The Induction of Systemic Resistance and Systemic Susceptibility in *Sphaeropsis sapinea* inoculated *Pinus nigra*

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Abstract—This study was conducted to test if inoculation of Austrian pines with *Sphaeropsis sapinea* results in systemic induced resistance and/or systemic induced susceptibility to subsequent colonization by the pathogen. Inoculation at the base of stems stimulated an increase in various phenolics in the phloem further up the stems. Inoculation at the stem base significantly induced resistance in the upper stem. However, inoculation at the stem base significantly induced susceptibility in shoot tips. This study demonstrates that in the same pine host either systemic induced resistance or systemic induced susceptibility can occur, depending on the site of challenge infection.

Introduction:

Sphaeropsis sapinea (Fr.:Fr.) Dyko and Sutton [syn. *Diplodia pinea* (Desmaz.) J. Kickx fil.] causes shoot blight, cankers, crown wilt, collar rot, and root diseases in at least eight coniferous genera throughout the world (Farr and others 1989; Gibson 1979; Nicholls and Ostry 1990; Stanosz and Cummings Carlson 1996; Swart and Wingfield 1991). Diseases caused by *S. sapinea* have resulted in extensive losses in nurseries, Christmas tree and ornamental plantings, and forest stands (Gibson 1979; Nicholls and Ostry 1990; Palmer and Nicholls 1985; Swart and Wingfield 1991). Two types of this pathogen (groups A and B) were originally differentiated by their appearance in culture (Palmer 1997). However, these two groups can be differentiated more clearly using molecular methods (Zhou and others 2001; Zhou and Stanosz 2001). On several conifer species, inoculations of shoot tips with group A isolates result in larger shoot cankers than inoculations with group B isolates (Blodgett and Stanosz 1999).

Systemic induced resistance (SIR) is used in this paper to describe resistance that is induced in noninfected parts of the host by prior infections elsewhere in the plant, irrespective of specific induction pathways. Recent developments have advanced our understanding of disease resistance in model herbaceous species. In contrast, relatively little is known about the biochemical and molecular basis of disease resistance in pines. Resistance mechanisms in pine could be different from those described to date.

The objectives of this study were to: 1) test if inoculation of Austrian pines (Pinus nigra) with S. sapinea results in systemic induced resistance and/or systemic induced susceptibility to subsequent colonization by S. sapinea; and 2) correlate the systemic resistance/susceptibility to changes in the secondary metabolism.

Materials and Methods:

Six-year-old, greenhouse-grown Austrian pines were wounded at the stem base and treated with either the A or B isolate group of S. sapinea (inducing inoculum); control trees were mock inoculated. At 3 wks, the pines were challenge inoculated with either an A isolate or mock inoculated, on either: the stem, 25 cm above the initial treatment sites; or branch tips. Symptoms were recorded 3 wks after the second inoculation for shoot challenges, and 5 wks after the second inoculation for stem challenges. Phloem samples were collected in both experiments for later HPLC analysis of methanol extracts. Five replications were used for each combined treatment (upper/lower treatment combinations), and two independent trials were used in both experiments.

Lesion lengths were analyzed by three-way analysis of variance with interactions. Factors used as main effects were: inducing treatment, challenge treatment, and trial. If significant differences were found ($P < 0.05$), means were separated using Fisher's least significant difference (LSD) at $P = 0.05$. Analysis of variance (using general linear model procedure) were performed using Minitab for

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Windows, release 10.2 (Minitab Inc., State College, PA).

Results:

Cankers were only observed on inoculated trees. Inoculation at the stem base with either isolate group significantly ($P < 0.001$) induced resistance in the upper stem (fig. 1). However, inoculations at the stem base significantly ($P < 0.001$) induced susceptibility in shoot tips, with the less aggressive B isolate group stimulating greater susceptibility (fig. 2). HPLC analysis of methanol extracts showed several differences among treatments. As an example, inoculations significantly ($P < 0.001$) elicited accumulation of pinosylvin in stems at both the inoculation (fig. 3), and 25 cm above the inoculation site (fig. 4).

Summary:

A novel phenomenon was observed in which inoculation at the stem base induced systemic: resistance in the upper stem, and susceptibility in shoot tips. The less aggressive B isolate group appears to stimulate greater susceptibility in the tips. This is the first time that this phenomenon has been observed, in trees or any other plants. The inducing inoculum also stimulated the accumulation of many soluble secondary compounds further up the stems.

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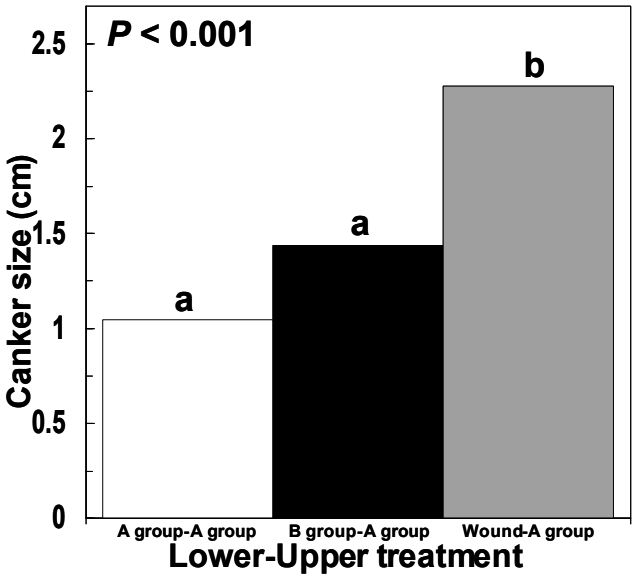

Figure 1. Canker size caused by the A isolate on upper stems for trees with three lower stem treatments. Bars with the same letter are not significantly different based on Fisher's least significant difference (LSD) at $P = 0.05$.

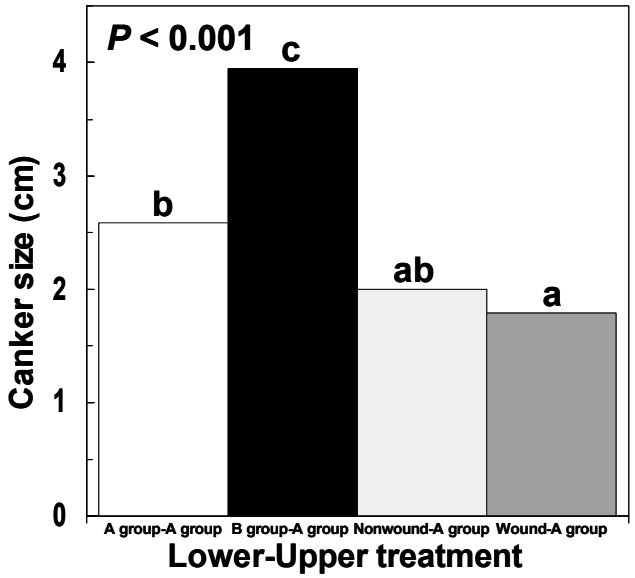

Figure 2. Canker size caused by the A isolate on branch tips for trees with four lower stem treatments. Bars with the same letter are not significantly different based on Fisher's least significant difference (LSD) at $P = 0.05$.

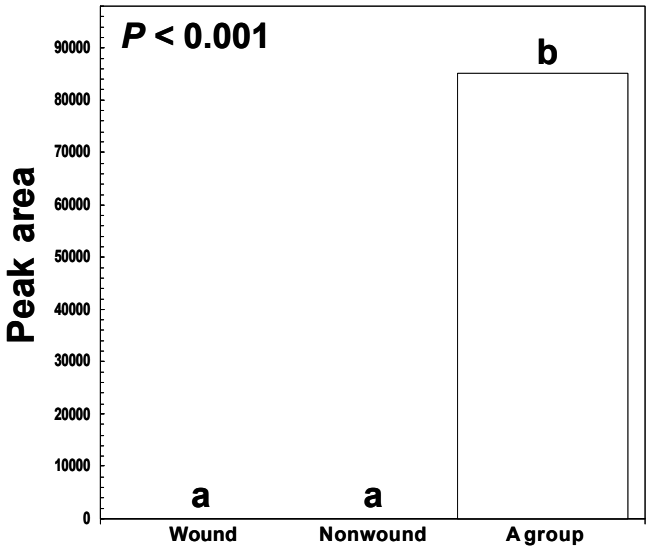

Figure 3. Relative concentration of pinosylvin at the inoculation site for three treatments. Bars with the same letter are not significantly different based on Fisher's least significant difference (LSD) at $P = 0.05$.

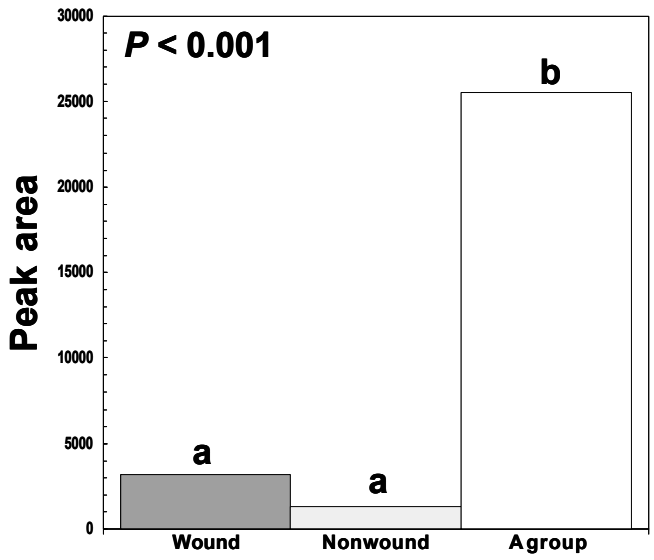

Figure 4. Relative concentration of pinosylvin 25 cm above the inoculation site for three treatments. Bars with the same letter are not significantly different based on Fisher's least significant difference (LSD) at $P = 0.05$.