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The aggressiveness of *Sphaeropsis sapinea* on Austrian pine varies with isolate group and site of infection

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Summary

The aggressiveness of the A and B isolate groups (morphotypes) of *Sphaeropsis sapinea* on shoot tips and lower stems of Austrian pine (*Pinus nigra*) was compared. The distinct differences in aggressiveness between the two *S. sapinea* groups, previously reported for other conifers, are confirmed for Austrian pine. However, the relative aggressiveness of the B group isolate varied by site of infection. Although the B group isolate of *S. sapinea* was not aggressive on shoot tips of Austrian pine, it was aggressive on stems of this host.

1 Introduction

Sphaeropsis shoot blight and stem canker diseases, caused by *Sphaeropsis sapinea* (Fr.: Fr.) Dyko and Sutton [syn. *Diplodia pinea* (Desmaz.) J. Kickx fil.], affect hosts in several coniferous genera (Gibson 1979; Farr et al. 1989). These diseases have resulted in extensive damage to conifers throughout the world. Pines are affected from the seedling stage to mature size, and damage occurs in nurseries, Christmas tree and ornamental plantings, plantations and natural stands. The disease has caused extensive losses of Austrian pine (*Pinus nigra* Arnold) in the northeast and central US limiting the use of this species in ornamental plantings.

In the US there are two groups, A and B, (previously called morphotypes) within *S. sapinea,* and they can be consistently differentiated using molecular methods (Smith and Stanosz 1995; Zhou et al. 2001; Zhou and Stanosz 2001). While isolates of the A group can be aggressive shoot blight pathogens, the B group is less aggressive or non-aggressive on shoots of some conifers (Blodgett and Stanosz 1997, 1999). However, it is not known whether these groups differ in aggressiveness on Austrian pine, or if the site of infection affects symptom severity on this or on any other host.

The objectives of this study were to: (i) compare the aggressiveness of the A and B groups on Austrian pine and (ii) test the relative aggressiveness of the two groups on shoot tips and lower stems of this host. Shoot tips (shoot-blight experiment) or lower stems (stem-canker experiment) of Austrian pine were inoculated in separate greenhouse experiments.

2 Materials and methods

Dormant, 5-year-old Austrian pine trees were obtained from Ridge Manor Nursery (Madison, OH) in spring 2001. Trees were grown in an organic mix (5% hardwood bark, 60% pine bark and 35% peatmoss) in 14.8 l (28 cm diameter × 24 cm deep) plastic pots.

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The pines had a mean stem height of 89.5 ± 0.6 cm (SE) and a mean stem diameter of 3.5 ± 0.03 cm. They were placed in a greenhouse with a mean temperature of 24 ± 0.04°C and a mean relative humidity of 49 ± 0.5%. The maximum recorded ambient greenhouse photon flux density was 995 μE s^{-1} m^{-2}. The trees were watered to field capacity twice daily.

In the first experiment (shoot-blight experiment), the pines were wounded on elongating shoot tips. Wounds were made by removing a single needle fascicle (by a sterile scalpel cut flush to the branch) approximately 2 cm below the shoot apex. A 4-mm diameter potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) plug colonized with either the A or B isolate group 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. The representative A and B group monoconidial isolates (isolate number 3AP and 124, respectively) originated from pines from central Ohio and central Wisconsin, respectively. Non-colonized PDA plugs were applied to wounded controls. Parafilm (American National Can Co., Chicago, IL) was wrapped around the shoots at the inoculation site and removed after 3 days.

In the second experiment (stem-canker experiment), the pines were wounded on the stem base, 10 cm above the soil. Stem wounding involved removing the bark/phloem with a sterile cork borer (1-cm diameter). The trees were inoculated in a similar manner as in the shoot-blight experiment, but the Parafilm was not removed until lesion lengths were scored.

Ten trees were used in each of the two experiments (shoot-blight and stem-canker) for each isolate group and for the corresponding controls. Treatments were assigned to trees in a random complete block design. Canker lengths were measured and recovery of *S. sapinea* was attempted from surface disinfested shoots or bark of each tree at 4 and 6 weeks after shoot and stem inoculations, respectively. The bark around the inoculation site was removed with a sterile knife to expose the canker margin for measurements. Measurements were then made basipetally on the shoots and acropetally on the stems (i.e. only below or above the wounds, respectively).

The effects of isolate on canker size were compared using ANOVA in each experiment. Fisher's least significant difference (LSD) at $p = 0.05$ was used to compare mean canker lengths. ANOVA (using the general linear model procedure) were performed with the Minitab for Windows program (release 10.2; Minitab Inc., State College, PA).

3 Results

For shoot tip inoculations, cankers were produced on 100% of the shoots inoculated with the A isolate, but on only 60% of the shoots inoculated with the B isolate. Cankers were never produced on wounded controls. Inoculations with the A isolate resulted in abundant necrotic needles, dead shoots, and resin at the wound site (Fig. 1A). Symptoms induced by inoculation with the B isolate ranged from no symptoms to three necrotic needle fascicles at the wound site (Fig. 1B). Cankers on shoots inoculated with the A isolate were significantly ($p < 0.001$) longer than those on shoots inoculated with the B isolate, but cankers on shoots inoculated with the B isolate were not significantly longer than controls (Fig. 2).

For lower-stem inoculations, both isolate groups caused cankers 100% of the time. Cankers were never produced on wounded controls. Inoculations with both isolate groups resulted in resinous cankers that expanded both basipetally and acropetally, with little horizontal spread. Cankers resulting from inoculation with the A isolate (Fig. 1C, D) tended to be more diffuse and appeared less resinous within the canker than those resulting from inoculations with the B isolate (Fig. 1E), which tended to have resinous centres and more distinct margins. Cankers produced by the A isolate were significantly

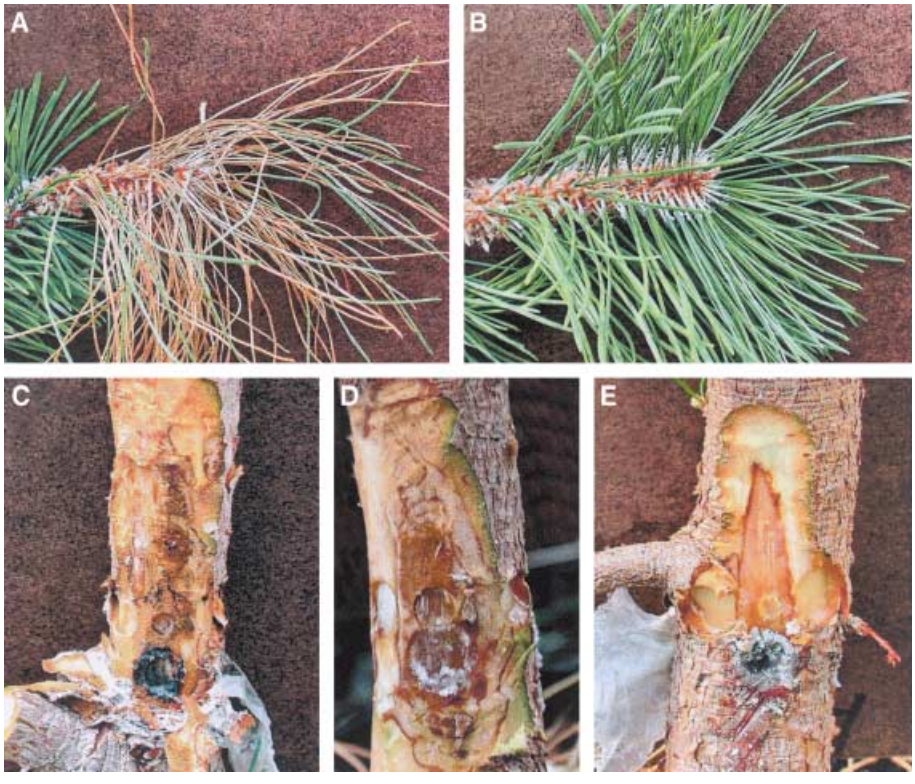

Fig. 1. Symptoms on greenhouse-grown Austrian pines that were wounded and inoculated with A or B isolates of *Sphaeropsis sapinea*. Shoot-blight experiment: symptoms 4 weeks after inoculation with the A (A) and B (B) isolate groups on elongating shoot tips 2 cm below the shoot apex. Stem-canker experiment: symptoms 6 weeks after inoculation with the A (C and D) and B (E) isolate groups on the stem base 10 cm above the soil. The two cankers shown for the A isolate represent the range of symptoms resulting from inoculation with this isolate

($p < 0.001$) longer than those produced by the B isolate and cankers produced by both the A and B isolates were significantly ($p < 0.001$) longer than controls (Fig. 2).

Sphaeropsis sapinea was recovered from both symptomatic and asymptomatic trees. The pathogen was recovered from 100% of the inoculated trees in both experiments, and from 10 and 0% of the control trees in the shoot-blight and stem-canker experiments, respectively.

4 Discussion

Isolates of the B group previously have been shown to cause significant disease on the shoots of a few conifers, namely jack pine (Blodgett and Stanosz 1997), blue spruce (Blodgett and Stanosz 1999) and American and European larch (Stanosz et al. 1997a). In the prior studies, however, a distinct difference in aggressiveness between the two known groups of *S. sapinea* was found, with A isolates being significantly more aggressive than B isolates on shoots of several conifers (Blodgett and Stanosz 1997, 1999). In our study, the aggressiveness of an A group isolate on both shoots and stems

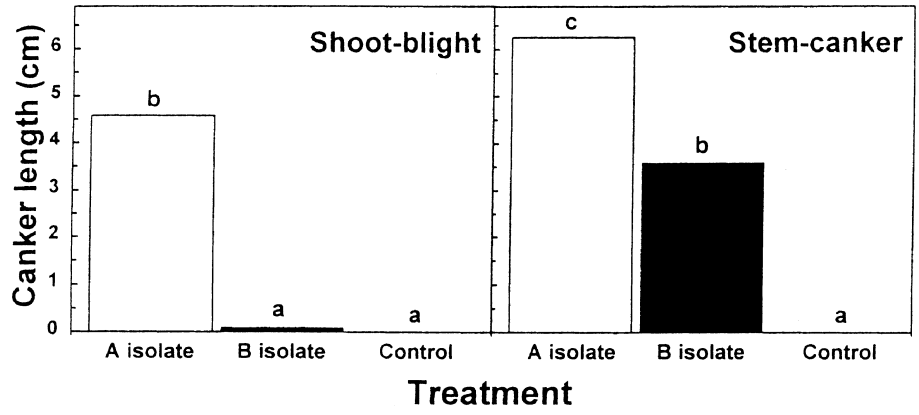

Fig. 2. Canker length on greenhouse-grown Austrian pines that were wounded and inoculated with A or B isolates of *Sphaeropsis sapinea*. Shoot-blight experiment: cankers 4 weeks after inoculation on elongating shoot tips 2 cm below the shoot apex. Stem-canker experiment: cankers 6 weeks after inoculation on the stem base 10 cm above the soil. Within each experiment, bars with the same letter are not significantly different based on Fisher's least significant difference (LSD) at $p = 0.05$

was confirmed. In addition, the site of infection significantly affected the relative aggressiveness of the B isolate. Although inoculations with the B isolate result in little or no measurable symptoms on shoots, the potential for the B group isolates to cause significant cankers on the main stems of Austrian pine was demonstrated. Consequently, while the A group is confirmed as an aggressive shoot blight and stem canker pathogen, the B group appears only to be an aggressive stem canker pathogen on this host.

The A group of *S. sapinea* has been shown to persist asymptomatically on or in shoots of naturally infected pines (Stanosz et al. 1997b), including Austrian pine (Flowers et al. 2001). In the current study, a B isolate was able to persist asymptomatically in shoots of Austrian pine for at least 4 weeks following wound inoculation. Recovery from 10% of control shoots and all asymptomatic shoots inoculated with the B isolate might be explained by latent infection (Stanosz et al. 1997b, 2001; Flowers et al. 2001). This asymptomatic persistence might be one survival strategy for both *S. sapinea* groups.

Planting conifer species resistant to *S. sapinea* is an important management strategy in ornamental plantings in areas where the pathogen is found, where conditions favour disease development and where there is little tolerance for damage. Because both groups of *S. sapinea* can cause significant cankers on stems of Austrian pine, both groups should be considered when determining the cause of symptoms attributed to *S. sapinea* in regions where each occurs. In addition, the potential for the B group to cause severe disease in woody stems of other conifer hosts should be investigated and both groups should be used in future studies of resistance.

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Résumé

L'agressivité de Sphaeropsis sapinea varie selon son groupe morphologique et le site d'infection chez le pin noir d'Autriche

L'agressivité des groupes morphologiques A et B de *Sphaeropsis sapinea* a été comparée sur des extrémités de pousses et à la base des tiges chez le pin noir d'Autriche. Les nettes différences d'agressivité entre ces deux groupes, déjà mentionnées chez d'autres conifères, ont été confirmées chez le pin noir. Cependant, l'agressivité relative du groupe B variait selon le site d'infection. Ce groupe n'était pas agressif sur l'extrémité des pousses, mais l'était au niveau du tronc des pins noirs d'Autriche.

Zusammenfassung

Die Aggressivität von Sphaeropsis sapinea auf Schwarzkiefer variiert in Abhgängigkeit von Morphotyp und Infektionsort

Es wurde die Aggressivität von Isolaten der Gruppe A und B (Morphotypen) von *Sphaeropsis sapinea* auf Triebspitzen und im Stammbereich von *Pinus nigra* untersucht. Die von anderen Koniferen beschriebenen ausgeprägten Unterschiede in der Aggressivität dieser beiden *S. sapinea*-Gruppen, wurden an Schwarzkiefer bestätigt. Die relative Aggressivität der B-Gruppe variierte jedoch in Abhängigkeit vom Infektionsort. Obwohl die B-Gruppen an Triebspitzen von *P. nigra* weniger Nekrosen verursachte, war sie an Stämmen dieses Wirtes aggressiv.

References

Blodgett, J. T.; Stanosz, G. R., 1999: Differences in aggressiveness of *Sphaeropsis sapinea* RAPD marker group isolates on several conifers. Plant Dis. **83,** 853–856.

Blodgett, J. T.; Stanosz, G. R., 1997: *Sphaeropsis sapinea* morphotypes differ in aggressiveness, but both infect nonwounded red or jack pines. Plant Dis. **81,** 143–147.

Farr, D. F.; Bills, G. F.; Chamuris, G. P.; Rossman, A. Y., 1989: Fungi on Plants and Plant Products in the United States. St Paul, MN: American Phytopathological Society.

Flowers, J.; Nuckles, E.; Hartman, J.; Vaillancourt, L., 2001: Latent infection of Austrian and Scots pine tissues by *Sphaeropsis sapinea*. Plant Dis. **85,** 1107–1112.

Gibson, I. A. S., 1979: Diseases of Forest Trees Widely Planted as Exotics in the Tropic and Southern Hemisphere. Part II. The Genus *Pinus*. Kew, UK: Commonwealth Mycological Institute.

Stanosz, G. R.; Blodgett, J. T.; Smith, D. R.; Kruger, E. L., 2001: Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings. New Phytologist **149,** 531–538.

Stanosz, G. R.; Smith, D. R.; Guthmiller, M. A., 1997a: Pathogenicity of A and B morphotypes of *Sphaeropsis sapinea* confirmed on American larch (tamarack) and European larch. Eur. J. For. Path. **27,** 301–307.

Stanosz, G. R.; Smith, D. R.; Guthmiller, M. A.; Stanosz, J. C., 1997b: Persistence of *Sphaeropsis sapinea* on or in asymptomatic shoots of red and jack pines. Mycologia **89,** 525–530.

Smith, D. R.; Stanosz, G. R., 1995: Confirmation of two distinct populations of *Sphaeropsis sapinea* in the north central United States using RAPDs. Phytopathology **85,** 699–704.

Zhou, S.; Smith, D. R.; Stanosz, G. R., 2001: Differentiation of *Botryosphaeria* species and related anamorphic fungi using inter simple or short sequences repeat (ISSR) fingerprinting. Mycol. Res. **105,** 919–926.

Zhou, S.; Stanosz, G. R., 2001: Relationships among *Botryosphaeria* species and associated anamorphic fungi inferred from analyses of ITS and 5.8s rDNA sequences. Mycologia **93,** 516–527.