

## Differences in Occurrence and Co-occurrence of *Sirococcus conigenus* and *Diplodia pinea* on Blighted Red Pine Shoots

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**Abstract** – Blighted red pine shoots, either having symptoms only on the most recent year's growth or having symptoms present both on most recent and previous year's growth, were examined for pycnidia of *Sirococcus conigenus*, *Diplodia pinea*, or both. On shoots having symptoms only on the most recent year's growth, *S. conigenus* was detected more frequently than *D. pinea*. On shoots having symptoms on both the most recent and the previous year's growth, however, *D. pinea* was detected more frequently than *S. conigenus* on the most recent year's growth. In addition, on those shoots having symptoms on both the most recent and the previous year's growth, *D. pinea* was detected almost eight times more frequently on the previous year's growth than the most recent year's growth. Whether only one pathogen was detected or the two pathogens were detected co-occurring on a single year's growth also varied with shoot condition and shoot year of growth, but the two fungi co-occurred on shoots of each condition.

*Pinus resinosa* / *Sirococcus strobilinus* / *Sphaeropsis sapinea*

**Kivonat** – **Eltérések a *Sirococcus conigenus* és a *Diplodia pinea* előfordulása és együttes előfordulása között *Pinus resinosa* pusztuló hajtásain.** Vagy csak a folyó évi, vagy a folyó és előző évi növedékeken is tünetet viselő, pusztuló *Pinus resinosa* hajtásokon vizsgáltunk a *Sirococcus conigenus* és/vagy *Diplodia pinea* piknidiumok jelenlétét. A csak folyó évi tünetes hajtásokon a *S. conigenus* gyakoribb volt, mint a *D. pinea*. A folyó, és előző évi növedéken is tünetes hajtásokon viszont a legújabb növedéken a *D. pinea* gyakoribb volt, mint a *S. conigenus*. Ráadásul a legújabb és az előző évi növedéken is tüneteket hordozó hajtások esetében az előző évi növedéken a *D. pinea* csaknem nyolcszor gyakoribbnak bizonyult, mint a folyó évi növedéken. Csak az egyik, vagy mindkét kórokozó együttes előfordulása az egyes növedékeken a hajtás állapotától és a növedék évétől függően változott, de a két gomba minden állapotú hajtáson együttesen fordult elő.

*Pinus resinosa* / *Sirococcus strobilinus* / *Sphaeropsis sapinea*

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## 1 INTRODUCTION

Red pine (*Pinus resinosa*) is an ecologically and commercially important tree species of the northern United States and southern Canada, where it extends from the Atlantic coast westward through the Great Lakes region. Rudolf (1990) reviewed the silvical characteristics of red pine, which may exist naturally in relatively pure stands or in mixtures. Associated species on the coarser soils on which red pine is most common include jack pine (*Pinus banksiana*), eastern white pine (*P. strobus*), and scrubby oak species (e.g., *Quercus ellipsoidalis*). Natural regeneration is sporadic, apparently dependent on an abundant seed crop and a coincidence of local conditions that may include a preceding fire, opening of the overstory canopy, reduction of seed predators, favorable weather providing moisture for seedling establishment, and subsequent absence of fire for several decades. Because silvicultural techniques that might allow consistent achievement of adequate natural regeneration on most sites have not yet been mastered (Farnsworth 2002), planting is the primary means of stand replacement following commercial clear cut harvests. For example, almost 250 million red pine seedlings have been produced in state-operated nurseries during the last 25 years in Wisconsin (personal communication, Gregory Edge, Wisconsin Department of Natural Resources), and red pine plantations in Wisconsin occupy approximately 213,000 ha (almost three-fourths of the total area of planted forest in this state) (Schmidt 1997). Mechanical and/or chemical treatments to reduce herbaceous and woody competition with this relatively intolerant species may be applied both before and after planting. Seedlings may require 4-10 years to reach breast height (1.37 m), with height growth thereafter usually ranging from 0.3-0.5 m per year. Three to four thinnings beginning at age 30-40 years until a final harvest at age 60-80 years are common in stands commercially managed for pulpwood, poles, and small sawtimber. Left to grow, however, red pines may be long-lived and exceed 300 years of age.

Among the diseases that can severely damage red pines is shoot blight caused by the fungus *Sirococcus conigenus* (DC.) P. Cannon and Minter (syn. *Sirococcus strobilinus* G. Preuss). This pathogen occurs widely in Europe and both the eastern and western United States and Canada, attacking a variety of conifers, especially spruces and pines (Farr et al. 1989, Smith et al. 2003, Sutton 1980). In northern Wisconsin and the upper peninsula of Michigan, a disease often characterized by drooping and retention of needles on diseased shoot tips was first noticed in 1959, but over a decade passed before *S. conigenus* (as *S. strobilinus*) was identified as the causal agent (O'Brien 1973). Pycnidia of the pathogen develop on killed shoots, especially at the bases of necrotic red pine needles. Ostry et al. (1990) trapped conidia dispersed from diseased red pines during periods of wet weather in spring and early summer when they are carried by rainfall to young, susceptible shoots. Small red pine seedlings may be killed within one growing season, while growth of saplings is reduced or they are killed from cumulative effects of infection in successive years, especially in the understory of infested stands (Bronson - Stanosz 2006, Ostry et al. 1990). The "die up" of lower branches to result in progressive crown reduction and the probability of mortality of large, overstory red pine trees was noted by O'Brien (1973).

Red pine also is among the forest tree species most severely damaged by a second shoot blight pathogen, *Diplodia pinea* (Desmaz.) J. Kickx. fil. (syn. *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton). This fungus reportedly attacks over 30 pine species, and less commonly other conifers, both in their native ranges and where introduced in the southern hemisphere (Farr et al. 1989, Gibson 1979, Punithalingam – Waterston 1970). In the northcentral United States *D. pinea* has been particularly damaging to red pine seedlings in nurseries with infested red pine windbreaks providing inoculum (Palmer – Nicholls 1985, Palmer et al. 1988, Stanosz et al. 2005). Conidia are disseminated from pycnidia borne in necrotic needles, stems, and

cones during periods of rain (Palmer et al. 1988). Infection of red pines has been achieved experimentally through wounds and by conidial application to nonwounded, young, expanding shoot tips (Blodgett – Stanosz 1997). Virulent strains of this pathogen persist on or in red pines in nurseries and in the forest in the absence of symptoms (Stanosz et al. 1997, Stanosz et al. 2001, Stanosz et al. 2005). The potential for *D. pinea* to subsequently proliferate and rapidly cause disease, including mortality, under conditions that induce host stress is supported by greenhouse experiments (Stanosz et al. 2001). Epidemics characterized by high frequencies of mortality of red pine seedlings and saplings, and branch dieback, stem cankers, and top-kill on larger trees have been associated with drought (Nicholls – Ostry 1990, Palmer 1991, Stanosz – Cummings Carlson 1996). The role of water stress in enhancing colonization of red pine shoots by water stress was demonstrated using potted seedlings and established plantation trees (Blodgett et al. 1997a, Blodgett et al. 1997b).

## 2 MATERIALS AND METHODS

Red pine shoots were collected in mid-winter from four sites (replicates) in the Northern Highland-American Legion (NHAL) State Forest in Vilas County of northern Wisconsin (46°05'N, 89°40'W). Planted red pine saplings were present at each site and scattered red pine overstory trees had been deliberately retained (for aesthetic reasons) during the previous harvest. The soils are nutrient-poor sands on glacial outwash. Preliminary observations revealed shoot blight symptoms and indicated presence of both *S. conigenus* and *D. pinea* at each site.

Shoots were obtained at five sampling points located at intervals along transects across each site. At each sampling point, five shoots of each of two different shoot conditions were collected, either having symptoms present only on the most recent year's growth or having symptoms present both on most recent year's and previous year's growth (25 shoots of each condition at each of the four sites, or 200 shoots total). Shoots were individually bagged, carried to the laboratory, and refrigerated until they were examined further.

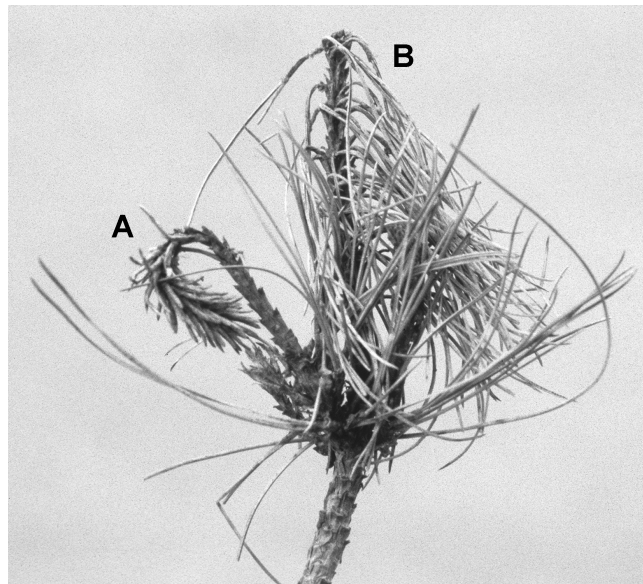
A dissecting microscope was used to inspect needles and stems of the symptomatic most recent year's growth and symptomatic previous year's growth for pycnidia. If pycnidia were present, spores were examined at up to 400x using a light microscope. The shoot blight pathogens *S. conigenus* and *D. pinea* were identified based on presence of characteristic spores obtained from pycnidia (Sutton 1980). Although not used during this study, molecular methods for identification of each of these fungi (as differentiated from closely related species) (Smith – Stanosz 2006, Smith – Stanosz submitted) have been employed on multiple samples obtained on other occasions in the vicinity of the collection sites.

Occurrence of each pathogen was recorded for shoots of each of the two conditions by shoot year of growth (most recent or previous year's growth). The means and standard errors of the frequencies of occurrence of pycnidia of each pathogen for each shoot condition-year of growth combination were calculated. Comparisons were made using the Kruskal-Wallis test of equality of medians. Analyses were performed using Minitab for Windows version 14 (Minitab Inc., State College, PA).

## 3 RESULTS

Blighted shoots exhibited symptoms that have been considered characteristic of *Sirococcus* shoot blight or *Diplodia* shoot blight on red pine (Palmer - Nicholls 1985, Ostry et al. 1990). These symptoms included brown to gray discoloration of needles, retention of dead needles

on dead stems, death of shoots either before or after full needle elongation, curl or crook of the stem of the most recent year's growth on shoots killed during expansion (a symptom commonly associated with *Diplodia* shoot blight), and droop of killed, fully expanded needles (considered characteristic of *Sirococcus* shoot blight). The particular symptoms were not, however, always indicative of whether *S. conigenus* or *D. pinea* was detected on a particular shoot. For example, *S. conigenus* was detected on crooked or curled dead shoots, and *D. pinea* was detected on killed shoots with drooped, killed fully-expanded needles (*Figure 1*).



*Figure 1. Blighted red pine shoots exhibiting: (A) curl of the stem of a shoot killed during expansion (a symptom commonly associated with Diplodia shoot blight); and (B) droop of dead fully-expanded needles (considered characteristic of Sirococcus shoot blight). However, pycnidia with conidia of S. conigenus were found on the shoot at (A), and pycnidia with conidia of D. pinea were found on the shoot at (B)*

Although either pathogen (at least one of these two fungi) was detected on the majority of the shoots collected at each site, the frequency of detection of each pathogen varied with shoot condition and shoot year of growth (*Table 1a, b*). *Sirococcus conigenus* or *D. pinea* were detected on 77/100 of the shoots having symptoms present only on the most recent year's growth and 91/100 of the shoots having symptoms present both on most recent year's and previous year's growth. On shoots having symptoms present only on the most recent year's growth, *S. conigenus* was detected more frequently than *D. pinea*. On shoots on which symptoms were present on both the most recent year's growth and the previous year's growth, however, *D. pinea* was detected more frequently than *S. conigenus* on the most recent year's growth. In addition, on shoots on which symptoms were present on both the most recent and the previous year's growth, *D. pinea* was detected almost eight times more frequently on the previous year's growth than the most recent year's growth.

Table 1a. Total occurrence of pycnidia of *Sirococcus conigenus* (Sc) or *Diplodia pinea* (Dp) on blighted most recent and previous years' growth of red pine shoots. Values are means  $\pm$  standard error of the mean for four sites (replicates); 25 shoots of each condition at each site were examined. Uppercase letter under each value identifies that cell for pairwise comparison with other cells (see Table 1b).

Shoot condition	Year of growth of needles or stems on which the indicated pathogens were detected	Total no. of shoots (out of 25) on which the indicated pathogens were detected	
		Sc	Dp
Symptoms present only on most recent year's growth	Most recent	15.75 $\pm$ 1.49 A	9.25 $\pm$ 2.29 D
	Previous	NA	NA
Symptoms present both on most recent year's and previous year's growth	Most recent	13.75 $\pm$ 1.11 B	18.75 $\pm$ 1.80 E
	Previous	1.25 $\pm$ 0.63 C	9.75 $\pm$ 1.94 F

Table 1b. Values of *p* for pairwise comparisons using the Kruskal-Wallis test of equality of medians. Upper case letters correspond to those in cells in Table 1a. Comparisons that were not of interest were omitted (-).

	A	B	C	D	E
B	0.31				
C	0.02	0.02			
D	0.06	-	-		
E	-	0.03	-	0.02	
F	-	-	0.02	1.00	0.02

Whether one pathogen was detected and not the other, or whether the two pathogens were detected co-occurring on a single year's growth of a shoot also varied with shoot condition and shoot year of growth (Table 2a, b). On shoots having symptoms present only on the most recent year's growth, *S. conigenus* only tended to be detected more frequently than *D. pinea* only or both pathogens together. On the most recent year's growth of shoots on which symptoms were present on both the most recent year's growth and the previous year's growth, *D. pinea* only or both pathogens together were detected more frequently than *S. conigenus* only. On the previous year's growth of shoots on which symptoms were present on both the most recent and previous year's growth, *D. pinea* only was much more frequently detected than *S. conigenus* only or both pathogens together.

Table 2a. Occurrence of pycnidia of *Sirococcus conigenus* (Sc) only, *Diplodia pinea* (Dp) only, or both on blighted most recent and previous years' growth of red pine shoots. Values are means  $\pm$  standard error of the mean for four sites (replicates); 25 shoots of each condition at each site were examined. Uppercase letter under each value identifies that cell for pairwise comparison with other cells (see Table 2b).

Shoot condition	Year of growth of needles or stems on which the indicated pathogens were detected	No. of shoots (out of 25) on which the indicated pathogens were detected		
		Sc only	Dp only	Both
Symptoms present only on most recent year's growth	Most recent	10.00 $\pm$ 2.48 A	3.50 $\pm$ 0.50 D	5.75 $\pm$ 1.93 G
	Previous	NA	NA	NA
Symptoms present both on most recent year's and previous year's growth	Most recent	3.50 $\pm$ 1.19 B	8.50 $\pm$ 1.94 E	10.25 $\pm$ 0.25 H
	Previous	0.25 $\pm$ 0.25 C	8.50 $\pm$ 2.06 F	1.00 $\pm$ 0.48 I

Table 2b. Values of *p* for pairwise comparisons using the Kruskal-Wallis test of equality of medians. Upper case letters correspond to those in cells in Table 2a. Comparisons that were not of interest were omitted (-).

	A	B	C	D	E	F	G	H
B	0.08							
C	0.02	0.03						
D	0.05	-	-					
E	-	0.06	-	0.02				
F	-	-	0.02	0.04	0.88			
G	0.19	-	-	0.35	-	-		
H	-	0.02	-	-	0.24	-	0.18	
I	-	-	0.16	-	-	0.02	0.03	0.02

#### 4 DISCUSSION

Plant disease is often studied in the growth chamber, greenhouse, or the field as the product of a single pathogen with its host. For woody plants in complex ecosystems such as forests, however, there are likely to be many situations in which fungi (including plant pathogens) could exist in communities with other microorganisms within the same trees and parts of trees. Recognition that *S. conigenus* and *D. pinea* may co-occur, not just in the same location and tree but on the same shoot, has implications for surveys to assess incidence and severity of disease and also for further studies of their potential interactions in shoot blight disease cycles.

Reliance on symptoms and even the utilization of fruiting structures as indicators of occurrence of a fungus have great limitations. Our experience indicates a lack of specificity in blight symptoms, or at least that a symptom considered characteristic of one pathogen (e.g., droop of red pine needles caused by *S. conigenus*) does not exclude the possibility of other pathogens also being present. Therefore, symptom-based surveys that fail to substantiate

presence of particular pathogens must be viewed with skepticism. For example, a visual survey of red pines for shoot blight damage attributed to *S. conigenus* was conducted in the Northern Highland-American Legion State forest in 1995 (Prey et al. 1995). "Level of infection" was categorized as heavy or light at each of 49 widely distributed sites, but no information was presented regarding frequency of identification of *S. conigenus* (or other pathogens) on even a subset of shoots. Conversely, while fruiting structures can substantiate presence of a fungus of interest, a lack of pycnidia with conidia of *S. conigenus* or *D. pinea* does not support the conclusion that they are absent. Sporulation may not always occur even on symptomatic shoots. And because sporulation of these pathogens occurs only on necrotic tissues, fruiting structures are not useful indicators of their association with asymptomatic needles or stems. Therefore, our data present a very conservative estimate of the frequency of the co-occurrence of *S. conigenus* and *D. pinea*. Future studies might employ both species-specific molecular methods (Smith – Stanosz 2006; Smith – Stanosz submitted) and cultural techniques to detect and confirm viability of *S. conigenus* and *D. pinea* in a variety of host organs prior to, during, and after disease development.

Co-occurrence of *S. conigenus* and *D. pinea* on shoots examined in this study does validate two previous observations by our group of the co-occurrence of *S. conigenus* and *Diplodia* shoot blight fungi on red pine shoots. Smith and Stanosz (submitted) attempted to culture *S. conigenus* from blighted red pine shoots on which pycnidia with conidia of this pathogen (i.e., *S. conigenus*) had been observed. Fungi identified as either *D. pinea* or the similar fungus *D. scrobiculata* (De Wet et al. 2003) on the basis of characteristics of pycnidia with spores that were produced in culture, were obtained in 43 of 180 attempts from needles, bark, or wood of these shoots. Bronson and Stanosz (2006) found pycnidia and spores identified as *S. conigenus* and *D. pinea* occurring together on red pine seedlings that became blighted after being planted in the understory of a mature red pine plantation. After just one summer, fruiting of both pathogens on current year's shoot growth was observed on as many as 31% of the seedlings that had become blighted, and fruiting of both pathogens on previous year's shoot growth was observed on as many as 10% of the seedlings that had become blighted. As in our current report, Bronson and Stanosz (2006) found *S. conigenus* (alone or with *D. pinea*) more frequently on the most recent year's shoot growth, and found *D. pinea* (alone or with *S. conigenus*) more frequently on the previous year's shoot growth.

The natural co-occurrence of *S. conigenus* and *D. pinea* on red pine shoots may stimulate further studies of their possible interactions before, during, and after development of disease. The potential for each fungus to inhibit or stimulate spore germination, infection, or colonization by the other is unknown. Similarly, each could influence pycnidial production and sporulation, or survival of the other fungus in shoots in which they compete for substrate. The relative prevalence of *S. conigenus* on the most recent year's shoot growth and *D. pinea* on previous year's growth suggest the potential for resource partitioning. These differences reflect the relative invasiveness of these fungi and differences in progression of the diseases they cause. Damage by *S. conigenus* to pines is associated with invasion of succulent new growth on branch tips, although it may extend into 1-year-old twigs (Smith 1973, Nicholls – Robbins 1984, Sinclair – Lyon 2005). In contrast, although *D. pinea* readily infects expanding needles and shoots, it is much more invasive, colonizing bark and sapwood of large branches and main stems of pines, including red pine (Nicholls – Ostry 1990, Palmer 1991).

Prevalence of *D. pinea* on blighted shoots of red pine collected in the Northern Highland-American Legion State Forest also supports the hypothesis that *D. pinea* is an invasive pathogen that has greatly expanded its range and activity in the northcentral United States in the last 30 to 40 years. A single instance of a fungus referred to as *Sphaeropsis ellisii* was reported from eastern white pine (*P. strobus*) in Wisconsin in the 1930's (Crandall 1938), but the identity of this fungus cannot be confirmed. However, reports of economic damage did

not soon follow, and *D. pinea* (or synonyms) was not included on comprehensive lists of fungi occurring on Wisconsin's native pines for several decades (Greene 1965). Further, during extensive studies of Sirococcus shoot blight in the Northern Highland-American Legion State Forest in the 1970's that were described by Ostry et al. (1990), *D. pinea* was not encountered (personal communication Michael Ostry, USDA Forest Service; Thomas Nicholls, USDA Forest Service, retired). Reports of economic damage to jack and red pines in seedling nurseries and plantations in Wisconsin began to appear only during the 1970's (Renlund 1977, Renlund 1979). The probable extension of the geographic range of *D. pinea* and its current prevalence at sites in the Northern Highland-American Legion State Forest today is not surprising, however, given knowledge of the persistence of virulent strains of *D. pinea* on asymptomatic red pine seedlings in nurseries and the many years of planting and subsequent disease of seedlings from infested nurseries (Stanosz – Cummings Carlson 1996, Stanosz et al. 1997, Stanosz et al. 2001, Stanosz et al. 2005).

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