Proc. First IUFRO Rusts of Forest Trees WP Conf., 2–7 Aug, 1998, Saariselkä, Finland
Finnish Forest Research Institute, Research Papers 712: 305–309, 1998

Endophytic fungal flora from eastern white pine needles and apple tree leaves as a means of biological control for white pine blister rust

J.A. Bérubé[1], J.G. Trudelle[2], O. Carisse[3], and M. Dessureault[4]

[1]Laurentian Forestry Centre, Canadian Forest Service, 1055 du PEPS, CP 3800, Ste-Foy, QC, Canada, G1V 4C7. E-mail: jaberube@cfl.forestry.ca
[2]Centre de Recherche en Biologie Forestière, Université Laval, Ste-Foy, QC, Canada, G1K 7P4.
[3]Agriculture and Agri-food Canada, Horticulture Research Station, 430 Gouin, St-Jean-sur-Richelieu, QC, Canada, J3B 3E6.
[4]Faculté de Foresterie et Géomatique, Université Laval, Ste-Foy, QC, Canada, G1K 7P4.

Summary

We are studying the biodiversity of the endophytic fungal flora from eastern white pine (*Pinus strobus*) across its Canadian range. 3200 needles from 6 Canadian ecoregions were plated on growth media to determine qualitatively and quantitatively the biodiversity of its endophytic flora. Preliminary results indicate that more than 80 species of fungal endophytes are to be isolated from eastern white pine. Another large collection of previously isolated endophytic fungi from white pine needles and apple tree (*Malus domestica*) leaves were tested for their potential to inhibit *Cronartium ribicola* the causal agent of white blister rust. *Coniothyrium* spp. isolates from apple leaves was very effective in controlling *C. ribicola* on *Ribes* leaves, the alternate host of the disease. *Coniothyrium* caused a dramatic reduction in the number of teliospores, infecting and killing the fungus at the uridial stage. From the 99 white pine needle endophytes tested, three have shown potential to inhibit the infection of *C. ribicola* basidiospores on white pine needles. Preliminary results of these tests will be presented and discussed.

Key words: *Cronartium ribicola,* endophytes, *Microsphaeropsis arundinis, Pinus strobus*

1 Introduction

Eastern white pine (*Pinus strobus* L.) is one of the most valuable timber species in eastern Canada, and it is highly susceptible to white pine blister rust caused by *Cronartium ribicola* J.C. Fisch. Seedlings and young saplings are most susceptible to infection, especially under cool, wet environmental conditions such as those found in the northeastern American continent.

A biocontrol agent effective against *C. ribicola* would be a useful tool for the management of young white pine plantations and naturally regenerating stands. Since teliospores of *C. ribicola* produce the infectious propagules

leading to blister rust on white pine, inhibition of teliospore production or its uredium precursor could be an effective way to control this disease. Alternatively, inhibition of blister rust in the early infection stages on pine needles would yield the same result.

We report here on the biodiversity of fungal endophytes found in needles of *P. strobus*, nursery tests of 63 isolates of needles endophytes to inhibit infection on white pine seedlings, and *in vivo* tests of two isolates of *Microsphaeropsis arundinis* (Ahmad) Sutton, against *C. ribicola* on *Ribes glandulosum* Grauer leaves.

2 Biodiversity of fungal endophytes of *Pinus* strobus

2.1 Material and methods

To evaluate the biodiversity of fungal endophytes of *P. strobus*, 320 needles on eight trees were sampled in four stands in the region near Québec City. Needles were surface sterilized and plated on potato dextrose agar (PDA) and maltextract agar (MA). Fungal endophytes usually emerged from needles after one week but up to one month.

2.2 Results

Pinus strobus has a very diverse endophytic fungal flora. Some 73 putative species of endophytes were isolated. After sampling three sites, 95% of the fungal endophytes of a given region had been found. The most common endophytes found were *Leptostroma* spp. (32.7%), *Pseudotypella translucens* (Gordon) Reid & Minter, which is a parasite of *Leptostroma* spp (17.8%), Dematiaceous 1 (15.5%), *Hormonema* spp. (12.4%), and *Acremonium kiliense* Grutz (6.2%) (Table 1). *Apiospora montagnei* Sacc., *Pestaliopsis funerea* Steyert, *Phacidiopycnis* spp., *Myrioconium* spp., *Phacidiopycnis* spp., *Cladosporium* spp., and *Coniothyrium* spp. were found at lesser frequencies.

Table 1. Frequency of the most common fungal endophytes of *Pinus strobus*.

Isolate number	Name	Frequency
QS-10	*Pseudotypella. translucens*	17.8%
QS-24	Dematiaceous 1	15.5%
QS-7	*Hormonema* 1	8.5%
QS-18	*Leptostroma* 6	7.0%
QS-1	*Leptostroma* 2	6.2%
QS-40	*Leptostroma* 1	6.2%
QS-88	*Acremonium kiliense*	6.2%
QS-85	*Leptostroma* 3	4.7%
QS-108	*Leptostroma* 4	4.7%
QS-6-3	*Leptostroma* 5	3.9%
QS-6-1	*Hormonema* 4	3.9%

3 Inhibition of *C. ribicola* on *Pinus strobus* needles using fungal endophytes

3.1 Material and methods

A collection of 63 fungal endophyte isolates previously collected from healthy asymptomatic needles of white pine were tested for their biocontrol potential. The isolates were grown on PDA in the dark at 20° C until the media was covered with spores. A spore suspension was made by gently crushing the collected surface mycelium in sterile distilled water containing Tween 20.

The 63 fungal endophyte isolates were inoculated onto 2000 one-year-old seedlings in July 1997. Thirty days later, in August 1997, the seedlings were exposed to *C. ribicola* in a nursery compound and in a wooded lot where the disease is common. The seedlings were placed in a greenhouse in December 1997 to accelerate symptom expression. Results were scored by counting yellow spots on treated and control seedlings as an indicator of blister rust infection.

3.2 Results

Fourteen isolates representing some seven putative fungal endophyte species (Table 2) were found to inhibit infection by *C. ribicola*. The most interesting of fungal endophyte species, labeled Species A, is represented by three isolates in our collection. Control seedlings developed an average of more than 10 yellow spots, whereas seedlings treated with Species A averaged less than 0.2 spots per seedling.

Table 2. Potential* for needle endophyte isolates to inhibit infection by *Cronartium ribicola* on white pine seedlings.

S-1	–	Species E	?	S-13	–	S-18	–
S-22	–	S-26	–	S-32	–	S-40	–
S-41	–	S-43	–	S-52	–	S-68	–
S-73	–	S-89	–	S-95	–	S-96	–
Species B	?	S-101	–	S-102	–	S-104	–
Species G	?	Species C	?	S-115	–	S-116	–
Species C	?	S-142	–	S-143	–	S-155	–
S-162	–	S-164	–	Species F	+	S-175	–
S-186	–	S-192	–	S-195	–	S-198	–
Species C	?	S-209	–	Species C	+	S-229	–
S-230	–	S-233	–	S-234	–	S-237	–
S-238	–	Species B	?	S-254	–	Species D	?
S-268	–	S-271	–	S-276	–	S-277	–
Species C	++	S-286	–	S-288	–	S-290	–
Species A	++	Species A	+	S302-1	–	S-314	–
Species A	++	S-318	–	S-321	–		

*Almost complete elimination (+++), major reduction (+), marginal reduction (?), and no difference (–) in number of yellowspots on white pine needles, as compared with controls.

4 Inhibition of *C. ribicola* on *Ribes* spp. using *Microsphaeropsis arundinis*

4.1 Material and methods

Isolates P-176 and P-130 of *Microsphaeropsis arundinis*, which have shown great potential in controlling apple scab (Bernier *et al.* 1996), were isolated and grown on PDA in the dark at 20° C until the media was covered with mycelium. A mycelial suspension was made by gently crushing the collected surface mycelium in sterile distilled water containing Tween 20.

Mature leaves of red currant (*R. glandulosum*) were surface sterilized by dipping them for 2 min in 20% Javex, then 1 min in 70% EtOH, and then rinsed in distilled water. Leaf disks, 37 mm in diameter, were punched from the leaves and transferred onto 2% water agar in petri dishes. The disks were then inoculated with previously frozen (–80° C) *C. ribicola* aeciospores. Inoculation of biocontrol agents P-176 and P-130 was done 0, 7, and 14 days following inoculation with *C. ribicola*. The inoculum density of P-130 was 8.1 colony-forming-units (cfu) per cm^2 and 9.2 cfu for P-176.

Leaf disks were transferred to a growth chamber at 18° C with eight hours of light for 21 days. At this time, uredia with urediospores were present on the surface of leaf disks. The disks were then transferred to another growth chamber at 13° C with eight hours of light for 14 days.

4.2 Results

Microsphaeropsis arundinis strains P-176 and P-130 showed great potential to inhibit *C. ribicola* on red currant leaves. The controls developed an average of 440.3 (±80.5) uredia. Strain P-130 infected an average of 253.8 (96.4%) uredia per leaf disk, leading to an average of only three healthy uredia per leaf disk (Table 3). Strain P-176 was similarly effective, with 148.1 infected uredia per leaf (89.2%) and only 7.7 healthy uredia per leaf disk.

Table 3. Incidence of symptoms of two *Microsphaeropsis arundinis* isolates on *Cronartium ribicola* uredia grown on *Ribes* leaf discs inoculated 0, 7, and 14 days after inoculation with *C. ribicola*.

	14 days	7 days	0 days
P-130	0.6% healthy (1.6±1.6)*	0.0% healthy	2.2% healthy (7.4±6.6)
	0.7% infected (1.7±1.3)	5.8% infected (11.8±11.8)	1.4% infected (4.6±3.4)
	98.7% dead (246.1±48.9)	94.2% dead (191.1±53)	96.4% dead (324.5±33.9)
P-176	5.0% healthy (8.7±4.5)	10.5% healthy (14.9±13.7)	0.0% healthy
	4.7% infected (8±5.4)	6.0% infected (8.5±3.8)	6.2% infected (11.2±7.3)
	90.3% dead (155.2±30.2)	83.5% dead (118.4±17)	93.8% dead (170.8±24)
Controls	94.3% healthy		
	5.7% infected		
	0.0% dead		

*Condition of *C. ribicola* uredia.

5 Discussion

Pinus strobus needles have a very diversified endophytic fungal flora, with some 73 putative species isolated from its needles in this study.

Those fungal endophytes were tested for their capacity to inhibit infection by *C. ribicola*. Seven species of endophytes were shown to have potential for biocontrol of white pine blister rust. Fungal endophyte Species A successfully inhibited the development of yellow needles spots on white pine seedlings, showing efficacy similar to the fungicide triadimefon (Bérubé 1996). Potential use includes systematic inoculation of seedlings in nurseries to protect them during the production period. Annual inoculation for the first six years in the field would allow seedlings to escape blister rust in their most vulnerable life stage. Field trials are planned for the 1998–2004 period.

Since the telia originating from uredia are the structures responsible for producing basidiospores, which are the propagules for infection of white pine, we wanted to see if the biocontrol agent *Microsphaeropsis arundinis* was able to inhibit their production. Isolates P-179 and P-130 inoculated after infection by *C. ribicola* efficiently inhibited formation of uredia and telia on *Ribes*. In practical field treatments, this means that biocontrol agents would have to be applied in late June or early July for maximum efficacy. Since basidiospores can travel a few kilometers, the area around the plantation must also be treated. *Microsphaeropsis arundinis* makes possible the biocontrol of *C. ribicola* on *Ribes* spp., an important control option for commercial currant growers in areas where there is quarantine restriction on such a culture.

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