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Effects of fertilization on red pine defense chemistry and resistance to *Sphaeropsis sapinea*

James T. Blodgett [a,*], Daniel A. Herms [b], Pierluigi Bonello [a]

[a] *Department of Plant Pathology, The Ohio State University, 201 Kottman Hall, 2021 Coffey Road, Columbus, OH 43210, USA*
[b] *Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691, USA*

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Abstract

Sphaeropsis sapinea (Fr.:Fr.) Dyko & Sutton in Sutton is a fungal pathogen that causes shoot blight and canker disease throughout the world on conifers predisposed by stress. Fertilization is often recommended to reduce stress, and presumably increase disease resistance. We examined the effects of fertilization on red pine (*Pinus resinosa* Aiton) resistance to *S. sapinea*, and on concentrations of constitutive and pathogen-induced secondary metabolites putatively involved in disease resistance. Wounded branch tips were inoculated with agar plugs colonized by the pathogen; noncolonized plugs were used for controls. Fertilization increased lesion size ($P < 0.05$) and foliar nitrogen ($P < 0.01$), and decreased foliar C:N ratio ($P < 0.01$), constitutive lignin levels both locally ($P < 0.05$) and distally ($P < 0.05$), and total soluble phenolics distal to the inoculation point ($P < 0.01$). At the infection court, inoculation significantly increased accumulation of total soluble phenolics ($P < 0.01$), and the induction or depletion of several individual phenolics ($P < 0.05$). Distally, inoculation was also a significant factor ($P < 0.05$) in the accumulation or depletion of some of the individual phenolics investigated. These results show that fertilization decreases resistance of red pine to *S. sapinea*, and suggest that lignin and soluble phenolics might be involved in host defense.

Keywords: *Diplodia pinea*; High pressure liquid chromatography; Host defense; HPLC; Lignification; Predisposition; Secondary metabolism

1. Introduction

Sphaeropsis shoot blight and canker, caused by *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton in Sutton, has caused extensive damage to conifers throughout the world, including *Pinus resinosa* Aiton (red pine). Pines are affected from the seedling stage to mature size and damage occurs in nurseries, Christmas tree and ornamental plantings, plantations, and natural stands (Chou, 1976; Gibson, 1979; Stanosz and Cummings Carlson, 1996). Severe disease levels have frequently been attributed to different abiotic stressors. Environmental conditions such as nutrient,

* Corresponding author. Present address: USDA-Forest Service, Forest Health Management, 1730 Samco Road, Rapid City, SD 57702, USA. Tel.: +1 605 394 6191; fax: +1 605 394 6627.
E-mail address: jblodgett@fs.fed.us (J.T. Blodgett).

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temperature, and water extremes have been shown to affect many plant-fungus associations, although those conditions are generally poorly defined. For example, water stress is associated with enhancement of disease development on several tree species (Bagga and Smalley, 1974; Schoeneweiss, 1981; Pusey, 1989), including *S. sapinea* on red pine (Blodgett et al., 1997). However, water stress also may have a neutral or negative role on the development of certain woody plant diseases (Biggs et al., 1983; Jacobi and Riffle, 1989).

Fertilization of pines is often recommended, especially in nurseries, Christmas tree plantations, and ornamental settings, to boost and enhance overall tree health (Smith, 1978; Neely and Himelick, 1987; Iles, 2000). The expectation is that vigorous growth reduces stress and thus susceptibility to disease and insect damage. However, the majority of evidence suggests an association between fertilization and increased susceptibility of ornamental woody plants to insect attack (Herms, 2002). Much less work has been conducted on the effects of fertilization on disease development in trees, and no detailed studies simultaneously linking fertilization, measures of disease susceptibility, and expression of constitutive or induced defense responses are known for canker diseases of conifers in ornamental landscapes. Therefore, a better understanding of the potential effect of fertilizer use on disease development and host defense in pines is important.

Little is known about how soil nutrients might affect disease caused by *S. sapinea* or other pine canker fungi. In a survey of red and jack (*P. banksiana* lamb.) pines, tree mortality attributed to *S. sapinea* was as high as 30% for red pine and 51% for jack pine (Nicholls and Ostry, 1990). Such high mortality levels were attributed to, among other factors, poor site conditions. However, in another field survey, red pine mortality was correlated with paper-mill waste application and was linked to higher foliar nitrogen in the waste-treated stands (Stanosz et al., 2004). Van Dijk et al. (1992) also correlated increased disease development by *S. sapinea* with high soil nutrient concentrations. Although losses to *S. sapinea* have been associated with certain nutrient conditions, results are based on field observations and field surveys. These observations and surveys do not provide information on the quantitative effects of nutrition on disease development and/or cannot separate the effects of nutrients from many other possible environmental factors.

The defensive chemistry of coniferous trees has been investigated extensively. Some compounds in secondary metabolism, particularly monoterpenes, soluble and cell wall-bound phenolics, and lignin are thought to be involved in host defense (Bonello et al., 1993). These chemicals may be affected qualitatively and quantitatively by pathogen invasion (Strobel and Sinclair, 1986; Klepzig et al., 1996; Blodgett and Stanosz, 1998; Bonello et al., 2003), and can have fungistatic or toxic effects in vitro at concentrations observed in pines and other conifers (Alcubilla et al., 1971; Bonello et al., 1993; Klepzig et al., 1996; Blodgett and Stanosz, 1997a). This evidence has led to speculation that these compounds are involved in the restriction of infection and colonization of the host by pathogens (Blanchette and Biggs, 1992). Some evidence suggests that these chemical defense responses may be compromised under host stress, resulting in lower concentrations of these chemicals and greater disease (Entry et al., 1991; Klepzig et al., 1995). However, little research has been conducted on the potential effects of fertilization on these putative defense mechanisms of pines when challenged by canker pathogens such as *S. sapinea*.

This controlled field study addresses the effects of fertilization of red pine on disease development and the expression of chemical defense responses. Consequently, the objectives of this study were to: (i) test if fertilization of field-grown red pine at recommended levels influences symptom severity of red pine inoculated with *S. sapinea*, and (ii) characterize and quantify the relationship between fertilization and concentrations of constitutive and pathogen-induced secondary metabolites (lignin, soluble phenolics, and cell wall-bound phenolics) putatively involved in disease resistance.

2. Materials and methods

Plots consisted of collections of individual, physically isolated soil cells (phytotrons) each containing one 13-year-old red pine established at the Ohio Agricultural Research and Development Center in Wooster, OH. Each phytotron was con-