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

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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,

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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 constructed by slicing a narrow trench 1 m deep around plots of 4 m^2. The trench was lined with 30-mil PVC landfill liner extending 1 m vertically into and 20 cm above the ground. The liner isolated the soil environment without affecting drainage, allowing for replicated, randomized soil environments. The native soil is a Canfield silt loam (29% sand, 42% silt, 29% clay). One red pine tree was transplanted in each phytotron in October 1997. Before transplanting, trees were grown together under the same conditions in an Ohio nursery for 8 years prior to being transplanted, at which time they ranged in height from 2.0 to 2.5 m.

Beginning in April 1998, half of the 20 trees were randomly selected to be fertilized and the other half served as untreated controls. Fertilizer was applied at a rate of 200 kg N ha^{-1} yr^{-1}, with half the annual amount applied in late April and half in early October from 1998 through 2001. The fertilizer used was 18-5-4 N:P:K, with 56% of the N in slow release form (methylene urea), and 44% of the N in fast release form (17% ammonium nitrate and 27% water-soluble urea). Fertilizer was applied directly to the soil surface, which was maintained weed-free by application of the pre-emergence herbicides Gallery (isoxaben) for broadleaves in combination with Surflan (oryzalin) or Barricade (prodiamine) for grasses, augmented with cultivation and spot-treatment with the nonselective herbicide Roundup (glyphosate). The fertilization treatment represents an intermediate rate based on standard recommendations for woody plants in ornamental landscapes. These recommended rates range from 147 to 294 kg ha^{-1} yr^{-1} of actual N, with N:P:K applied in a ratio of about 3:1:1 (ANSI, 1998).

To determine effects of the fertilization on incidence and severity of *S. sapinea*, elongating shoot tips were experimentally inoculated with the fungal pathogen using methods previously described (Blodgett and Stanosz, 1997b). For each of the 20 trees, two branches were selected from the same mid-canopy whorl, and their elongating, terminal shoots were wounded by removing a single needle fascicle approx. 3 cm below the shoot apex. One of the shoot tips on each branch (selected randomly) was inoculated with *S. sapinea* and the other served as a wounded control. *S. sapinea* used in the inoculations was a monoconidial isolate (3AP) (de Wet et al., 2003) originating from an Austrian pine tree (*Pinus nigra* Arnold) growing in central Ohio. The isolate was previously confirmed as virulent by wound-inoculating both red pine and Austrian pines (Blodgett and Bonello, 2003). A 4 mm diameter 1.5% water agar (WA; Difco Laboratories, Detroit, MI) plug colonized with the 3AP isolate was placed mycelium-side-down on the wounds. Plugs were cut from margins of actively growing cultures incubated for 6 days in the dark at 23 °C. Parafilm (American National Can Co., Chicago, IL) was wrapped around the shoots at the inoculation site and removed after 3 days. The wounded control shoot was treated as above except that the water agar plug had not been colonized by *S. sapinea*. One half of the trees from the fertilized and unfertilized treatments (selected randomly) were inoculated on 22 May 2001, and the other half were inoculated on 29 May 2001.

Symptom incidence and severity were recorded 4 weeks after inoculations. Severity was based on lesion length and this was assessed indirectly by measuring the maximum distance below the inoculation site at which necrotic needles were observed. Shoot segments were collected at both 0–3 cm (proximal site) and 15–18 cm (distal site) from the inoculation site. These two segments allowed comparison of locally and systemically induced chemistry of colonized and non-colonized tissues on the same branch (80 total shoot segments). Needles were removed and a small amount of each segment was excised to confirm the presence or absence of *S. sapinea* using methods described previously (Blodgett et al., 2003). The remainder of the shoot segments were flash-frozen in liquid nitrogen and stored at −80 °C for later analyses of soluble and cell-wall bound secondary metabolites and lignin concentrations.

The frozen shoot segments were ground to powder in liquid nitrogen and processed for sequential extraction of soluble and cell wall-bound metabolites, and lignin as described previously (Bonello and Blodgett, 2003). Soluble and cell wall-bound secondary metabolites were analyzed by HPLC using a Waters (Milford, MA) 2690 separations module, 474 scanning fluorescence detector, 996 photo diode array detector, and the solvent system and settings as described previously (Bonello and Blodgett, 2003). The system was managed by a workstation running Waters Millennium HPLC software. A Waters Xterra^{TM} RP18, 5 μm, 4.6 mm × 150 mm column was used for sample separation. Injections consisted of 5 μl of either soluble or cell wall-bound metabolite extracts. The fluorescence detector was set at 300 nm excitation and 400 nm emission wavelengths. The photo diode array detector was set to scan between 237 and 376 nm, and two channels were selected for data processing, 280 and 308 nm. The former wavelength is commonly used for detection of secondary metabolites, but often generates poor peak separation with conifer samples. The 308 nm wavelength was selected based on preliminary 3D scans of actual pine phloem samples and various phenolic derivative standards because it offered clean peak separation/detection and stable and flat baselines. These wavelengths are designed to detect mostly phenolic compounds, thus we will refer to all secondary metabolites detected in this way as ‘‘phenolics".

The spectral characteristics and retention times of the individual peaks were compared to standards and quantified, wherever possible, using methods described previously (Bonello and Blodgett, 2003). Additionally, we considered the total soluble phenolics and total cell wall-bound phenolics fractions measured as sums of all peak areas in an HPLC chromatogram at 280 nm.

Other sampled data included foliar nitrogen content and foliar carbon:nitrogen (C:N) ratio. Needle fascicles were sampled randomly for nitrogen and carbon analysis in May 2001. Twenty fascicles of 1-year-old (formed in 2000) needles were sampled from throughout the canopy of each tree. Needles were dried at 60 °C to constant weight and then milled to pass a 40 mesh screen. Foliar nitrogen and carbon concentrations were then determined for each tree by analyzing duplicate aliquots (10 mg) of the foliage samples with a Carlo Erba Series II C:H:N analyzer Model NA1500 (Carlo Erba Elantech, Lakewood, NJ) (Daun and DeClerq, 1994).

2.1. Statistical analyses

Effects of fertilization on *S. sapinea* lesion length and phytochemical variables were analyzed using ANOVA (PROC GLM, SAS, 1999). All response variables were tested for normality of residuals and equality of variances among treatments. No data transformations were required. For most variables, the model was a split-plot with fertilizer treatment as main plot and inoculation treatment as the subplot. For certain variables where the wounded control treatment was not relevant (e.g., *S. sapinea* lesion length, foliar N and C concentrations, and specific phloem phenolic compounds that were not detectable in the majority of wounded control shoots), inoculation treatment was excluded from the model, and one-way ANOVA was used to test their response to fertilization. Date of inoculation was treated as a blocking factor, but was insignificant ($P > 0.05$ in every case, >0.2 in most cases), and was excluded from the analyses. If significant differences ($P \geq 0.05$) were found, means were separated by Fisher’s least significant difference (LSD) at $P = 0.05$.

Simple linear regression (PROC GLM, SAS, 1999) was used to confirm the linearity of the standard calibration curves for lignin and the phenolic compounds, and to assess relationships between accumulation/depletion of individual secondary metabolites (e.g., catechin, pinosylvin) or classes of secondary metabolites (e.g., lignin, total soluble phenolics, etc.) and lesion size. The latter regressions were conducted only for inoculated samples, i.e., those that had lesions.

3. Results

Lesions were first observed at the inoculation site on all inoculated branches at 13 days post-inoculation. The fertilization treatment resulted in approx. 50% longer lesions ($F_{1,19} = 4.47$, $P = 0.049$; Fig. 1) on inoculated branches after 4 weeks compared with the no-fertilization treatment. Symptoms were not observed on the noninoculated control branches. *S. sapinea* was recovered from all inoculated branches at the branch tips, from 20% of the control branches at the branch tips for both tree treatments, and never from either inoculated or control branches 15 cm from the inoculation site. The fertilization treatment resulted in higher foliar nitrogen content and decreased foliar C:N ratio compared with the no-fertilization treatment: percent nitrogen content—fertilized: 1.8, nonfertilized: 1.4, $F_{1,19} = 13.16$, $P < 0.01$; C:N ratio—fertilized: 30, nonfertilized: 39, $F_{1,19} = 13.89$, $P < 0.01$).

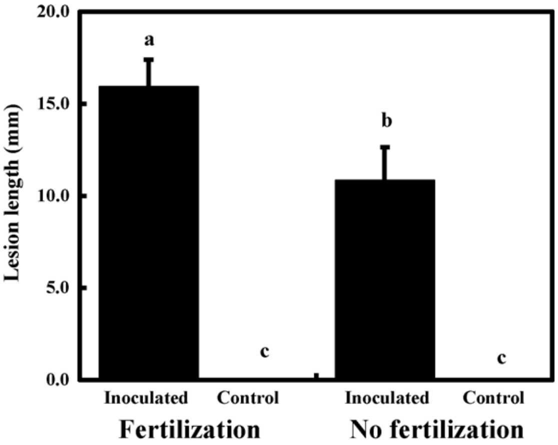

Fig. 1. Lesion length measured after 4 weeks on wounded red pine (*P. resinosa*) branches inoculated with agar plugs colonized by *S. sapinea*; noncolonized agar plugs were used for wounded controls. Trees were either fertilized at recommended levels or not fertilized. Bars with the same letters are not significantly different ($P \leq 0.05$) based on Fisher's least significant difference test.

3.1. Proximal stem sections

Fertilization decreased lignin concentration in the control inoculations compared with the no-fertilization treatment, but the inoculation treatment had no effect (Table 1). Significant accumulation of total soluble phenolics occurred in response to the inoculation treatment, but not to the fertilization treatment (Table 1). Individual soluble phenolic compounds also significantly responded to the inoculation treatment, but most did not respond to the fertilization treatment (Table 1). However, fertilization lowered the concentration of taxifolin ($P = 0.054$). Total cell-wall bound phenolics and individual cell-wall bound phenolics did not show a response to either the inoculation or the fertilization treatments (results not shown).

3.2. Distal stem sections

The fertilization treatment resulted in decreased ($P = 0.042$) lignin concentration compared with the no-fertilization treatment, but the inoculation treatment did not affect concentrations (Table 1). In contrast to the stem sections collected at 0–3 cm from the inoculation site, total soluble phenolics accumulation was negatively affected by the fertilization treatment, but did not respond to the inoculation treatment (Table 1). Some of the individual soluble phenolic compounds that had significant responses at 0–3 cm from the branch tips also significantly responded to the inoculation treatment, but none of the soluble phenolic compounds responded to the fertilization treatment (Table 1). Total cell-wall bound phenolics and individual cell-wall bound phenolics did not show a response to either the inoculation or the fertilization treatments (results not shown).

Three phenolic peaks were identified based on spectral characteristics and retention times. All standard calibration curves had strong correlations ($R^2 > 0.990$), and all had significant linear regressions ($P < 0.001$). The identified peaks were: taxifolin with an average retention time of 19.06 min ± 0.01 S.E. (detected at 308 nm); pinosylvin with an average retention time of 27.51 min ± 0.01 S.E. (fluorescence detection); and pinosylvin monomethyl ether with an average retention time of 30.73 min ± 0.01 S.E. (fluorescence detection).

3.3. Regressions

Linear regressions against lesion lengths were significant for only four variables (Table 2).

4. Discussion

Our controlled field study shows that fertilization of red pine at levels recommended for ornamental and shade trees can influence symptom development, resulting in decreased resistance of the host to *S. sapinea*. While the lesions were small, fertilization resulted in a significant, 50% increase in lesion size. This is important biologically for two reasons. First, this result is contrary to the notion that vigorous growth reduces stress and thus susceptibility to disease (Smith, 1978; Neely and Himelick, 1987; Iles, 2000). Second, an increase of this magnitude in lesion length can tip the balance in favor of significant lesion, i.e., symptom development. In a study on a similar pathosystem, i.e., pitch canker of Monterey pine (*Pinus radiata* D. Don), Gordon et al. (1998) demonstrated that, if lesions lengths caused by *Fusarium circinatum* Nirenberg and O'Donnell remained below approx. 2 cm, the branches never

Table 1
Lignin, normalized peak areas of unknown soluble phenolics, and actual amounts of known soluble phenolics in red pine (*P. resinosa*) branches from 20 trees[a] that were either fertilized at recommended levels or not fertilized, and that were inoculated with *S. sapinea*, or noncolonized agar plugs as controls

Location/variable	Fertilization treatment/inoculation treatment				Significance of factors (*P* values)[b]	
	Fertilization		No fertilization			
	Inoculated	Control	Inoculated	Control	Inoculation	Fertilization
Branch segments collected 0–3 cm from the inoculation site						
Lignin (mg g^{-1} FW)	16.00 ab[c]	13.56 a	17.19 b	17.35 b	0.353	0.187
Total soluble phenolics	13×10^6 a	5×10^6 b	12×10^6 a	4×10^6 b	<0.001	0.631
Phenolic 10.07[d] FL[e]	23×10^4 ab	33×10^4 c	19×10^4 a	31×10^4 bc	0.002	0.388
Phenolic 12.20 308	11×10^4 a	10×10^4 a	14×10^4 b	10×10^4 a	0.002	0.253
Taxifolin 19.06 308 nmol g^{-1} FW	498 ab	365 a	631 ab	432 a	0.002	0.054
Phenolic 23.75 308	25×10^3 a	–[f]	30×10^3 a	–		0.448
Pinosylvin 27.51 FL nmol g^{-1} FW	333 a	–	315 a	–		0.906
Phenolic 28.42 308	13×10^4 a	–	4×10^4 a	–		0.142
Phenolic 29.68 308	79×10^3 a	–	21×10^3 a	–		0.127
Pinosylvin monomethyl ether 30.73 FL nmol g^{-1} FW	9.5 a	–	6.1 a	–		0.429
Phenolic 31.68 308	92×10^3 a	30×10^3 b	85×10^3 a	18×10^3 b	<0.001	0.475
Phenolic 33.52 FL	81×10^4 a	-	82×10^4 a	–		0.977
Phenolic 34.24 308	58×10^3 a	–	33×10^3 a	–		0.333
Phenolic 36.40 280	44×10^5 a	5×10^5 bc	26×10^5 ac	3×10^5 b	<0.001	0.212
Branch segments collected 15–18 cm from the inoculation site						
Lignin (mg g^{-1} FW)	13.84 a	14.80 ac	16.91 bc	16.77 c	0.583	0.042
Total soluble phenolics	25×10^5 a	26×10^5 a	33×10^5 bc	29×10^5 ac	0.348	0.013
Phenolic 10.07 FL	27×10^4 a	43×10^4 b	26×10^4 a	39×10^4 b	<0.001	0.662
Phenolic 12.20 308	69×10^3 a	81×10^3 a	74×10^3 a	76×10^3 a	0.371	0.982
Taxifolin 19.06 308 nmol g^{-1} FW	219 ab	183 a	242 b	176 a	0.020	0.644
Phenolic 31.68 308	17×10^3 ab	14×10^3 a	24×10^3 b	12×10^3 a	0.032	0.221
Phenolic 36.40 280	21×10^4 a	25×10^4 a	33×10^4 b	27×10^4 ab	0.668	0.150

[a] Forty branch samples per measured variable for each branch segment. Except for lignin, values in columns are absorbance or fluorescence peak areas per 5 μl injection.

[b] *P* values based on ANOVA.

[c] Values in a row followed by the same letters are not significantly different ($P \leq 0.05$) based on Fisher's least significant difference test.

[d] Average peak retention time (min).

[e] Detector channel for quantification: FL = fluorescence; 308 = 308 nm; 280 = 280 nm.

[f] Not detected in the majority of, or in all cases. In this instance, the effect of inoculation was not tested because the control treatments had too many zeroes.

became girdled by the pathogen and never became symptomatic, effectively representing a resistant response in that system. Thus, if a similar threshold behavior could be demonstrated for the red pine/*S. sapinea* pathosystem, fertilization of a borderline resistant tree would render that tree effectively susceptible to this pathogen. While this remains to be demonstrated, the implications of our results for management of this and other pine species through fertilization are significant. Indeed, in another study susceptibility of red pine to Sphaeropsis blight and canker was significantly higher in soils that provided increased available nitrogen to the hosts (Stanosz et al., 2004). Similar outcomes were observed in the Corsican pine (*P. nigra* ssp. *laricio* Maire)/*S. sapinea* pathosystem (van Dijk et al., 1992).

The existence of a relationship between soil fertility and plant disease susceptibility has been confirmed in many studies, most of which were conducted with agricultural crop plants (Agrios, 1997). In most cases this work has shown a positive correlation between disease susceptibility and soil

Table 2
Significant linear regressions between lesion size and selected biochemical variables

Variable	Function[a]	R^2	Significance (*P* value)
Total soluble phenolics			
Proximal	$y = 9 \times 10^{-7}x + 10.241$	0.282	0.016
Distal	$y = -4 \times 10^{-6}x + 25.362$	0.220	0.037
Phenolic 12.20			
Proximal	$y = -10^{-4}x + 27.74$	0.406	0.003
Distal	N.S.[b]		
Phenolic 34.24			
Proximal	$y = 5 \times 10^{-5}x + 11.213$	0.202	0.047
Distal	N/A[c]		
Phenolic 36.40			
Proximal	$y = 9 \times 10^{-7}x + 10.241$	0.282	0.016
Distal	$y = -2 \times 10^{-5}x + 18.993$	0.263	0.021

[a] x = peak area; y = lesion length in mm.
[b] Not significant.
[c] Not applicable: the compound was detected in only one sample.

fertility. The literature is far less developed for conifers. Overall the evidence with diseases caused by pathogens affecting conifer shoots and branches is consistent with studies on crop plants and the work reported here. For example, phosphorus fertilization increased the susceptibility of young maritime pine (*Pinus pinaster* Aiton) to twisting rust (*Melampsora pinitorqua* Rostr.), and this was associated with an increase in nitrogen content of shoots (Desprez-Loustau and Wagner, 1997). Similarly, increased susceptibility of Norway spruce (*Picea abies* (L.) Karsten) to Sirococcus shoot blight was correlated with high needle N/Mg and N/Ca ratios (Anglberger and Halmschlager, 2003). However, evidence linking elevated nutrient availability with increased host susceptibility is less clear with other pathogen groups. For example, there was only a moderate positive correlation with lesion lengths caused by *Ceratocystis polonica* Siem. Moreau (a wilt pathogen) on fertilized Norway spruce (Viiri et al., 2001b), and no correlation in a linked study (Kyto et al., 1996). Similarly, colonization by *Armillaria ostoyae* (Romagnesi) Herink (a pathogen causing root and butt rot) increased in several conifer species subjected to reduced light and nitrogen levels (Entry et al., 1991). This suggests that in some pathosystems increasing host vigor might have little or no effects on disease susceptibility, or even reduce disease incidence and severity.

Our results show that fertilization decreases lignin levels and total soluble phenolics, suggesting that these compounds may be involved in disease resistance in this pathosystem. On the other hand, for a compound or group of compounds to be considered as putative agents of resistance there should be at least a negative correlation between lesion size and concentration of those compounds in the tissue comprising the reaction zone to an infection (Bonello and Blodgett, 2003), i.e., the proximal tissues in this study. In this study, there was no correlation between lesion size and lignin concentration; the correlation between lesion size and total soluble phenolics in the proximal region was significant but positive (Table 2). In a related study of Austrian pine responses to the same pathogen (Bonello and Blodgett, 2003) we found that concentration of total soluble phenolics was not correlated to lesion size in the phloem of the main stem of young trees. Thus, the combined data from the two pathosystems strongly suggest that, although total soluble phenolics may locally accumulate following infection of pine shoot tissues, their role in actual resistance remains doubtful. Accumulation in response to infection is considered a defense response, but this response may be insufficient in combating host invasion by the pathogen, i.e., in conferring resistance. Interestingly, in this study the correlation between lesion size and accumulation of total soluble phenolics in tissues distal to the lesion was negative and significant. This suggests that there is a source/sink relationship in which demand for total soluble phenolics in the defense response at the infection court. Concentration of total soluble phenolics in proximal tissues was three-fold higher than in distal tissues on average (data not shown). Such relationship is postulated to deplete total soluble phenolic pools systemically, at least as far as the distal tissues we analyzed. These data provide circumstantial evidence for mass translocation of total soluble phenolics toward the infection court, a process that one other previous study suggested to involve individual phenolics (Bonello and Blodgett, 2003). To our knowledge, no evidence exists of mass translocation of phenolics in any other plant system. However, demonstration of this process would require pulse-chase experiments with labeled phenolic precursors that are beyond the scope of this study. One individual phenolic, phenolic 36.40, showed a behavior consistent with that observed for the total soluble phenolics (Table 2). The concentration of phenolic 34.24 was positively correlated with lesion size proximally, but was not consistently detected distally.

The only compound that was negatively and significantly correlated with lesion size proximally was phenolic 12.20 (Table 2). While this may suggest that this compound is potentially involved in resistance, it is entirely possible that *S. sapinea* is also capable of catabolizing this compound, resulting in lower concentrations in tissues supporting larger lesions. If this is demonstrated, then it is unlikely that a negative correlation between lesion size and this compound's concentration is indicative of a role in resistance for this compound.

To our knowledge, the only other detailed information on the relationships between fertilization, disease susceptibility, and induced defense responses based on host secondary metabolism in a conifer is available for the Norway spruce-*C. polonica* association. Viiri et al. (2001a) found that fertilization decreased pathogen-induced accumulation of stilbene aglycones and total terpenes, suggesting a depression of defense. Kyto et al. (1996) found no correlation between N fertilization and total phenolic content in phloem when inoculated with *C. polonica*. However, the lack of correlations with disease susceptibility should be considered cautiously in this pathosystem, as lesion length may not be an appropriate measure of fungal activity for vascular wilt pathogens (Parmeter et al., 1992). Studies on the relationship between susceptibility of conifer trees to Armillaria root and butt rot and host stress induced by reduced lighting and nitrogen availability also found that increased host stress resulted in reduced concentrations of total soluble phenolics (Entry et al., 1991). However, Shaw et al. (1998) found that increasing N availability and reducing K availability for containerized Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco) seedlings decreased the content of soluble phenolics and tannins in seedling root tips.

In addition to effects on phenolic-based defenses, fertilization might enhance host nutritive quality for the fungal pathogen, resulting in increased infection or colonization rates. For example, Lambert (1986) found that fertilization of *P. radiata* with high rates of N fertilization (enough to suppress tree growth) increased foliar arginine concentration. This was correlated with increased infection by the Dothistroma needle cast fungus. Arginine accumulation was linked to a deficiency of sulphur induced by a nutrient imbalance caused by high levels of N. Although we did not measure S or arginine concentrations, fertilization did increase foliar N levels, and probably phloem N levels, which may have increased the nutritional quality of host tissue for *S. sapinea* in our study.

5. Conclusion

Two major hypotheses can explain increased susceptibility of plants to disease with increased nitrogen availability. The first hypothesis suggests a direct effect of increased nitrogen in plant tissues on the invading pathogen by providing a better growth environment and triggering genes involved in pathogenicity (Snoeijers et al., 2000). The second hypothesis deals with tradeoffs between growth and defense. Kyto et al. (1996) and Herms (2002) concluded that fertilization generally decreases tree resistance to insects by increasing host nutritional quality and/or decreasing concentrations of constitutive secondary metabolites, as both host responses to fertilization have been widely observed (Herms and Mattson, 1992; Koricheva et al., 1998). Although both hypotheses are plausible, our data appear to support the second. Disease resistance was lower in fertilized plants and constitutive lignin content (in the inoculation controls) was reduced by fertilization at both 0–3 cm and 15–18 cm (Table 1). Constitutive levels of total soluble phenolics responded less predictably, because they were reduced only at 15–18 cm, and not significantly. However, systemic effects of fertilization on induced secondary metabolites were also apparent, with significantly reduced levels of lignin and total soluble phenolics in fertilized trees at 15–18 cm in the inoculation treatment. These systemic effects on secondary metabolism are significant because modulation of systemic induced resistance or susceptibility to pathogens (Bonello et al., 2001; Blodgett and Bonello, 2002) by nutrient availability has not been investigated in conifers. Locally, any effects of fertilization on host defenses were presumably masked by the intense cellular response against the pathogen. As expected, effects of fertilization and inoculation, both locally and systemically, on individual secondary metabolites were much less predictable; their accumulation depends as much on internal tradeoffs in carbon allocations as on demands on individual biosynthetic pathways by selected metabolic processes (Koricheva et al., 1998), in our case defense responses against a specific pathogen.

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