Forest Pathology

For. Path. 40 (2010) 458–464 doi: 10.1111/j.1439-0329.2009.00623.x

Pathogenicity of *Ceratocystis resinifera* to Norway spruce

By Paal Krokene[1,3], Jolanda Roux[2], Halvor Solheim[1] and Michael J. Wingfield[2]

[1]Norwegian Forest and Landscape Institute, Høgskoleveien 8, N-1432 Ås, Norway;
[2]Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa;
[3]E-mail: paal.krokene@skogoglandskap.no (for correspondence)

Summary

The blue-stain fungus *Ceratocystis resinifera* colonizes wounds on living *Picea* spp. and other conifers in Europe and North America. Little is known regarding the pathogenicity of this fungus and consequently, four Norwegian *C. resinifera* isolates were inoculated on to Norway spruce (*Picea abies*) using two different techniques. These included single-point inoculations on young trees (two inoculations per tree on 14-year-old trees) and mass-inoculations on older trees (~200 inoculations per tree on 34-year-old trees). In both experiments, *C. resinifera* induced minor symptoms that in most cases did not differ significantly from inoculation with sterile agar. The virulent blue-stain fungus *C. polonica*, which was inoculated for comparative purposes, induced extensive symptoms, causing 83% dead cambium circumference and 82% blue-stained sapwood, and long necrotic lesions in the phloem. The results suggest that *C. resinifera* is non-pathogenic or only mildly pathogenic to Norway spruce and does not present a threat to these trees.

1 Introduction

The genus *Ceratocystis* includes seven closely related species in the *Ceratocystis coerulescens* complex that have conifers as their primary host (Harrington and Wingfield 1998). One of these species, *Ceratocystis resinifera* Harrington & Wingfield, has relatively recently been recognized as a species distinct from *Ceratocystis coerulescens* (Münch) Bakshi (Harrington and Wingfield 1998). *Ceratocystis resinifera* seems to be more common in wounds of living spruce trees, whereas *C. coerulescens* is more common as a blue-stain fungus on dead wood and cut timber (Harrington and Wingfield 1998). *Ceratocystis resinifera* has been found mainly on *Picea* spp. in Europe and North America (Harrington and Wingfield 1998), but more recently it was also isolated from freshly cut logs of *Pinus* spp. and balsam fir [*Abies balsamea* (L.) Mill.] in Canada (Morin et al. 2004). *Ceratocystis resinifera* does not appear to have specific insect vectors (Morin et al. 2007) and cultures produce a characteristic fruity odour (Hanssen 1993). This is usually interpreted as an adaption to fungus- or sap-feeding insect vectors such as flies and nitidulid beetles (Malloch and Blackwell 1993).

Among the seven *Ceratocystis* species in the *C. coerulescens* complex that colonize conifers, three species (*Ceratocystis laricicola* Redfern & Minter; *Ceratocystis polonica* (Siemaszko) C. Moreau and *Ceratocystis rufipenni* Wingfield, Harrington & Solheim) are closely associated with different tree-killing bark beetles in the genera *Ips* and *Dendroctonus* (Harrington and Wingfield 1998). These fungi are relatively virulent to the host trees of their bark beetle vectors when artificially inoculated into the phloem (Horntvedt et al. 1983; Redfern et al. 1987; Solheim and Safranyik 1997; Krokene and Solheim 1998) and are thought to aid their vectors in overcoming host defences. The remaining four species, including *C. resinifera*, do not appear to have consistent vector relationships, but are considered important saprophagic blue-stain agents in conifer timber (Harrington and

Received: 20.9.2009; accepted: 21.9.2009; editor: S. Woodward

Wingfield 1998). Within this group, *C. resinifera* appears to be the species with the highest potential for being pathogenic, due to its rapid growth and its role as an invader of fresh stem wounds (e.g. Roll-Hansen and Roll-Hansen 1980). In this respect, an extensive study of several *C. resinifera* isolates from across Canada showed that this species may be pathogenic to *Picea* spp., but the outcome varied with experimental conditions (Morin et al. 2007).

The objective of this study was to test the pathogenicity of *C. resinifera* to Norway spruce [*Picea abies* (L.) Karst.] under Norwegian conditions, using both single point inoculation of young trees and mass-inoculation of older trees.

2 Material and methods

2.1 Fungal isolates

Because *C. resinifera* was only recently distinguished from *C. coerulescens*, very few verified European *C. resinifera* isolates were available for experimentation. In addition to isolates from two Norwegian surveys of wound colonizing fungi in Norway spruce (Solheim and Selås 1986; Solheim 1989), some of the isolates listed as *C. coerulescens* by Roll-Hansen and Roll-Hansen (1980) were later shown by Harrington and Wingfield (1998) to be *C. resinifera*. Thus, we were able to include four *C. resinifera* isolates from Norway in this study, including the holotype of the species (Table 1). Three of these isolates were also included in an earlier study where species in the *C. coerulescens* complex were delimited (Harrington et al. 1996). In addition to the *C. resinifera* isolates, an isolate of *C. polonica* that is known to be virulent to Norway spruce was included for comparative purposes (Table 1). The *C. resinifera* isolates were maintained at 3°C in the culture collection of the Norwegian Forest and Landscape Institute and subcultured onto new agar plates at regular intervals until 2000, when they were transferred to an ultrafreezer and stored at −150°C until the start of the experiments.

2.2 Inoculation of 14-year-old trees

Norway spruce trees, planted as 2-year-old seedlings in 1992 at Hogsmark, Ås, SE Norway, where used for single-point inoculations. The trees were planted in a randomized

Table 1. **Fungal isolates used in single-point inoculation of 14-year-old trees and mass-inoculation of 34-year-old Norway spruce (*Picea abies*) trees.**

Species	Isolate no.	Host	Collected	Young trees	Older trees
Ceratocystis resinifera	1986-434/9	Wounded *Picea abies*	1986, Akershus (Ås), Norway	x	
	1986-434/15[1]	Wounded *Picea abies*	1986, Akershus (Ås), Norway	x	
	1986-434/16	Wounded *Picea abies*	1986, Akershus (Ås), Norway	x	x
	1966-157/21	Wounded *Picea abies*	1966, Akershus (Hurdal), Norway	x	
Ceratocystis polonica	1993-208/115	*Polygraphus poligraphus* in *P. abies*	1993, Akershus (Ås), Norway	x	x

All isolates are from the culture collection of the Norwegian Forest and Landscape Institute, Norway.
[1]Holotype.

block design, with 16 full-sib families per block and 1.5 m between trees. On 21 June 2004, two trees per family were inoculated at about 1.3 m height with the four isolates of *C. resinifera*, *C. polonica* or malt agar as a control (Table 1; n = 32 trees per treatment). Inoculations were made by removing a bark plug with a 5-mm cork borer, inserting inoculum into the wound and replacing the plug. Inoculum consisted of actively growing mycelium of the test fungus on malt agar (2% malt and 1.5% agar) or malt agar alone. Each tree was inoculated twice, on opposite sides of the stem. Five weeks after inoculation, the outer bark over one of the two inoculation sites per tree was removed and the full length of the necrotic lesion in the inner bark was measured to assess the success of fungal colonization. Ten weeks after inoculation, the lesion lengths associated with the remaining inoculations on each tree were measured. At the same time, re-isolation of *Ceratocystis* spp. was attempted from the phloem of 63 of the inoculated trees.

2.3 Mass-inoculation of large trees

On 1–2 July 2004, three trees from each of six clones were selected from a plantation of 34-year-old Norway spruce clones close to Årungen, Ås, SE Norway. One tree per clone was randomly assigned to be inoculated with *C. resinifera* (isolate NFLI 1986-434/16), *C. polonica* (isolate NFLI 1993-208/115) or sterile malt agar control. The inoculum consisted of actively growing fungal mycelium on malt agar (2% malt and 1.5% agar). Trees were mass-inoculated at a density of 400 inoculations/m^2 spread evenly over a 0.8-m section of the stem from about 1.0 to 1.8 m above ground. Inoculations were made as described above. The trees were felled 6 months after inoculation and two thin stem discs were taken from within the inoculated section of each tree. To quantify fungal colonization of host tissues, the proportion of the sapwood that was blue-stained by the fungus, the proportion of desiccated sapwood and the proportion of cambium circumference that was killed on the discs were measured, as described in Krokene and Solheim (1998). For each tree, the stem disc with the most extensive symptoms was used in the statistical analyses. At the time of harvesting, the full vertical extension of six of the uppermost phloem lesions on each tree was also measured. On some trees inoculated with *C. polonica*, necroses coalesced within the mass-inoculated section and we, therefore, measured necrosis lengths upwards from the centre of the inoculation wounds. Re-isolation of *Ceratocystis* spp. was attempted from six phloem and sapwood samples per tree at the time of harvesting.

2.4 Statistical analyses

Data were subjected to ANOVA, using the JMP 7.0 software package (SAS Institute, Cary, NC, USA). Where treatments were significantly different ($p < 0.05$), means were separated by Tukey's HSD test. Data for the lengths of the necrotic lesions were log-transformed and percentage data were arcsine-transformed before ANOVA to correct for unequal variance and departures from normality.

3 Results

3.1 Inoculation of 14-year-old trees

On the 14-year-old Norway spruce trees, all four *C. resinifera* isolates induced relatively short necrotic lesions in the phloem that were significantly smaller than those induced by *C. polonica* (Fig. 1). Only data from the last sampling point 10 weeks after inoculation are presented, as lesions were most fully developed at that time. Two of the *C. resinifera* isolates induced significantly longer lesions than control inoculation with sterile malt agar,

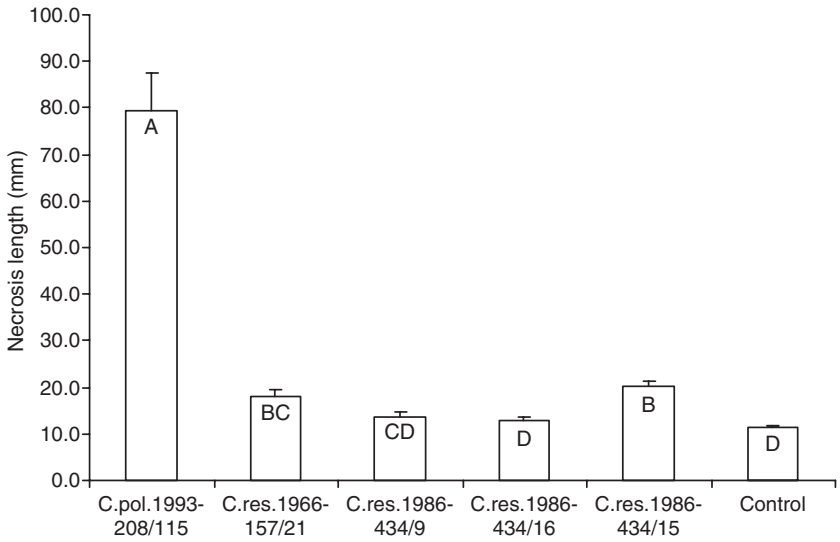

Fig. 1. Necrosis length in the phloem of 14-year-old Norway spruce trees 10 weeks after inoculation with four different isolates of *Ceratocystis resinifera*, *Ceratocystis polonica* or sterile agar control. Values are means of 32 trees per treatment +SE. Columns not sharing the same letters are significantly different (Tukey's HSD test at $p < 0.05$, following one-way ANOVA; $F_{5,184} = 95.71$, $p < 0.0001$).

but for these isolates, lesion lengths were only about 1.7 times longer than those in the control treatment, compared with the more than sevenfold difference between *C. polonica* and the controls. The *C. resinifera* isolate (NFLI 86-434/16) that was also used in the mass-inoculation experiment did not differ significantly from the control, and induced only 1.1-fold longer average lesion length in the young trees (Fig. 1). *Ceratocystis resinifera* and *C. polonica* were reisolated from 36% and 23% of the trees respectively.

3.2 Mass-inoculation of large trees

In the mass-inoculation experiment, *C. resinifera* did not cause significantly more severe symptoms than the sterile agar control (Table 2). Compared with *C. resinifera*, *C. polonica* induced 10-fold more necrotic cambium, sixfold longer necrotic lesions in the phloem and 2.5-fold more desiccated or stained sapwood area (Table 2). *Ceratocystis resinifera* did not cause any blue-stain in the mass-inoculated trees 6 months after inoculation, compared with 82% blue-stained sapwood area in trees inoculated with *C. polonica*. *Ceratocystis resinifera* and *C. polonica* were not re-isolated from lesions on any of the trees 6 months after mass-inoculation. Instead, *Nectria fuckeliana* Booth was isolated from all but one of the trees. The reason for the low re-isolation success is probably that the *Ceratocystis* species, being poor saprophytes, had been excluded by other fungi at the time of re-isolation.

4 Discussion

Results of this study suggest that Norwegian *C. resinifera* isolates are non-pathogenic or only mildly pathogenic to Norway spruce. Because the *C. resinifera* isolate used in the mass-inoculation study was slightly less virulent than some of the other isolates in the

Table 2. **Symptoms in Norway spruce 6 months after mass inoculation of two species of blue-stain fungi and sterile agar.**

Treatment	% Sapwood area		% Dead cambium	Phloem necrosis length (mm)
	Blue-stained	Blue-stained and desiccated		
Ceratocystis polonica	82.1 ± 14.28 a	88.2 ± 9.51 a	83.4 ± 12.09 a	132.9 ± 31.50 a
Ceratocystis resinifera	0.0 b	36.1 ± 6.28 b	8.2 ± 2.39 b	22.3 ± 3.13 b
Control inoculations	0.0 b	30.0 ± 6.77 b	8.2 ± 3.25 b	24.9 ± 6.97 b
F-ratio[1]	97.48	31.89	50.71	12.9
p	<0.0001	<0.0001	<0.0001	0.0006

Values in a column not sharing the same letter are significantly different (Tukey's HSD test at $p < 0.05$, following one-way ANOVA). Values are means ± SE, n = 6.
[1]Degrees of freedom = 2, 9 for all tests, except necrosis length where d.f. = 2, 15.

single-point inoculation experiment, the results of the mass-inoculation experiment may underestimate the virulence of this species. However, all *C. resinifera* isolates tested in the single-point inoculations produced symptoms that were less than two times stronger than the sterile agar controls, indicating a very low level of virulence.

The results of this study are in contrast to those from North America, where *C. resinifera* was shown to be mildly to moderately virulent to white spruce *Picea glauca* (Moench) Voss and black spruce *Picea mariana* (Mill.) BSP (Morin et al. 2007). The different level of virulence of *C. resinifera* in North America and Norway could be attributed to different host trees or to genetic differences between the fungal populations from the two areas. *Ceratocystis resinifera* has been demonstrated to have low genetic variability across Canada (Morin et al. 2004), but there are no genetic studies comparing European and North American populations, making it impossible to assess this possible anomaly. The low genetic variability of *C. resinifera* in Canada suggests that it could be an introduced species (Morin et al. 2004). If this is true, it could explain the higher level of virulence of *C. resinifera* to North American spruce species, which may not have had an evolutionary history with this fungus.

Ceratocystis resinifera has several characteristics suggesting that it might be a relatively virulent tree colonizer, whereas other characteristics point towards a more saprophagic life style. Among the characteristics that it shares with many successful early colonizers is a relatively high growth rate, both in culture and in live trees. *Ceratocystis resinifera* grows twice as fast as *C. coerulescens* on growth medium at 25°C (Harrington and Wingfield 1998), and up to 12-fold faster than other ophiostomatoid fungi in lodgepole pine (*Pinus contorta* Dougl. ex Loud.) billets (Fleet et al. 2001). *Ceratocystis resinifera* is also one of the earliest and most rapid colonists of fresh stem wounds in Norway spruce. Wounding experiments on live Norway spruce stems have shown that *C. resinifera* grows about 70 cm/year on average in the sapwood, and it has been isolated from sapwood up to 485 cm above wounds 4 years after wounding (Roll-Hansen and Roll-Hansen 1980; Solheim and Selås 1986; Solheim 1989). On the other hand, *C. resinifera* does not appear to be particularly well adapted to grow in the oxygen-poor environment prevailing in live sapwood, as its growth was more strongly inhibited by low oxygen levels than that of three other ophiostomatoid fungi (Morin et al. 2007). Solheim (1991) has suggested that an ability to grow under low oxygen levels is typical for effective sapwood invaders, and a relationship between tolerance to low oxygen levels and ability to colonize fresh sapwood has been demonstrated for fungi associated with several aggressive bark beetle species (Solheim 1991, 1992, 1995a,b; Solheim and Krokene 1998a,b).

Morin et al. (2007) found that *C. resinifera* colonization of black spruce sapwood after experimental inoculations extended much further axially than radially, with sapwood necrosis extending 66 cm upwards from the inoculation site but <2.5 cm into the sapwood. They suggested that this pattern of shallow sapwood colonization can be explained by growth inhibition of *C. resinifera* at low oxygen levels, as oxygen levels are likely to be more favourable in the outer sapwood. The limited ability of *C. resinifera* to penetrate into the sapwood of healthy trees may also explain the apparent contradiction that *C. resinifera* is both a rapid sapwood colonizer in wounded Norway spruce trees and a relatively weak pathogen.

The vector relationships of *C. resinifera* are not clear, and no specific insect vectors are mentioned in the literature (Morin et al. 2007). In wounding experiments on live Norway spruce trees, *C. resinifera* is consistently isolated only from wounds made in the summer (Roll-Hansen and Roll-Hansen 1980; Solheim and Selås 1986). This pattern suggests that *C. resinifera* relies on insect vectors for dispersal to suitable habitats. The fruity odours that this fungus produces in culture (Hanssen 1993) further suggest that its main vectors are non-specific insects that are attracted to fermenting organic material and that also visit wounds on trees (Kile 1993). This includes fungus- or sap-feeding insects, such as flies and nitidulid beetles, which tend to be secondary colonizers of wounded or dying trees. Such vector relationships could explain why *C. resinifera*, at least in Norway, appears to be only mildly virulent to Norway spruce and serve as a necrotrophic invader of stem wounds.

Acknowledgements

Funding for this work was provided by the South Africa – Norway Programme on Research Co-operation (project no. 152266/V10). We thank Øystein Johnsen and Tore Skrøppa for providing experimental trees and Olaug Olsen for help with the fungal isolates.

References

Fleet, C. A.; Breuil, C.; Uzunovic, A., 2001: Nutrient consumption and pigmentation of deep and surface colonizing sapstaining fungi in *Pinus contorta*. Holzforschung, **55,** 340–346.

Hanssen, H.-P., 1993: Volatile metabolites produced by species of *Ophiostoma* and *Ceratocystis*. In: *Ceratocystis* and *Ophiostoma*. Taxonomy, Ecology, and Pathogenicity. Ed. by Wingfield, M. J.; Seifert, K. A.; Webber, J. F. St Paul, MN: APS Press, pp. 117–125.

Harrington, T. C.; Wingfield, M. J., 1998: The *Ceratocystis* species on conifers. Can. J. Bot., **76,** 1446–1457.

Harrington, T. C.; Steimel, J. P.; Wingfield, M. J.; Kile, G. A., 1996: Isozyme variation and species delimitation in the *Ceratocystis coerulescens* complex. Mycologia, **88,** 104–113.

Horntvedt, R.; Christiansen, E.; Solheim, H.; Wang, S., 1983: Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. Medd. Nor. Inst. Skogforsk., **38,** 1–20.

Kile, G. A., 1993: Plant diseases caused by species of *Ceratocystis sensu stricto* and *Chalara*. In: *Ceratocystis* and *Ophiostoma*. Taxonomy, Ecology, and Pathogenicity. Ed. by Wingfield, M. J.; Seifert, K. A.; Webber, J. F. St Paul, MN: APS Press, pp. 173–183.

Krokene, P.; Solheim, H., 1998: Phytopathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. Phytopathology, **88,** 39–44.

Malloch, D.; Blackwell, M., 1993: Dispersal biology of the Ophiostomatoid fungi. In: *Ceratocystis* and *Ophiostoma*. Taxonomy, Ecology, and Pathogenicity. Ed. by Wingfield, M. J.; Seifert, K. A.; Webber, J. F. St Paul, MN: APS Press, pp. 195–206.

Morin, C.; Breuil, C.; Bernier, L., 2004: Genetic variability and structure of Canadian populations of the sapstain fungus *Ceratocystis resinifera*. Phytopathology, **94,** 1323–1330.

Morin, C.; Couturier, S.; Bernier, L., 2007: Pathogenicity of wild-type and albino strains of the fungus *Ceratocystis resinifera*, a potential biocontrol agent against bluestain. Can. J. For. Res., **37,** 919–930.

Redfern, D. B.; Stoakley, J. T.; Steele, H.; Minter, D. W., 1987: Dieback and death of larch caused by *Ceratocystis laricicola* sp. nov. following attack by *Ips cembrae*. Plant Pathol., **36,** 467–480.

Roll-Hansen, F.; Roll-Hansen, H., 1980: Microorganisms which invade *Picea abies* in seasonal stem wounds. II. Ascomycetes, Fungi imperfecti, and Bacteria. General discussion, Hymenomycetes included. Eur. J. For. Path., **10,** 396–410.

Solheim, H., 1989: Discoloration and stem rot following wounding of Norway spruce in thinned stands (in Norwegian with English summary). Aktuelt fra NISK, **1,** 21–26.

Solheim, H., 1991: Oxygen deficiency and spruce resin inhibition of growth of fungi associated with *Ips typographus*. Mycol. Res., **95,** 1387–1392.

Solheim, H., 1992: The early stages of fungal invasion in Norway spruce infested by the bark beetle *Ips typographus*. Can. J. Bot., **70,** 1–5.

Solheim, H. (1995a). A comparison of blue-stain fungi associated with the North-American spruce beetle *Dendroctonus rufipennis* and the Eurasian spruce bark beetle *Ips typographus*. In: Forest Pathology Research in the Nordic Countries 1994, Vol. 4. Ed. by Aamlid, D., pp. 61–67. Aktuelt fra Skogforsk 4, Norwegian Forest Research Institute, Ås, Norway.

Solheim, H., 1995b: Early stages of blue-stain fungus invasion of lodgepole pine sapwood following mountain pine beetle attack. Can. J. Bot., **73,** 70–74.

Solheim, H.; Krokene, P., 1998a: Growth and virulence of *Ceratocystis rufipenni* and three blue-stain fungi isolated from the Douglas-fir beetle. Can. J. Bot., **76,** 1763–1769.

Solheim, H.; Krokene, P., 1998b: Growth and virulence of mountain pine beetle associated blue-stain fungi, *Ophiostoma clavigerum* and *O. montium*. Can. J. Bot., **76,** 561–566.

Solheim, H.; Safranyik, L., 1997: Pathogenicity to Sitka spruce of *Ceratocystis rufipenni* and *Leptographium abietinum*, blue-stain fungi associated with the spruce beetle. Can. J. For. Res., **27,** 1336–1341.

Solheim, H.; Selås, P., 1986: Discoloration and microflora in wood of *Picea abies* (L.) Karst. after wounding. I. Spread after two years (in Norwegian with English summary). Rapp. Norsk Inst. Skogforsk. **7/86,** 16.