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# Horizontal transmission of the *Picea glauca* foliar endophyte *Phialocephala scopiformis* CBS 120377

*J. David MILLER*[a,*], *Hafsa CHERID*[a], *Mark W. SUMARAH*[a], *Gregory W. ADAMS*[b]

[a]*Ottawa-Carleton Institute of Chemistry, Carleton University, 228 Steacie Building, Ottawa, Ontario K1S 5B6, Canada*
[b]*JD Irving Limited, Sussex Tree Nursery, 181 Aiton Road, Sussex, New Brunswick E4G 2V5, Canada*

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ABSTRACT

We have studied the presence of the foliar endophtye of *Picea glauca* (white spruce) *Phialocephala scopiformis* CBS 120377 and its affect on the growth of *Choristoneura fumiferana* (spruce budworm). Here we examine the transmission of this fungus from 50 trees planted in a test field site to 250 *P. glauca* seedlings planted under the emerging canopies. After 3 y, the endophyte spread to 40 % of these trees (now 20–30 cm) with an average rugulosin (an anti-insect toxin) concentration of 1 $\mu g\, g^{-1}$. All woody plants within 2 m of the test trees were collected. These were all shown to be negative for *P. scopiformis* except for some spruce seedlings that arose from seeds (natural generation). This is positive evidence for the horizontal transmission of *P. scopiformis* and its apparent specificity to *P. glauca* under field conditions.



Foliar fungal endophytes produce symptomless infections in plant leaves and sometimes the vascular tissue (Arnold 2007, Sánchez Márquez *et al.* 2008, Huang *et al.* 2008, Hyde & Soytong 2008). The best understood endophyte-plant system is in various grasses that can be colonized by the *Balansia* endophytes (Sánchez Márquez *et al.* 2007; Wei *et al.* 2007). The fungus is found inside leaf tissue and produces a number of alkaloids that result in serious health problems for cattle and horses when in pastures but also in various insect pests (Clay & Schardl 2002). Endophyte-positive cultivars used for lawns and golf courses are much more insect tolerant, but also have increased drought and fungal pathogen resistance (Clay & Schardl 2002). We have been studying the foliar endophytes of white spruce (*Picea glauca*) and their effects on the eastern spruce budworm (*Choristoneura fumiferana*). Spruce budworm is the most serious forest pest in eastern Canada and the northeast USA. The last major epidemic in Canada occurred from the 1970's to the mid 1980's which resulted in severe defoliation, except where sprayed with pesticide in an effort to keep the forest green (Irland 1980; MacLean *et al.* 2002).

We have demonstrated that *P. glauca* seedlings can be colonized with strains of the foliar endophyte *Phialocephala scopiformis* (DAOM 229536, CBS 120377) isolated from surface-sterilized needles of *P. glauca* on the border of Quebec and New Brunswick. The dominant anti-insect toxin produced by this fungus is rugulosin. The needles of infected seedlings grown in growth chambers, under nursery conditions and in a long-term field test contain rugulosin in concentrations that affect the growth of spruce budworm (Miller *et al.* 2002, 2008; Sumarah *et al.* 2008a). We have also demonstrated that spruce budworm growth rates are impaired by rugulosin in infected needles in growth chambers (Miller *et al.* 2002) and under nursery conditions (Miller *et al.* 2008).

* *Corresponding author.*
E-mail address: david_miller@carleton.ca (J.D. Miller).
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The available ecological literature data, though sparse, indicated that unlike grass endophytes which are mostly vertically transmitted, foliar endophytes of woody plants are transmitted horizontally to seedlings planted immediately under infected trees (Bayman *et al.* 1998; Frohlich *et al.* 2000). The *Xylaria* endophytes of the tropical woody angiosperm *Manilkara bidentata* (Sapotaceae) were demonstrated to be transmitted horizontally (Bayman *et al.* 1998). Endophytes of another tropical woody angiosperm, *Theobroma cacoa* (Malvaceae) have also been shown to be similar. In this case, specialization of its endophytes appears to occur. Factors that might contribute to this include nutrients and secondary metabolites of the leaves of particular plants (Arnold & Herre 2003; Arnold *et al.* 2003). Data on foliar endophytes of north temperate species of conifers has been largely inferential to date. Miller *et al.* (2002) examined the needles of a large number of seed-grown white spruce seedlings from a large production nursery and found them free of endophytes (see also Sumarah *et al.* 2005). Ganley & Newcombe (2006) tested seeds and needles of *Pinus monticola* (western white pine) for its endophytes and obtained similar results.

The strain of *P. scopiformis* discussed in the present report was isolated from surface-sterilized needles. The fungus has previously been reported to be isolated from apparently healthy roots, branches or twigs of spruce species in the north temperate region (Kowalski & Kehr 1995; Ahlich & Sieber 1996; Barklund & Kowalski 1996). From limited field evidence, its transmission in white spruce was horizontal (Sumarah *et al.* 2008a). The purpose of this report is to expand these studies and examine the transmission of *P. scopiformis* to white spruce seedlings, and to look for colonization in adjacent woody plants other than white spruce.

## Materials and methods

### *Seedlings*

Descriptions of the trees and inoculation methods used in these experiments are given in Sumarah *et al.* (2005, 2008a). Briefly, in early September 2003, 300 (some of the original 330 positives died) endophyte/toxin-positive trees were planted at 2 m × 2 m spacing in a test field site *ca.* 30 km from Sussex, NB, Canada. One year later in July 2004, 250 (15 m old) un-inoculated seedlings were obtained as previously described from the JD Irving, Limited genetic improvement program. Five of these seedlings (20–30 mm tall) were planted around each of 50 randomly selected trees from the 300 planted on this field site. The seedlings were planted in close proximity to the infected trees (50 cm away from the stems). Following that, fibreglass screens comprising an area of 0.25 $m^2$ were placed around a further 50 test trees to collect cast needles.

In October of 2007, 4 y after planting the test trees and 3 y after planting the small seedlings, all ''small seedlings'' were collected (244). At this time, they were all under or immediately beside the test tree (~2 m tall) and were typically 20–30 cm tall. In addition all other woody plants from natural regeneration within 2 m of each infected tree were collected. This included *Abies balsamea* (17 plants), *Pinus strobes* (18 plants), *Picea rubens* (11 plants), 34 unidentifiable (due to the age of the tree) *Picea* species (likely mostly white spruce), *P. glauca* (eight plants), 20 *Salix* species, eleven *Betula papyrifera* plants, five *Viburnum cassinoides* plants and three *Tsuga canadensis* plants, as well as cast needles from the screens. These trees/shrubs were 20–30 cm tall. All samples were frozen, freeze-dried and ground to a fine powder (Sumarah *et al.* 2008a).

### *Analysis*

An ELISA (Enzyme-Linked ImmunoSorbent Assay) analysis was carried out to determine the amount of *P. scopiformis* in the needles (or leaves). Analysis was performed using a polyclonal antibody test that is specific and sensitive to *P. scopiformis*. The method limit of detection (LOD) for cell mass was 60 ng $g^{-1}$ (i.e. 60 ppb) and the limit of quantification (LOQ), i.e. a positive, was 120 ng $g^{-1}$ dry weight of needle (Sumarah *et al.* 2005, 2008a)

Rugulosin concentration was determined by High Performance Liquid Chromatography with a Diode Array Detector. Briefly, 300 mg of freeze-dried, ground needle was extracted in ice-cold petroleum ether. The suspension was filtered and the extract discarded. The needles were then re-extracted with 10 ml of chloroform. The chloroform extract was washed with 5 % $NaHCO_3$. The first chloroform layer was discarded while the aqueous fraction was acidified to pH 3 and extracted with a further 10 ml of chloroform. The chloroform layer was removed and dried in an amber vial under nitrogen. The dried extracts were re-dissolved in acetonitrile. Analysis was done with an 1100 series Agilent Technologies HPLC-DAD with a 250 × 4.6 RP column with a gradient elution. Samples were analyzed at 389 nm, the maximum UV/VIS absorption for rugulosin and peak identity was confirmed by full spectrum data from the diode array detector. The LOD and LOQ for rugulosin were both 150 ng $g^{-1}$ (150 ppb; Sumarah *et al.* 2008a).

For statistical purposes, values negative by ELISA and below the analytical detection limit were entered at half the detection limit for rugulosin (as is normal for exposure characterization). Needles that were negative for rugulosin but positive by ELISA were entered at the detection limit because there was positive evidence for the presence of the fungus in the needle.

## Results and discussion

Of the small seedlings planted immediately beside and currently underneath the infected trees, approximately 40 % were infected, supporting the first limited observation made at 2 y post planting (six small trees; Sumarah *et al.* 2008a). The arithmetic mean concentration of the positives was 1.1 μg $g^{-1}$ with a geometric mean of 0.6 μg $g^{-1}$ (Fig 1). This is similar to data from previous studies (Sumarah *et al.* 2005, 2008a; Miller *et al.* 2008). Biomass of the fungus was present in high concentrations in the cast needles but no toxin was detected, which was also similar to samples collected over two previous years (Sumarah *et al.* 2008a).

*P. scopiformis* was not detected in non-target plants. Of the 53 spruce seedlings collected, five (9 %) were positive for *Picea scopiformis* (DAOM 229536, CBS 120377) or a very closely related