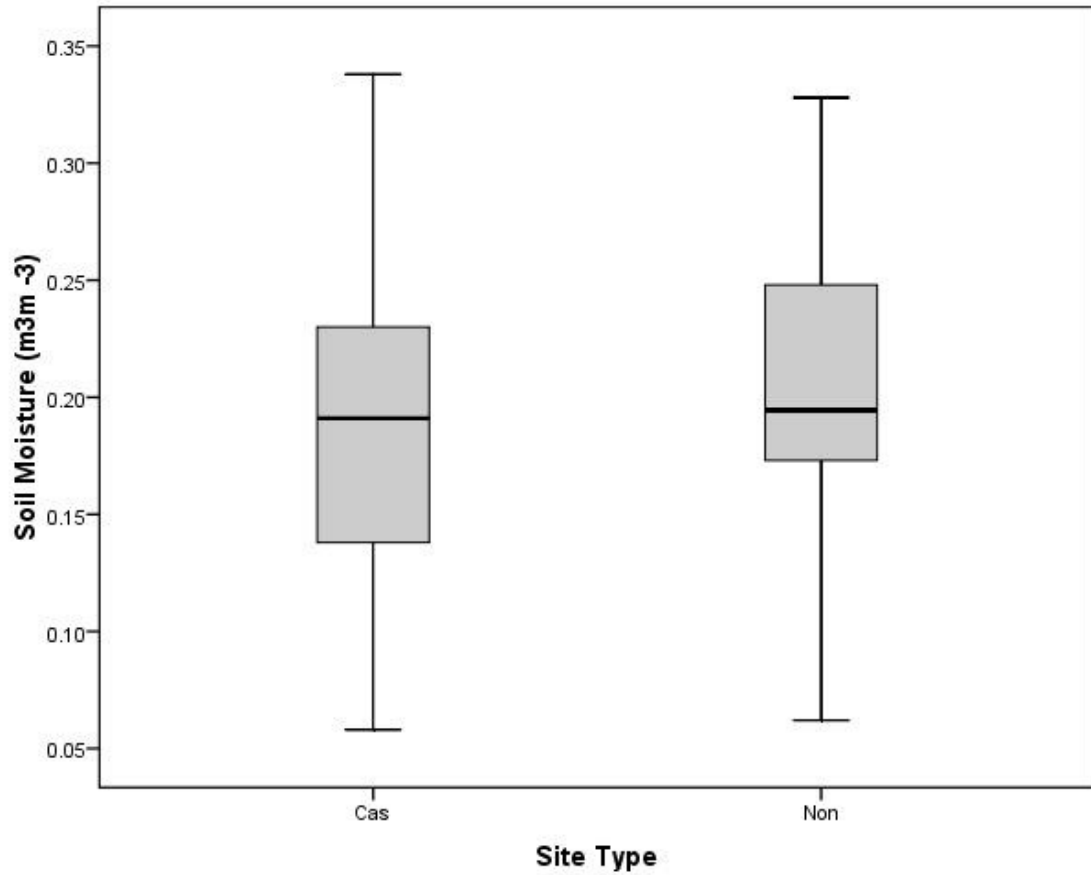


Figure 8 Soil moisture comparisons between Casuarinas dominated sites (Cas) and sites devoid of Casuarinas (Non).

Phosphorous concentration was significantly ( $P= 0.002$ ) less in sites dominated by Casuarinas (Figure 9).

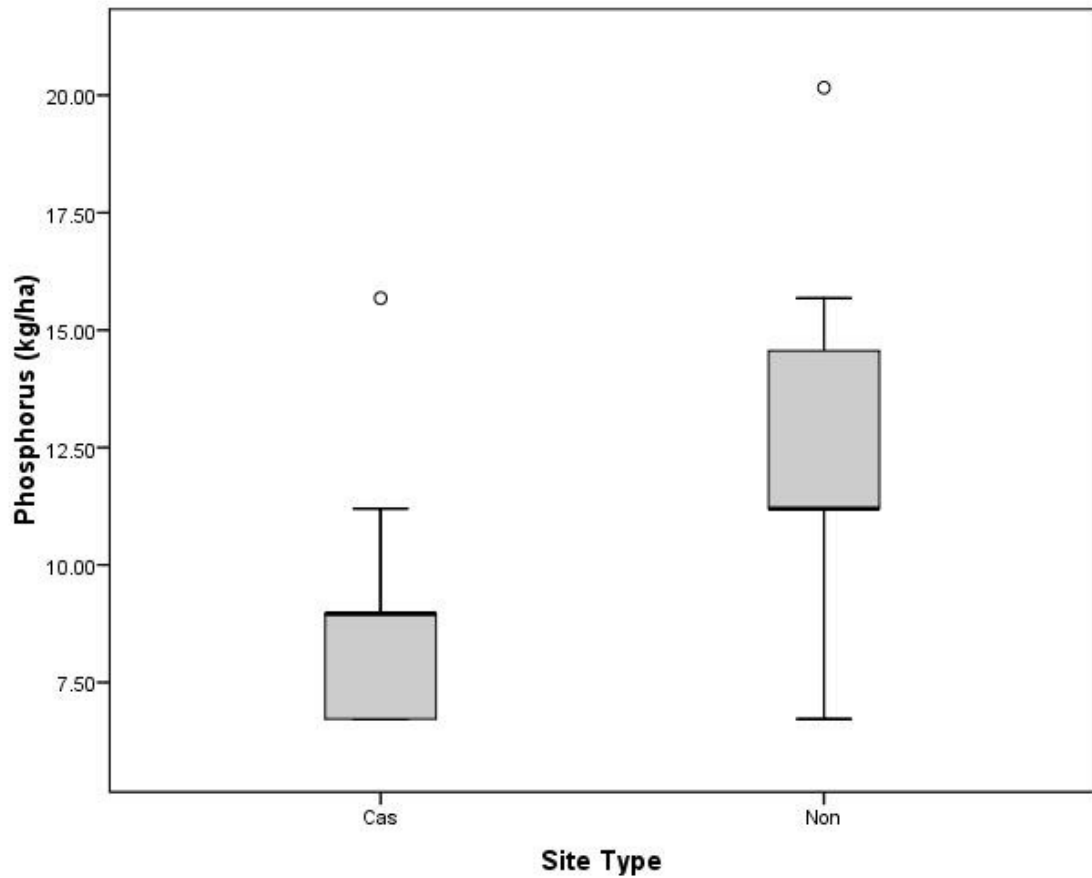


Figure 9 Comparison of phosphorous concentration in sites dominated by Casuarinas (Cas) and sites devoid of Casuarinas (Non).

Potassium concentration in sites devoid of Casuarinas was significantly greater than sites dominated by Casuarinas ( $p < 0.05$ ; Figure 10).

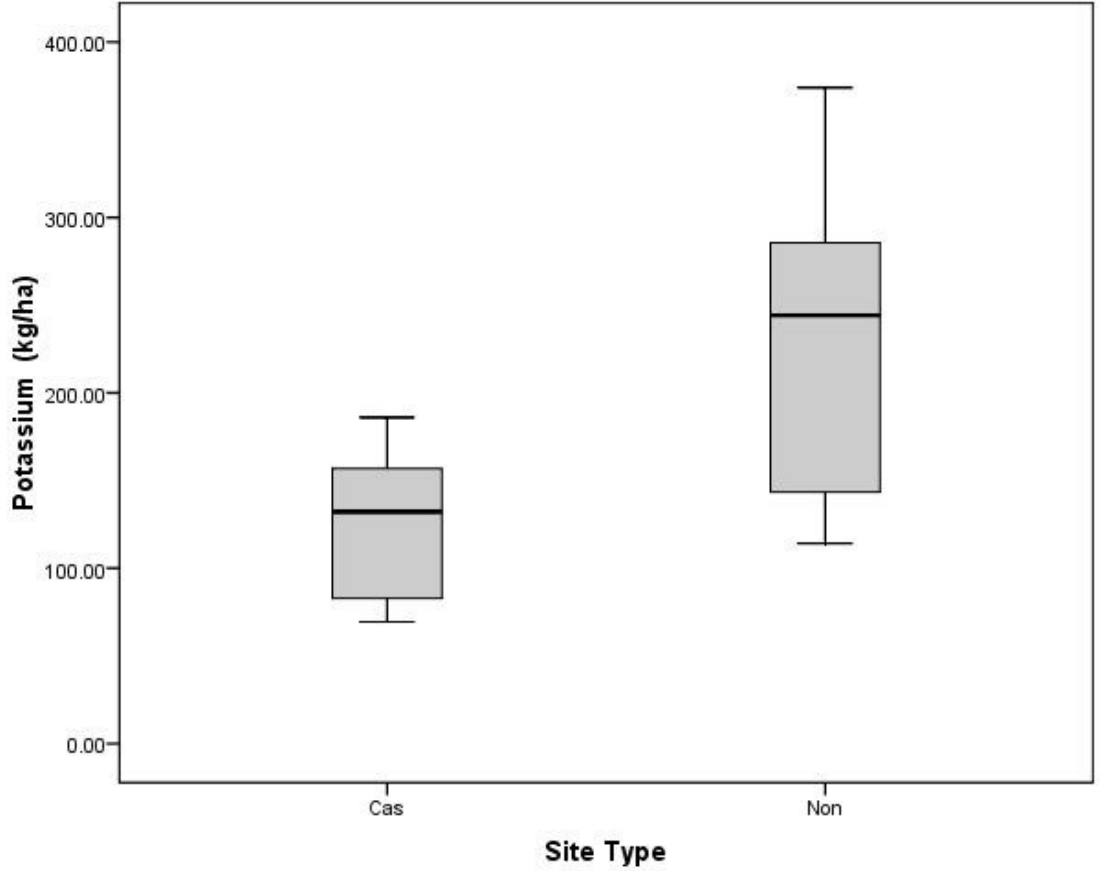


Figure 10 Comparison of potassium concentration between sites dominated by Casuarinas (Cas) and sites devoid of Casuarinas (Non).

Calcium concentration in sites dominated by Casuarinas compared to sites devoid of Casuarinas was not significant ( $p = 0.9675$ ; Figure 11).

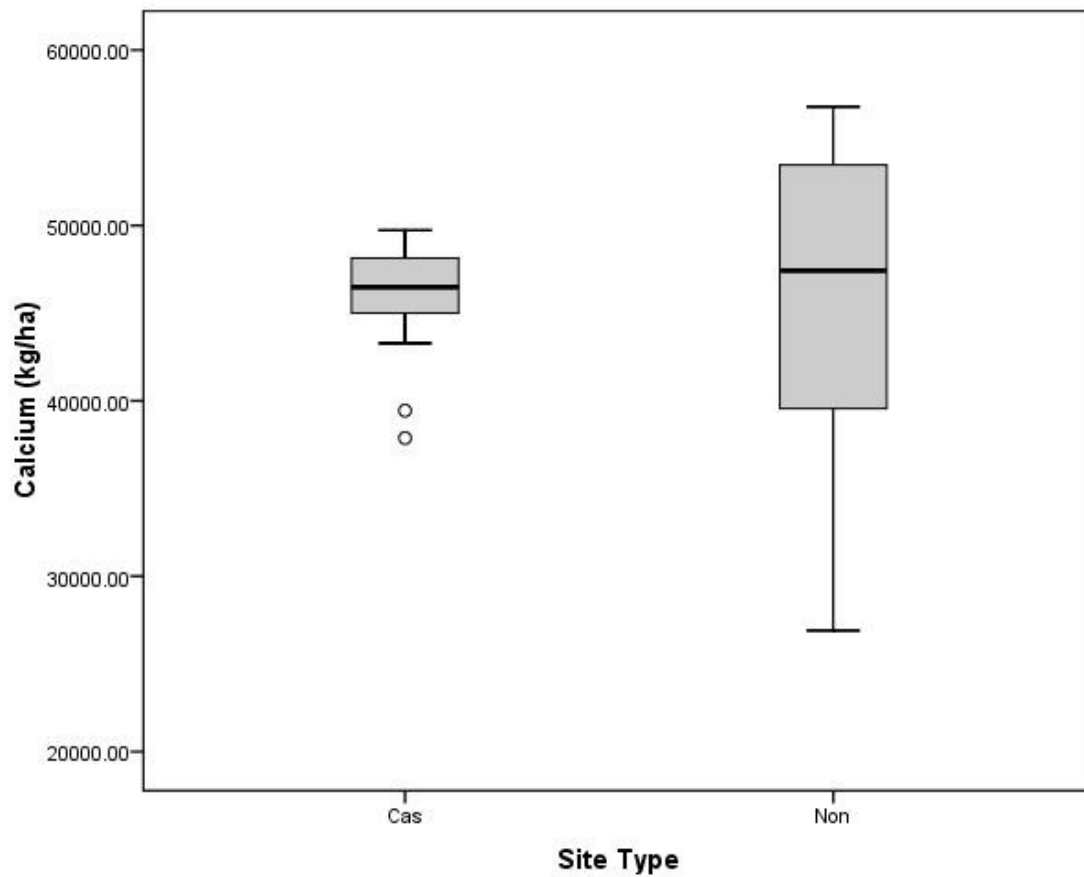


Figure 11 Comparison of calcium between sites dominated by Casuarinas and sites devoid of Casuarinas (Non).

Magnesium concentration was not significantly different between Casuarinas and non-Casuarinas sites ( $p= 0.8555$ ; Figure 12).

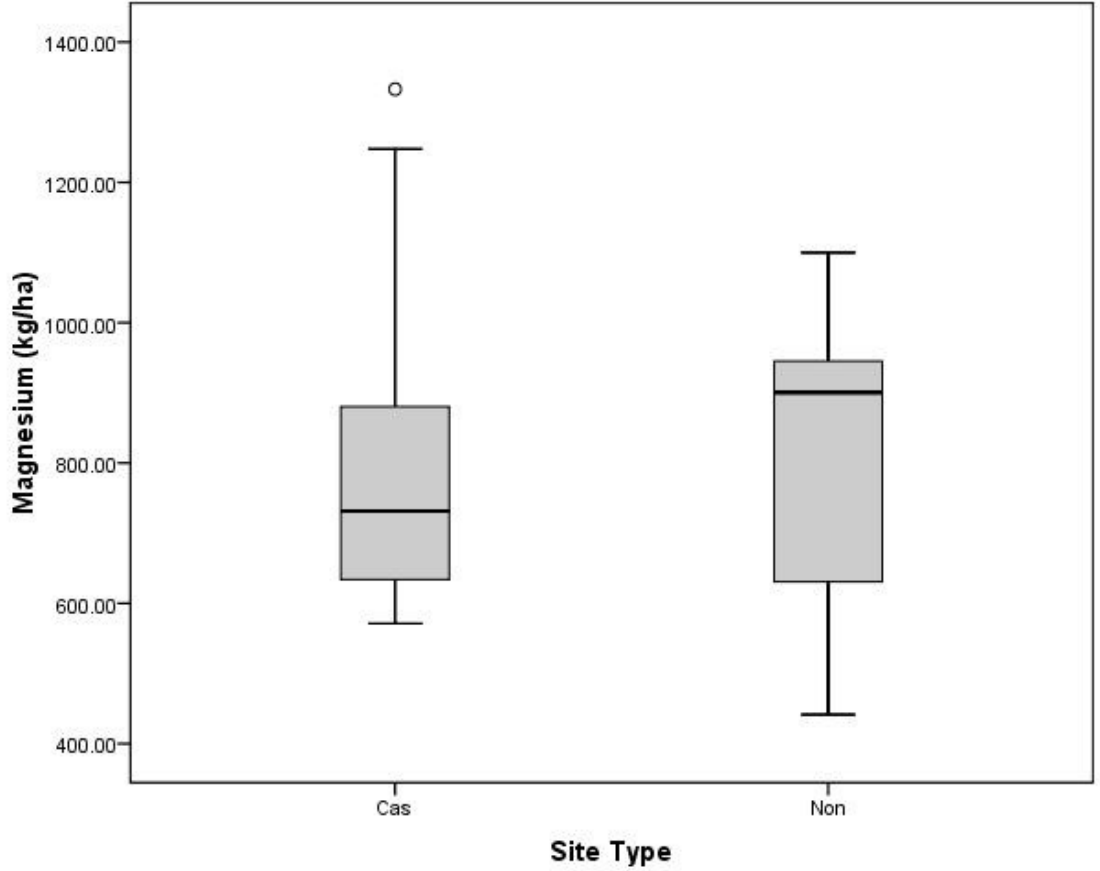


Figure 12 Comparison of magnesium concentration between Casuarinas (Cas) dominated sites and sites devoid of Casuarinas (Non).

Organic matter was significantly in greater quantities in sites devoid of Casuarinas compared to sites dominated by Casuarinas ( $p = 0.007$ ; Figure 13).

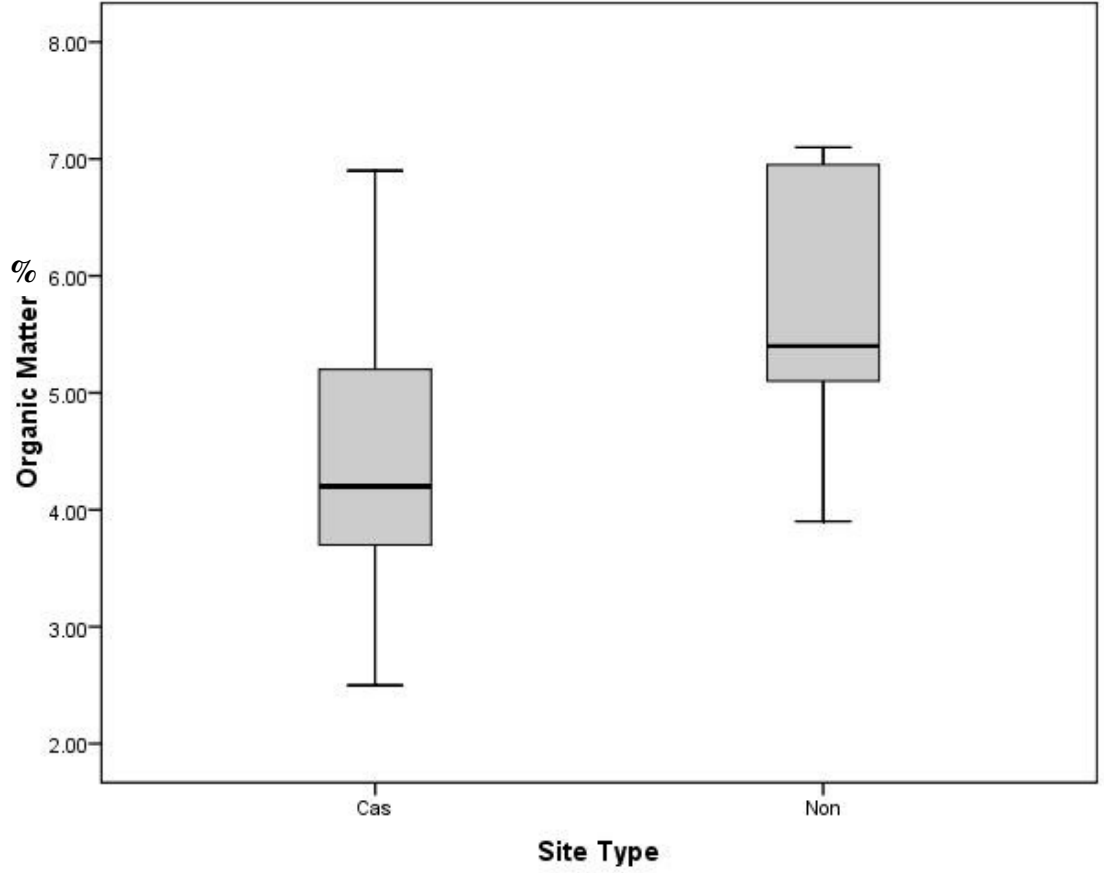


Figure 13 Comparison of percent organic matter between Casuarinas dominated sites (Cas) and sites devoid Casuarinas (Non).

Nitrate-nitrogen concentration was not significantly different between sites ( $p=0.525$ ) (Figure 14).



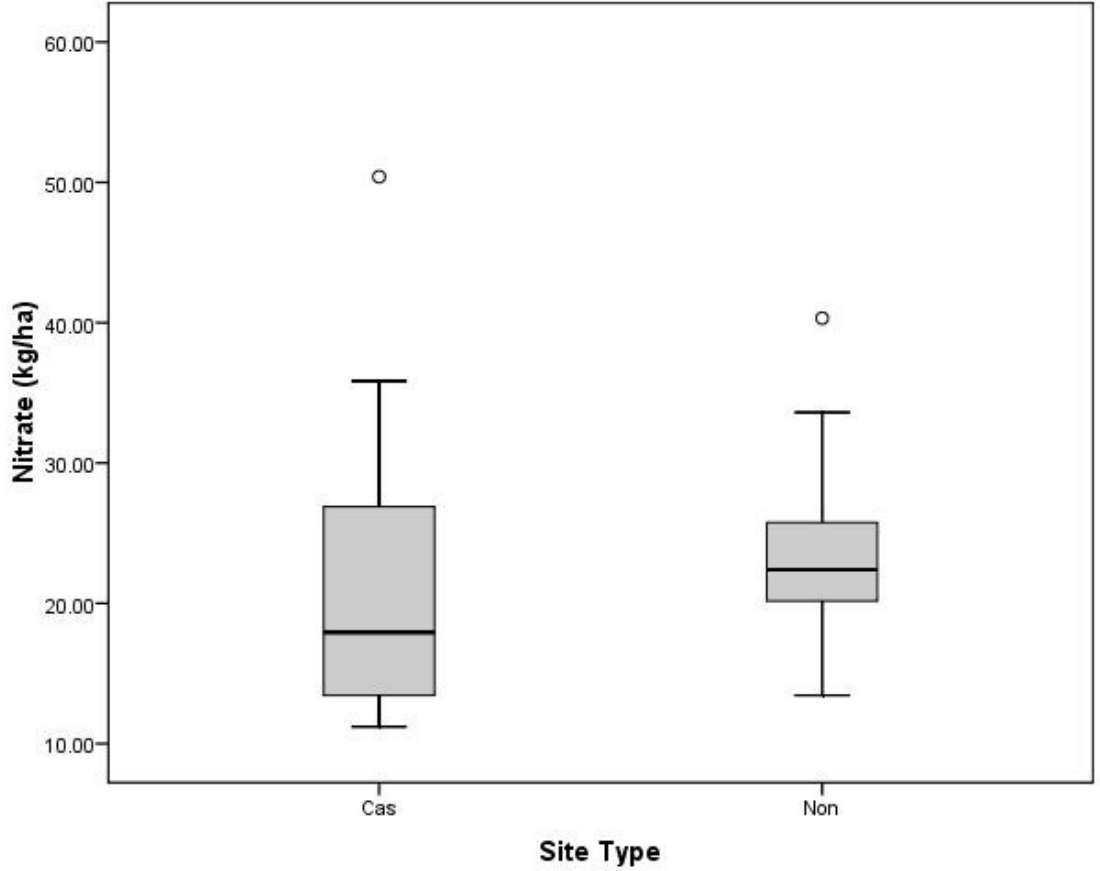


Figure 14 Comparison of nitrate-nitrogen concentration in Casuarinas dominated sites (Cas) and sites devoid Casuarinas (Non).

Cation exchange capacity (CEC) was not significantly different between sites ( $p=0.996$ ) (Figure 15).

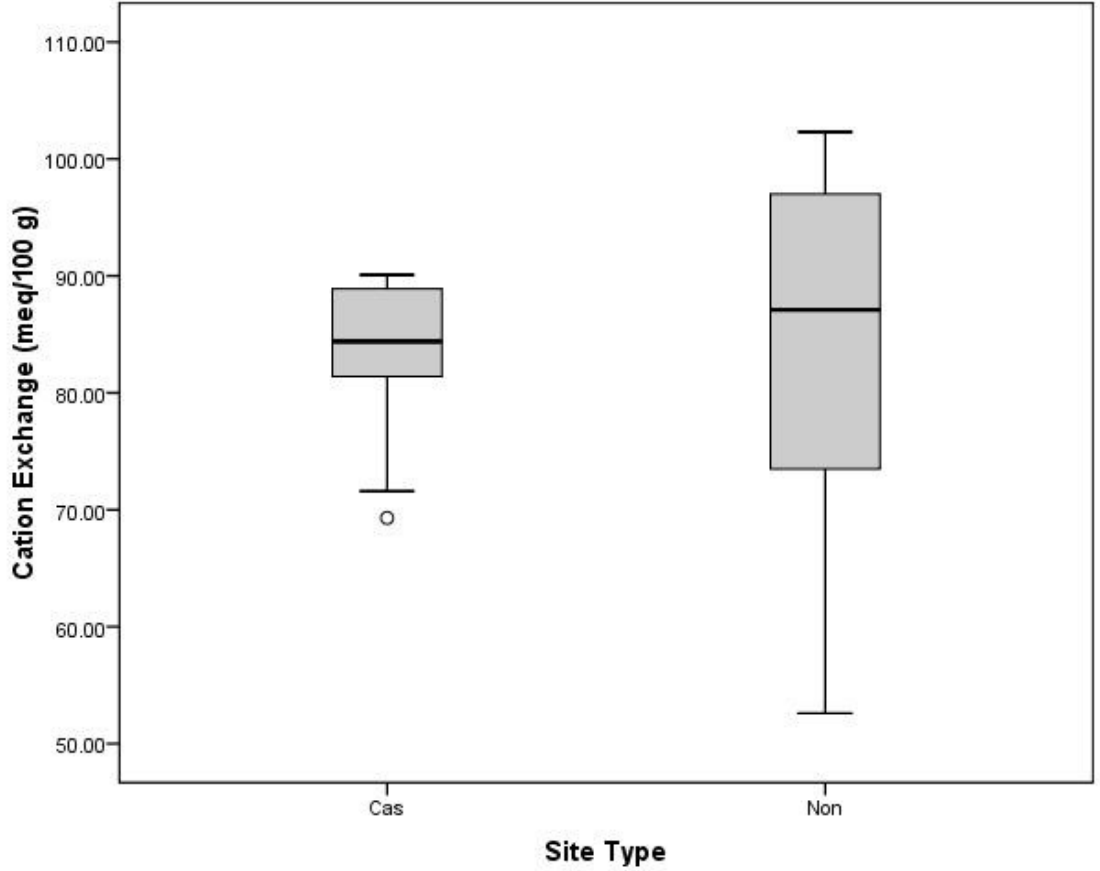


Figure 15 CEC comparison between Casuarinas dominated sites (Cas) and sites devoid of Casuarinas (Non).

The potassium: magnesium ratio was significantly greater in sites devoid of Casuarinas ( $p < 0.01$ )(Figure 16).

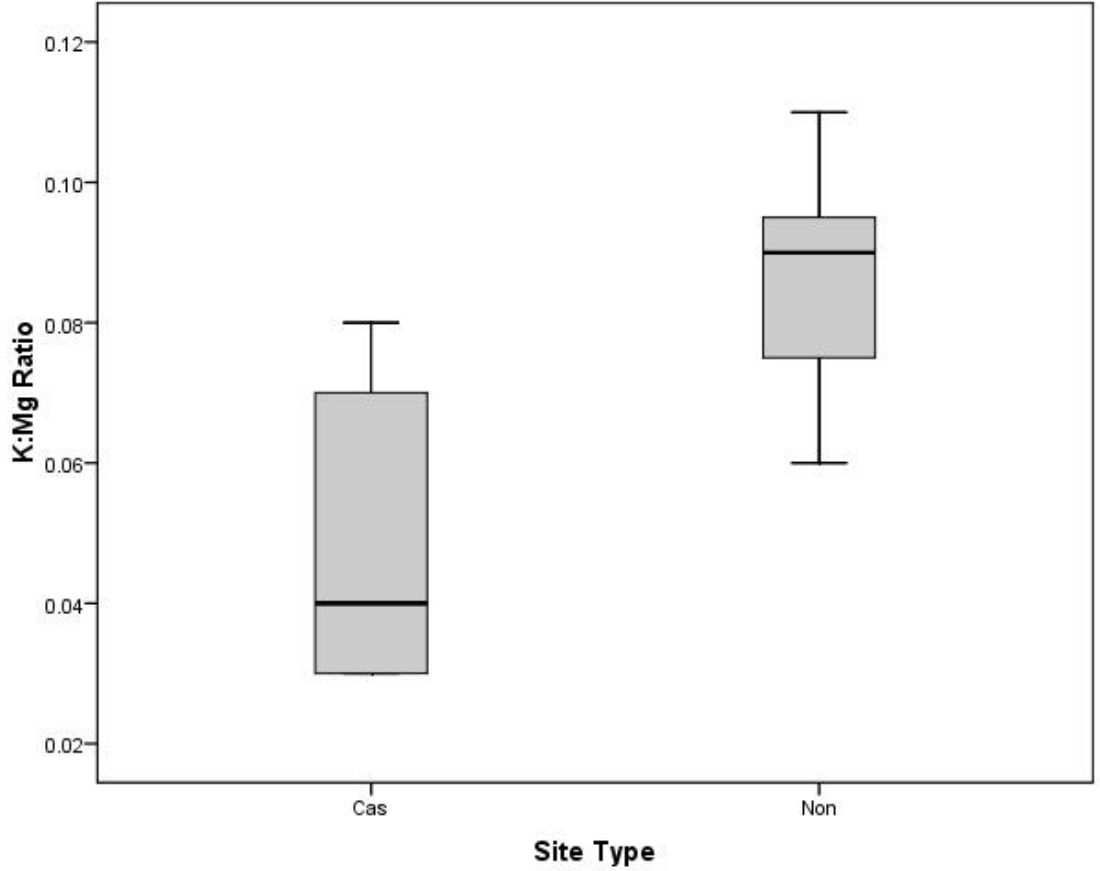


Figure 16 Comparison of K:Mg ratio between Casuarinas dominated sites and sites devoid of Casuarinas (Non).

### Discussion

Surface soils with Casuarinas were significantly different than surface soils devoid of Casuarinas indicating that the presence of the invasive Casuarinas may modify their host environment. Data indicate the physical soil properties which were significantly different included leaf litter depth and organic matter. Chemical

characteristics found to be significantly different included soil pH, concentrations of K<sup>+</sup> and P, and the Potassium: Magnesium ratio.

Investigating how certain soil properties differ between Casuarinas sites and sites devoid of Casuarinas paints an important picture that reflects overall chemical, hydrological, biogeochemical processes occurring among sites (Dixon and Schulze, 2002). Researchers contend that introduced plant species do not necessarily deplete nutrients and water from the soil or inhibit native vegetation from growing in their understory asserting that evidence in support that introduced plants do so is rare (Harrington and Ewel 1997; Loumeto and Huttel 1997; Parrotta and Knowles 1999). Increasingly, however, studies suggest that introduced species do affect soil properties. Lonsdale et al. (1989) suggests *Mimosa pigra* forms mono-specific communities and contributes to increased sediment accumulation along stream banks, *Tamarix* spp is known to use vast amounts of water which contributes to riparian areas dearth of soil moisture thus affecting native plant growth (Invasive 2006). *Eichhornia crassipes*, *Hydrilla verticillata*, and *Pistia stratiotes* are known to decrease dissolved oxygen, pH, and phosphorous contents while increasing dissolved carbon dioxide, and turbidity (Schmitz et al. 1993), and *Imperata cylindrica* (cogongrass) maintains a significantly faster decomposition rate compared to native grasses leading to increased nutrient cycling which can facilitate *I. cylindrica* occurrence (Holly 2008).

Plants require at least sixteen elements in order to achieve normal growth (Hodges). Carbon, hydrogen, and oxygen compose the non-mineral elements which are essential to plants while nitrogen, phosphorous, and potassium serve as mineral elements

and as essential primary nutrients (Hodges). Plants need greater quantities of primary nutrients such as carbon, nitrogen, potassium and phosphorous than they do secondary nutrients such as calcium, magnesium, and sulfur (Hodges). Primary nutrients must be continuously available to support photosynthesis and other plant metabolic activity (Hodges). However, the availability of nutrients to plants is often determined by the chemical property of the element, soil pH, microbial activity, and physical conditions like temperature and soil moisture (Hodges).

As hypothesized by Swearingen (1997), Batish (1998), and Hammerton (2001), Casuarinas may have the capability (as part of competitive exclusion) to take up an increased amount of nutrients from the soil whereby reducing nutrient availability to neighboring native plants. Soil data from this study revealed sites devoid of Casuarinas contained 46% more  $K^+$  and 20% more P compared to soil from sites dominated by Casuarinas. Thus the possibility exists that Casuarinas are depleting primary soil elements. Because Casuarinas are known to establish in nutrient poor soil (Swearingen 1997) a competitive advantage may be created if they have the ability to remove nutrients hence depleting nutrient sources for neighboring native vegetation. Yet, this research did not specifically examine nutrient cycling or which nutrients were taken in by neighboring vegetation which is why more research is needed to assess the affects Casuarinas may have on their environments.

Phosphorous is a primary plant nutrient released from decaying plant and animal material and microorganisms and is a required nutrient for plant developmental and growth (Biology 1992). The presence of phosphorous is also essential for various

biochemical reactions like photosynthesis, respiration, and cell division. Occurring in both inorganic and organic forms, the dominate form of phosphorous is usually determined by soil pH (Biology 1992). When soil is very acidic phosphorous can react and bind with aluminum and iron making it virtually unavailable for plant use (Biology 1992; Hodges).

In sites dominated by Casuarinas, phosphorous was consistently in lower concentrations compared to sites devoid of Casuarinas. Why Casuarinas sites with even more abundant leaf litter than non-Casuarinas sites, and therefore a greater susceptibility for leaf litter to release P into the soil, contained lesser abundances of phosphorous is one indication that the presence of Casuarinas somehow modifies soil nutrient abundances and reveals a more complex soil interaction occurring. Because Casuarinas are known to thrive in nutrient poor soils (Elfers 1988; Swearingen 1997; Hammerton 2001) it is no surprise that it may possess better inherent capabilities for nutrient uptake which enable it to dominate nutrient poor areas.

Potassium, like phosphorous, is required for chemical reactions such as photosynthesis and protein synthesis in plants (Biology 1992). Usually leached into the soil from leaf litter decay, potassium can be absorbed by plants where it is then transported to the active growing points of plants and immature seeds (Schlesinger 1997). The availability of potassium can improve plant resistance to disease and improve plants tolerance to drought. When potassium is limited, however, root growth is stunted causing premature defoliation and plant death (Hodges).

Soil from sites dominated by Casuarinas showed overall lower concentrations of potassium than sites devoid of Casuarinas. The presence of leaf litter in non-Casuarinas sites may be correlated with potassium and phosphorous concentrations. In fact, Dixon and Schulze (2002) state that organic matter influences the physical, chemical, and biological properties in soil along with providing nutrients such as phosphorus, potassium, and nitrogen for plant growth. The data illustrate that soil organic matter content was significantly greater in sites devoid Casuarinas ( $p = 0.007$ ). Sites devoid of Casuarinas also contained greater concentrations of potassium and phosphorous. Zhang et al. (2008) indicated that phosphorous concentrations for soil under Casuarinas in Fujian Province, China ranged from 4.84-8.8 kg/ha. Similarly phosphorous concentration in soil from Casuarinas sites in this study were at most 10.45 kg/ha with non-Casuarinas sites containing at roughly 15.84 ka/ha.

Potassium and phosphorous are significant soil nutrients in that their presence is essential for plant activities such as photosynthesis, respiration, cell division, protein synthesis, and metabolic activity (Biology 1992). Availability of nutrients to plants is also determined by soil pH, microbial activity, and physical conditions like temperature and soil moisture (Hodges). Why sites dominated by Casuarinas contain very little native plant species can potentially be related to a combination of these factors. Not only can it be recognized that potassium and phosphorous abundances can be related to organic matter content present in soil, but also the abundance of potassium and phosphorous can be influenced by soil pH ( Biology 1992). As illustrated, sites devoid of Casuarinas contained a slightly higher soil pH and also greater abundances of  $K^+$  and P.

Gochenaur's (1975) study on soil pH in sites containing Casuarinas on New Providence Island, The Bahamas showed Casuarinas soil was more acidic than soil under *Cocos nucifera* (coconut groves). Gochenaur (1975) obtained a pH of 7.3-7.5 for soil in Casuarinas dominated locations while this study obtained a slightly lower pH of 6.61 for soil from Casuarinas location. Soil from Gochenaur's (1975) coconut groves had a pH of 8.4-8.6 while this study's non Casuarinas sites had an average pH of 6.81. These results corroborate with Gochenaur (1975) that soil acidity in Casuarinas is decreased to some degree compared to soil either from coconut groves or soil from sites absent of Casuarinas.

Another interesting aspect of Gochenaur's (1975) study on microfungi in Bahamian soil from sites dominated by Casuarinas suggests certain phytopathogenic microfungi dominate Casuarinas forested areas. These microfungi may play a vital role in shaping soil properties like pH (Gochenaur 1975). From field observations, a white fungus was found growing ~5cm deep within Casuarinas leaf litter (Figure 17) and not observed in sites absent of Casuarinas. Soil acidity in sites dominated by Casuarinas may, then, also be a result from the presence of soil fungi, however, more research would need to be conducted to make a true correlation.

Soil chemistry is heavily influenced by plants and their production of organic acids in the soil profile (Schlesinger 1997). In general, chemical reactions among soil nutrients and biota determine the rate of cation removal which, in turn, effects nutrient availability and increases soil acidity (Schlesinger 1997). Cation exchange is a useful indicator for soil fertility because it is a measure of the soil's ability to hold cations which



plants absorb and use for energy (Lines-Kelley 2003). Soil with greater abundances of organic matter normally have higher cation exchange capacity as more colloids are available with negative charges to bind with cations such as  $K^+$  (Soil 2002). With this in mind, sites devoid of Casuarinas had significantly greater soil organic matter content but CEC was not significantly different between sites ( $p > 0.05$ ).



Figure 17 Tropical soil fungi (white) present in Casuarinas leaf litter at a depth of approximately 5cm. Specific fungi species were not identified.

This could possibly be due to the thick matt of leaf litter creating anoxic conditions that inhibit decomposition. Another reason the CEC may not have been different between Casuarinas sites and sites devoid of Casuarinas may be the abundance of sandy soil on San Salvador Island instead of clay soil. Soil deficient in clay contains fewer binding sites for cations such as  $\text{Ca}^{2+}$  and  $\text{K}^+$  thereby leaving cations to be more readily leached by percolating soil water (Biology 1992). Nevertheless, more research is needed to understand these processes.

Leaf litter, however, also has potential to influence soil moisture content. Hammerton (2001) proposes the abundance of leaf litter in Casuarinas sites inhibits rainwater infiltration thereby reducing overall soil moisture content. However, dense leaf litter and the forest canopy inhibit evaporation, protect the ground from wind and irradiation, and therefore potentially aid in retaining soil moisture content in sites with abundant leaf litter, like Casuarinas areas (Gochenaur 1975). More exposed soils may be subjected to increased evaporation as well as longer periods of solar exposure, so, Casuarinas sites should then, according to Gochenaur (1975), exhibit greater soil moisture content because of the abundance of leaf litter. Although leaf litter was significantly greater by 75% in sites dominated by Casuarinas ( $p < 0.001$ ) soil moisture in sites devoid of Casuarinas was similar to soil moisture in Casuarinas dominated sites and was not overall statistically significant ( $p = 0.6355$ ). The fact that similar soil moisture values were present in both protected soil (Casuarinas) and exposed soil (non-Casuarinas) indicates more a complex soil moisture interaction taking place.

Magnesium abundance was slightly greater in sites dominated by Casuarinas, however it was not statistically significant ( $p > 0.05$ ). Schlesinger (1997) studied a forest in Tennessee and found using a mass-balance approach that Mg in vegetation was directly related to decreases in content of Mg in the soil. According to Environmental Testing & Consulting (ETC) soil report (2009) higher Mg levels may reduce  $K^+$  uptake. These smaller  $K^+$  abundances would yield smaller Potassium: Magnesium ratio and, as indicated by ETC soil report (2009), K:Mg ratios below 0.2 indicate a  $K^+$  uptake reduction. As seen from the data in this study, sites dominated by Casuarinas had smaller abundances of  $K^+$  and larger  $Mg^{2+}$  abundances resulting in a K: Mg ratio of 0.05. Although sites devoid of Casuarinas had greater  $K^+$  abundances than  $Mg^{2+}$  the ratio was 0.08, still below the desirable K:Mg threshold as designated by ETC (2009). These K:Mg ratios may be attributed to the low cation exchange capacity as demonstrated in both Casuarinas and non-Casuarinas sites.

Analysis of soil data revealed Nitrate-nitrogen abundances were not statistically different ( $p = 0.525$ ) between Casuarinas sites and sites devoid of Casuarinas. Interestingly, sites devoid of Casuarinas actually had a 9% greater abundance of Nitrate-nitrogen, which is unusual as Casuarinas are  $N_2$ -fixing species—which are known to increase nitrogen input into the soil (Salisbury 1985). For plants, however, that cannot fix  $N_2$ , nitrate ( $NO_3^-$ ) and ammonium ( $NH_4^+$ ) are the important available nitrogen sources (Salisbury 1985). The process of converting organic nitrogen to  $NH_4^+$  is known as ammonification (Salisbury 1985). When soils are warm, moist, and at near neutral pH,  $NH_4^+$  is further converted to  $NO_3^-$  by oxidizing bacteria (Salisbury 1985). These

processes are essential for plants to obtain nitrogen as, depending on the type of soil and what plants are growing in that soil,  $\text{NH}_4^+$  can be readily oxidized leaving only  $\text{NO}_3^-$  to be absorbed (Salisbury 1985). Sites dominated by Casuarinas, therefore, should theoretically exhibit greater Nitrate-nitrogen abundances than sites absent of Casuarinas as Casuarinas are  $\text{N}_2$ -fixing plants. Parrotta (1999) indicates that nitrogen fixing trees have been considered beneficial to the soil for their nitrogen production and are intentionally planted to increase soil nitrogen content. The notion that Casuarinas improve soil fertility by adding Nitrate-nitrogen to the soil can be refuted as Casuarinas sites contain similar abundances of Nitrate-nitrogen compared to sites devoid of Casuarinas. Conversely, some plant communities like conifer forests absorb nitrogen as  $\text{NH}_4^+$  because nitrification is thought to be inhibited by lower soil pH or tannins and phenolic compounds (Salisbury 1985; Schlesinger 1997). Casuarinas are suggested to contain tannin compounds (Elfers 1988), yet more research is needed to investigate if tannin compounds from Casuarinas contribute to inhibition of nitrification. While Casuarinas are  $\text{N}_2$ -fixing plants, data from this study indicates that overall Nitrate-nitrogen abundance was not significantly greater in Casuarinas dominated locations. It is recognized, however, that although nitrate-nitrogen was not found to be in greater concentrations in sites dominated by Casuarinas, nitrogen may be instead occupied in another form such as ammonium (Biology 1992).

Other soil nutrients such as magnesium and calcium did not show any significant differences ( $p > 0.05$ ) in concentration in soil collected from sites dominated by Casuarinas compared to sites devoid of Casuarinas. The predominate soil type on the

island of San Salvador is a protosol of calcium carbonate (Carew and Mylroie 1995; Sealey 2006) which corroborates the similar  $\text{Ca}^{2+}$  abundance between Casuarinas and non-Casuarinas sites. Magnesium, like potassium and phosphorous, is also necessary for plant protein synthesis and phosphorous reactions. Since no significant differences in abundance exist between sites, it is possible that the presence Casuarinas does not affect the abundance this soil nutrient.

These studies comparing soil from Casuarinas dominated sites and soil from sites absent of Casuarinas on San Salvador Island, The Bahamas confirm that Casuarinas do indeed modify their environment and corroborates with other studies (Elfers 1988; Jiqin 1991; Swearingen 1997; Batish 1998; Hammerton 2001). As hypothesized, soil from sites dominated by Casuarinas compared to soil from sites devoid of Casuarinas showed different physical and chemical soil properties. Sites with Casuarinas had greater leaf litter abundance, slightly lower soil acidity, and lower concentrations of  $\text{K}^+$  and P, organic matter, and smaller K :Mg ratios.

### Conclusion

An investigation was conducted to measure differences in soil properties between Casuarinas dominated sites and sites devoid of Casuarinas in order to assess if this noxious invasive plant significantly modifies its environment, and by extension, creates changes to perpetuate its own kind. Measurements from a soil moisture probe indicate that soil from sites devoid of Casuarinas had more soil moisture, though it was still not statistically significant. Soil characteristics that also differed included leaf litter

abundance and organic matter. Soil chemical analysis showed differences in phosphorus and potassium but not nitrate-nitrogen, magnesium, or calcium. Thus, there are indications that *Casuarinas* do modify its environment. These quantitative analyses are important because preliminary results signify that *Casuarinas* have the potential to alter soil properties. Understanding the mechanisms by which this invasive species modifies soil chemistry is an important step to management and possible eradication.

Undeniably, discrepancies within the data have yet to be exposed as more research is necessary in order for data comparisons. It is important to note that there may have been some confounding errors. For example, the soil moisture probe used may not have given consistent data due to a malfunction it experienced while in the field. Soil moisture could also vary deepening on the season in which the samples were collected. Depths at which soil was collected may not have been deep enough or perhaps the selected sites were already too heavily influenced by urban factors. Statistical analysis of the data, however, is consistent and signifies some modification of the soil is taking place with the presence of *Casuarinas*. Limitations of this research include a paucity of soil samples from various field seasons. Identifying how soil properties vary depending on wet and dry seasons should be tested as well as how soil properties compare from neighboring islands in the Bahamian Archipelago.

Understanding the impact of the invasive *Casuarina equisetifolia* provides a model case study to assess the role of invasive species on the environment. Because there is very little existing literature available and because this plant is undeniably detrimental to the environment, there is an essential need for more research-- which may

lead to increased awareness and development of better management strategies for this invasive species.

## CHAPTER III

### ALLELOPATHY ANALYSIS OF *CASUARINA EQUISETIFOLIA*

#### Overview

Casuarinas are a noxious invasive species to the West Indies and have been suggested to exhibit allelopathy. Growth chamber experiments and laboratory bioassays indicated bean and radish seeds applied with Casuarinas leachate were significantly suppressed in germination and radicle growth by at least 32-97% and 70-90%, respectively, compared to the control. However, radish seedlings applied with leachate did not show any overall significant growth suppression nor any differences in wet/dry mass compared to the control. Liquid chromatography mass spectrometry (LC-MS) analysis of Casuarinas leachate suggests the secondary metabolite chalepin, which is a known allelochemical, is present within the leachate. Thus, because Casuarinas leachate inhibits seed germination and because there is a known allelochemical potentially present in the plant tissue, allelopathy is likely. The possibility exists, therefore, for Casuarinas to chemically reduce competition from other species which may help them form monocultures and environments that have been described as ecologically sterile.



## Introduction

A new dimension of invasive species biology includes investigating how invasive species alter the biochemistry of an area via allelopathy, which is the release of biochemicals that affect germination and growth of native plants or alter native soil biota (Callaway and Ridenour 2004). Callaway and Ridenour (2004) suggest allelochemicals, which they also term novel weapons, interfere with plant-soil interactions and aid some invasive plant species in dominating their communities. As an example, the invasive Casuarinas in the Bahamas form monospecific stands and are suggested to create ecological sterile environments (Swearingen 1997, Hammerton 2001). It has been suggested that Casuarinas also employ allelopathy (Batish 1998; Jiqin 1991; Langui 1996) and its associated suppression of seed germination and seedling growth may be a reason why few native plants are present in the understory of Casuarinas dominated areas.

Interestingly, the idea that plants have the ability to release toxic chemicals in order to sustain themselves in a community has been documented as early as 300 BC by Greek and Roman philosophers (Weir et al. 2004; Borz and Vivanco 2006). Such observations were seen in *Cicer arietinum* (chickpea) and *Juglans nigra* (walnut) where no other vegetation was observed to grow their vicinity (Weir et al. 2004). However, it was not until the early twentieth century that allelopathy was formally defined within the scientific community. In 1937 Hans Molisch first used the term allelopathy to describe certain biochemical interactions (Broz and Vivanco 2006). Appropriately, allelopathy literally translates from the Latin root *allelon* to mean ‘of each other’ while *pathos*

translates to 'feel or suffer.' (Weir et al. 2004). In current literature, the term allelopathy is most often used in reference to toxic chemical interactions, either indirectly or directly, between plants (Willis 2004). One example of allelopathy is from mixed plantation forests consisting of *Juglans nigra* L. (black walnut) and *Betula papyrifera* (birch). Studies indicate birch died prematurely suggesting allelochemical influences from walnut (Rietveld et al. 1983, Raven 1992). *Lycopersicon esculentum* (tomato) and *Medicago sativa* (alfalfa) are also shown to have leaf wilting and seed death when grown in close proximity to walnut (Raven 1992). The invasive *Centaurea diffusa* and *Centaurea maculosa* are known to release the allelochemical catechin (Callaway and Ridenour 2004) and studies have demonstrated native North American grass species were significantly suppressed in germination and growth when applied with this allelochemical (Bias et al. 2003; Hierro and Callaway 2003).

Allelopathy has been studied extensively (Bias et al. 2003; Ervin and Wetzel 1999, 2003; Fitter 2003; Hierro and Callaway 2003; Callaway and Ridenour 2004; Weir et al. 2004; Orr et al. 2005; Broz and Vivanco 2006; Broeckling and Vivanco 2008; and Inderjit et al. 2008) and research has sought to explain how exactly allelopathy alters soil chemistry which results in suppression or inhibition of plant germination and growth. Consequently, the significance of this investigation of how native plants are suppressed from growing as a result of allelopathy is important, as *Casuarina equisetifolia* is suggested to be a species which employs this mechanism (Batish 1998; Langui 1996) to create a feedback loop facilitating its existence in its host community. The principle objectives of this investigation were to:

- 1.) Quantitatively determine how germination and growth of seedlings from common cultivars of bean (*Phaseolus vulgaris*) and radish (*Raphanus sativus*) were affected when applied with a known concentration of Casuarinas leachate. Although bean and radish are not representative of native Bahamian flora, they are relatively easy to grow in a controlled growth chamber, and at a minimum they will allow investigation to determine if reduced germination and growth induced from allelopathy is possible.
- 2.) Identify through a liquid chromatography mass spectrometry (LC-MS) any potential compounds within Casuarinas leachate that are known allelochemicals.

The invasive Casuarinas is suggested to be allelopathic (Jiqin 1991; Langui 1996; Batish 1998; Hammerton 2001) so studying if this invasive species employs allelopathy will help determine how the presence of Casuarinas in the West Indies may have detrimental effects on native vegetation by modifying soil biogeochemistry and altering ecosystem dynamics. Attempts to control its introduction and proliferation, not only to the Bahamian Archipelago but to coastal areas of the United States, should be of utmost concern especially as Casuarinas have the ability to disperse easily without the aid of people (Swearingen 1997; Hammerton 2001; Rodgers 2005). Identifying if Casuarinas also contain an allelochemical may also contribute to new research being conducted on herbicides and agrochemicals (Vyvyan 2002). According to Vyvyan (2002) the widespread use of synthetic herbicides has resulted in herbicide resistant weeds and major concerns on the impact these synthetic herbicides have on human health as well as

the environment. Because allelopathy is the direct or indirect inhibitory effect by one plant to another, certain chemicals can be studied from plants that employ allelopathy that may help facilitate research of natural herbicide (Vyvyan 2002).

## Background

Callaway and Ridenour (2004) developed the novel weapons hypothesis to explain how some plant species change from being harmless in their native environment into invasive aggressors in their new community by releasing allelochemicals. These allelochemicals have inhibitory effects on plants and soil microbes that have not adapted to certain biochemicals compared to native plants, which have adapted over time (Rabotnov 1982; Mallik and Pellisier 2000). Since 2004, Broz and Vivanco (2006), and Broeckling and Vivanco (2008) have further investigated novel weapons and have attempted to identify plant species that employ this mechanism. The following section describes in more detail the invasive species allelopathy literature.

### *Allelopathy*

*Sorghum bicolor* (sorghum) is one of the most studied allelopathic plants because it releases the phytotoxin sorgoleone. Sorgoleone is known to inhibit the process of photosynthesis and oxygen evolution, which are essential processes for plants growth (Weir et al. 2004). *Gypsophila paniculata* (baby's breath) is known to modify microbial dynamics in the rhizosphere of *Trifolium subterraneum* (clover) through the release of an allelochemical (Weir et al. 2004). *Centaurea maculosa* (spotted knapweed) contains the

allelochemical catechin which has demonstrated strong allelochemical properties in laboratory simulations and field conditions (Broz and Vivanco 2006). Catechin has been further investigated to determine if it can be detected in soil from sites containing the invasive *C. maculosa*. It was concluded, however, catechin is difficult to extract *in situ* due to its rapid breakdown process whereby making it difficult to accurately identify (Broz and Vivanco 2006).

Other examples of proposed allelopathy included Gentle and Duggin (1997) and Sharma et al. (2005) who suggest *Lantana camara* (verbena) reduces native seedling growth. Inderjit and Foy (1999) found that *Artemisia vulgaris* (mugwort), which is native to Europe, Northern Africa, and Asia but invasive in Eastern North America, suppresses seedling growth of *Trifolium pretense* (red clover). Further investigation by Inderjit and Foy (1999) indicate that mugwort appeared to release phenolic allelochemicals, however, Barney et al. (2005) suggest from testing volatiles released from mugwort that no phytotoxicity exists and propose that perhaps an untested compound or a more complex chemical interaction is present.

A plant known to cause severe damage to disturbed forests and crops is *Chromolaena odorata* (Jack in the Bush), which is native to Central and South America and has spread throughout tropical areas including the Caribbean, India, the Philippines, and Australia. Reports of *C. odorata* allelopathy from Adetayo et al. (2005) suggest extracts affect germination and seedling growth of *Vigna unguiculata* (cowpea), *Zea mays* (maize), *Glycine max* (soybean), and even itself (Adetayo et al. 2005). Bousquet-Mélou et al. (2005) have studied the invasive shrub *Medicago arborea* (moon trefoil) and

suggest that it also demonstrates allelopathy as leaf extracts were shown to inhibit seed germination and seedling growth of *Linum strictum* (upright flax), *Lactuca sativa* (garden lettuce) and *Lepidium sativum* (garden cress pepperweed).

Feo et al. (2002) studied the allelopathic properties of *Ruta graveolens* L. (rue) on radish seed germination and found allelopathic activity inhibited germination by 60% and radicle growth was reduced by 53%. Compounds suggested to be in the rue oil were used in bioassay experiments in which valeric acid inhibited germination 100%, salicylate inhibited germination by 90%, and  $\alpha$ -pinene by 68%. The compound furanocoumarin xanthotoxin was also identified as being important in explaining the allelopathic activity of rue (Aliotta et al. 1996; Feo 2002).

Ervin and Wetzel (1999) studied the allelochemical potential of the wetland macrophyte *Juncus effusus*. The authors found *Eleocharis obtusa* and *Scirpus cyperinus* seeds when applied with an aqueous extract of *J. effusus* were not significantly suppressed, however, biomass-specific chlorophyll *a* concentration was significantly reduced in *E. obtusa*. Interestingly, *J. effusus* seedlings experienced significant suppression in shoot development and reduction in biomass and chlorophyll *a* concentrations indicating that *J. effusus* suppresses its own species through releasing allelochemicals (Ervin and Wetzel 1999).

Livitt and Lovett (1984) studied *Datura stramonium* (thorn apple), which is known to have allelopathic potential against barley, wheat, linseed and sunflower. Allelopathy was attributed to tropane alkaloids present in *D. stramonium*'s seed coat. Another study by Lovett and Potts (1987) indicated that *D. stramonium* also released

scopolamine and hypscyamine. Overland (1966) found that although tobacco germination was not suppressed its growth was inhibited by gramine, a compound found in barley. Barley husks are also known to contain the allelochemicals coumarin and hydroxycinnamic acid (Sumere et al., 1958).

Langui (1996) and Batish (1998) suggest allelochemicals are released from Casuarinas which show to inhibit the germination and growth of *Bidens pinnata* and *Parthenium hysterophorus*. These allelochemicals released from Casuarinas leaf litter include kaempferol-3- $\alpha$ -rhamnoside, quercetin-3- $\alpha$ -araboside, luteolin-3', 4'-dimethoxy-7- $\beta$ -rhamnoside, kaempferol-3- $\beta$ -dirhamnoside and quercetin-3- $\beta$ -glucoside (Langui 1996).

Some invasive plants release particular biochemicals into the soil that interact and modify native plant growth (Borz and Vivanco 2006). Hierro and Callaway (2003) are sure, however, to state that allelopathy is probably not the dominant way plants interact-- but they also stress non-resource competition should be reconsidered as an important mechanism which may explain why some invasive species achieve significant success in new territory.

### *Study Organism*

The presence of *Casuarina equisetifolia* is problematic for a variety of reasons: they form dense thickets that crowd out native vegetation, produce an abundant amount of leaf litter, and are hypothesized to release allelopathic chemicals (Swearingen 1997; Hammerton 2001). Land occupied by Casuarinas can also become ecologically sterile

where little or no germination and growth exist of other plant species (Swearingen, 1997).

Native to tropical areas of the Indian Ocean and the South China Sea, *Casuarinas* was intentionally introduced to the West Indies in the late 1800's as an ornamental tree because of its ability to thrive in saline beach environments (Elfers 1988; Hammerton 2001). Since its introduction, *Casuarinas* has spread across much of the West Indies including to central and south Florida, U.S.A. (Elfers 1988). Because of its rapid colonization, particularly to already disturbed urban areas (Elfers 1988; Rodgers 2005), it is important to understand if *Casuarinas*' pervasiveness is related to allelopathy to help with management strategies.

*Casuarinas* were at one time also thought to stabilize eroding beaches, which unfortunately has now been proved as a faulty assumption (Sealey 2006). Although *Casuarinas* resemble coniferous trees in appearance, they are actually in the class Magnoliopsida (flowering plants). Pollinated by the wind, *Casuarinas* produce multiple flowers throughout the year and disperse thousands of seeds (Elfers 1988). According to Elfers (1988) eighty-two species of *Casuarinaceae* exist, however, the most common in The Bahamas are *Casuarina equisetifolia* (L.) and *Casuarina glauca* (Sieb.) with the island of San Salvador dominated by *Casuarina equisetifolia*. This species also has the ability to colonize in various habitats from beaches, dunes, rocky cliffs, and mangroves because it can fix nitrogen allowing them the ability to thrive in a wide range of soil types (Swearingen 1997).



It is essential, therefore, because of Casuarinas rapid colonization, pervasiveness, and potential allelopathy, to understand how this introduced species modifies its environment. This study is important as it shall provide novel quantitative evidence as to how and to what degree Casuarinas suppress or reduce cultivar seed and seedlings and though LC-MS analysis, provide information about potential compounds which may be allelochemicals.

## Methods

### *Study Area*

The Commonwealth of the Bahamas is a chain of twenty-nine islands and numerous cays in the Caribbean located in the North Atlantic Ocean southeast of Florida, USA and northeast of Cuba. The northwest-southeast trending archipelago extends 1400 km from the Florida peninsula to the tectonically active Caribbean Plate (Carew and Mylroie 1995). The combined land area of The Bahamas is 10,010 km<sup>2</sup>.

Lithified coral sediments, oolitic sediments, grapestone, pellet mud, and clay and silt make up the carbonate Bahamas Platform (Sealey 2006). The trade winds contribute to the warm waters and tropical marine climate of The Bahamas, however, as a consequence of its geographic location, The Bahamas can receive up to a quarter of its rainfall from tropical storms and hurricanes (Sealey 2006). Temperatures with normal trade winds in the Spring usually lie between 19-29 °C and Autumn temperatures lie between 24-30 °C (Sealey 2006).

*San Salvador Island, The Bahamas*

San Salvador's geology, as described by Carew and Mylroie (1995), is a complex array of eolianite limestones deposited in the middle to late Quaternary. Soil on San Salvador Island, as well as on other islands in The Bahamas, is alkaline with the texture ranging from sandy to stony with loam (a mixture of sand and clay) being absent because the soils are so poorly developed (Sealey 2006). The soil is considered azonal, or immature, though a red clay soil and organic soil can be recognized and classified in some areas (Sealey 2006).

The vegetation on the island grows in three main zones: the inland zone, the nearshore zone, and the coastal zone (Smith 1993). The inland zone contains dense thickets with mangrove communities that surround inland hypersaline lakes (Rodgers 2005). The nearshore zone is the transition area between dune grasses and shrub communities of the coastal zone and the more dense thickets of the inland zone (Rodgers 2005). Coastal vegetation grows in nutrient poor-carbonate rich sand with vegetation inland growing in older more organic soil.

San Salvador Island is estimated to receive 100 cm of rain annually with evaporation rates exceeding precipitation rates (Sealey 2006). The beginning of the rainy season lasts from June to December with October being San Salvador Island's wettest month (Gerace 2009). Northern islands in the archipelago can receive up to 75-100 cm more rain per year than San Salvador Island (Gerace 2009).

### *Field Collection*

Fresh leaves were clipped January 4, 2009 from the Field Station site, which contains a dense 30 m x 40 m stand of *Casuarinas* approximately 50 years old (Figure 18), and transported back to Mississippi State University, Starkville, MS. Because of stringent international regulations on transporting plant material, *Casuarinas* leaves were transported in plastic bags (accessible to Bahamian customs agents for inspection).

### *Laboratory Methods*

Fresh leaves were collected on San Salvador Island, The Bahamas opposed to already decomposing leaf litter. As suggested by Zhang et al. (2008), senescent leaves have less extractable condensed tannin content and overall fewer phenolics than young leaf parts. Indeed, in order to simulate leaf litter decay occurring on the forest floor on San Salvador Island, The Bahamas, fresh leaves were stored for nine months at room temperature until they turned from green to brown. This storage duration emulated

## Casuarinas and Non-Casuarinas Study Sites San Salvador Island, The Bahamas

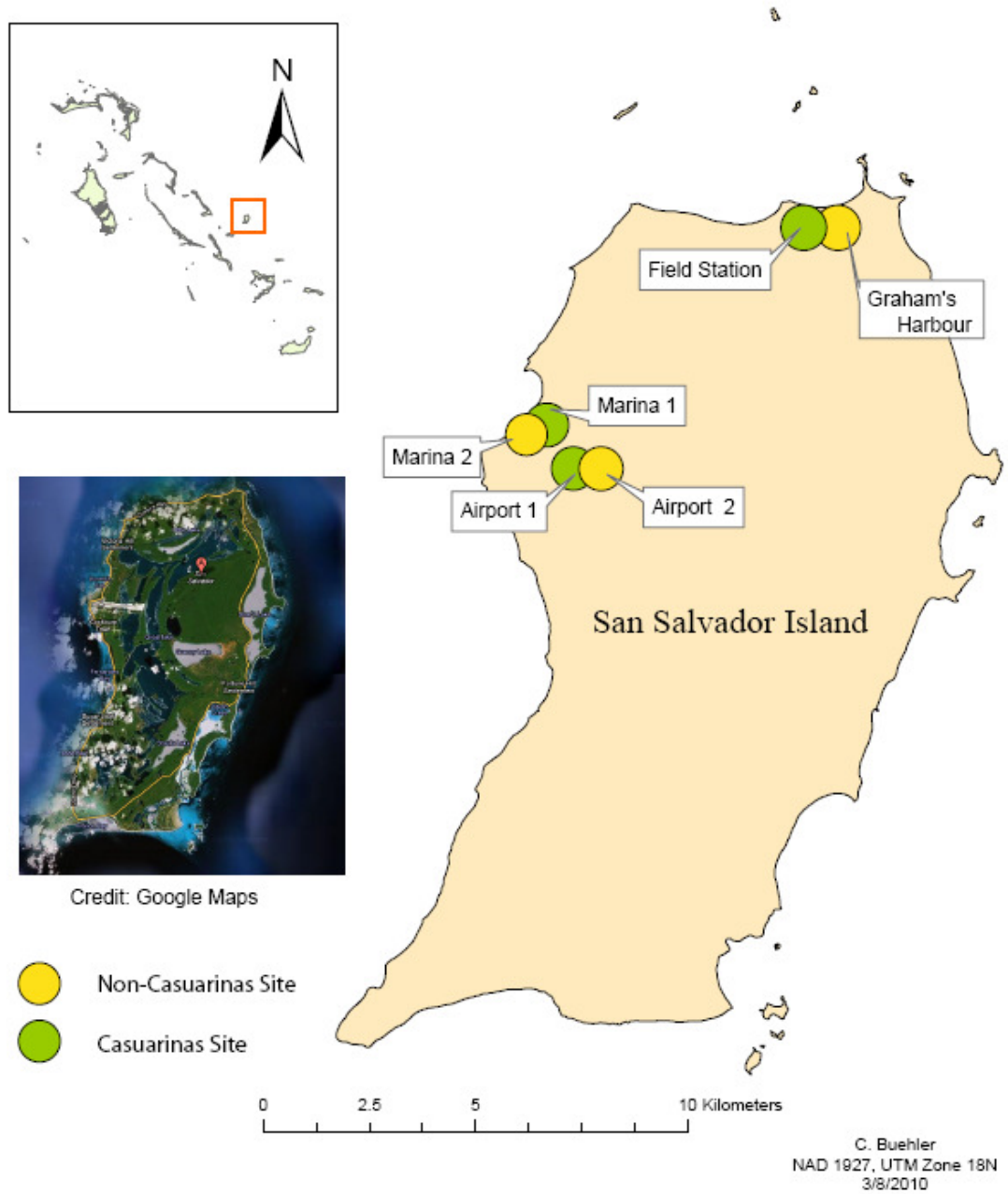


Figure 18 Map of San Salvador Island, The Bahamas depicting study site locations.

active decomposition processes similar to leaf litter decomposition on San Salvador Island. As this allelopathy study was not conducted in natural field conditions, it was impossible to simulate exactly the decomposition processes occurring. It is recognized that the collected fresh leaves were not subjected to natural rainwater and leaching conditions nor microbial decomposition in the soil litter, however, collecting fresh leaves (Batish 1998) or leaf branchlets (Langui 1996) for simulating allelopathy experiments and determining the presence of allelochemicals has been demonstrated in the literature.

*Quercus stellata* Wangenh. (post oak) leaf litter was also collected from Starkville, Oktibbeha County, Mississippi, USA to provide a baseline comparison with Casuarinas leaf litter. *Quercus* was used as a comparison in this study as it is not known to be allelopathic inhibiting radish or bean seed germination. Nevertheless, Abraham et al. (2003) suggest it does contain the allelochemical  $\alpha$ -pinene, which is known to reduce primary root growth in maize, however, not much is known about its affect on other plant species (Singh et al. 2006).

For bioassay extractions, ~200 g leaf material was extracted in 1800 mL RO (reverse osmosis) water in an Erlenmeyer flask (McPherson and Muller 1969). Leaves were not ground prior to addition of water (Ervin and Wetzel 1999). After a 7 day leaching period leachate was passed though filterpaper (Fisherbrand), 510  $\mu$ m-medium porosity, flow rate slow, diameter 15.0 cm to remove particulate material and stored in containers at room temperature until use 24 hours later.

### *Seed Germination*

*Raphanus sativus* (radish) and *Phaseolus vulgaris* (bean) seeds were obtained from Burpee Company and not sterilized prior to use. While bean and radish seeds are not representative of native Bahamian flora, they are relatively easy to grow in a laboratory growth chamber, and at a minimum they will allow to observe if reduced germination and growth induced from allelopathy is possible. Bean seeds were transferred to each well of a sterile 24-well plate that contained 1.0 mL of ultrafiltered UV water (control), 1.0 mL Casuarinas leachate, and 1.0 mL Quercus lechate. Radish seeds were also transferred to each well of a sterile 24-well plate, however, 0.5 mL the test solutions were applied as radish seeds are smaller in size than bean and less solution was required to immerse seeds.

Seed plates were maintained in a Lab-Line Biotronette Plant Growth Chamber (Melrose Park, IL) for the duration of the experiment in the Department of Biological Sciences, Mississippi State University. The growth chamber was maintained at 27°C with light supplied by 13-W Bright Effect fluorescent bulbs and 20-W wide spectrum plant and aquarium fluorescent bulbs at a range of 42-58  $\mu\text{mol}/\text{m}^2/\text{sec}$  photosynthetically active radiation (PAR) with 12 h light/12 h dark at the level of the plates. After 72 hours, radish seeds were observed for germination and radicle growth. Bean seeds were observed for germination and radicle growth after 7 days. In the first two round of growth experiments seeds only applied with control and Casuarinas treatments were studied. In the consecutive four rounds, Quercus was added in order to serve as a comparison to examine and gauge how seeds applied with Casuarinas leachate compared

to seeds applied with another type of leaf leachate not known to be allelopathic. The seeds were considered germinated once the radical emerged beyond the seed coat (Ervin and Wetzel 1999).

### *Seedling Growth*

Seeds of radish and bean for seedling growth experiments were also obtained from Burpee Company and not sterilized prior to use. Seeds were sown in sterile cups filled with a mixture of potting soil and sand totaling a mass of 45 g. The growth chamber was maintained at 27°C with light supplied by 13-W Bright Effect fluorescent bulbs and 20-W wide spectrum plant and aquarium fluorescent bulbs at a range of 42-58  $\mu\text{mol}/\text{m}^2/\text{sec}$  photosynthetically active radiation (PAR) with 12 h light/12 h dark at the level of the plates. Seedlings were applied with 10 mL sterilized ultra filtered UV water every 1-2 days for 7 days.

In the first set of seedling growth experiments, only the control and Casuarinas treatment seedlings were studied. To examine how seedlings applied with Casuarinas leachate compared to seedlings applied with another leaf extract not known to be allelopathic, Quercus was included in the second set of seedling growth experiments.

Once all seedlings were  $\geq 1$  cm in length, the sample was randomly divided into thirds and seedlings were applied with either 10 mL ultrafiltered UV water (control), 10 mL Casuarinas leachate, or 10 mL Quercus leachate. Growth of seedlings were monitored and measured with a handheld ruler from the base of the seedling to the longest leaf every 24 hours for 3 days. After three days of vertical growth, seedlings

expanded horizontally. As a result, no noticeable differences in vertical growth occurred so after three days and the experiment was stopped.

Significant differences in seed germination and growth were tested by permutation tests utilizing CRAN (Comprehensive R Archive Network) because it provides a variety of statistical techniques and as well as produces publication-quality plots ([www.r-project.org](http://www.r-project.org)). Permutations tests are considered a natural generalization of the Wilcoxon-Mann-Whitney test, in which given two samples measuring the same variable data can be compared for possible differences in magnitude (Wilks 2006). Permutation tests, compared to analysis of variance (ANOVA), do not have an assumption of normality so this test can be beneficial for many kinds of ecological data (Taylor 1961; Gaston and McArdle 1994). Instead of normalizing data using transformation matrixes or liner models, permutation tests utilize the re-ordering (permutations) of observations (this study utilized 2000 iterations and sampled with replacement). A  $p$ -value was calculated as the proportion of values obtained under the permutation that were greater than or equal to the observed value (Anderson and Braak 2003). For this study, data were considered significant if  $p < 0.05$ .

#### *Chloroform Extraction/GC-MS Analysis and SPE/LC-MS Analysis*

Compounds from Casuarinas and Quercus leachates were extracted using chloroform (Optima grade, Fisher) (500  $\mu$ L chloroform and 500  $\mu$ L leachate) and analyzed through Gas Chromatography-Mass Spectrometry (GC-MS) as the Mississippi State Chemical Analysis Laboratory. A Varian 3400 Gas Chromatograph and Saturn



2000 Mass Spectrometer were used with a Restek Stabilwax-DA 30 m × 0.25 mm i.d column with a film thickness of 0.25 μm. The Quercus leachate showed several prominent compounds upon preliminary GC-MS analysis, however, the Casuarinas leachate was too dilute to yield relevant compounds for mass spectral library identification. Solid Phase Extraction (SPE) was utilized to concentrate the Casuarinas leachate 100x. The Quercus leachate was also concentrated 10x using SPE. Supelco Supelclean LC-18 3-mL columns were activated with 10 mL of methanol (Optima grade, Fisher) followed by a wash of 10 mL of water (Optima grade, Fisher). Casuarinas leachate (100 mL) and Oak leachate (10 mL) were passed through the columns and then washed with 10 mL water followed by elution of retained compounds using 3 × 1 mL methanol. GC-MS analysis of the concentrated leachate samples showed no retention of distinct compounds suggesting that analysis by Liquid Chromatography-Mass Spectrometry (LC-MS) needed to be performed due to lack of volatile components. An Agilent 1100 Liquid Chromatograph and Bruker Esquire~LC Mass Spectrometer were used with a Phenomenex Kinetex 150 x 4.6 mm C18 2.6 μm column.

## Results

### *Seed germination/seedling growth*

Results indicate that bean and radish seeds applied with both Casuarinas treatment and Quercus treatment had reduced seed germination (Figure 19).

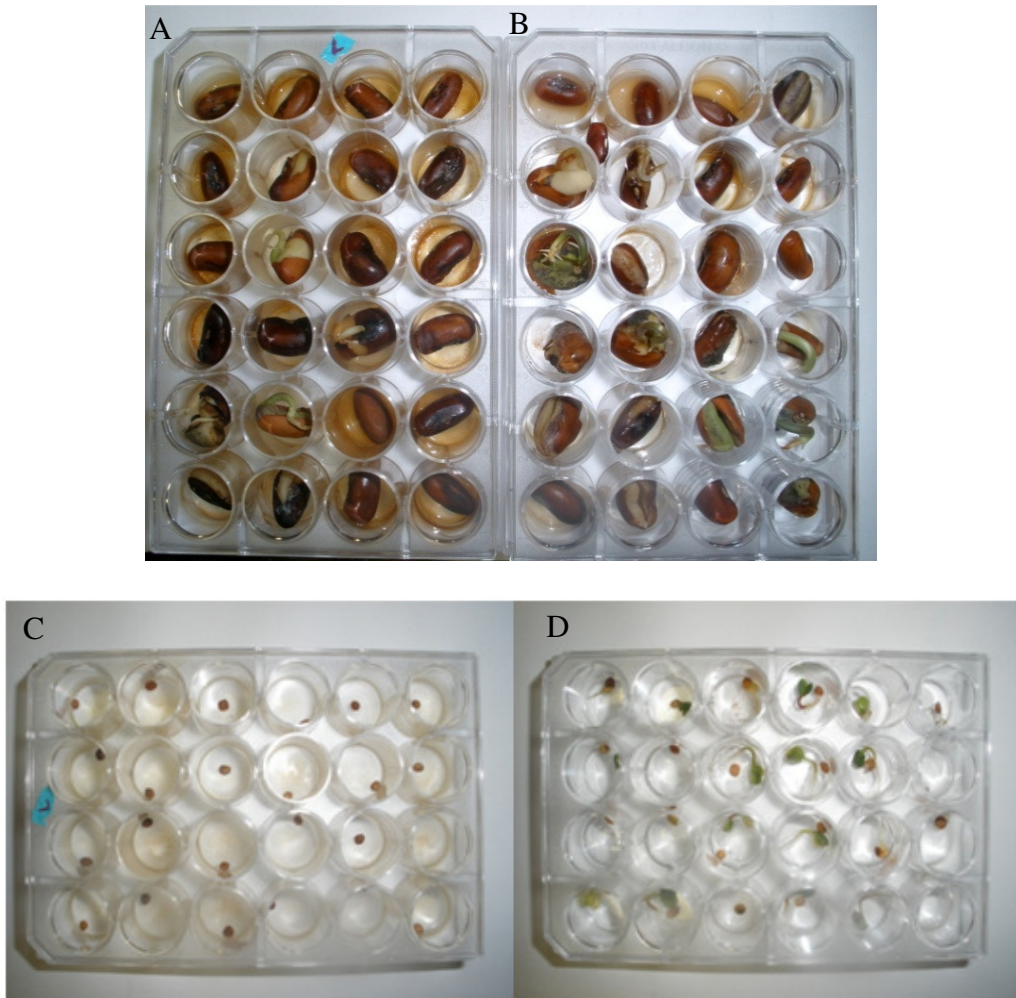


Figure 19 Bean seeds applied with Casuarinas treatment (A) after 7 days compared to bean seeds applied with control (B). Radish seeds applied with Casuarinas treatment (C) after 3 days compared to radish seeds (D) applied with control.

Seeds applied with treatment demonstrated noticeable germination reduction.

Specifically, results from the seed germination study indicated only 8.3-25% of the bean seeds applied with Casuarinas treatment over the six trials germinated compared to 20.8-37.5% germination of seeds applied with the control treatment over six trials (Figure 20).

Trial 1 and 2 demonstrated the largest germination by roughly 20% for the bean seeds applied with Casuarinas treatment compared to Trial 3 and 6 which demonstrated 15% germination. Trial 4 showed no germination of bean seeds when applied with Casuarinas treatment.

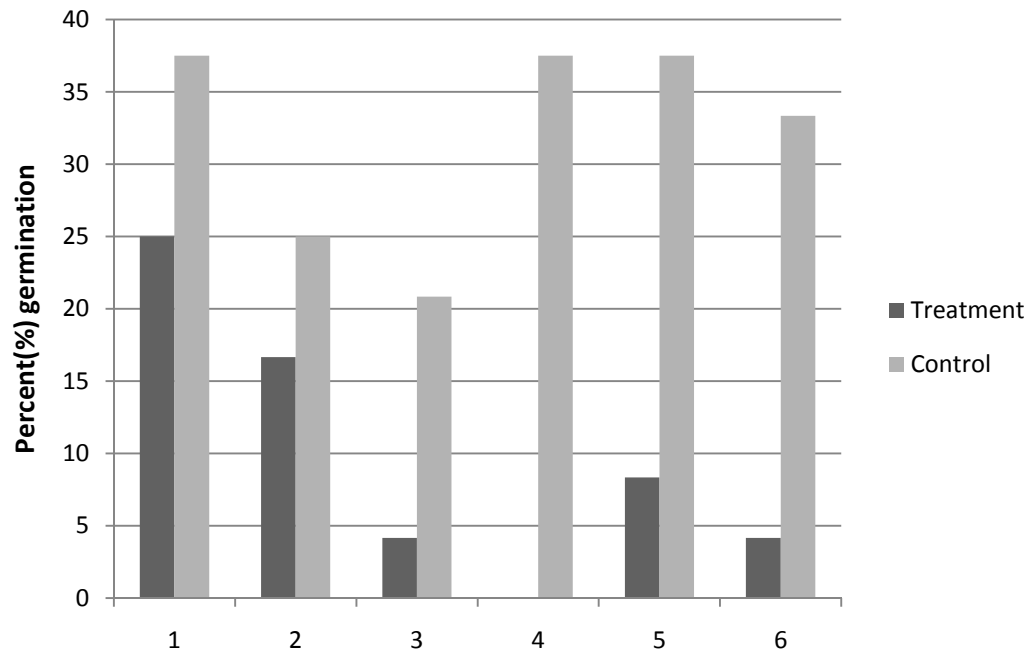


Figure 20 Percent germination of bean seeds when applied with Casuarinas treatment and control treatment during six trials.

Radish seeds applied with Casuarinas treatment had 0-12.5% germination over six trials compared to 70.8-87.5% germination of the seeds applied with the control (Figure 21). Trial 3 had the most seeds applied with Casuarinas treatment germinate compared to

trials 4, 5, and 6. No germination of radish seeds applied with Casuarinas treatment occurred in trials 1 and 2 compared to the control.

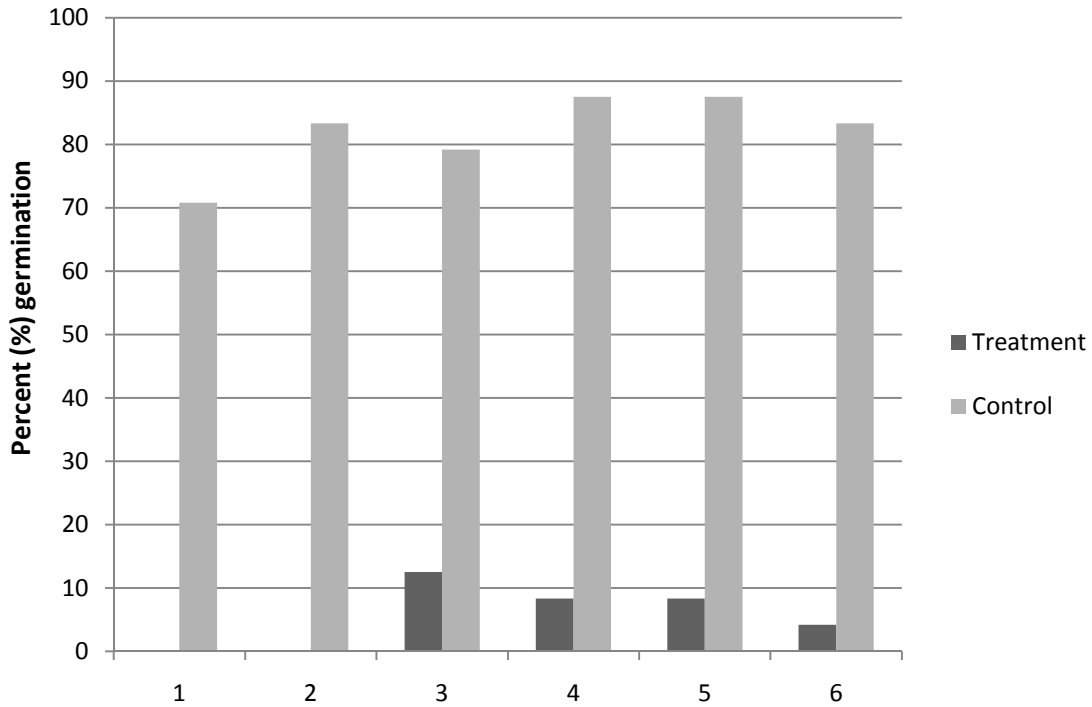


Figure 21 Percent germination of radish seeds applied with Casuarinas treatment and control during six trials.

Interestingly, radish seeds applied with *Quercus leachate* also showed reduced germination. Only 0-8.3% of radish seeds applied with *Quercus leachate* germinated compared to the control in which 70.8-87.5% germinated (Figure 22). Trial 5 showed 4% more germination of the seeds applied with *Quercus* treatment compared to the other

six trials. Trials 1 and 4 showed no germination of seedling applied with Quercus treatment compared to the control.

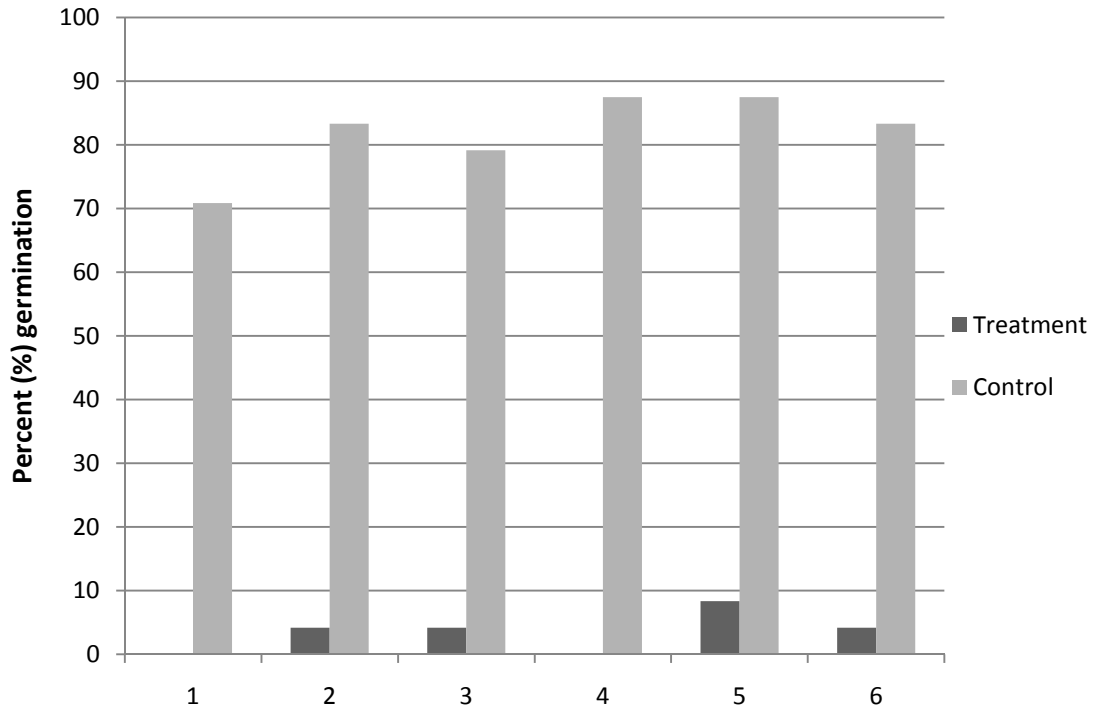


Figure 22 Percent germination of radish seeds applied with Quercus treatment compared to seeds applied with control treatment with six trials.

Similarly, Quercus had the same effect on reducing bean seed germination. Bean seeds applied with Quercus treatment had germination of 8.3-20.8% compared to the control in which bean seeds had a germination of 20.8-37.5% (Figure 23). Bean seeds applied with Quercus treatment had more germination than radish seeds applied with Quercus treatment by an average of 14%. Trial 1, 3, and 4 showed the greatest percent germination of the seeds applied with Quercus treatment, however, Trials 5 and 6

demonstrated the greatest difference in germination between seeds applied with Quercus treatment and control treatment by roughly 70%.

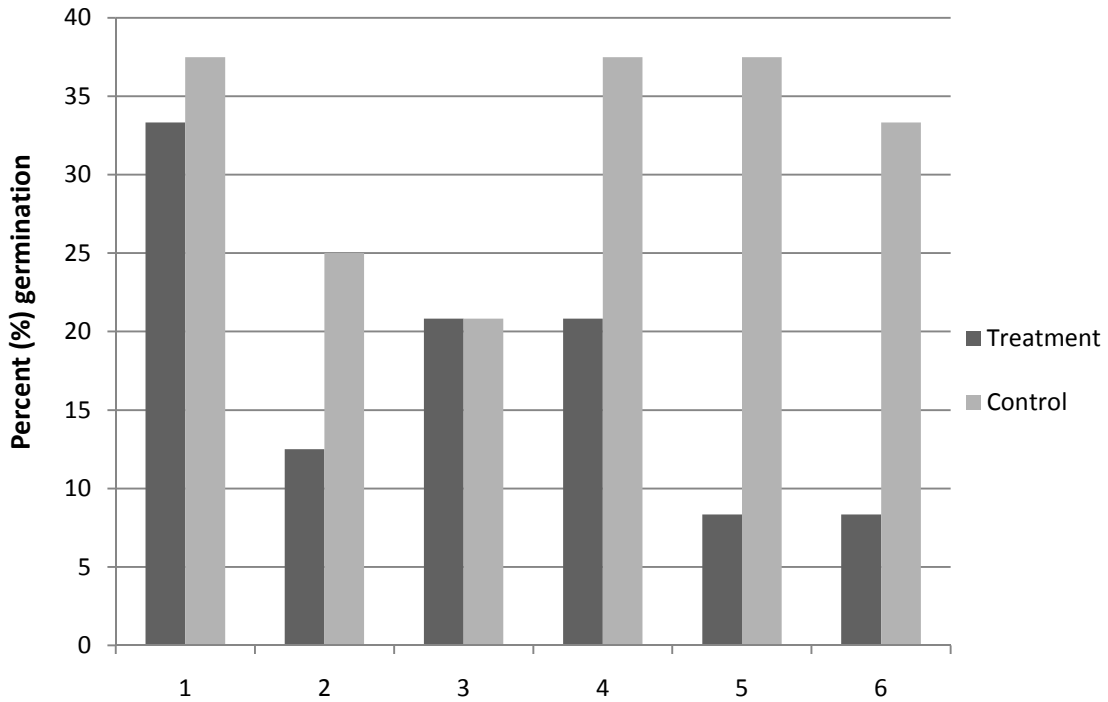


Figure 23 Percent germination of bean seeds when applied with Quercus treatment and control with six trials.

Radish seedlings applied with Casuarinas leachate demonstrated 10% suppression in growth during the first 24 hrs of observation (T1), however, it was not statistically significant ( $p > 0.05$ ). Radish seedlings applied with Casuarinas treatment in the second 24 hours of observation (T2) demonstrated a 26% suppression in growth compared to the control, however, this was also not statistically significant ( $p > 0.05$ ) (Figure 24). Growth

of radish seedlings applied with Casuarinas treatment in the third 24 hours of observation (T3) was suppressed by 6% and also not significant ( $p > 0.05$ ) compared to the control.

The greatest radish seedling growth for seedling applied with Casuarinas treatment and applied with the control treatment during the duration of the experiment occurred during the first 24 hour interval (T1). In fact, seedlings applied with Casuarinas leachate had 50% more growth in T1 than in either T2 or T3 ( $p < 0.05$ ). By the last interval, however, T3 seedlings applied with Casuarinas leachate showed the least difference when compared to the control and this variation was not significant ( $p > 0.05$ ). Overall, no significant differences were observed between seedlings applied with Casuarinas treatment and seedlings applied with control treatment for each time interval, however, differences were present in growth suppression comparing growth in T1 to growth in T3 ( $p < 0.05$ ).

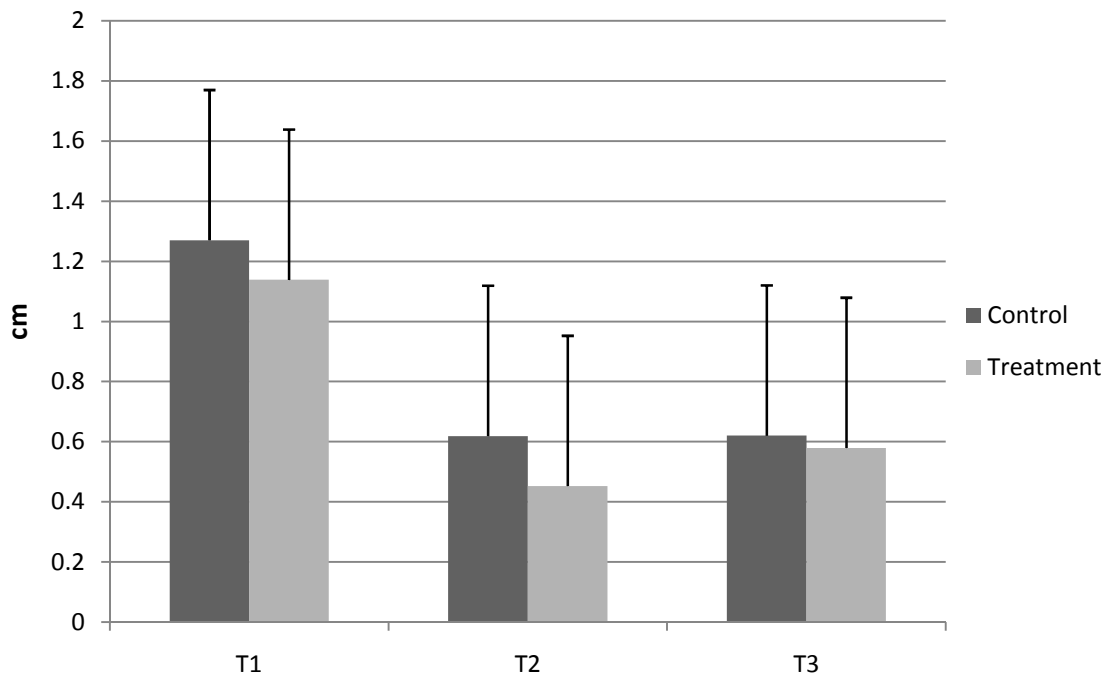


Figure 24 Comparison of growth between radish seedlings applied with control treatment and seedlings applied with Casuarinas treatment. No significant difference ( $p > 0.05$ ) of seedling growth existed between control treatments and Casuarinas treatment for each time interval.

Much like the radish seedlings applied with Casuarinas treatment, the radish seedlings applied with Quercus treatment appeared to have suppression in growth which was only minimally significant when compared to seedlings applied with the control treatment ( $P = 0.052$ ; Figure 25). Seedlings applied with Quercus leachate illustrated 50% more growth in T1 than in either T2 or T3, which was statistically significant ( $p < 0.05$ ). However, by the last interval, T3 seedlings applied with Quercus leachate showed the least variability when compared to the control and this difference was not significant ( $p > 0.05$ ). Overall, a very slight difference may be present ( $p = 0.052$ )



between seedling growth of those seedlings applied with Quercus treatment and seedlings applied with control treatment for each time interval. A significant difference ( $p < 0.05$ ) was also present in growth suppression comparing seedling growths in T1 to seedling growths in T3.

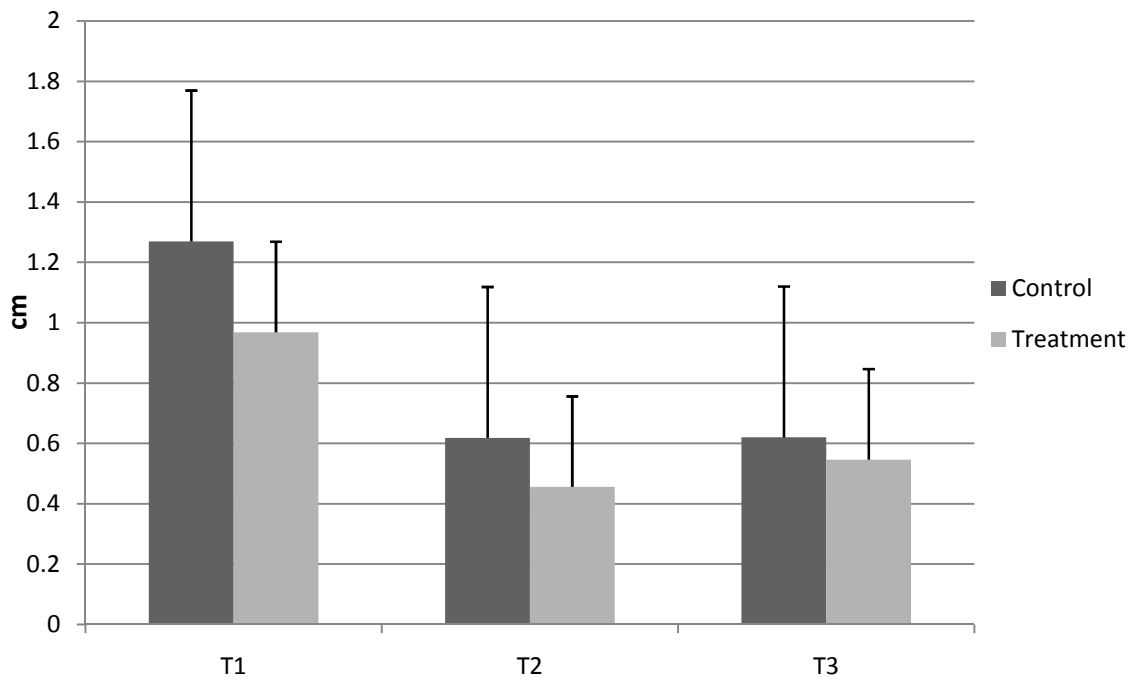


Figure 25 Average radish seedling growth applied with Quercus treatment at 24 hr intervals (T1, T2, T3) for 72 hrs. Only a slight difference of seedling growth existed between control and treatment per time interval ( $p > 0.05$ ). Seedling growth in T1 was significantly greater than T3 ( $p < 0.05$ ).

Bean seedlings were also applied with Casuarinas treatment, however, the sample size for bean seedlings (N=10) was too small to compute meaningful statistics, thus suppression in growth is demonstrated in centimeter (cm) and percent difference between

samples. In the first 24 hours of observation (T1) and in second 24 hours of observation (T2) seedlings applied with Casuarinas treatment appeared to be suppressed by almost 40% compared to the control (Figure 26). Conversely, T3 seedlings applied with treatment were observed to have grown roughly 30% more than the control. The difference in growth comparing the control in T1 and the control in T3 is markedly different by about 60%. The seedling applied with Casuarinas treatment did not seem to have as much variability in growth as the seedlings applied with control. Overall, the seedling applied with the control grew roughly 70% more in T1 and T2 than the seedlings applied with the Casuarinas treatment.

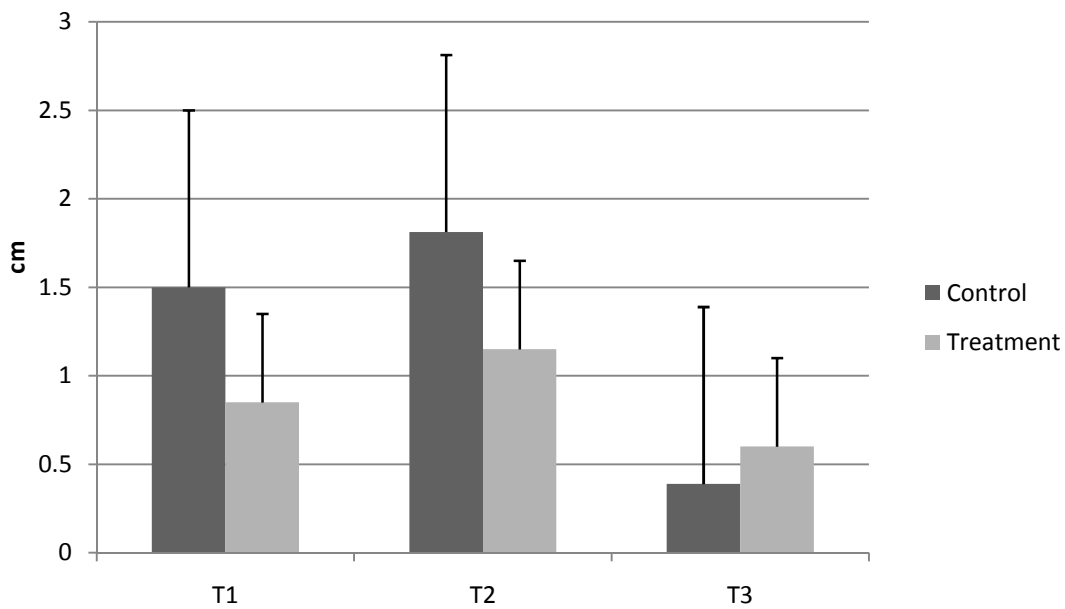


Figure 26 Comparison of growth between bean seedlings applied with control and seedling applied with Casuarinas leachate. T1 and T2 show more growth of seedlings applied with control compared to seedlings applied with Casuarinas treatment. T3 demonstrates more growth.

Bean seedlings were also applied with Quercus treatment, however, the sample size for bean seedlings (N=10) was also too small to do meaningful statistics, so, suppression in growth is demonstrated in centimeters (cm) and percent difference between samples. In the all of the time intervals of observation (T1, T2, and T3) seedlings applied with Quercus treatment appeared to be suppressed on average by roughly 50% compared to the control (Figure 27). The difference in growth comparing the control in T1 and T2 to the control in T3 is markedly different by at least 60%. The seedlings applied with Quercus treatment did not seem to have as much variability in growth as the seedlings applied with control. T1 demonstrated the greatest growth of seedlings applied with Quercus treatment followed consecutively by T2 and T3. Overall, the seedling applied with the control demonstrated to grow slightly more than the seedlings applied with the Quercus treatment.

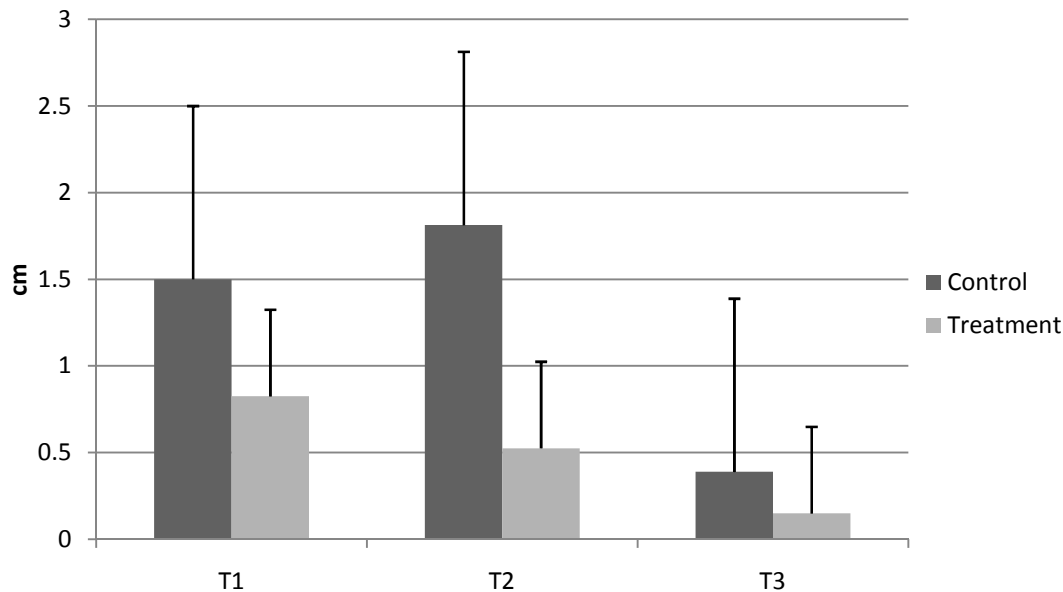


Figure 27 Comparison of growth between bean seedlings applied with control treatment and seedlings applied with Quercus treatment. T1 and T2 show more growth of seedlings applied with control treatment compared to seedlings applied with Quercus treatment.

To further investigate how seedlings differed, wet and dry mass measurements were taken in order gauge how biomass content differed between seedlings applied with Casuarinas treatment, seedling applied with Quercus treatment, and seedling applied with the control treatment. Radish seedlings applied with Casuarinas treatment showed no significant differences (wet minus dry) in mass from seedlings applied with Quercus treatment ( $p= 0.337$ ) or the control ( $p= 0.329$ ; Figure 28). In terms of wet mass, no significant differences existed between the seedlings applied with Casuarinas treatment and the control ( $p = 0.335$ ), or Quercus treatment ( $p= 0.993$ ), or between the control and Quercus treatment ( $p= 0.299$ ). However, seedlings applied with Quercus treatment did

show a slightly greater dry mass by 30% than either Casuarinas ( $p= 0.029$ ) or the control ( $p= 0.027$ ). Although differences exist in dry mass between seedlings applied with Quercus treatment and seedlings applied with Casuarinas treatment and control, the difference between wet and dry mass measurements were not statistically different between seedlings.

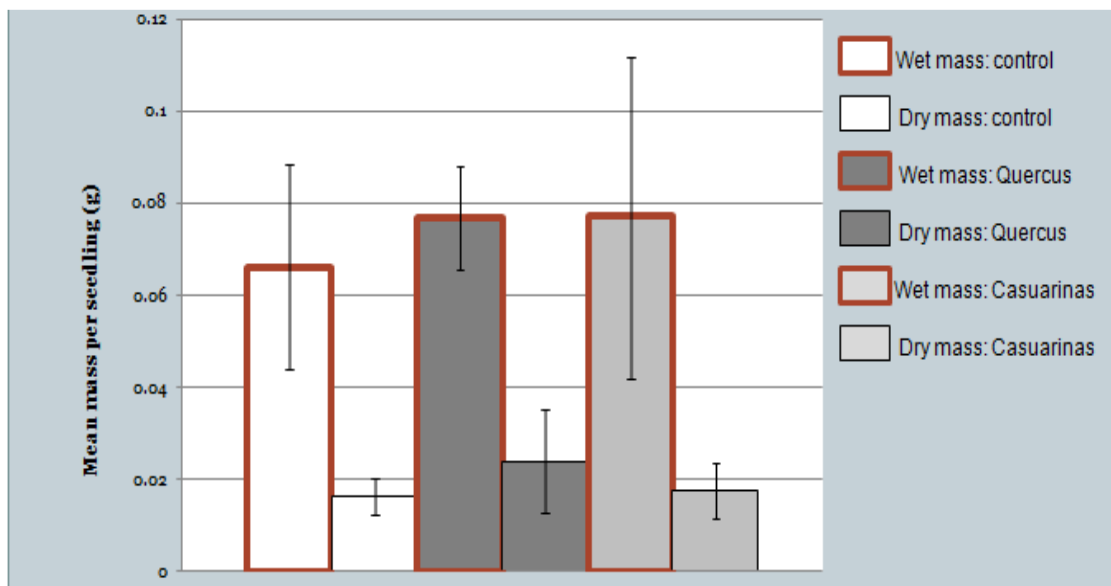


Figure 28 Fresh and dry mean mass per radish seedling. Dry mass of Casuarinas treatment seedlings was not significantly different from the dry mass of the control treatment seedlings ( $p= 0.328$ ) or Quercus treatment seedlings ( $p= 0.336$ ). Wet mass of the control treatment seedlings was also not significantly different from the wet mass of the seedlings applied with Casuarinas treatment ( $p=0.335$ ) or Quercus treatment ( $p= 0.298$ ).

### *LC-MS Analysis*

Various compounds were detected by Liquid Chromatography-Mass Spectrometry (LC-MS) within Casuarinas leachate but were absent within Quercus leachate (Table 2). Compounds present in leachates were identified by their mass-to-charge ratios ( $m/z$ ), which is the relationship between the ion mass and the number of charges that a given ion carries (Communication with Holmes and McDaniel, 2010). LC-MS only reports mass-charge ratio. Seven unidentified compounds were unique, based on the mass-to-charge ratio, to Casuarinas leachate while five compounds were unique to Quercus leachate.

Table 2 LC-MS analysis of compounds detected in Casuarinas leachate and Quercus leachate. Six compounds found in Casuarinas leachate were not present in Quercus leachate. The leachates shared one compound in common, however, it was 100x more intense in Casuarinas leachate.

<i>m/z</i>	Casuarinas	Intensity	Quercus	Intensity
<b>217.1</b>	--		X	
<b>267.2</b>	X		--	
<b>303.2</b>	X		--	
<b>305.4</b>	--		X	
<b>311.3</b>	--		X	
<b>315.3</b>	X		--	
<b>317.4</b>	X		--	
<b>350.4</b>	--		X	
<b>451.1</b>	X	100	X	1
<b>472.3</b>	X		--	
<b>473.4</b>	X		--	

## Discussion

Data show *Casuarinas equisetifolia* have potential for allelopathy. As this investigation illustrated, radish and bean seed germination with *Casuarinas* treatments were significantly suppressed compared to a control. Because laboratory germination experiments demonstrated significant bean and radish germination reduction when applied with *Casuarinas* treatment, it may be evidence to suggest how *Casuarinas* exude potential allelochemicals which enhances its ability to achieve dominance and facilitate its existence in invaded communities.

Although bean and radish seeds showed suppression in germination when applied with *Casuarinas* leachate, radish and bean also showed suppression in germination when applied with *Quercus* leachate. While *Quercus* is not known to be allelopathic to other plant species (Singh et al. 2006) a study conducted by Abraham et al. (2003) suggests *Quercus* contains the allelochemical  $\alpha$ -pinene which is known to suppress primary root growth in maize. It is possible, therefore, *Quercus* also has the potential to suppress radish and bean seed germination through the release of biochemicals.

As some quantified analyses have been already conducted on the allelopathic potential of *Casuarinas* on certain cultivar, data do exist for a comparison. The results from this study can be corroborated with Langui (1996), Batish (1998) and Srinivasan et al. (1996) who suggest that *Casuarinas* have the potential to modify soil properties and exude allelochemicals which inhibit the growth of vegetation. These studies also suggest the certain allelochemicals released from *Casuarinas* include kaempferol-3- $\alpha$ -rhmanoside,



quercetin-3- $\alpha$ -araboside, luteolin-3', 4'-dimethoxy-7- $\beta$ -rhamnoside, kaempferol-3- $\beta$ -dirhamnoside, and quercetin-3- $\beta$ -glucoside (Langui 1996).

Other invasive species which have aggressive colonization habits are also suggested to employ allelopathy-- which lends evidence to the idea that Casuarinas may also employ this mechanism to achieve dominance in its host community. For example, *Centaurea maculosa* (spotted knapweed) contains the allelochemical catechin which has demonstrated strong allelochemical properties in laboratory simulations and field conditions (Broz and Vivanco 2006). Inderjit and Foy (1999) found that *Artemisia vulgaris* (mugwort) suppresses seedling growth of *Trifolium pretense* (red clover). Further investigation by Inderjit and Foy (1999) indicate that *T. pretense* appear to also release phenolic allelochemicals. Reports of *Chromolaena odorata* (Jack in the Bush) from Adetayo et al. (2005) suggest extracts effect germination and seedling growth of *Vigna unguiculata* (cowpea), *Zea mays* (maize), *Glycine max* (soybean), and even itself. Bousquet-Mélou et al. (2005) have studied the invasive shrub *Medicago arborea* (moon trefoil) and suggest that it also demonstrates allelopathy as leaf extracts were shown to inhibit seed germination and seedling growth of *Linum strictum* (upright flax), *Lactuca sativa* (garden lettuce) and *Lepidium sativum* (garden cress pepperweed).

Regarding the radish seedling growth study, data show the inhibitory effects of Casuarinas leachate were not significant compared to the control. The fresh and dry mass of radish seedlings applied with Casuarinas treatment was also measured and results indicated Casuarinas seedling wet mass and dry mass were not significantly ( $p > 0.05$ ) different from the wet mass and dry mass of the seedlings applied with the control

treatment. However, seedlings applied with Quercus treatment did show to have a slightly greater dry mass by 30% than either Casuarinas dry mass or the control dry mass.

While radish seeds showed significant reduction in germination, radish seedlings did not show markedly differing growth rates. According to McPherson and Muller (1969) and their study of the allelopathic effects of *Adenstoma fasciculatum*, vegetation in the soil of mature *A. fasciculatum* stands is thought to be suppressed from physical factors such as light, water, and nutrient availability. If this is indeed true, then radish seedlings applied with Casuarinas treatments would not necessarily demonstrate growth suppression in growth chamber conditions-- as light, water, and nutrient availability from the soil were not limited during the study. This hypothesis provides a possible explanation as to why radish *seedling growth* in the laboratory was not suppressed when applied with Casuarinas treatment because the seedling environment was not stressed. This hypothesis does not, however, explain why radish *seed germinations* were suppressed when applied with Casuarinas treatment. One idea is that because radish seedlings were sown in standard potting soil, compared to radish seeds which were sown in sterile 24-well plates with only treatment solutions, significant reduction in seed germination may have been because no soil was present to interact with the leachate compounds. Tang et al. (1995) suggest nutrient poor habitats often have greater abundances of secondary metabolites (and therefore a greater potential for the presence of allelochemicals) compared to nutrient rich site and also suggest that under stressful environments such as drought or limited nutrients, allelochemicals may suppress the growth of other plants. Consequently, because seedlings in this growth chamber study

were grown in standard nutrient rich potting soil instead of in nutrient poor soil, potential allelochemical interactions from Casuarinas leachate may have been suppressed due to the nutrient in the potting soil.

In natural field conditions soil from sites dominated by Casuarinas is suggested to be nutrient poor (Swearingen 1997), which may be a reason why native plants are inhibited from growing in areas dominated by Casuarinas. Nutrient poor soils are also suggested (Tang et al. 1995) to have greater abundances of secondary metabolites. This may explain why Casuarinas have the ability to form dense monocultures (Swearingen 1997; Hammerton 2001). This hypothesis that Casuarinas dominate areas which also have lower abundances of soil nutrients is corroborated by the soil analyses (conducted in Chapter II) as well as the idea that Casuarinas are N<sub>2</sub> fixers—species which containing nitrogen fixing bacteria in its root system which allow it to thrive in nutrient poor soil.

Because the laboratory environment differs inherently from field conditions in which allelochemicals interact within the soil and with soil microorganisms, obtaining accurate results can be problematic (Blanco 2007). Although laboratory conditions do not exactly mimic *in situ* conditions, various studies do suggest allelopathy is observable in bioassay and greenhouse experiments (Feo 2002; Ervin and Wetzel 2003; Fitter 2003; Hierro and Callaway 2003; Callaway and Ridenour 2004; Weir et al. et al. 2004; Orr et al. 2005; Broz and Vivanco 2006; Broeckling and Vivanco 2008; and Inderjit et al. 2008). Feo et al. (2002) studied the allelopathic properties of *Ruta graveolens* L. (rue) on radish seed germination and found allelopathic activity inhibited germination by 60% and radicle growth was reduced by 53% (Aliotta et al. 1996; Feo 2002). *Chromolaena*

*odorata* (Jack in the Bush) is known to exude biochemicals which inhibited germination and seedling growth of *Vigna unguiculata* (cowpea) by 14%, *Glycine max* (soybean) by 8%, and even itself by 87% (Adetayo et al. 2005). Fernandez et al. (2006) studied *Pinus halepensis* which showed to inhibit germination of *Linum strictum* (upright flax) by 40-60%. Similarly, Bousquet-Mélou et al. (2005) studied the invasive shrub *Medicago arborea* (moon trefoil) and suggest that it also demonstrated allelopathy as leaf extracts where shown to significantly inhibit seed germination and seedling growth of *Linum strictum* (upright flax), *Lactuca sativa* (garden lettuce) and *Lepidium sativum* (garden cress pepperweed). As evidence from the studies, research done in the laboratory on seed and seedling germination and growth suppression are numerous and provide evidence that allelopathy can be observable in laboratory simulations which can then be applied to the understanding of the allelopathy in natural conditions.

Few quantitative studies exist on the potential for Casuarinas to suppress germination and growth of seeds and seedlings in a laboratory, however, the studies that so exist which suggest leachate from Casuarinas inhibits germination of common cultivars or radish and bean (Langui 1996; Batish 1998) seeds corroborates with this study's findings.

LC-MS analyses of Casuarinas leachate indicate Casuarinas may employ allelopathy as the presence of a known allelochemical was potentially detected. A compound of that of chalepin, a coumarin compound, was potentially identified. The molecular formula for chalepin is  $C_{19}H_{22}O_4$  with a molecular weight of 314.37 g/mol. It is recognized that coumarin and its derivatives are ubiquitous in plants as secondary

metabolites (Vyvyan 2002; Orlita et al. 2008) and have shown to inhibit seed germination and growth of various plants such as radish, zucchini, cauliflower, and tomato (Vyvyan 2002; Anaya et al. 2005). Feo et al. (2002) suggests radicle growth of radish seeds, when applied with an aqueous extract of *Ruta graveolens* (rue), may have been inhibited by coumarin as well as other compounds including valeric acid which inhibited germination 100%, salicylate inhibited germination by 90%, and  $\alpha$ -pinene which inhibited germination by 68%. As a potential allelochemical detected in *Casuarinas leachate*, chalepin may be a compound which assisted in suppressing radish and bean germination and radicle growth.

It is known the soil environment influences residence times of chemicals, their fate, and how they interact with microbes in the rhizosphere (Interjit et al. 2008). For example, the compounds glucose and phenylalanine are not known to be allelopathic, however, when they interact with *p*-coumaric acid released from *Ipomea violacia* (morning glory) the allelopathic ability of *p*-coumaric acid is known to increase (Weir et al. 2004).

These results show that *Casuarinas* could limit the establishment of other species by affecting the growth of species already present within the environment by exuding allelochemicals. It is recognized that biochemical interactions within the soil are complex and determining the presence of specific allelochemicals can be challenging as chemicals are continuously influenced by various factors such as acidity, soil moisture, organic matter content, and soil microbes (Barrilleaux and Grace 2000; Inderjit and Mallik 1997; Leicht-Young 2009).

## Conclusion

An investigation was conducted to measure differences in germination and growth of radish and bean seed and seedlings applied with Casuarinas treatment, Quercus treatment, and control treatment. Data indicated that germination of bean and radish seeds was significantly suppressed by Casuarinas treatment as well as Quercus treatment. Radish seedlings applied with Casuarinas leachate did not show significant ( $p > 0.05$ ) suppression in growth for each 24 hour interval compared to the control nor did seedlings applied with Quercus treatment demonstrate statistically significant ( $p > 0.05$ ) suppression in growth for each 24 hour interval compared to the control. While bean seedlings did not comprise a large enough sample size for statistical analysis, the experiment did pose as an initial study which suggests bean seedlings, like radish seedlings, may not be markedly suppressed in growth when applied with Casuarinas treatment. The difference in wet and dry mass of radish seedling applied with Casuarinas treatment was not significant ( $p > 0.05$ ) from the control. These laboratory studies, while not describing native plant species interaction with Casuarinas, are still an effective measure for assessing the potential affect the presence of Casuarinas have on vegetation. It would have been ideal in this study to have obtained native plant species on San Salvador, The Bahamas to better evaluate how Casuarinas influence their germination and growth, however, due to international regulations banning the transport of native flora, this was not possible.

Ecological holes are present in this research, as no native flora were used in the laboratory growth simulations. Because allelochemicals also interact within the

rhizosphere, it is recognized that soil conditions *in situ* differed markedly than the soil utilized in laboratory. The small scale nature of this study also inherently affected the sample size of the greenhouse experiments. Using more and different types of common cultivars for comparison and testing how seedlings differed in growth with varying light and treatment concentrations should also be investigated.

These quantitative analyses are important because results signify that *Casuarinas* have the potential to reduce seed germination and even perhaps reduce seedling growth. Understanding the mechanisms by which this invasive species modifies its environment by potentially suppressing germination and growth of native vegetation is an important step to implement better management and eradication strategies. Understanding the impact of the invasive *Casuarinas equisetifolia* provides a model case study to assess the role of invasive species on the environment. There is an essential need for more research as this plant undeniably has a detrimental effects on its environment and few studies exist identifying how *Casuarinas* modify their environment-- particularly in the West Indies where it is considered especially noxious and considered to alter the Bahamian landscape.

## CHAPTER IV

### CONCLUSION

*Casuarina equisetifolia* (Australian pine) is an invasive species which is known to be particularly noxious. Little is known about the impact it may have on the environment in the West Indies so this investigation's focus was to quantitatively compare the physical and chemical soil differences between sites dominated by Casuarinas and sites devoid of Casuarinas located on San Salvador Island, The Bahamas. It was demonstrated that sites dominated by Casuarinas had significant ( $p < 0.05$ ) differences in some soil properties compared to sites devoid of Casuarinas. It is suggested, therefore, that Casuarinas have the potential to modify their environments to create a positive feedback in which they perpetuates their own existence.

This investigation also conducted a series of greenhouse experiments in order to determine Casuarinas potential for allelopathy on *Phaseolus vulgaris* (bean) and *Raphanus sativus* (radish) seed and seedlings. Greenhouse experiments indicated a significant ( $p < 0.05$ ) reduction of radish and bean seeds germination when applied with Casuarinas treatment. However, radish and bean seedlings applied with Casuarinas treatment did not demonstrate significant suppression ( $p > 0.05$ ) in growth compared to the control. The difference in wet and dry masses of radish seedlings applied with



Casuarinas leachate was also not significant ( $p>0.05$ ). While the seedling laboratory simulation did not demonstrate suppression in growth, it was observed that leachate from Casuarinas did reduce seed germination. With these results, Casuarinas may have the potential to modify their environments whereby reducing or suppressing native flora from germination and growing.

Casuarinas are not the only invasive species suggested to modify its environment. *Tamarix* spp., with its rapid regeneration rate, is recognized to change nutrient cycling as well as contribute to geomorphological changes along stream banks (Invasive, 2006). *Myrica faya* (myrica) colonizes young volcanic soil whereby changing nutrient availability and community stand structure (Vitousek 1990). *Eichornia crassipes* and *Pistia stratiotes* have shown to increase siltation rates by slowing water flows (Schmitz et al. 1993). *Centaurea diffusa* inhibits native species ability to acquire phosphorous in the soil and is also suggested to suppress growth of native species by means of releasing biochemicals into the soil (Callaway and Ridenour 2004). *Chromolaena odorata* (Jack in the Bush) is suggested to effect germination and seedling growth of *Vigna unguiculata* (cowpea), *Zea mays* (maize), *Glycine max* (soybean), and even itself (Adetayo et al. 2005). Bousquet-Mélou et al. (2005) have studied the invasive shrub *Medicago arborea* (moon trefoil) and suggest that it also demonstrates allelopathy as leaf extracts were shown to inhibit seed germination and seedling growth of *Linum strictum* (upright flax), *Lactuca sativa* (garden lettuce) and *Lepidium sativum* (garden cress pepperweed). *Ruta graveolens* L. (rue) inhibited radish seed germination and allelopathic activity was found to suppress germination by 60% and radicle growth by 53% (Feo et al. 2002). These

studies illustrate that invasive species can modify their environments and leading to the exclusion of native species.

Understanding how Casuarinas alter their environment will ultimately provide information for better management and eradication strategies. For example, management techniques could be implemented in which leaf litter is collected and disposed of instead of left to leach into the soil. Ideally, not allowing Casuarinas to spread into new host communities would be best option for management. Perhaps making the accessibility more stringent for obtaining Casuarinas seedlings for landscaping would help decrease its spread. Nevertheless, Casuarinas are an invasive force without the aid of humans to facilitate their spread; therefore, eradication techniques would be critical to have in place for areas that are already colonized.

Future research should investigate nutrient cycling in Casuarinas dominated areas which may lead to a better understanding of rhizosphere dynamics between soil microbes and native vegetation. Also simulating more greenhouse experiments in which native vegetation is utilized to gauge seed and seedling germination and growth is necessary. Having simulations more like *in situ* soil characteristics would produce more accurate results that are more apropos to natural field conditions. Overall, this research is significant because understanding the roles of invasive species, like the Casuarinas, may provide new case studies for understanding how invasive species contribute to environmental modification. Understanding more about Casuarinas' effects on soil and its potential to employ allelopathy will provide essential information as to how and to what extent Casuarinas modify communities. Studying invasive plant dispersal and their

affect on the environment is pertinent to the study of Geosciences as these investigations are spatial in nature. The environmental harm associated with invasive species like Casuarinas will, hopefully, lead to increased awareness about how invasive species can create disturbances that result in permanent changes to an ecosystem.

## REFERENCES

- Abraham, D., L.Takahashi, A.M. Kelmer-Bracht, E.L. Ishii-Iwamoto. 2003. Effects of phenolic acids and monoterpenes on the mitochondrial respiration of soybean hypocotyls axes. *Allelopathy*. 11:21-30.
- Adetayo O.B., Lawal I.O., Alabi B.S., O.F. Owolade. 2005. Allelopathic effect of siam weed (*Chromolaena odorata*) on seed germination and seedling performance of selected crop and weed species. In: *Proceedings of IV World Allelopathy Congress, Australia*. 348–351.
- Aliotta, G., Cafiero G., De Feo, V., Palumbo ,A.D., Strumia, S., 1996. Infusion of rue for control of purslane weed: biological and chemical aspects. *Allelopathy Journal*. 3: 207–216.
- Anaya, Ana Luisa, Martha Macías-Rubalcava, Rocío Cruz-Ortega, Clara García-Santana, Perla N. Sánchez-Monterrubio, Blanca E. Hernández-Bautista, Rachel Mata. 2005. Allelochemicals from *Stauranthus perforatus*, a Rutaceous tree of the Yucatan Peninsula, Mexico. *Phytochemistry*. 66: 487-494.
- Anderson M.J., Braak, C.F.J.T. 2003. Permutation tests for multi-factorial analysis of variance. *Journal of Statistical Computation and Simulation*. 73(2): 85-113.
- Bais, H.P., Vspachedu R., Gilroy,S., Callaway, R.M., Vivanco, J.M. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*. 301:1377–1380.
- Barney, J.N., Hay, A.G., Weston, L.A. 2005. Isolation and characterization of allelopathic volatiles from mugwort (*Artemisia vulgaris*). *Journal of Chemical Ecology*. 31:247–265.
- Barrilleaux, T.C., and J.B. Grace. 2000. Growth and invasive potential of *Sapium sebiferum* ( Euphorbiaceae) within the coastal prairie region: the effects of soil and moisture regime. *American Journal of Botany*. 87(8): 1099-1106.

- Batish, D.R., and H.P. Singh. 1998. International Union of Forestry Research Organization Conference on Environmental Forest Science, Division 8.
- Biochemistry: an analysis of global change. 1997. 2<sup>nd</sup> Ed. Schlesinger, W.H: Ed. Academic Press: San Deigo, California.
- Biology of Plants. 1992. 5<sup>th</sup> ed. Ed: Raven, P.H., Evert, R.F., and Eichhorn, S.E. Worth Publishers, Inc.: New York.
- Blaisdell, R., R. Conant, W. Dick , Dobermann, A.C. Izaurrealde, M. Ransom, C. Rice, P. Robertson, J. Stuth, M. Thompson. 2003. Recommended procedures for collecting, processing, and analyzing soil samples in CASMGS research plots. CASMGS 4.1 Working Group. 1-7.
- Blanco, J.A. 2007. The representation of allelopathy in ecosystem-level forest models. *Ecological Modeling*. 209:65-77.
- Blumenthal, D.M. 2006. Interaction between resource availability and enemy release in plant invasion. *Ecology Letters*. 9:887-895.
- Bousquet-Mélou A., L. Sophie, C. Robles, S. Greff, S. Dupouyet, C. Fernandez. 2005 Allelopathic potential of *Medicago arborea*, a Mediterranean invasive shrub. *Chemoecology*. 15(4): 193–198 .
- Broeckling, C.D., and J.M. Vivanco. 2008. A selective, sensitive, and rapid in-field assay for soil catechin, an allelochemical of *Centaurea maculosa*. *Soil Biology and Biochemistry*. 40: 1189-1196.
- Broz, A.K. and J. M. Vivanco. 2006. Secondary Metabolites and Allelopathy in Plant Invasions: A Case Study of *Centaurea maculosa* L. Companion to Plant Physiology. 4<sup>th</sup> Ed. Chpt 13, Essay 13.7. Sinauer Associates Inc.
- Callaway, R.M. and W.M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers of the Ecological Environment*. 2(8): 436-443.
- Cameron, G.N. and S.R. Spencer. 1989. Rapid leaf decay and nutrient release in a Chinese tallow forest. *Oecologia*. 80: 222–228.
- Carew, J. and J. Mylroie. 1995. Depositional model and stratigraphy for the Quaternary geology of the Bahamas Islands. In Geological Society of America special paper 300: Terrestrial and shallow marine geology of the Bahamas and Bermuda, eds. H.A. Curran and B. White. Boulder, CO: Geological Society of America.

- Catford, J.A., Jasson, R., Nilsson, C. 2009. Diversity and Distributions (Diversity Distribution). 15: 22-40.
- Holmes, W. and A. McDaniel. Mississippi State Chemistry Laboratory. Personal communication, January 2010.
- Davis, M. A., J. P. Grime, K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*. 88: 528–534.
- Deaton, A. 1994. Shoreline monitoring at Long Key. *Resource Management Notes* 6(4):13–14.
- Duever, M.J., J.E. Carlson, J.F. Meeder, L.C. Duever, H. Gunderson, L.A. Riopelle, T.R. Alexander, R.L. Myers, D.P. Spangler. 1986. The Big Cypress National Preserve, Research report No. 8. National Audubon Society, New York, New York, USA.
- Elfers, S.C. 1988. Element Stewardship Abstract for *Casuarinas equisetifolia*. The Nature Conservancy. Retrieved Oct 9. 2008.
- El-Ghareeb, R.M. 1991. Suppression of Annuals by *Tribulus terrestris* in an Abandoned Field in the Sandy Desert of Kuwait. *Journal of Vegetation Science*. 2(2) 147-154.
- Ervin G.N. and R.G. Wetzel. 2000. Allelochemical autotoxicity in the emergent wetland macrophyte *Juncus effuses* (Juncaceae). *Journal of Botany*. 87(6) 853-860.
- Ervin, G.N. and R.G. Wetzel. 2003. An ecological perspective of allelochemical interference in land-water interface communities. *Plant and Soil*. 256: 13-28.
- ETC Environmental Testing & Consulting Inc., 2009. Soil Analysis Report.
- Feo, V.D., F.D. Simone, F. Senatore. 2002. Potential allelochemical from the essential oil of *Ruta graveolens*. *Phytochemistry*. 61:573-578.
- Fernald, R.T. and B.S. Barnett. 1988. Establishment of native hammock vegetation on spoil islands dominated by Australian pine (*Casuarina equisetifolia*) and Brazilian pepper (*Schinus terebinthifolius*). *Proc. Symposium on Exotic Pest Plants*, Miami, Fl. 19.

- Fernandez, C. B. Lelong, B. Vila, J. Mévy, C. Robles, S. Greff, S. Dupouyet, A. Bousquet-Mélou. 2006. Potential allelopathic effect of *Pinus halepensis* in the secondary succession: an experimental approach. *Chemoecology*. 16:97-105.
- Fitter, A. 2003. Making allelopathy respectable. *Science*. 301: 1337-1338.
- Gaston, K. J. and B.H. McArdle. 1994. The temporal variability of animal abundances: measures, methods and patterns. *Philos. Trans. R. Soc. London Ser. B*, 345: 335–358.
- Gentle, C.B. and J.A. Duggin. 1997. Allelopathy as a comparative strategy in persistent thickets of *Lantana camara* L. in three Australian forest communities. *Plant Ecology*. 132: 85–95.
- Gerace, D.T., G.K. Ostrander, G.W. Smith. 2005. San Salvador, Bahamas. Coastal region and small island papers. 3:1-16.
- Gochenaour, S.E. 1975. Distributional patterns of mesophilous and thermophilous microfungi in two Bahamian soils. *Mycopathologia*. 57: 155-164.
- Gordon, D.R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: lessons from Florida. *Ecological applications*. 8(4): 975-989.
- Hammerton, J.L., 2001. *Casuarinas* in The Bahamas: a clear and present danger. *Bahamas Journal of Science*. 9: 2-14.
- Harrington, R.A. and J.J. Ewel. 1997. Invasibility of tree plantations by native and non-indigenous plant species in Hawaii. *Forest Ecology and Management*. 99: 153-162.
- Hierro, J.L. and R.M. Callaway. 2003. Allelopathy and exotic plant invasion. *Plant and Soil*. 256: 29-39.
- Hodges, S.C. (no date). Soil fertility basics. Soil Science Extension of North Carolina State University. Retrieved 28, February 2010.  
<<http://www.soil.ncsu.edu/programs/nmp/Nutrient%20Management%20for%20CA>>.

- Holly, C.H. 2008. Multi scale evaluation of mechanisms associated with the establishment of a model invasive species in Mississippi: *Imperata cylindrical*. Dissertation, Mississippi State University, Department of Biological Sciences.
- Holmes, W. and A. McDaniel, 2010. Mississippi State Chemistry Lab. Personal communication.
- Hornik, K. 2010. Frequently asked questions on R. The R project for Statistical Computing. Retrieved 12, February 2010. <<http://www.r-project.org/index.html>>.
- Inderjit and J. Weiner. 2001. Plant allelochemical interference or soil chemical ecology? Perspectives in Plant Ecology, Evolution and Systematics. 4: 4-12.
- Inderjit, and C. L. Foy. 1999. Nature of the interference mechanism of mugwort (*Artemisia vulgaris*). Weed Technology. 13:176–182.
- Inderjit and A.U. Mallik. 1997. Effect of phenolic compounds on selected soil properties. Forest Ecology and Management. 92: 11-18.
- Inderjit, T.R. Seastedt, R.M. Callaway, J.L. Pollock, J. Kaur. 2008. Allelopathy and plant invasions: traditional, congeneric, and bio-geographical approaches. Biological Invasions. 10: 875-890.
- Inderjit, R.M. Callaway, J.M. Vivanco. 2006. Can plant biochemistry contribute to understanding of invasion ecology. Trends in Plant Science. 11(12): 574-580.
- Mallarino, A. 1999. Interpreting Mehlich-3 soil test results. Iowa State University Extension. Retrieved 15, February 2010. <<http://www.ipm.iastate.edu/ipm/icm/1999/2-15-1999/mehlich3.html>>.
- Invasive species. 2006. National Invasive species Information Center. Retrieved 20 February, 2010. <<http://alic.arid.arizona.edu/invasive/sub3/index.shtml>>.
- Jiqin, S., Z. Daxian, F. Zuguan, X. Jiaoru, H. Yixiong. 1991. The decline in soil pH values under, (sic) the Casuarina Equisetifolia trees and its effects on availability of trace elements Cu, Zn, B, Mo. Journal of Fujian Normal University (Natural Science Edition).



- Karachi, M. and R.D. Peiper. 1987. Allelopathic effects on kochia on blue grama. *Journal of Range Management*. 40(4): 380-381.
- Langui, D., K. Chuihua, L. Shiming 1996. Isolation and identification of extract from *Casuarinas equisetifolia* branchlet and its allelopathy on seedling growth. *Chinese Journal of Applied Ecology*.
- Leicht-Young, S.A., H. O'Donnell, A.M. Latimer, J.A. Silander Jr. 2009. Effects of an invasive plant species, *Celastrus orbiculatus*, on soil composition and processes. *The American Midland Naturalist*. 161: 219-231.
- Levitt, J. and J.V. Lovett. 1984. Activity of allelochemicals of *Datura stramonium* L. (Thorn-apple) in contrasting soil types. *Plant and Soil*. 79:181-189.
- Lonsdale, W.M., I.L. Miller, I.W. Forno. 1989. The biology of Australian weeds, 20. *Mimosa pigra* L. *Plant Protection Quarterly* 4:119-131.
- Loumeto, J.L. and C. Huttel. 1997. Understory vegetation in fast-growing tree plantations on savanna soil in Congo. *Forestry Ecology and Management*. 99: 65-81.
- Lovett, J.V. and W.C. Potts. 1987. Primary effects of allelochemicals of *Datura stramonium* L. *Plant and Soil*. 98: 137-144.
- Mack, R.N. 2000. Biotic invasions: causes epidemiology, global consequences and control. *Ecological Applications*.10: 689–710.
- Mallik, A.U., Pellissier, F. 2000. Effects of *Vaccinium myrtillus* on spruce regeneration: testing the notion of coevolutionary significance of allelopathy. *Journal of Chemical Ecology*. 26: 2197–2207.
- McPherson J.K. and C.H. Muller. 1969. Allelopathic effects of *Adenostoma fasciculatum* “Chamise”, in the California chaparral. *Ecological Monographs*. 39:177-198.
- Otila A., M. Sidwa-Gorycka, R. Malinski, M. Czerwicka, J. Kumirska. 2008. Effective biotic elicitation of *Ruta graveolens* L. shoot cultures by lysates from *Pectobacterium atrosepticum* and *Bacillus* sp. *Biotechnology Letters*. 30: 541-545.
- Overland, L. 1966. The role of allelopathic substances in the ‘smother crop’ barley. *American Journal of Botany*. 53:423-432.

- Parrotta, J.A. 1999. Productivity, nutrient cycling, and succession in single-and mixed-species plantations of *Casuarinas equisetifolia*, *Eucalyptus robusta*, and *Leucaena leucocephala* in Puerto Rico. *Forest Ecology and Management*. 124: 45-77.
- Parrotta, J.A. and O.H. Knowles. 1999. Restoring tropical moist forests on bauxite mined lands in the Brazilian Amazon: a comparative analysis of afforestation techniques. *Restoration Ecology*. 7.
- Pimentel, D. 2005. Update on the environmental and economic costs associated with alien invasive species in the United States. *Ecological Economics*. 52 (3): 273-288.
- Pollen-Bankhead, N., A. Simon, K. Jaeger, E. Whol. 2009. Destabilization of streambanks by removal of invasive species in Canyon de Chelly National Monument, Arizona. *Geomorphology*. 103(3): 363-374.
- Rabotnov, T.A. 1982. Importance of the evolutionary approach to the study of allelopathy (translated from Russian). *Ekologia*, 3: 5–8.
- Rietveld, W.J., Schelinger, R.C., Kessler, K.J., 1983. Allelopathic effects of black walnut on European black alder coplanted as a nurse species. *Journal of Chemical Ecology*. 9: 1119–1133.
- Rodgers, J.C., 2005. The distribution of *Casuarinas* on San Salvador Island, The Bahamas. *Southeastern Geographer*. 42(2) 222-238.
- Plant Physiology*. 1985. 3<sup>rd</sup> ed. Ed: Salisbury F.B. and C.W. Ross. Wadsworth Publishing Company: Belmont, California.
- San Salvador Island. 9 September 2009.  
<<http://www.geraceresearchcentre.com/sansal.html>>
- Schmitz, D. C., J. D. Schardt, A. J. Leslie, F. A. Dray, J. A. Osborne, B. V. Nelson. 1993. The ecological impact and management history of three invasive alien aquatic species in Florida. Ed: B. N. McKnight. *Biological pollution: the control and impact of invasive exotic species*. Indiana Academy of Science, Indianapolis, Indiana, USA. 173–194.

- Soil Mineralogy with Environmental Applications. 2002. J.B. Dixon and D.G. Schulze: Ed. Number 7 in the Soil Science Society of America Book Series. Soil Science of America, Inc.: Madison, WI, USA.
- Sealey, N. 2003. The cycle of Casuarinas-induced beach erosion; A case study from Andros. Proceedings of the 10th Symposium on the Natural History of The Bahamas.
- Sealey, N.E. 2006. Bahamian Landscapes. 3<sup>rd</sup> ed. Macmillan Publishers: Oxford.
- Sharma, G.P., A.S. Raghubanshi, J.S. Singh. 2005. Lantana invasion: an overview. Weed Biology Management. 5:157–163.
- Singh, B.K., L. Nazaries, S. Munro, I.C. Anderson, C.D. Campbell. 2006. Use of multiplex terminal restriction fragment length polymorphism for rapid and simultaneous analysis of different components of the soil microbial community. Applied Environmental Microbiology. 72: 7278-7285.
- Smith, R. 1993. Field Guide to the Vegetation of San Salvador Island, The Bahamas, 2nd Edition. Bahamian Field Station. 120.
- Srivastava, A. K. and R. S. Ambasht. 1996. Litterfall, decomposition, and nitrogen release in two age groups of trees in *Casuarina equisetifolia* plantations in the dry
- van Sumere, C.F., H. Hilderson, L. Massart. 1958. Coumarins and phenolic acids of barley and malt husk. Naturwissenschaften. 45:292.
- Suresh, K.K. and Rai, R.S.V. 1987. Studies on the allelopathic effects of some agroforestry tree crops. Int.Tree Crops J. 4: 109-115.
- Swearingen, J.M. 1997. Australian pine; Casuarinas equisetifolia L. Plant Conservation Alliance's Alien Plant Working Group. Last accessed 11 March 2010 at <<http://www.nps.gov/plants/alien/>>
- Tang, C.H., W.F. Cai, K. Kohl, R.K. Nishimote. 1995. Plant stress and allelopathy. In: Inderjit, Dakshini, K.M.M., Einhellig, F.A. (Eds.), Allelopathy, Organisms, Processes and Applications. American Chemical Society, Washington, DC. 142–157.
- Taylor, L. R. 1961. Aggregation and the mean. Nature. 189: 732–735.

The R project for Statistical Computing. Retrieved 24 February, 2010.  
<<http://www.r-project.org/>>

Vitousek, P. M. 1986. Biological invasions and ecosystem properties: can species make a difference? Ecology of biological invasions of North America and Hawaii. Ed. H. A. Mooney and J.A. Drake. Springer: New York. 163-176.

Vitousek, P.M. 1990. Biological invasion and ecosystem processes: towards an interaction of population biology and ecosystem studies. *Oikos*. 57:7-13.

Vyvyan, J.R. 2002. Allelochemicals as leads for new herbicides and agrochemicals. *Tetrahedron*. 58: 1631-1646.

Weir, T.L., S-W. Park, J.M. Vivanco. 2004. Biochemicals and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology*. 7:472-479.

Wilks D.S. 2006. Statistical methods in the atmospheric sciences. 2nd Ed. Elsevier: Burlington, MA.

Zhang, L, Y. Lin, G.Ye, X. Liu, Lin, G. 2008. Changes in the N and P concentrations, N:P ratios, and tannin content in *Casuarina equisetifolia* branchlets during development and senescence. *Journal of Forest Research*. 13: 302-311.