CHAPTER 4

Etiology of Diplodia Tip Blight Outbreak on Slash Pine in Florida

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INTRODUCTION

Abiotic stressors such as drought, tree wounding, nutritional imbalances, and/or improper management practices generally predispose trees to disease outbreaks caused by endemic pathogens. These factors can weaken trees, increasing their susceptibility to infection by pathogens (Ayres and Lombardero 2000). Nutrient imbalances and excessive fertilizer applications have been implicated as major predisposing factors in certain tree disease outbreaks (Blakeslee and others 1999, Panique and others 1997, Swart and others 1987). Of all plant nutrient imbalances, excessive nitrogen is most associated with elevated disease incidence and severity (Lopez-Zamora and others 2007). Numerous studies have demonstrated the predisposition of trees to outbreaks in relation to nitrogen fertilization rates (Vandijk and others 1992). An excess of nitrogen induces succulent shoot and foliar growth, which decreases lignin concentration and facilitates wounding from insects, hail, or wind (Hesterberg and Jurgensen 1972), as well as decreases the concentration and toxicity of phenols used by plants against pathogens (Torres-Olivar and others 2014). For example, high levels of foliar and soil nitrogen are known to enhance disease severity of pitch canker (*Fusarium circinatum*) in pines (*Pinus* spp.; Lopez-Zamora and others 2007), as well as fire blight (*Erwinia amylovora*) in pear (*Pyrus* spp.) and rust (*Puccinia graminis* f. sp. *Tritici*) on wheat (*Triticum* spp.; Agrios 2005). Likewise, pine trees in the Netherlands severely affected by *Diplodia sapinea* showed approximately 60 percent more nitrogen than healthy trees (Vandijk and others 1992).

This study focused on an outbreak of *D. sapinea* and *D. scrobiculata*, which caused extensive dieback and mortality of slash pines (*P. elliottii*) along a highway in central Florida starting in 2012 (Paez and Smith 2018). The presence of an unusually large and uniform outbreak led to concern about what factors were responsible in the development of the outbreak. The objectives of this study were to (1) examine the effect of nutrients in this outbreak and (2) investigate the role of nitrogen in disease severity using inoculated saplings.

METHODS

Field Foliar Nutrient Analysis

In the summer of 2014, live foliar samples were taken from three slash pine stands in the outbreak area in central Florida. Each stand was categorized into healthy (<10 percent of trees with symptoms), medium (25–50 percent), or high (>50 percent) disease incidence of diplodia tip blight. Each sample was dried to a constant weight in a conventional oven at 55 °C for 2 days. The dry samples were sent to Micro Macro International Laboratory (MMI Labs, Athens, GA) to be ashed (AOAC 900.02B) and analyzed for nutrient analysis using inductively coupled plasma (ICP-EPA 6010a; MMI Labs, Athens, GA). Foliar nutrient analysis was compared among the three levels of disease incidence. These comparisons examined potential relationships between nutrient imbalances and diplodia disease incidence in the field.

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Effect of Nitrogen on Disease Severity

Under controlled conditions, one greenhouse study of loblolly pine (*P. taeda*), slash pine (*P. elliottii* var. *elliottii*), and south Florida slash pine (*P. elliottii* var. *densa*) was performed twice to evaluate the effect of different levels of nitrogen fertilization on disease severity of diplodia tip blight caused by the two fungal pathogens (*D. sapinea* and *D. scrobiculata*). Each experiment was designed as a randomized complete block design, with three blocks. The treatments were arranged in a factorial design with fungal species (*D. sapinea*, *D. scrobiculata*, and mock-inoculated control) and three nitrogen fertilization rates, for a total of nine treatments.

The fertilization treatments were preceded by a base application of Nutricote® (18:6:8; Florikan®) at the recommended dose, based on operational silvicultural commercial standards (Jokela 2004) downsized for the surface area and volume of the tree pot. The fertilization treatments were then imposed after a month and were applied as (1) no added nitrogen (0X N), (2) twice the recommended nitrogen dose (112.1 kg/ha; 2X N), and (3) three times the recommended nitrogen dose (168.1 kg/ha; 3X N). The nitrogen source was urea (46:0:0) and the dose was estimated for the pot size (11.4 L) used in the study. Stems of 3-year-old loblolly pine, slash pine, and south Florida slash pine saplings were wounded and inoculated using a 6-mm sterile cork borer at approximately 25 cm below the terminal shoot tip. A 6-mm colonized agar plug (from the marginal growth of each in vitro fungal culture) was inserted in the wounded area facing the cambial tissue of the stem and wrapped with Parafilm® M (Pechiney Plastic). For mock-inoculated controls, the inoculation was made with a sterile (noncolonized) potato dextrose agar plug (full strength) wrapped with Parafilm® M (Pechiney Plastic). Parafilm was not removed until the end of the study. External symptoms and internal lesion lengths were evaluated 11 weeks after inoculation. Lesion length was evaluated by removing the bark with a sterile scalpel blade to expose discolored sapwood and all bark surrounding the healthy tissue on margins of the discolored tissue. The lesions were then measured in centimeters. To complete Koch's Postulates, the inoculated *Diplodia* strains were reisolated from the discolored tissues and identification was confirmed by internal transcribed spacer (ITS) sequencing.

Statistical Analysis

To determine if differences existed among the levels of macronutrients and micronutrients in the slash pine stands, a Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) test was used with the disease incidence level (healthy, medium, or high) of the sampled trees as the independent variable. A Steele-Dwass nonparametric multiple comparison procedure was used to test which incidence level differed among the stands.

To analyze lesion lengths in pine saplings treated with different nitrogen levels, a two-factor linear mixed model was used to test for differences, with fungi (*D. sapinea* and *D. scrobiculata*) and nitrogen level (0X N, 2X N, and 3X N) set as the independent variables. A mixed model was used to account for the blocks in the experimental design and for variability between the fungi treatments. After performing the analysis, Tukey's multiple means comparison honestly significant difference (HSD) was conducted when the lesion length was significant. The significance was evaluated at $p \leq 0.05$. All analyses were performed using the statistical software SAS version 14.

RESULTS AND DISCUSSION

The foliar analysis of samples from tree stands with different disease incidence indicates that among 16 nutrients, only nitrogen and molybdenum were significantly different (fig. 4.1). Mean nitrogen levels were significantly higher in plots exhibiting high disease incidence (~2.3 percent) versus plots with healthy trees (1.2 percent) and those showing a medium disease incidence (1.5 percent) (fig. 4.1A). The level of foliar nitrogen in the high disease incidence plots (~2.3 percent) is considered high (nearly double) when compared to published values for healthy slash pine trees (Jokela 2004). This was expected, as the nitrogen applied in these plots by highway maintenance crews—ranging between 55 and 142 kg/ha (based on 1,568–3,913 trees/ha) every year for 4 years—was much higher than what is recommended in commercial forestry plantations using the same species (45–56 kg/ha of nitrogen at time of planting only (Jokela 2004). Numerous factors are known to cause stress to pine trees, including drought, competition, and overfertilization (Bachi and Peterson 1985, Blodgett and others 1997, Johnson and others 1997, Nicholls and Ostry 1990, Paoletti and others 2001).

In the greenhouse experiment, higher nitrogen levels (3X N) significantly increased disease effects in slash pine and south Florida slash pine when inoculated with *D. sapinea* compared to those with low nitrogen levels (0X N). However, the nitrogen level did not cause a severity difference with any pathogens in inoculated loblolly pine (fig. 4.2). When inoculated with *D. scrobiculata*, no significant differences in disease severity were found among the different nitrogen levels for any tree species ($p > 0.05$). Previous studies show that if environmental conditions become unfavorable for pines (causing host stress), *D. sapinea* turns pathogenic and moves from the bark to living internal tissues (Flowers and others 2001, Smith and others 2002). Also, previous research has shown that nitrogen increases the susceptibility of pines to *D. sapinea* infection (Blodgett and others 2005).

CONCLUSIONS

As landscapers use native trees in diverse, often very intensively maintained landscapes, more education about the effects of specific stresses created in these situations, such as excessive nitrogen, is needed. Some forestry and horticulture practitioners tend to adopt a philosophy that if the goal is fast plant growth, then the method to achieve this goal is to apply more fertilizers, especially nitrogen. This study highlights a case analysis providing detailed evidence of the potential risk to a specific pathogen resulting from overfertilization. In future management of the southern pines, this risk could be considered by examining nutrition management in relation to disease outbreaks.

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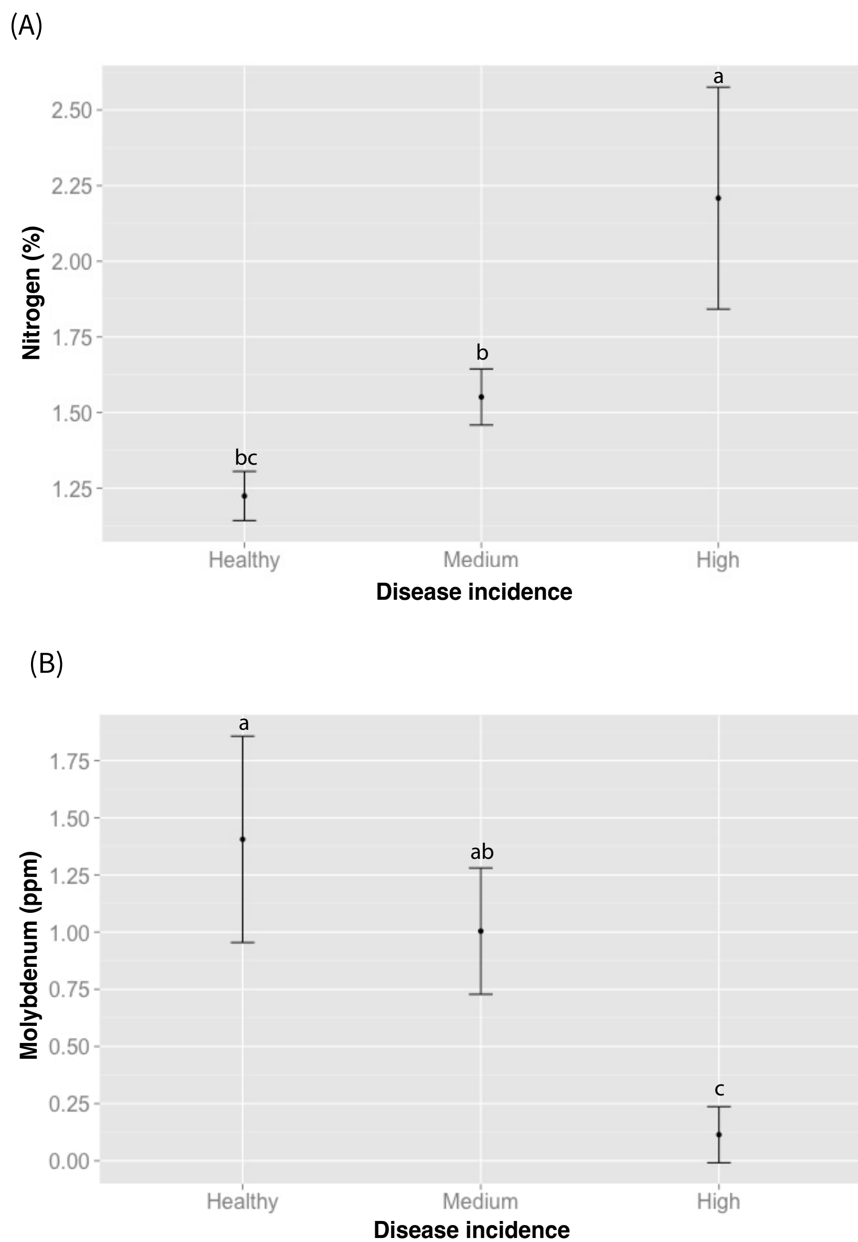

Figure 4.1— Foliar nitrogen concentration (percent) (A) and foliar molybdenum concentration (parts per million) (B) of slash pine trees from stands with different diplodia disease incidence (Paez 2017). Different letters indicate statistical significance (p ≤0.05).

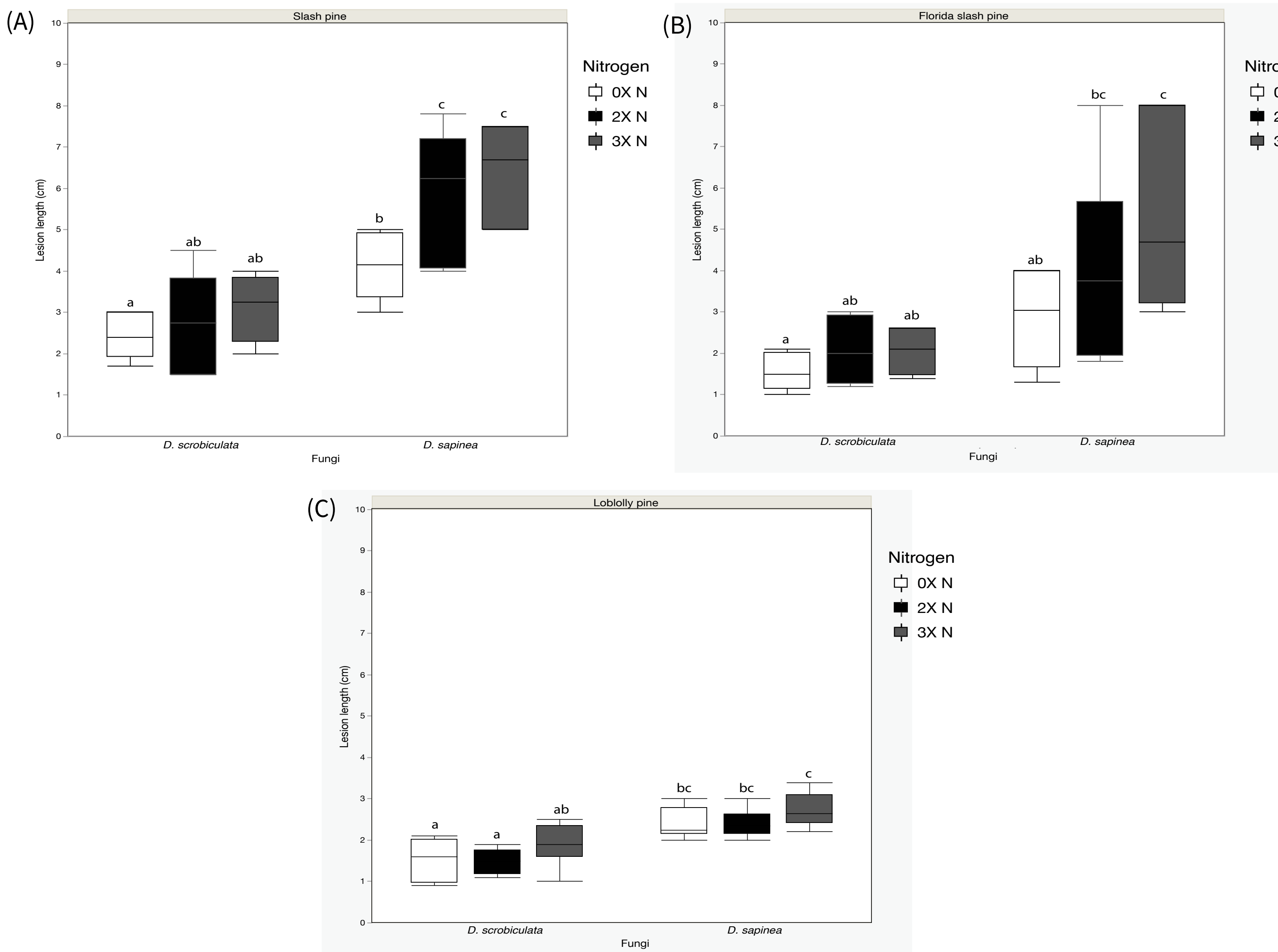

*Figure 4.2 —Lesion length (cm) caused by inoculation with two fungal pathogens (*Diplodia sapinea *and* D. scrobiculata*) in slash pine (A), south Florida slash pine (B), and loblolly pine (C) saplings treated with three levels of nitrogen fertilizer (Paez 2017). Different letters indicate statistical significance (*p *≤0.05) within tree species among nitrogen treatments.*

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