Virulence of the entomopathogenic fungus *Lecanicillium* (Deuteromycota: Hyphomycetes) to *Pissodes strobi* (Coleoptera: Curculionidae)

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Abstract—The widely occurring fungal genus *Lecanicillium* Zare & W. Gams (formerly *Verticillium*) includes species that are pathogenic to insects. We collected 27 *Lecanicillium* isolates from soil and from dead adult *Pissodes strobi* (Peck) in British Columbia, Canada, and assessed their virulence against this host. Eighteen isolates were identified as *L. longisporum* (Petch) Zare & W. Gams and six as *L. muscarium* (Petch) Zare & W. Gams, while three isolates could not be identified to the species level. We assayed a subset of these isolates (14 *L. longisporum*, 3 *L. muscarium*, and 1 *Lecanicillium* sp.) as well as the fungal component of the commercial products Mycotal® (*L. muscarium*) and Vertalec® (*L. longisporum*) and a herbarium isolate (*Lecanicillium* sp.). When adult weevils were inoculated with a conidial suspension (1×10^7 conidia/mL), mycosis-related mortality at the end of a 17-day incubation period varied between 20% and 100%, depending on the isolate tested. Eight of the isolates killed >75% of weevils: a *Lecanicillium* sp. isolate, PFC19, which displayed the lowest LT_{50} value; five indigenous *L. longisporum* isolates; and both commercial products. In a goodness-of-fit test comparing isolate virulence among species, the unidentified PFC19 isolate was found to be more effective than either *L. longisporum* or *L. muscarium*, while *L. longisporum* caused somewhat greater mortality than *L. muscarium*. In a similar analysis, isolates extracted from soils tended to be more effective than those obtained from cadavers. Horizontal transmission to live *P. strobi* was observed using different isolates of *Lecanicillium* species. Notwithstanding the variability in virulence, the indigenous *Lecanicillium* species that we isolated and assayed are confirmed as pathogenic to *P. strobi* in British Columbia.

Résumé—Le genre de champignons *Lecanicillium* Zare & W. Gams (précédemment *Verticillium*) à large répartition géographique contient des espèces pathogènes pour les insectes. Nous avons recueilli 27 isolats de *Lecanicillium* dans le sol et sur des cadavres d'adultes de *Pissodes strobi* (Peck) en Colombie-Britannique, Canada, et nous avons évalué leur virulence vis-à-vis cet hôte. Dix-huit des isolats ont été identifiés comme *L. longisporum* (Petch) Zare & W. Gams et six comme *L. muscarium* (Petch) Zare & W. Gams, alors que trois autres n'ont pu être identifiés à l'espèce. Nous avons évalué un sous-ensemble de ces isolats (14 *L. longisporum*, 3 *L. muscarium* et 1 *Lecanicillium* sp.), de même que la composante fongique des produits commerciaux Mycotal® (*L. muscarium*) et Vertalec® (*L. longisporum*) et un isolat d'herbier (*Lecanicillium* sp.). Après une inoculation avec une suspension de conidies (1×10^7 condidies/mL), les charançons adultes subissent après une période d'incubation de 17 jours une mortalité due à la mycose qui varie de 20 % à 100 % selon l'isolat utilisé. Huit des isolats tuent >75 % des charançons, dont un isolat de *Lecanicillium* sp. (PFC19) qui possède le LT_{50} le plus bas, cinq isolats indigènes de *L. longisporum* et les deux produits commerciaux. Un test d'ajustement qui compare la virulence de l'isolat en fonction de l'espèce montre que l'isolat non indentifié PFC19 est plus efficace que *L. longisporum* et que *L. muscarium* et que *L. longisporum* cause un mortalité un peu plus élevée que *L. muscarium*. Une analyse similaire montre que les isolats extraits des sols ont tendance à être plus efficaces que ceux récoltés sur les cadavres. Nous avons observé une transmission horizontale chez des *P. strobi* vivants à l'aide de différents isolats de *Lecanicillium.* Malgré la

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variabilité de leur virulence, les espèces indigènes de *Lecanicillium* que nous avons récoltées et évaluées se sont avérées pathogènes pour *P. strobi* en Colombie-Britannique.

[Traduit par la Rédaction]

Introduction

The white pine weevil, *Pissodes strobi* (Peck, 1817) (Coleoptera: Curculionidae), occurs as a pest of forest trees throughout Canada and the United States. In western Canada, it causes severe damage to natural stands and plantations of Sitka spruce (*Picea sitchensis* (Bong.) Carr.), Engelmann spruce (*Picea engelmannii* Parry ex Engelm.), and white spruce (*Picea glauca* (Moench) Voss), although black spruce (*Picea mariana* (P. Mill.) B.S.P.), Norway spruce (*Picea abies* (L.) Karst.), and lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) are occasionally attacked. In eastern Canada, the weevil feeds on white pine (*Pinus strobus* L.), red pine (*Pinus resinosa* Ait.), jack pine (*Pinus banksiana* Lamb.), Scots pine (*Pinus sylvestris* L.), white spruce, red spruce (*Picea rubens* Sarg.), black spruce, Norway spruce, and Colorado blue spruce (*Picea pungens* Engelm.).

In British Columbia (BC), *P. strobi* adults overwinter (from October to March) in the forest duff and soil, at depths down to 10 cm, usually near the tree from which they emerged during the previous summer and fall (July to September) (Alfaro 1994). In spring (April to May), the weevils mate and lay eggs under the bark in the previous year's terminal leader. After hatching, the larvae mine downward, consuming the phloem and killing the leader. New terminal leader growth results in crooks and forks, which reduces wood quality and quantity. Wood quantity reductions of 15% to 35% have been reported in 40-year-old Sitka spruce stands for weevil attack rates of 10% to 35%, respectively (Alfaro *et al.* 1995).

Suggestions for potential management tools to regulate weevil populations include an integrated pest management strategy that would employ entomopathogenic fungi (Timonin and Morris 1974; Alfaro *et al.* 1985). However, little is known about the natural microbial enemies of forest weevils, although fungal entomopathogens of coleopteran forest pests are common and widespread and can cause spectacular epizootics (Hajek and St. Leger 1994). The entomopathogenic genus *Lecanicillium* Zare & W. Gams (formerly the *Verticillium* species complex) includes species that are parasitic on a wide range of hosts with a broad geographical distribution (Zare and Gams 2001). These ubiquitous entomopathogenic fungi have been isolated from dead adult *P. strobi* and from soil (Kope *et al.* 2000), making them potential agents for weevil control.

The aims of this study were to (*i*) collect indigenous isolates of *Lecanicillium* species occurring in BC, (*ii*) assess their efficacy against live adult weevils, and (*iii*) evaluate the virulence of secondary inoculum and its potential for horizontal transmission from infected cadavers to live adults.

Materials and methods

Isolation of *Lecanicillium* species

Lecanicillium isolates were obtained from infected *P. strobi* cadavers and from soil in Sitka spruce plantations infested by weevils. Live weevils were collected from four areas on Vancouver Island: Nimpkish (50°19′N, 126°54′W), Eve River (50°17′N, 126°18′W), Cowichan Lake (48°50′N, 124°06′W), and the arboretum at the Pacific Forestry Centre (48°28′N, 124°24′W). Weevils were held in rearing boxes, with Sitka spruce leaders for food, in the greenhouse at the Pacific Forestry Centre. Weevil cadavers were collected from the rearing boxes and were incubated on moistened filter paper in a sealed petri plate. Development of conidia on cadavers was monitored daily.

Soil samples were collected in 2000 at five sites on Vancouver Island: Glenroy (50°24′N, 126°01′W), Camp 4 (50°05′N, 125°23′W), Snowden (50°05′N, 125°21′W), Cowichan Lake, and Jordan River (48°24′N, 124°05′W). Five soil cores, extracted with a 2 cm diameter soil borer that was inserted 20 cm into the soil and cleaned with 70% ethanol between each sample, were collected from the base of 10 Sitka spruce trees at each site. Thoroughly mixed soil samples were air-dried for 48 h before being passed through a 0.48 µm mesh screen. A 0.5 g sample of each sieved soil sample was added to 20 mL of 0.1% water agar and agitated for 30 s; 1 mL of the suspension was then spread over the surface of three replicate

petri plates of a newly formulated *Lecanicillium*-selective medium (LSM). This medium was prepared as follows: 2.0 g of L-sorbose, 2.0 g of L-asparagine, 1.0 g of K_2HPO_4, 0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of FeNaEDTA, and 20.0 g of agar were added to 1 L of water. The mixture was autoclaved and then cooled to 40 °C, and the following were added aseptically: 0.3 g of streptomycin SO_4, 0.05 g of chlorotetracycline HCl, 0.8 g of pentachloronitrobenzene, and 1.0 g of $NaB_4O_7 \cdot 10H_2O$. Finally, the pH was adjusted to 4.0 with 10% H_3PO_4. The selective medium excluded bacteria and actinomycetes and held in check the growth of contaminating fungi (unpublished observations). After 4 days of incubation, colonies of *Lecanicillium* spp. were compact and formed characteristic conidiophores with viable conidia. The plates were incubated at 20 °C and monitored during 21 days for the presence of *Lecanicillium*.

Purification of single-spore isolates of *Lecanicillium* species

Pure isolates of *Lecanicillium* spp. were produced by suspending a drop of sterile water from the end of a hypodermic needle and then touching this drop onto conidiophores, resulting in the hydrophilic conidia (Hajek 1997; Inglis *et al.* 2001) migrating into the droplet. The droplet was spread over the surface of an LSM plate; single germinated conidia were removed and subcultured on Sabouraud dextrose agar (SDA), grown for 14 days, and then stored at 5 °C until needed.

Identification of isolates of *Lecanicillium* species

Twenty-seven isolates of *Lecanicillium* spp. that are indigenous to BC were collected. Identification of the different isolates to the species level followed the keys of Zare and Gams (2001). Species were differentiated by colony growth rates on potato–dextrose agar (PDA, Oxoid) after 10 days of growth at 20 °C in darkness (Zare *et al.* 2000) and by the morphology of the phialides and conidia. Most isolates were assigned to either *L. longisporum* (Petch) Zare & W. Gams or *L. muscarium* (Petch) Zare & W. Gams, but there were three *Lecanicillium* isolates that could not be identified to species (Table 1). Molecular characterization of the isolates is currently in progress and will be reported elsewhere.

Bioassay of the efficacy of a conidial suspension of *Lecanicillium* spp. against *P. strobi*

Weevils were submerged in 30% H_2O_2 for 3 min to remove surface contaminants and then rinsed in three changes of sterile distilled water. Washed weevils were allowed to dry and reacclimatize for 24 h in a sterile container before being used in an experiment. A section of a Sitka spruce leader to be used as a food source was scrubbed with 30% H_2O_2 and then rinsed in three changes of sterile distilled water. The leader was aseptically cut to 5 cm lengths and further cut into rounds or longitudinal halves and quarters. To test whether weevils were carriers, internally or externally, of *Lecanicillium* conidia, 10 randomly sampled weevils were individually squashed: 5 were placed on LSM agar and 5 were placed on SDA. Squashed weevils were observed for 14 days for presence of *Lecanicillium*.

Of the 27 indigenous isolates collected (Table 1), a subset of 18 isolates was tested for entomopathogenic efficacy. We also tested the fungal component only of two commercial products, Mycotal® (*L. muscarium*) and Vertalec® (*L. longisporum*) (Koppert Canada Ltd., Scarborough, Ontario), and one *Lecanicillium* isolate from the Canadian National Mycological Herbarium ((DAOM 216596, preserved as *Verticillium lecanii* (Zimm.) Viégas, originating on *Marcosiphoniella* sp. (Aphididae)). In total, 21 isolates were tested.

Single-spore colonies of the 21 isolates were grown for 21 days on SDA at 20 °C in the dark. Inocula for the bioassay were prepared by flooding the plates with 5 mL of sterile distilled water and agitating the colonies with a sterile glass rod to dislodge conidia. Conidial suspensions of 1×10^7 conidia/mL were made for each of the 18 indigenous and 3 additional isolates (Table 1). Concentrations were determined using a Neubauer haemocytometer. Conidia viability was determined by spreading a drop (0.5 mL) of inoculum on the surface of an SDA plate: conidia germination was counted after a 24 h incubation at 20 °C in the dark.

Inoculation of weevils was carried out by simultaneously placing 24 weevils into a conidial suspension for 10 s and then pouring them out onto a filter paper to drain off excess liquid. The 24 weevils treated with each isolate were placed in 9 cm plastic petri plates with moistened filter paper on the bottom to maintain a

Table 1. Isolates of *Lecanicillium* species: substrate of origin, location in British Columbia (see text for details), and year of isolation.

Isolate No.	Substrate of origin	Location and year of isolation
	Lecanicillium longisporum	
PFC 1	Adult *P. strobi*	PFC Arboretum, 2003
PFC 3	Adult *P. strobi*	Eve River, 2003
PFC 5	Adult *P. strobi*	Eve River, 2003
PFC 6	Adult *P. strobi*	Eve River, 2003
PFC 9	Adult *P. strobi*	PFC Arboretum, 2003
PFC 10	Adult *P. strobi*	Nimpkish, 2003
PFC 12	Adult *P. strobi*	Cowichan Lake, 2003
PFC 13	Adult *P. strobi*	Nimpkish, 2003
PFC 14	Adult *P. strobi*	PFC Arboretum, 2003
PFC 16	Adult *P. strobi*	PFC Arboretum, 2003
AFS 5	Soil	Camp 4, 2000
AFS 6	Soil	Glenroy, 2000
AFS 7	Soil	Jordan River, 2000
AFS 8	Soil	Snowden, 2000
AFS 9	Soil	Cowichan Lake, 2000
AFS 10	Soil	Cowichan Lake, 2000
AFS 11	Soil	Cowichan Lake, 2000
AFS 12	Soil	Cowichan Lake, 2000
	Lecanicillium muscarium	
PFC 4	Adult *P. strobi*	Eve River, 2003
PFC 11	Adult *P. strobi*	Nimpkish, 2003
AFS 1	Adult *P. strobi*	Eve River, 2000
AFS 2	Adult *P. strobi*	Eve River, 2000
AFS 3	Adult *P. strobi*	Eve River, 2000
AFS 4	Adult *P. strobi*	Eve River, 2000
	***Lecanicillium* sp.**	
PFC 17	Adult *P. strobi*	PFC Arboretum, 2003
PFC 18	Adult *P. strobi*	PFC Arboretum, 2003
PFC 19	Adult *P. strobi*	PFC Arboretum, 2003

high water activity (>0.98 a_w) and a piece of disinfected Sitka spruce leader for food. Control weevils were inoculated with sterile distilled water. The bioassay petri plates were incubated at 20 °C under a 12L:12D photoperiod.

Weevil mortality was recorded daily for a total of 17 days; dead weevils were removed from the incubation plates and transferred to humid petri plates and the resulting fungal growth was subcultured on SDA plates.

Horizontal transmission of *Lecanicillium* spp. in *P. strobi*

A subsample of six indigenous *Lecanicillium* isolates (AFS1, 2, 4, 5, 7, and 8) and the DAOM isolate were tested to determine the potential of colonized weevil cadavers as an inoculum source. One colonized weevil cadaver was placed among eight surface-disinfected live adult *P. strobi* in a petri plate with moistened filter paper and a piece of Sitka spruce leader, and the plate was incubated at 20 °C. Controls had eight surface-disinfected live adult weevils and one surface-disinfected weevil cadaver. Three replicate plates were made for each of the seven isolates tested. Plates were checked daily, and dead weevils were counted and removed to petri plates with moistened filter paper; the resulting fungal growth was subcultured on SDA plates.

Statistical analysis

Variability in weevil mortality was corrected for the natural mortality in the control using Abbott's (1925) formula, which calculates the proportion of insects killed by the entomopathogen alone. To test differences in mortality between the control and the treatments, the log-likelihood ratio goodness-of-fit test (*G* test) (Sokal and Rohlf 1995) was calculated for two types of association: (1) that among *Lecanicillium* species and (2) that between the substrates from which *Lecanicillium* was originally isolated. In this analysis, data transformation (+1) was necessary owing to the presence of a zero value, which does not compute in a *G* test. For the first type of association, the null hypothesis was that the proportion of *P. strobi* surviving the fungal treatment is independent of the *Lecanicillium* species used; for the second type of association, the null hypothesis was that the level of *Lecanicillium*-related mortality is independent of the substrate from which the source of inoculum was originally obtained. How quickly *P. strobi* died because of a *Lecanicillium* sp. infection was estimated using PROBIT transformation of Abbott-corrected mortality data (Throne *et al.* 1995). Median lethal times (LT_{50} values; time required for 50% mortality), with lower and upper 95% fiducial limits, were reported only for those isolates that killed >75% of the *P. strobi* adults (Robertson and Preisler 1992). Calculations were performed with the MINITAB® statistical package (Minitab Inc. 2003). Differences in mortality in the horizontal transmission assay were calculated with ANOVA, and multiple comparisons of means were made using the Student–Newman–Keuls post hoc test (Zar 1999).

Results

Identification of isolates of *Lecanicillium* spp.

The 18 isolates identified as *L. longisporum* all displayed the long (6.4 to 9.9 µm, median 7.9 µm) and wide (1.5 to 2.5 µm) conidia characteristic of this species. *Lecanicillium longisporum* was isolated from both soil and adult weevil cadavers (Table 1). In comparison, the 6 isolates identified as *L. muscarium* displayed shorter (3.7 to 6.6 µm, median 5.0 µm) and narrower (1.0 to 2.0 µm) conidia. These isolates were found only on adult weevil cadavers. The three unidentified *Lecanicillium* isolates were collected from adult weevil cadavers (Table 1).

Bioassay of the efficacy of a conidial suspension of *Lecanicillium* spp. against *P. strobi*

No contaminating surface or internal propagules of *Lecanicillium* spp. were found on either LSM or SDA plates of prewashed and squashed weevils. Conidia viability was ≥98% for the conidial suspensions of the 21 isolates of *Lecanicillium* spp. tested.

Eighteen indigenous isolates of *Lecanicillium* spp., as well as the fungal isolates from the DAOM, Mycotal®, and Vertalec®, were tested for their virulence against live adult weevils. Mycosis-related mortality, expressed as a percentage using Abbott's correction, was recorded after 17 days of incubation (Fig. 1).

Under the assay conditions used, isolates of *L. longisporum* displayed a wide range of virulence, causing from ~20% to 100% mortality at the end of the 17-day incubation period (Fig. 1). Six isolates of *L. longisporum* caused ≤50% mortality, whereas nine others, as well as the commercial formulation Vertalec®, induced ≥50% mortality. Of these nine isolates, the three that appeared most effective — PFC6, PFC12, and PFC14 — were isolated from dead weevils, whereas the other six were isolated from soil.

Although the commercial isolate Mycotal® yielded a high level of mortality under the conditions used here, the three *L. muscarium* isolates that we isolated (AFS1, AFS2, and AFS4) caused <50% mortality (Fig. 1). In comparison, the level of mortality caused by the DAOM and PFC19 (*Lecanicillium* sp.) isolates were at the low and high ends of the spectrum, respectively (Fig. 1).

LT_{50} values were calculated for the 21 isolates of *Lecanicillium* spp.; results shown here (Table 2), however, are only for those isolates that killed >75% of *P. strobi* adults (Robertson and Preisler 1992). The LT_{50} estimates ranged from 6.91 to 10.35 days for the 8 isolates considered here. Six of these isolates were *L. longisporum* (PFC6, PFC12, PFC14, AFS 7, AFS8, and Vertalec®), while the other two were *L. muscarium* (Mycotal®) and *Lecanicillium* sp. (PFC19).

In a goodness-of-fit test, in which the results obtained for the various *Lecanicillium* isolates were pooled according to species or species associations, significantly higher mortality was observed among treated weevils (all *Lecanicillium*

Fig. 1. Cumulative mycosis-related mortality (%) of adult *Pissodes strobi* inoculated with isolates of *Lecanicillium* spp. by soaking in a conidial suspension of 1×10^7 conidia/mL, at the end of a 17-day incubation period.

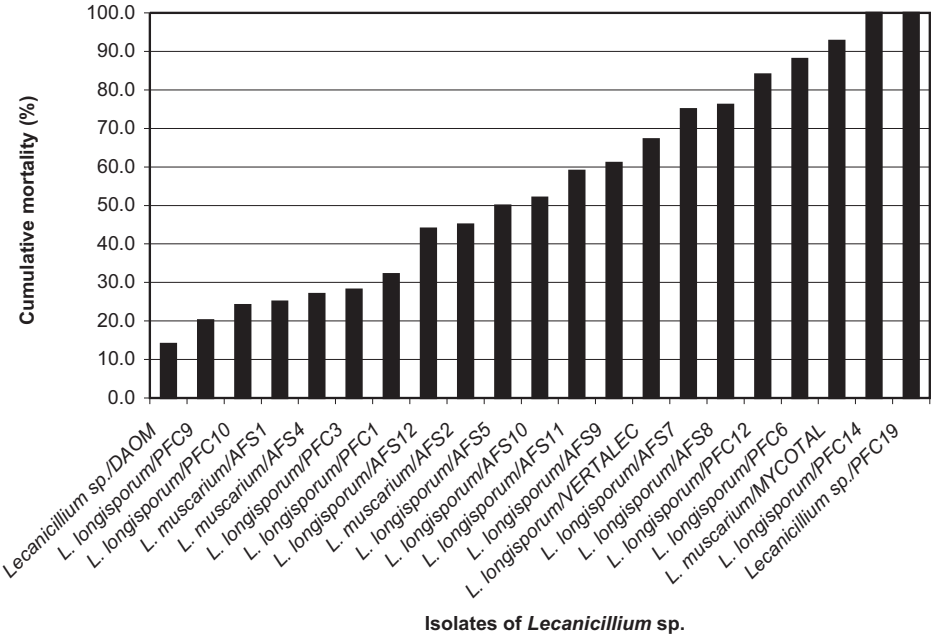


Table 2. Median lethal time (LT_{50}) and 95% fiducial limits, in days, for the eight *Lecanicillium* isolates that caused >75% mortality among *Pissodes strobi* adults soaked in a conidial suspension (1×10^7 conidia/mL).

Species / isolate No.	Lower fiducial limit	LT_{50}	Upper fiducial limit
Lecanicillium sp. / PFC19	6.35	6.91	7.49
L. longisporum / PFC 14	6.45	7.02	7.63
L. muscarium / Mycotal	7.22	7.85	8.54
Lecanicillium longisporum / PFC 6	7.42	8.04	8.70
Lecanicillium longisporum / PFC 12	8.29	8.98	9.72
L. longisporum / Vertalec	8.42	9.12	9.86
L. longisporum / AFS 7	9.31	10.09	10.93
L. longisporum / AFS 8	9.57	10.35	11.18

Note: Analysis of Abbott-corrected data after PROBIT transformation.

isolates pooled) than among controls; however, the three species groups were not homogeneous in their ability to cause weevil mortality (Table 3). This lack of homogeneity was attributable mostly to the greater effectiveness of *Lecanicillium* sp. (PFC19) at causing beetle death compared with either *L. longisporum* or *L. muscarium* ($P \leq 0.001$ for both comparisons; Table 3), although *L. longisporum* also caused significantly higher mortality ($P \leq 0.05$) than *L. muscarium* (Table 3).

Isolates of *Lecanicillium* spp. that were collected from the soil were statistically more effective at causing mortality to adult *P. strobi* than isolates collected from adult *P. strobi* cadavers (Table 4). All soil isolates were identified as *L. longisporum*, whereas isolates collected from cadavers included *L. longisporum*, *L. muscarium*, and *Lecanicillium* sp.

Horizontal transmission of *Lecanicillium* spp. in *P. strobi*

At the end of a 21-day incubation, *L. longisporum* isolate AFS7 was found to be the most effective at causing beetle mortality (76.3%) through horizontal transmission, followed by

Table 3. Log-likelihood ratio goodness-of-fit test (*G* test) for differences in *Lecanicillium*-related mortality (*a*) of *Pissodes strobi* adults as a function of *Lecanicillium* species or species associations (*b*).

(*a*) Mortality of *P. strobi* adults.			
	No. of insects		
Entomopathogenic species	Alive	Dead	Total
Control	21	3	24
L. longisporum	170	190	360
L. muscarium	57	39	96
Lecanicillium sp.	0	24	24
Totals	248	256	504 (*n*)
(*b*) Log-likelihood ratio goodness-of-fit test.			
	G test	df	*P*
Control versus *L. longisporum*, *L. muscarium*, *Lecanicillium* sp.	14.79	1	≤0.001
Homogeneity among *L. longisporum*, *L. muscarium*, *Lecanicillium* sp.	71.52	2	≤0.001
Homogeneity within *L. longisporum*, *L. muscarium*	4.42	1	≤0.05
Homogeneity within *L. longisporum*, *Lecanicillium* sp.	110.69	1	≤0.001
Homogeneity within *L. muscarium*, *Lecanicillium* sp.	30.60	1	≤0.001

Table 4. Log-likelihood ratio goodness-of-fit test (*G* test) for differences in *Lecanicillium*-related mortality of *Pissodes strobi* adults (*a*) as a function of the source (soil or cadavers) of the *Lecanicillium* isolates (*b*).

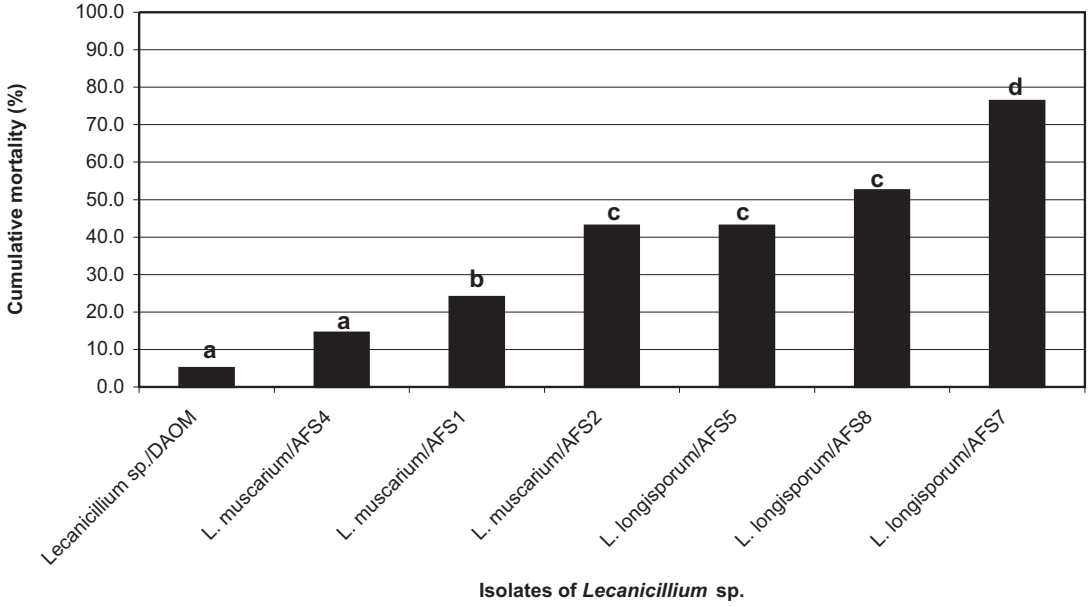
(*a*) Mortality of *P. strobi* adults.			
	No. of insects		
Collection substrate	Alive	Dead	Totals
Soil	74	94	168
Cadaver	142	122	264
Totals	216	216	432 (*n*)
(*b*) Log-likelihood ratio goodness-of-fit test.			
	G test	df	*P*
Soil versus cadaver	3.90	1	≤0.05

three species that caused statistically similar levels of mortality (43%–52.5%): two other *L. longisporum* isolates, AFS8 and AFS5, and an *L. muscarium* isolate, AFS2 (Fig. 2). In terms of effectiveness, *L. muscarium* isolate AFS1 was intermediate (24%) between these latter three isolates and the least effective isolates tested, *L. muscarium* isolate AFS4 and the *Lecanicillium* sp. DAOM isolate, both of which caused <15% mortality (Fig. 2). When virulence of the conidial suspension inoculum (Fig. 1) was compared with that assessed under conditions where the inoculum was provided by infected cadavers (Fig. 2), the rankings of the isolates tested were observed to be similar.

Discussion

Along with earlier reports documenting the colonization of forest insect pests by entomopathogenic Hyphomycetes (Harney and Widden 1991*a*, 1991*b*; Ahmed and Leather 1994), the present work provides clear evidence that species of *Lecanicillium* can cause disease in *P. strobi*. Our study confirms the pathogenicity of *L. longisporum*, *L. muscarium*, and *Lecanicillium* sp. isolates against *P. strobi* under laboratory conditions. When inoculation was carried out by placing weevils in a conidial suspension of 1×10^7 conidia/mL, percent mortality ranged from ~20% to 100% and the LT_{50}

Fig. 2. Cumulative mycosis-related mortality (%) of adult *Pissodes strobi* using *Lecanicillium*-infected *P. strobi* adult cadavers as the inoculum source, at the end of a 21-day incubation period. Bars with the same letter are not significantly different (ANOVA, df = 6, $F = 16.505$, $P = 0.0001$; Student–Newman–Keuls, $P = 0.05$).



values calculated for the isolates that killed more than 75% of their hosts ranged from 6.91 to 10.35 days. Under the experimental conditions employed here, indigenous isolates of *L. longisporum* and *Lecanicillium* sp. tended to display greater virulence than those of *L. muscarium*. Among the indigenous *Lecanicillium* isolates that we identified to the species level, those that caused >50% mortality were of the long conidia type (*i.e.*, *L. longisporum*), irrespective of the inoculation method used (Figs. 1, 2). Hall (1984) reported a similar finding for *Lecanicillium* (*V. lecanii*) isolates: large conidia were associated with a stronger epizootic potential. Hall suggested that the larger conidia might contain more nutrients for growth and have a higher infective potential than smaller conidia.

Zare and Gams reported that *L. longisporum* (2003*a*) and *L. muscarium* (2003*b*) have cosmopolitan distributions: collections were made from both insect hosts and soil. Here, multiple isolates of *L. longisporum*, *L. muscarium*, and *Lecanicillium* sp. were obtained from infected weevils and from soil where *P. strobi* overwinter, demonstrating that a fungal inoculum reservoir is naturally present in the BC forest environment. That forest soils constitute natural reservoirs of entomopathogenic fungi has been observed before (Widden 1979; Harney and Widden 1991*a*, 1991*b*; Brownbridge *et al.* 1993; Vänninen 1995); indeed, microclimatic conditions within forest soils favour the saprophytic growth of facultative pathogens (Bidochka *et al.* 1998). Whether soils are a good source of virulent isolates, however, remains unclear. Although the analysis presented in Table 4 suggests that, overall, isolates that were extracted from soil caused greater mortality than those obtained from weevil cadavers, it must be noted that the isolates that caused the highest (PFC19, PFC14, PFC6, PFC12) and the lowest (PFC9, PFC10, AFS1, AFS4) mortality (Fig. 1) were all isolated from dead weevils (Table 1). Thus, the source of the *Lecanicillium* isolate cannot, at this stage, be used as a reliable predictor of virulence against *P. strobi*.

The important differences in virulence among the *Lecanicillium* isolates that we compared likely reflect innate differences in pathogenicity against *P. strobi*. However, it will be essential to assess the robustness of the present ranking by conducting additional assays under both the same and alternative experimental conditions. Only those isolates that consistently cause high levels of mortality under varying experimental conditions should be considered for further development as biocontrol agents.

The present work indicates that diseased weevils can effectively transmit *Lecanicillium* spp. to healthy individuals, at least under the moist conditions of our bioassays (Fig. 2). Inoculum dispersal is required for a fungal pathogen to cycle and induce an epizootic (Kish and Allen 1978). Primary inoculum, which initiates the disease, can cause secondary inoculum formation in infected individuals. This movement of the inoculum from cadavers to live hosts is known as horizontal inoculum transfer. The mechanism of conidial transfer remains undefined, but it is assumed that the hydrophilic *Lecanicillium* conidia migrate via water droplets and that infection involves the capillary action of a water droplet depositing conidia into crevasses on the weevil cuticle. The behaviour of the infected host, such as its tendency to aggregate with other hosts or to remain isolated, will affect the rate and effectiveness of inoculum spread. Whether an epizootic could develop under field conditions following the introduction of a substantial *Lecanicillium* inoculum into the duff layers, where the weevil overwinters (Silver 1968), is a question that needs to be addressed, particularly if an isolate of this fungus is to be developed as a biocontrol agent.

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