Brodal, Bye, Høst, Pettersson, Fløistad, Edvardsen and Talgø (2020).
Seed Science and Technology, **48**, 1, 33-39.
https://doi.org/10.15258/sst.2020.48.1.05

Research note

Management of seed-borne *Sirococcus conigenus* on Norway spruce by fungicide seed treatment

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(Submitted November 2019; Accepted January 2020; Published online February 2020)

Abstract

Seedling blight caused by *Sirococcus conigenus* was recently reported on Norway spruce (*Picea abies*) from Norwegian forest nurseries. The inoculum source was found to be infected seeds. In a Petri dish assay, the fungicide fludioxonil + difenoconazole was, among other fungicides, found to inhibit mycelial growth of *S. conigenus*. This fungicide is formulated as a seed treatment and registered for cereals in Norway, and was chosen for an experiment to control *S. conigenus* on Norway spruce seeds. Samples from two naturally infected seed lots were treated with half, normal and double dose of the recommended rate for cereals. Together with untreated control samples, treated seeds were tested in the laboratory for efficacy against *S. conigenus* on potato dextrose agar (PDA) in Petri dishes and for germination potential on filter paper. We also recorded seed emergence in soil of one of the seed lots in a growth chamber and in a forest nursery. On agar, the fungus was not detected after seed treatment with fludioxonil + difenoconazole at any of the three dosages, but it was present in the control. Germination on filter paper and emergence in soil was high in both treated and untreated control seeds with no signs of detrimental effects from any of the three fungicide doses.

Keywords: conifers, germination, fungus, phytotoxicity, *Picea abies*, seed-borne, shoot-blight

Experimental and discussion

The fungus *Sirococcus conigenus* (DC.) P. Cannon and Minter causes disease on a number of conifer species in temperate and boreal forests in Europe and North America, as reviewed by Smith *et al*. (2003). It can affect various stages of the host plants; however, the most commonly reported damage is seedling and shoot blight in forest nurseries (e.g.

Sutherland, 1987; Lilja *et al*., 2005). The fungus has been detected on seeds from several spruce species (*Picea* spp.) in Canada (Sutherland *et al*., 1981), and on Norway spruce [*P. abies* (L.) H. Karst.] in Italy (Motta *et al.*, 1993) and Finland (Himanen *et al*., 2013). In Norway, *S. conigenus* was detected on Norway spruce seeds (2% infected seeds) in 1992 (Sutherland, 1992). Later, the fungus was detected in a Norwegian seed lot of noble fir (*Abies procera* Rehder), with 31% infected seeds (Talgø *et al*., 2010).

Seedling blight caused by *S. conigenus* has been reported to be more prevalent in container-grown than in bare root spruce seedlings (e.g. Sutherland *et al*., 1981; Lilja *et al*., 2005). The same has been experienced in Norway where considerable damage by *S. conigenus* was observed on Norway spruce seedlings in Norwegian forest nurseries when some seed lots harvested in 2015 were sown in microplant trays. These trays have very small soil volumes per plug ($1.4 \times 1.4 \times 3.3$ cm = 6.5 cm^3) and therefore require frequent irrigation, commonly applied overhead. This provides optimal humidity for fungal development. We found that *S. conigenus* formed pycnidia containing mature conidia on dead tissue shortly after emergence (figure 1A). Such conidial spores may easily spread in nurseries by water splash during overhead irrigation, and we observed seedling mortality in patches surrounding infected seedlings (figure 1B). We identified *S. conigenus* in seed lots from 2015 (figure 1C). Although, like Sutherland (1992) we found the highest infection level to be only 2%, it was sufficient to cause widespread disease.

Seeds are in short supply since seed production in Norway spruce usually occurs at 5-10 year intervals (Sarvas, 1957) and in some northern or higher altitude locations even less frequently. Therefore, even seed lots containing seed-borne pathogens must be saved to secure enough seeds from the required provenances. However, to avoid seedling losses seed infections should be controlled.

Fungicide seed treatments to control seed-borne pathogens are low-cost operations that are easy to carry out and require small amounts of chemicals compared with fungicide sprays after diseases have spread in nurseries. However, unlike agricultural and horticultural crops, treatments to control seed-borne pathogens do not seem to be widely practiced in forest seed production. Though in the past, treatment of seeds with fungicides (e.g. captan, thiram and benomyl), or by surface disinfecting agents [e.g. hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaOCl)] to control damping-off fungi, including *Fusarium* spp. in pine (*Pinus* spp.) and other conifer species, have been practiced in some North American nurseries (e.g. Carlson and Belcher, 1970; Lock *et al*., 1975). However, in some cases, phytotoxic effects on germination and variable efficacy reduced their use (Cayford and Waldron, 1967; Lock *et al*., 1975; Lamontagne and Wang, 1976; Runion *et al*., 1991). In the UK, Gosling (1997) reported that benomyl reduced seed germination of Corsican pine [*Pinus nigra* subsp. *maritima* (Ait.) Melville], and Cvjetkovic *et al*. (2013) reported that fungicides inhibited germination of Serbian spruce (*P. omorica* Pancic/Purkyne) seeds. Except for an Italian study, where Motta *et al*. (1996) found prochloraz effective against *S. conigenus* on Norway spruce seeds, we found no reports on seed treatment to control this fungus. The aim of our study was therefore to evaluate the possibilities to control seed-borne inoculum of *S. conigenus* on Norway spruce by fungicide seed treatment.

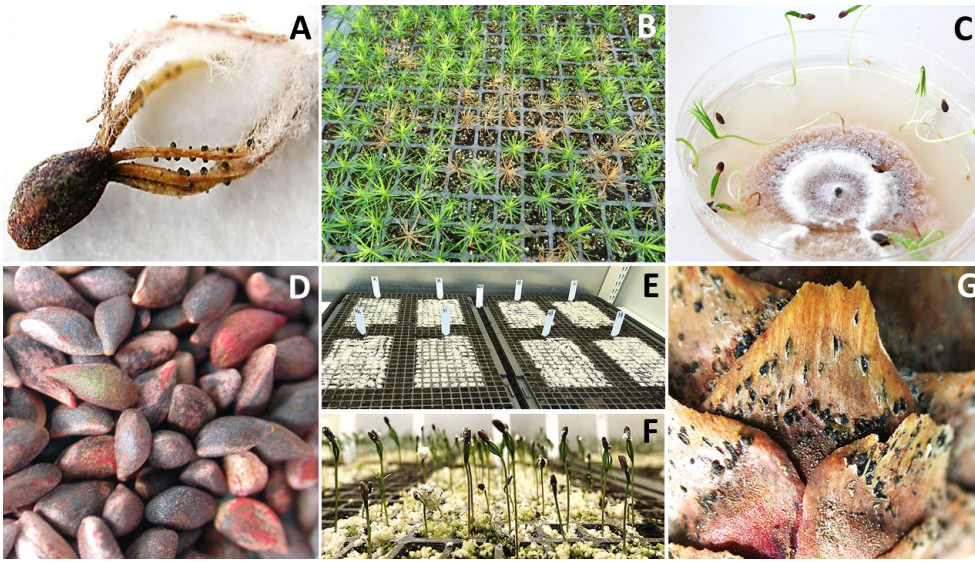
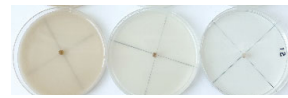
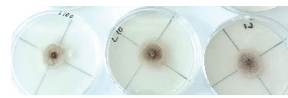
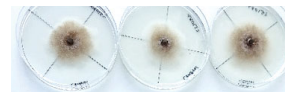


Figure 1. *Sirococcus conigenus* related to Norway spruce (*Picea abies*) seeds; (**A**) pycnidia and mycelium on a dead seedling shortly after emergence, (**B**) seedling blight spreading in a micro plant tray, (**C**) a culture growing from a dead seed, (**D**) red colour on seeds treated with fludioxonil + difenoconazole, (**E**) seeds treated with different doses of fludioxonil + difenoconazole sown in micro plant trays in a growth chamber, (**F**) high germination and no phytotoxicity after seed treatment with fludioxonil + difenoconazole, and (**G**) pycnidia on cone scales. Photos: Venche Talgø (A and C-G) and Eleonora Høst (B).

Prior to the seed treatment experiment, we studied the inhibition of five selected fungicides on mycelial growth of *S. conigenus* on potato dextrose agar (PDA) in 90 mm-diameter Petri dishes amended with the fungicides (table 1). Three different concentrations (100, 10 and 1% of maximum dose recommended by the manufactures) of each fungicide were obtained by diluting the fungicide in sterile distilled water and adding the solutions to liquid PDA just before pouring it into the Petri dishes. Agar plugs (5 mm diameter) with fresh growing isolates of *S. conigenus* from different Norway spruce seed lots, were placed on the fungicide amended agar plates (one piece in the centre of each plate) as well as on control plates without fungicide. We used four isolates, and three agar plates for each fungicide concentration per isolate. After ten days incubation at room temperature, we measured the diameter of the growing colonies. Of the fungicides tested, fludioxonil + difenoconazole, pyraclostrobin + boscalid and thiophanat-methyl inhibited *S. conigenus* growth at all three concentrations (table 1). Since the fungicide fludioxonil + difenoconazole is formulated as a seed treatment and registered for cereals in Norway, it was chosen for the spruce seed treatment experiment.

Seeds of two Norway spruce seed lots from 2015 naturally infected with *S. conigenus* were provided by the Norwegian Forest Seed Center. Samples, obtained by hand halving method as described in ISTA Rules (ISTA 2018), were treated with fludioxonil + difenoconazole in three doses; half, normal and double rate (1, 2 and 4 ml kg^{-1} seeds, respectively), together with 1 ml water kg^{-1} seeds, as recommended by the manufacturer

Table 1. Mycelial growth of *Sirococcus conigenus* on potato dextrose agar amended with fungicides. The growth is given as colony diameter after 10 days incubation (mean of three replicates per concentration and four isolates). The rate is the percentage of maximal recommended dosage of the respective fungicide.

Active ingredient(s) (concentration)	Trade name (manufacturer)	Dose[1] (ml l^{-1})	Mycelial growth, diameter (mm)			Images from the test[2]
			100% rate	10% rate	1% rate	
Fludioxonil + difenoconazole (25 + 25 g l^{-1})	Celest Extra Formula M (Syngenta)	1.50	0	0	0	
Pyraclostrobin + boscalid (67 + 267 g l^{-1})	Signum (BASF)	5.00	0	0	0	
Fenheksamid (500 g l^{-1})	Teldor (Bayer)	1.50	48	46	53	
Fluopyram (500 g l^{-1})	Luna privilege (Bayer)	1.00	51	53	52	
Thiophanat-methyl (500 g l^{-1})	Topsin (Bayer)	3.68	0	0	0	
Control				48		

[1] Maximum recommended dose of each product given in ml L^{-1} agar.
[2] Displays one replicate per product from one of the isolates. From left to right 100, 10 and 1% rate.
Photos: Venche Talgø.

for use in cereal seeds. The fungicide was applied to the seed samples (figure 1D) in a rotating seed treatment equipment. The effect of the fungicide was tested by plating seeds from each treatment as well as from the untreated control on PDA in 90 mm-diameter Petri dishes. The untreated control was surface disinfected (10 minutes in 1% NaOCl) to avoid overgrowing by superficial saprophytes.

After nine days incubation at 20 ± 2°C under alternating 12 hours NUV light and 12 hours darkness, we assessed the dishes for *S. conigenus*. Each seed was examined for characteristic mycelium growth, pycnidia and conidia of *S. conigenus* and results are given as percentage seed infection. The agar plate test was replicated three times with seed lot 1 (200 seeds each treatment and replicate) and four times with seed lot 2 (100 seeds each treatment and replicate). Infection levels in untreated seeds were 1.00 and 1.75% in the two seed lots respectively (table 2). We detected no *S. conigenus* infected seeds after treatment with fludioxonil + difenoconazole from any of the tested dosages.

Table 2. Percentage infected seeds, germination and emergence of two Norway spruce (*Picea abies*) seed lots naturally infected with *Sirococcus conigenus*, untreated and after seed treatment with fludioxonil + difenoconazole in three doses.

Treatment	Seed lot 1[1]				Seed lot 2[2]	
	% seed infection (lab.)[4]	% germination (lab.)[5]	% emergence (soil)[6]	% emergence (soil)[7]	% seed infection (lab.)[4]	% germination (lab)[5]
Untreated control[3]	1.00	96	93	97	1.75	96
1 ml/kg seed	0	96	92	95	0	95
2 ml/kg seed	0	96	92	97	0	95
4 ml/kg seed	0	95	90	97	0	94

[1] Mean of three replicates of 200 seeds.
[2] Mean of four replicates of 100 seeds.
[3] Surface disinfected in NaOCl-solution (1% available Cl) in 10 minutes before plating on potato dextrose agar.
[4] After nine days incubation.
[5] Percentage normal seedlings after 21 days germination in laboratory.
[6] Percentage emergence in soil in microplant trays in a growth chamber.
[7] Percentage emergence in soil in microplant trays at a forest nursery.

Despite relatively low *S. conigenus* levels in the used seed samples, we concluded that the fungicide was effective against the fungus on Norway spruce seeds.

Seed samples from each treatment as well as from the untreated control (no surface disinfection) were germinated according to ISTA Rules (ISTA, 2018), i.e. seeds were placed on top of filter paper in a Jacobsen germinator alternating between 20 ± 2°C for 16 hours and 28 ± 2°C for eight hours. Final assessments (counts) took place after 21 days. The germination test was replicated three times with seed lot 1 (200 seeds each treatment and replicate) and four times with seed lot 2 (100 seeds each treatment and replicate). Both seed lots had a high percentage of normal seedlings and there were no differences between untreated and treated seeds (table 2). No signs of detrimental (phytotoxic) effects on seed germination were observed. In addition to germination in the laboratory, seed lot 1 was also assessed for seedling emergence in soil. Two × 100 seeds from each treatment and untreated control (no surface disinfection) were sown in soil [peat mixed with sand (4:1)] in microplant trays, covered with perlite, and left for germination in a growth chamber at 16 ± 1°C (figure 1E). Final seedling emergence was counted after 55-56 days. Moreover, parallel samples were sown at a Norwegian forest nursery with final emergence counts after 63 days. Emergence in soil in growth chamber and in nursery was replicated three times. Both untreated and fungicide treated seeds in all three dosages showed high emergence (table 2) both in the growth chamber (figure 1F) and at the forest nursery. No differences in emergence were recorded between untreated and treated seeds.

To get more information of possible damage by fludioxonil + difenoconazole on seed germination, two additional Norway spruce seed lots were treated with the fungicide in the three dosages and germinated on filter paper as described above. All fungicide dosages resulted in high germination also with these seed lots; 95 and 96% normal seedlings, respectively, and no signs of phytotoxic effects were observed.

Sirococcus conigenus has been observed in Norway on cone scales of Norway spruce (figure 1G) and on pine seedlings in nurseries for decades (Roll-Hansen, 1967), but seed infection and possibilities to control seed-borne inoculum were not considered in the past. Neither did Sutherland (1992) when 2% seed infection was detected in a seed lot of Norway spruce, because the damage potential was considered to be low. However, the recent change to dense production in microtrays, have shown that even low amounts of seed-borne inoculum of *S. conigenus* may cause extensive damage (figure 1B). Therefore, a solution to the problem caused by *S. conigenus*-infection of seed lots was needed. Hence, the above described fungicide treatments with the promising results obtained with fludioxonil + difenoconazole, i.e. good effects with all three dosages of the fungicide against *S. conigenus* and no sign of phytotoxic effects on germination, were used in an application to The Norwegian Food Safety Authorities to gain permission to treat the infected seed lots. A permission was necessary since no seed treatment fungicide was approved in Norway for controlling fungi on conifer seeds. A temporary permission was given to The Norwegian Forest Seed Center for treatment of infected seed lots that were used in the spring of 2019. At NIBIO, we received one sample with diseased seedlings in 2019 where *S. conigenus* was suspected, and where the seed lot had been treated with fludioxonil + difenoconazole. However, the disease turned out to be grey mould (*Botrytis* sp.).

Seed lots 1 and 2 (table 2) had rather low infection levels (1.00 and 1.75% infected seeds, respectively). However, due to the potential rapid build-up of this pathogen in microplant tray productions, we consider the damage potential to be high. This is supported from Canadian investigations where an infection level of *S. conigenus* as low as 1% is considered significant in conifer seeds (Kolotelo *et al*., 2001).

Based on our promising results from the seed treatment experiments, which is supported by the inhibition of mycelial growth in the Petri plates described above, even at the lowest concentration, we suggest that the fungicide fludioxsonil + difenoconazole can be safely used to control seed-borne inoculum of *S. conigenus* on Norway spruce seeds.

Following an exceptional dry and warm 2018 growing season in Norway, the cone production from Norway spruce was extensive in 2019. The harvesting started in October and NIBIO in collaboration with The Norwegian Forest Seed Center will examine both cones and seeds for *S. conigenus* to be able to recommend treatment if necessary.

Acknowledgements

We want to thank Andrew Dobson, Trude Slørstad and Camilla Lilleåsen at NIBIO and personnel at Kimen Seed Laboratory for valuable technical assistance. The experiments were carried out in the project «Frøhygiene – friske frø er avgjørende for økonomien i moderne produksjon av skogplanter» [Seedhygiene – healthy seed is crucial for the economy in modern production of forest plants (2018-2019)] financed by a regional research council [Regionale Forskingsfond Innlandet (RFFINNL project no 289683)] which collaborates with The Research Council of Norway.

References

Carlson, L.W. and Belcher, J. (1970). Seed-treatment fungicides for control of conifer damping-off: Laboratory and greenhouse tests, 1968-1969. *Canadian Plant Disease Survey*, **50**, 63-73.

Cayford, J.H. and Waldron, R.M. (1967). Effects of captan on the germination of white spruce, jack and red pine seed. *Forestry Chronicle*, **43**, 381-384.

Cvjetkovic, B., Mataruga, M., Isajev, V., Levic, J., Lucic, A., Trkulja, V. and Kremenovic, Z. (2013). Variability in germination and germination dynamics of differently treated seeds of Serbian spruce (*Picea omorika* Pancic/Purkyne). *Genetica*, **45**, 109-119. DOI:10.2298/GENSR1301109C

Gosling, P.G. (1997). Germination of Corsican pine (*Pinus nigra* var. *maritima*) seeds after fungicidal treatment with benomyl or surface sterilization with 30% H_2O_2. *Proceedings of the Tree Seed Pathology Meeting*, (eds. Z. Procházková and J.R. Sutherland), pp. 46-48, Opocno, October 9-11, 1996. Forestry and Game Management Research Institute, Czech Republic.

Himanen, K., Lilja A., Rytkönen, A. and Nygren, M. (2013). Soaking effects on seed germination and fungal infection in *Picea abies*. *Scandinavian Journal of Forest Research*, **28**, 1-7.

ISTA (2018). *International Rules for Seed Testing 2019*, International Seed Testing Association, Bassersdorf, Switzerland.

Kolotelo, D., van Steenis, E., Peterson, M., Bennett, R., Trotter, D. and Dennis, J. (2001). *Seed Handling Guidebook*, British Columbia Ministry of Forests, Tree Improvement Branch publication.

Lamontagne, Y. and Wang, B.S.P. (1976). Germination of polyram treated white spruce seeds from various provenances. *Tree Planters' Notes*, **27**, 5-6+22.

Lilja, A., Poteri, M., Vuorinen M., Kurkela, T. and Hantula, J. (2005). Cultural and PCR-based identification of the two most common fungi from cankers on container-grown Norway spruce seedlings. *Canadian Journal of Forest Research*, **35**, 432-439.

Lock, W., Sutherland, J.R. and Sluggett, L.J. (1975). Fungicide treatment of seeds for damping-off control in British Columbia Forest Nurseries. *Tree Planters Notes*, **26**, 16-18.

Motta, E., Annesi, T. and Forti, E. (1993). First report of *Sirococcus conigenus* seedborne in Norway Spruce. *Plant Disease*, **77**, 1169.

Motta, E. Annesi, T. and Balmas, V. (1996). Seedborne fungi in Norway spruce: testing methods and pathogen control by seed dressing. *European Journal of Forest Pathology*, **26**, 307-314.

Roll-Hansen, F. (1967). On diseases and pathogens on forest trees in Norway 1960-1965. *Meddelelser fra Det Norske Skogforsøksvesen*, **21**, 175-262.

Runion, G.B., Kelley, W.D. and Land, D.H. (1991). Effects of triadimefon and thiram seed treatments on emergence of southern pines. *Seed Science and Technology*, **19**, 57-66.

Sarvas, R. (1957). Studies on the seed setting of Norway spruce. *Meddelelser fra Det norske Skogforsøksvesen*, **48**, 529-556.

Smith, D.R., Bronson, J.J. and Stanosz, G.R. (2003). Host-related variation among isolates of the Sirococcus shoot blight pathogen from conifers. *Forest Pathology*, **33**, 141-156.

Sutherland, J.R. (1987). Sirococcus blight. In *Cone and Seed Diseases of North American Conifers*, (eds. J.R. Sutherland, T. Miller and R.S. Quinard), pp. 34-41, North American Forestry Commission Publication Number 1, Victoria, British Columbia, Canada.

Sutherland, J.R. (1992). Preliminary study of seed-borne fungi on Norway spruce (*Picea abies*). Report based on studies as a visiting scientist at NISK (Norwegian Forest Research Institute), Ås, Norway, July 2 - September 30, 1990.

Sutherland, J.R., Lock W. and Farris, S.H. (1981). *Sirococcus* blight: a seed-borne disease of container-grown spruce seedlings in Coastal British Columbia forest nurseries. *Canadian Journal of Botany*, **59**, 559-562.

Talgø, V., Brodal, G., Klemsdal, S.S. and Stensvand, A. (2010). Seed borne fungi on *Abies* spp. *Seed Science and Technology*, **38**, 477-493. <https://doi.org/10.15258/sst.2010.38.2.20>