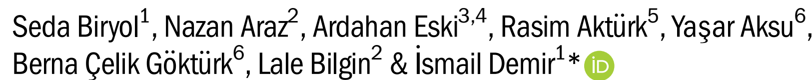
Entomologia Experimentalis et Applicata



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# Biodiversity and pathogenicity of entomopathogenic fungi associated with the lesser spruce sawfly, *Pristiphora abietina*

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**Abstract**

Outbreaks of lesser spruce sawfly, *Pristiphora abietina* (Christ) (Hymenoptera: Tenthredinidae), on conifers regularly occur in Turkey and other European countries. Chemical pesticides have been used to contain outbreaks, but those may damage the forest and disturb natural enemies. To minimize the use of chemical pesticides in the management of this forest pest, we identified the entomopathogenic fungi (EPF) that naturally occur on *P. abietina* and tested their efficacy against the pest under laboratory and semi-field conditions. Using morphological and molecular techniques, 13 EPF isolates were obtained from the pest and identified as *Beauveria bassiana* (Bals.-Criv.) Vuill., *Beauveria pseudobassiana* S.A. Rehner & Humber, and *Lecanicillium muscarium* (Petch.) Zare & W. Gams. A laboratory screening test at $10^6$ conidia $ml^{-1}$ showed that all isolates caused 59-100% mortality after 14 days. Further experiments were performed with the three most effective isolates, all belonging to *B. bassiana*. For these isolates, an increase in conidia dose increased pest mortality. Also, the ability of horizontal transmission in the pest population was shown. All three isolates were effective in killing *P. abietina* on spruce under semi-field conditions. These data will contribute to the development of an integrated pest management program for *P. abietina* based on microbial biological control.

## Introduction

Sawflies (Hymenoptera, suborder Symphyta) are major economic pests of forest trees and are widely distributed throughout the world. The largest family, Tenthredinidae, has over 5 000 species across ca. 430 genera (Boevé, 2008). The lesser spruce sawfly, *Pristiphora abietina* (Christ) (Tenthredinidae), damages 20- to 30-year-old spruce stands (*Picea* spp., Pinaceae), defoliating the top whorls, which negatively affects tree height. Repeated infestations also cause dieback of the leaders and deformation of the crown (Ohnesorge, 1961; Holuša & Holuša, 2002; Taut et al., 2011).

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In many regions of Europe, chemical insecticides and yellow sticky traps have been used to eliminate larvae and adults of the pest, respectively (Egger, 1989a; Murhead, 1991; Olenici & Olenici, 2005). In North America, pheromone traps were used to capture male individuals (Anderbrant, 1993). In the spruce forests of Austria, from 1981 to 1987, chemical insecticides were used, until the adverse effects on beneficial insects were detected (Egger, 1989b; Svestka & Holuša, 2000).

For biological control, *Formica rufa* L. (Hymenoptera: Formicidae), a predator also known as the redwood ant, was released in spruce forests in Turkey where *P. abietina* was widespread, but it has not succeeded in controlling the pest. Neither a chemical insecticide, nor the bio-insecticide *Bacillus thuringiensis* Berliner (Bt) has reduced *P. abietina* population densities in Turkey (Aksu & Göktürk, 2008). However, Bt strain PS86Q3 is effective against a German

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population of *P. abietina* larvae (Porcar et al., 2008). Also, a strain of cytoplasmic polyhedrosis virus (CPV) isolated from *Calliteara pudibunda* L. was effective against the pest (Lobinger & Skatulla, 1989), as well as the bio-insecticide spinosad (Brudea & Pei, 2006). Despite all of these studies, an effective integrated pest management (IPM) strategy has not yet been developed (Holuša & Drápela, 2003).

Entomopathogenic fungi (EPF) could be effective control agents for *P. abietina* (Schedl, 1953; Kolubajiv, 1958; Wiener, 1993; Schmied & Führer, 1996). The genera *Beauveria*, *Paecilomyces*, and *Metarhizium* attack the cocoon stage of the pest (Führer et al., 2001). In a recent study, fungi were isolated from *P. abietina* larvae and tested for virulence against the pest in a laboratory setting (İskender et al., 2017). However, the successful use of EPF under field conditions has yet to be investigated. An important aspect of biocontrol with EPF is the ability for horizontal transmission in insect communities. Autodissemination of fungal spores represents a new opportunity for IPM strategies and offers several advantages, such as reduction of application volume and minimization of the adverse effects on non-target organisms. The horizontal transmission capacity of *Beauveria bassiana* (Bals.-Criv.) Vuill. (Toledo et al., 2017), *Metarhizium anisopliae* (Metschn.) Sorokīn (Arthurs et al., 2001), and *Isaria fumosorosea* Wize (Avery et al., 2010) has already been demonstrated in various insect communities. However, there are no studies on fungal transmission between *P. abietina* larvae.

The average summer temperature of the Eastern Black Sea Region of Turkey is 25–30 °C and the average relative humidity is 70-80%, which is very suitable for the growth, germination, survival, and virulence of EPF. Considering the climatic conditions of this region and the biological properties of fungi, EPF could be the best choice for microbial biological control of *P. abietina*. The primary aims of the present study were to determine the biodiversity of EPF associated with *P. abietina* and to test the virulence of these EPF isolates under laboratory conditions. Subsequently, for the three isolates that were most effective, the dose-response relationship was examined, as well as horizontal transmission ability, and the insecticidal activity against *P. abietina* was tested under semi-field conditions.

## Materials and methods

### Collection of insects

*Pristiphora abietina* larvae were collected from trees in spruce forests in Artvin Forestry Directorate, Turkey. They were immediately brought to the laboratory in plastic boxes (30 × 20 × 15 cm) covered with gauze and containing fresh spruce shoots, and were kept at 18–20 °C under L12:D12 photoperiod. Spruce shoots were changed daily, and third instars were used for bioassays.

### Isolation of entomopathogenic fungi

The insects, which were collected as cadavers from the field or died while being reared on spruce shoots in the laboratory, were examined to determine whether the cause of death was a fungal infection. For surface sterilization, dead insects were treated with 1% sodium hypochlorite solution for 3 min and washed 3× with sterile distilled water. Cadavers were incubated in Petri dishes at 25 °C under L16:D8 photoperiod (Ali-Shtayeh et al., 2002). After the emergence of fungal hyphae and sporulation, they were inoculated onto potato dextrose agar supplemented with 1% yeast extract (PDAY) and 50 μg $ml^{-1}$ ampicillin (AppliChem, Darmstadt, Germany). A single colony from each isolate was subcultured to obtain pure cultures. Once the purity of the cultures was ensured, samples were stored in 20% glycerol (AppliChem) at −80 °C.

### Morphological identification

Fungal isolates obtained through isolation from insect samples were morphologically characterized using cultures on artificial media (PDAY). The monosporic pure cultures were characterized based on their colony appearance (shape, color, mycelium type, presence of striations, and colony height). A microscopic examination was performed to determine the shape and length of conidia. All isolates were identified based on the identification key of Humber (1997).

### DNA extraction and amplification

Aliquots of 100 μl of conidial suspension of each isolate were plated on PDAY medium and incubated at 25 °C for 14 days to select colonies originated from single conidia. Then, a colony was transferred to fresh medium and incubated at 25 °C for 14 days. DNA was extracted from the dried mycelium of each of the 13 isolates using the Quick-DNA Fungal/Bacterial MiniPrep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. Afterwards, the ITS1-5.8S-ITS2 region of each isolate was amplified using the following primer pairs: ITS5 (5′-GGAAGTAAAATCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). PCR reactions were performed by a T100 thermal cycler (Bio-Rad, Hemel Hempstead, UK). The reaction mixture (50 μl) consisted of 50 ng of DNA template, 10 μl 5× Phusion HF reaction buffer, 1 μl 10 mM dNTPs, 2.5 μl (10 μM) of each primer, and 0.5 μl 2-unit Phusion-DNA polymerase (New England Biolabs, Ipswich, MA, USA). The reaction parameters were: 35 cycles, denaturation at

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98 °C for 30 s, annealing at 55 °C for 1 min, followed by extension at 72 °C for 1 min; a final extension at 72 °C for 10 min was included after the last cycle. Amplified products (ca. 580 bp) were separated by electrophoresis on 1.0% agarose gels (stained with ethidium bromide) in 1× TAE buffer (40 mM Tris, 20 mM acetate, 2 mM EDTA) and then visualized under ultraviolet (UV) light. The amplified products were purified from the agarose gel using the NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Samples were quantified using a NanoDrop 2000 scanning spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and sent out for sequence analysis (Macrogen Europe, Amsterdam, The Netherlands). The sequences obtained were compiled and compared to those in the GenBank databases using BLAST (Altschul et al., 1990; Bischoff et al., 2009; Benson et al., 2012). Multiple sequence alignment was carried out using ClustalW2 in BIOEDIT v.7.0.5 software (Hall, 1999). Phylogenetic trees were built using the neighbor-joining (NJ) algorithm with MOLECULAR EVOLUTIONARY GENETICS ANALYSIS v.6.0 (MEGA6) software (Saitou & Nei, 1987; Tamura et al., 2013). The sequences of the ITS1-5.8-ITS2 gene of all isolates were deposited at the NCBI data library under accession numbers MK544077-MK544089.

#### Preparation of conidial suspension

The conidial suspensions of fungal isolates for bioassays were prepared by adding 10 ml of sterile 0.01% Tween 80 (AppliChem, Darmstadt, Germany) to 4-week-old cultures. The spore suspensions were filtered into sterile 50-ml plastic tubes (Falcon, Franklin Lakes, NJ, USA) using a sterile cotton gauze to remove fungal residue and vortexed for 5 min to homogenize the preparations. To determine the viability of conidia, the suspensions were spread on PDAY medium and germination was assessed after 24 h of incubation at 25 °C in the dark. Cultures with conidia viability above 95% were used for bioassay experiments.

#### Screening test

The number of conidia in the suspensions was determined using a Neubauer hemocytometer and the concentration was adjusted to $10^6$ conidia $ml^{-1}$. Larvae were dipped into 10 ml of suspension for 5 s and placed in plastic boxes (5 × 10 cm) containing fresh spruce shoots (30 larvae for each fungus per box). Control larvae were dipped in a control solution with 0.01% Tween 80. The experiment was conducted at 20 ± 1 °C and 70 ± 5% r.h. under L12: D12 photoperiod for 14 days. The boxes were checked daily and dead larvae were collected. The cadavers of larvae were surface sterilized with 70% alcohol and assessed for mycosis by placing them in a moisture chamber to stimulate fungal sporulation. All experiments were performed with 30 larvae, included three replicates, and were repeated 3× on separate days.

#### Dose-response test

Three *B. bassiana* isolates (Pa4, Pa5, and Pa6), which showed over 90% insecticidal effect in the screening tests, were used in dose-response experiments. Conidial suspensions were serially diluted and bioassays, assessing *P. abietina* mortality and mycosis, were conducted as described above with 10 ml of suspensions containing $10^9$, $10^8$, $10^7$, $10^6$, and $10^5$ conidia $ml^{-1}$. Control suspensions did not contain conidia. Dead larvae were collected daily for 14 days. $LC_{50}$ and $LC_{95}$ values of the isolates were calculated by probit analysis using IBM-SPSS v.25.0 statistical software (Finney, 1971).

#### Horizontal transmission test

Conidia transmission ability between treated and untreated *P. abietina* larvae were determined for *B. bassiana* isolates Pa4, Pa5, and Pa6. The suspension contained $10^6$ conidia $ml^{-1}$ and the following ratios of treated and untreated larvae were used: (1) 10 treated and 30 untreated larvae (25% treatment), (2) 20 treated and 20 untreated larvae (50% treatment), (3) 30 treated and 10 untreated larvae (75% treatment), (4) 40 treated larvae (100% treatment), and (5) 40 untreated larvae as control (0% treatment) (Kocacevik et al., 2016). Experiments were conducted in a climate-controlled cabinet at 25 ± 1 °C and 70 ± 5% r.h. under L12:D12 photoperiod for 15 days. After this time period, *P. abietina* mortality and mycosis was assessed as described above. Each treatment was replicated 3× on separate days.

#### Semi-field applications

The effectiveness of *B. bassiana* isolates Pa4, Pa5, and Pa6 was tested under semi-field conditions in the city of Artvin, in the Eastern Black Sea Region of Turkey, an area where pest infestation was high. For each isolate, five fresh shoots were selected on a spruce tree and 30 third instars were placed on each shoot. A spore suspension containing $10^8$ conidia $ml^{-1}$ was applied to each shoot using a backpack sprayer equipped with a hollow cone spray nozzle calibrated to deliver 200 l $ha^{-1}$ at 0.2 MPa (Blount, Portland, OR, USA). Control shoots were sprayed with a solution containing 0.01% Tween 80. Larvae were contained by tulle cages (25 × 35 cm). Treatments were applied in the evening to reduce confounding environmental factors. We used a completely randomized experimental design with five replicates per treatment that was repeated twice on separate days. Cages were checked once

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a week for 3 weeks until completion of the insect life cycle. After, shoots were cut with garden shears and insect mortality and mycosis were determined as described above. Larval mortality, pupal mortality, and adult survival were distinguished.

#### Statistical analysis

The mortality and mycosis values obtained from the screening test were corrected according to Abbott's formula: corrected % mortality = (observed % mortality – mean control % mortality) / (100 – mean control % mortality) (Abbott, 1925). Differences between the mortalities were evaluated by one-way ANOVA, followed by Duncan's post-hoc testing to compare means among fungi and the control group. To evaluate mortality as a result of horizontal transmission of fungal conidia, data were analyzed separately for 5, 10, and 15 days after treatment. Mortality data obtained from dose-response experiments were subjected to probit analysis to determine lethal concentrations ($LC_{50}$ and $LC_{95}$) of *B. bassiana* isolates Pa4, Pa5, and Pa6. Survival curves of five concentrations of *B. bassiana* isolates Pa4, Pa5, and Pa6 (and a control) were generated as a function of the observation time through the Kaplan–Meier method (Kaplan & Meier, 1958) using IBM-SPSS v.25.0 statistical software (IBM, Armonk, NY, USA).

## Results

#### Morphological characterization of fungal isolates

Thirteen isolates belonging to two genera from the parasitic fungi family Cordycipitaceae were obtained from *P. abietina*. The isolates belong to the genera *Beauveria* and *Lecanicillium*. Isolates Pa1, Pa2, Pa4, Pa5, Pa6, Pa7, Pa10, Pa12, and Pa13 were determined as *Beauveria* spp. These isolates formed white, smooth, rounded colonies with a fluffy-to-powdery appearance. The colonies were initially white, then turned pink-tan. The spores of these isolates were round, and the conidia-producing cells were characteristically forming a globular or bottle-like, mostly zigzagged, structure. Many small microconidia can form clusters around conidiophores. Isolates Pa3, Pa8, Pa9, and Pa11 were determined as *Lecanicillium* spp. These isolates produced colorless mycelia and prominent white colonies, as well as abundant, colorless, ellipsoidal-to-cylindrical conidia (Figure S1).

#### Molecular characterization of fungal isolates

Neighbor-joining trees based on ITS1-5.8-ITS2 gene regions revealed that the isolates Pa1, Pa2, Pa7, Pa10, and Pa12 were *Beauveria pseudobassiana* S.A. Rehner & Humber, isolates Pa4, Pa5, Pa6, and Pa13 were *B. bassiana*, and isolates Pa3, Pa8, Pa9, and Pa11 were *Lecanicillium muscarium* (Petch) Zare & W. Gams (Figure 1).

#### Insecticidal activity experiments

All fungal isolates were able to infect and kill *P. abietina* larvae, although there were differences in efficacy ($F_{13,56}$ = 12.339, P<0.05). Three *B. bassiana* isolates (Pa4, Pa5, and Pa6) were most effective, causing 92–100% mortality 14 days after application. Isolates Pa7, Pa12 (*B. pseudobassiana*), Pa9, and Pa11 (*L. muscarium*) were least effective, causing 59-63% mortality. In the control group, 3% mortality occurred. The mycosis ratios of dead larvae showed that mortality was predominantly caused by fungal infection (80–98%) (Figure 2).

*Beauveria bassiana* isolates Pa4, Pa5, and Pa6, which caused 90% or higher mortality in screening tests, were used in the dose-response experiment. Larval mortality rates increased as the conidia dose increased for Pa4 ($\chi^2$ = 294, d.f. = 5, P<0.001), Pa5 ($\chi^2$ = 273, d.f. = 5, P<0.001), and Pa6 ($\chi^2$ = 227, d.f. = 5, P<0.001). At the highest concentration ($10^9$ conidia $ml^{-1}$), all isolates caused 100% mortality after 14 days. *Beauveria bassiana* isolate Pa4 also caused 100% mortality at the second-highest conidia concentration. Also, this isolate caused higher mortality than the two other isolates at the lowest concentration ($10^5$ conidia $ml^{-1}$); no mortality was observed in the control treatment (Figure 3). The estimated $LC_{50}$ values of *P. abietina* larvae treated with isolate Pa4 were lower than those for larvae treated with isolates Pa5 and Pa6 (Table 1).

#### Horizontal transmission experiment

Mortality rates increased with the number of introduced treated larvae in a box (Table 2). No death was detected in the negative control group without initial fungal application (data not shown). When 10 out of 40 larvae received an initial fungal application (25% treatment), after 15 days, 100% mortality was detected with isolate Pa4, and 88 and 77% mortality with isolates Pa5 and Pa6, respectively. When 30 out of 40 larvae received an initial fungal application (75% treatment), the *P. abietina* mortality rates after treatment with isolates Pa4, Pa5, and Pa6 after 15 days were 100, 97, and 86%, respectively. Isolates Pa4 and Pa5 caused 100% mortality when 100% of the insects received a fungal application, whereas isolate Pa6 caused 89% mortality after 15 days.

#### Semi-field experiment

*Beauveria bassiana* isolates Pa4, Pa5, and Pa6 caused high mortality in *P. abietina* in semi-field experiments. Isolates Pa4 and Pa6 caused low mortality at the larval stage, whereas isolate Pa5 did not have an insecticidal effect at

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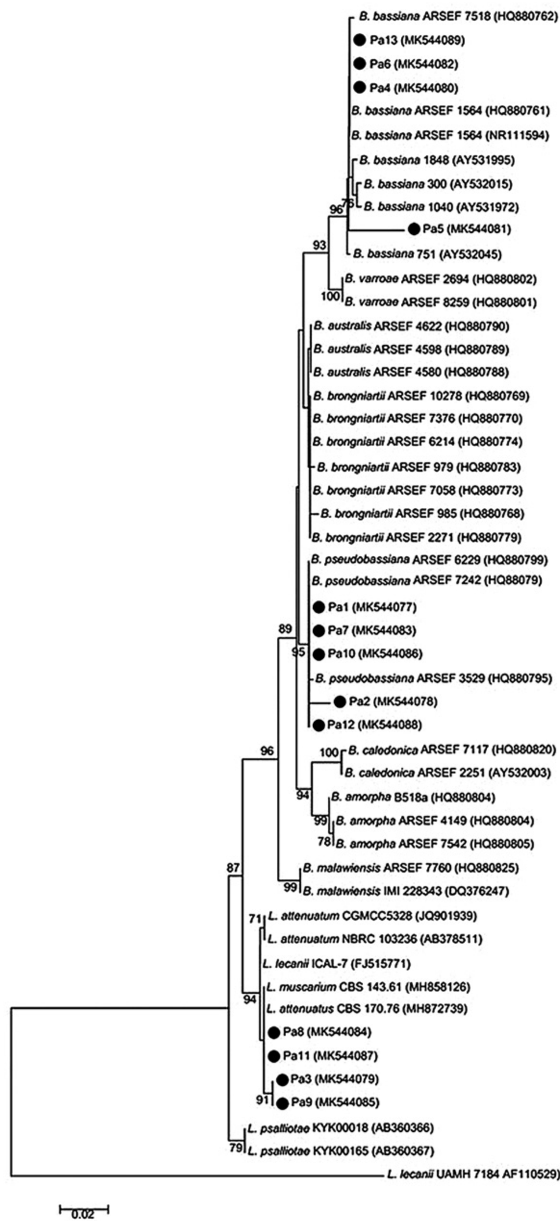


**Figure 1** Neighbor-joining tree of entomopathogenic fungi (*Beauveria* and *Lecanicillium* spp.) associated with *Pristiphora abietina* and closely related fungal species based on the sequence of the ITS gene. A ca. 580-bp sequence of the ITS1-5.8S-ITS2 gene region was used to construct the dendrogram. Fungal isolates from *P. abietina* larvae are indicated with a black dot (Pa1-Pa13). GenBank accession numbers are shown in parentheses. The numbers at the nodes are bootstrap percentages based on 1000 replicates; only nodes supported by the bootstrap values of 70% or over are shown. The scale bar represents the unit length of numbers of nucleotide substitutions per site.

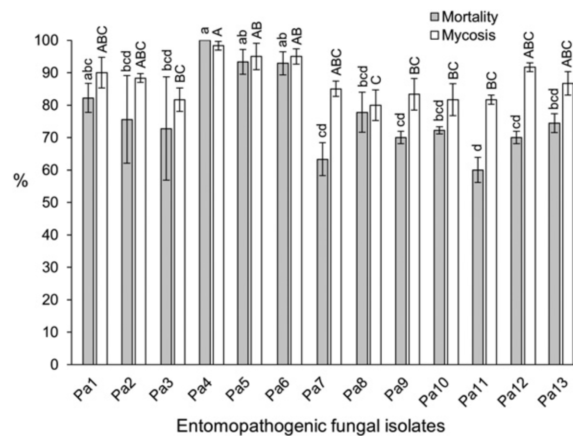


**Figure 2** Mean (+ SE; n = 270) mortality (%) of *Pristiphora abietina* larvae caused by the fungal isolates Pa1-Pa13 14 days after treatment and the percentage of mycosis of the dead larvae. Fungi were initially isolated from *P. abietina* larvae. Fungal isolates Pa4-6, and Pa13 were identified as *Beauveria bassiana*, Pa1, Pa2, Pa7, Pa10, and Pa12 were identified as *Beauveria pseudobassiana*, and Pa3, Pa8, Pa9, and Pa11 were identified as *Lecanicillium muscarium*. Means capped with different letters are significantly different (Duncan's multiple comparison test: $P<0.05$).

the larval stage. All three isolates caused 80-85% pupal mortality. Control larvae had a natural mortality rate of 5.5%; none of these insects displayed mycosis (Table 3). These results were consistent with the results of the laboratory experiments.

## Discussion

A great number of EPF have been isolated from various insect species and tested against important agricultural pests (Ansari et al., 2008; Sevim et al., 2010; Kocacevik et al., 2015; Sonmez et al., 2016, 2017; Yucel et al., 2018). However, investigations related to fungal control agents of *P. abietina* have been quite limited, especially field experiments. Considering the biology of *P. abietina* and the climatic conditions of the Eastern Black Sea Region, EPF were thought to be an effective microbial method to combat this pest in Turkey.

In previous studies, various fungal agents have been isolated from *P. abietina* and tested for their ability to control the pest. Schedl (1953) isolated several saprophytic fungi, and İskender et al. (2017) isolated 11 fungi belonging to different genera including *Beauveria*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, and *Trichoderma*. *Beauveria* spp. samples were pathogenic against the larvae of *P.*

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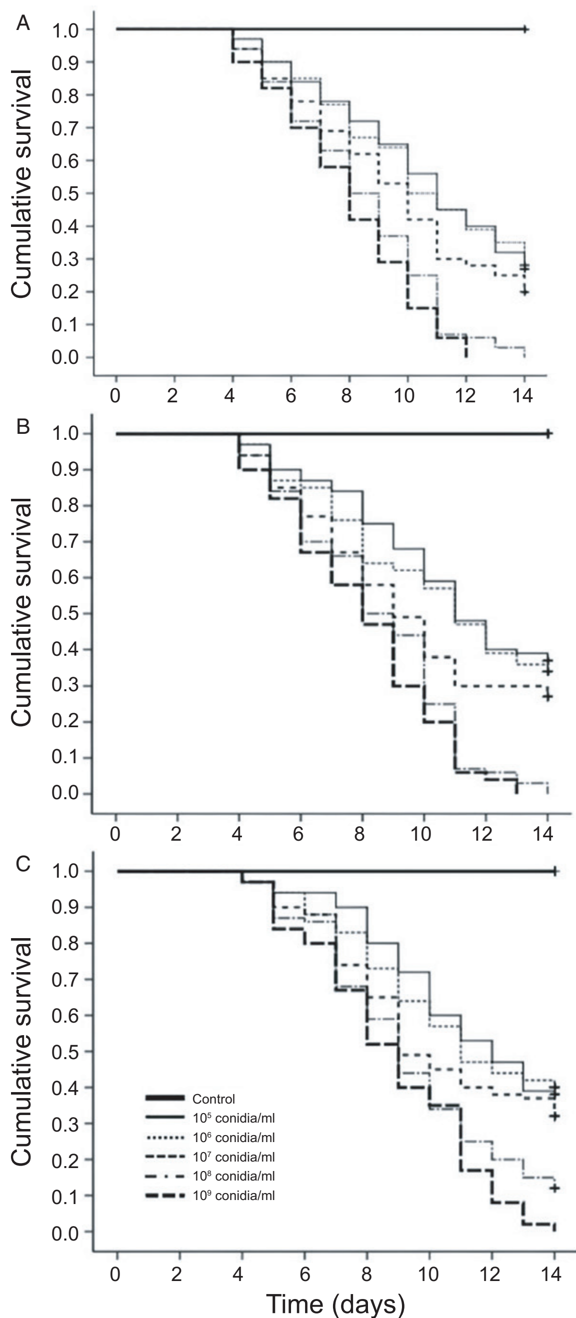


**Figure 3** Survival of *Pristiphora abietina* larvae treated with five doses of three isolates of *Beauveria bassiana*, (A) Pa4, (B) Pa5, and (C) Pa6, and a control treatment (sterile 0.01% Tween 80).

*abietina* at various humidity levels and doses of conidia under laboratory conditions, whereas the other samples were saprophytic. Therefore, these studies are not expected to encompass the complete EPF flora of *P. abietina*. In the current study, we isolated 13 EPF from the cadavers of naturally infected *P. abietina*, which belonged to two genera (*Beauveria* and *Lecanicillium*). Whereas five isolates were identified as *B. bassiana*, four isolates were identified as *B. pseudobassiana*, and four isolates were identified as *L. muscarium*.

All 13 isolates caused over 60% mortality of *P. abietina* larvae after 14 days at a dose of $10^6$ conidia $ml^{-1}$. *Beauveria bassiana* isolates Pa4, Pa5, and Pa6 caused over 90% mortality at this concentration, and mycosis rates confirmed that this was due to fungal infection. However, Pa13, another *B. bassiana* isolate, caused only 74% mortality. Similarly, Dhawan & Joshi (2017) tested four isolates of *B. bassiana* on cabbage butterfly, *Pieris brassicae* (L.), and showed that MTCC 6291 caused 46% mortality at a dose of $10^9$ conidia $ml^{-1}$ whereas MTCC 4495 caused 86% mortality at this concentration. Variation in mortality among isolates of *B. bassiana* can be correlated with differential expression of virulence-governing genes. *Beauveria pseudobassiana* isolated in this study caused 63–82% mortality 14 days after treatment, which was lower than *B. bassiana*. Sevim et al. (2013) found that *B. bassiana* KTU-24 caused 86% mortality on nymphs of *Corythucha ciliata* (Say), whereas *B. pseudobassiana* KTU-53 caused only 45% mortality. Romón et al. (2017) tested the infectivity of *B. bassiana* and *B. pseudobassiana* against the pine weevil *Pissodes nemorensis* Germar and found that *B. pseudobassiana* isolate UAMH301 had the lowest median lethal concentration ($1.18 \times 10^6$) whereas the highest $LC_{50}$ value ($2.8 \times 10^7$) was obtained with *B. bassiana* isolate LRC137. These results indicate that virulence of the EPF is not only related to genus, but also to the toxins and enzymes the fungi produce, as well as the susceptibility of the insect. *Lecanicillium muscarium* isolates (Pa3, Pa8, Pa9, and Pa11) had a relatively low insecticidal effect. Davari et al. (2015) tested the toxicity of *B. bassiana* and *L. muscarium* on German cockroach, *Blattella germanica* (L.), using a submersion method and the $LC_{50}$ for *B. bassiana* isolate PTCC5197 was 4.8 × lower than for *L. muscarium* PTCC 5184. Askary et al. (2014) tested the efficacy of spraying fungal entomopathogens on cotton whitefly, *Bemisia tabaci* (Gennadius), and found that *B. bassiana* isolate DEBI001 caused greater mortality than *L. muscarium* isolate DAOM 198499 after 5 days. In our study, *B. bassiana* isolates also caused higher mortality than other isolates.

A dose-response experiment of three *Beauveria* isolates (Pa4, Pa5, and Pa6) showed that mortality and mycosis ratio gradually increased when the concentrations of conidia increased. Isolates Pa4 and Pa5 caused 100% mortality at both $10^7$ and $10^8$ conidia $ml^{-1}$ 14 days after treatment. A similar study by Iskender et al. (2017) showed that two *B. bassiana* isolates caused over 90% mortality at $10^8$

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**Table 1** Lethal concentrations of three *Beauveria bassiana* isolates against *Pristiphora abietina* larvae

| Isolates | $LC_{50}$ | Slope ± SE | $LC_{95}$ | $\chi^2$ (d.f. = 3) | P |
|---|---|---|---|---|---|
| Pa4 | $1.7 \times 10^3$ | 0.06 ± 0.028 | $7.6 \times 10^6$ | 20.56 | <0.01 |
| Pa5 | $5.9 \times 10^3$ | 0.45 ± 0.057 | $2.7 \times 10^7$ | 18.24 | <0.01 |
| Pa6 | $8.2 \times 10^3$ | 0.28 ± 0.051 | $1.5 \times 10^8$ | 19.55 | <0.01 |

**Table 2** Horizontal transmission of fungal conidia of three isolates of *Beauveria bassiana* (Pa4, Pa5, and Pa6) from treated *Pristiphora abietina* larvae to fungus-free larvae at four treatment rates (%) on three time points (days) after treatment (DAT): mean (± SD) mortality (%)

| Time (DAT) | Treatment rate[1] (%) | Pa4 | Pa5 | Pa6 |
|---|---|---|---|---|
| 5 | 25 | 73.3 ± 4.72bA | 66.0 ± 2.03cA | 55.0 ± 4.58aB |
| | 50 | 82.0 ± 4.35bA | 69.0 ± 2.64bcB | 55.0 ± 2.00aC |
| | 75 | 94.0 ± 4.93aA | 75.0 ± 4.16abB | 52.0 ± 2.51aC |
| | 100 | 100.0aA | 77.0 ± 2.64aB | 50.0 ± 2.64aC |
| 10 | 25 | 91.0 ± 4.04bA | 75.0 ± 2.51dB | 63.0 ± 4.35cC |
| | 50 | 100.0aA | 86.0 ± 2.64cB | 75.0 ± 1.52bC |
| | 75 | 100.0aA | 91.0 ± 2.30bB | 77.0 ± 1.73bC |
| | 100 | 100.0aA | 100.0aA | 83.0 ± 2.30aC |
| 15 | 25 | 100.0aA | 88.0 ± 3.60bB | 77.0 ± 4.16bC |
| | 50 | 100.0aA | 94.0 ± 3.51abA | 83.0 ± 3.00abB |
| | 75 | 100.0aA | 97.0 ± 3.00aA | 86.0 ± 4.93abB |
| | 100 | 100.0aA | 100.0aA | 89.0 ± 3.51aB |

Means within a column and within a time point followed by different lowercase letters, and within a row followed by different uppercase letters, are significantly different (Duncan's multiple range test: P<0.05).

[1]Total no. of larvae was 40; 25% treatment involved 10 fungal-infested larvae and 30 non-infested larvae, 50% treatment involved 20 infested and 20 non-infested larvae, 75% treatment involved 30 infested and 10 non-infested larvae, and 100% treatment involved 40 infested larvae.

**Table 3** Mean (± SD) efficiency (%) of three *Beauveria bassiana* fungal isolates on *Pristiphora abietina* juvenile mortality and adult survival on spruce trees under semi-field conditions

| Isolates | Larval mortality | Pupae mortality | Adult survival |
|---|---|---|---|
| Pa4 | 12.04 ± 0.21a | 83.76 ± 5.58a | 4.2 ± 2.40a |
| Pa5 | 0.0c | 85.12 ± 1.41a | 14.87 ± 3.30b |
| Pa6 | 5.38 ± 2.11b | 80.23 ± 3.72a | 14.37 ± 1.06b |

Means within a column followed by different letters are significantly different (Duncan's multiple range test: P<0.05).

conidia $ml^{-1}$, suggesting that isolates Pa4 and Pa5 may be more effective against *P. abietina* than the isolates used in previous studies. Indeed, the $LC_{50}$ value was estimated as $1.7 \times 10^3$ conidia $ml^{-1}$ for isolate Pa4. In a previous study, the $LC_{50}$ of *B. bassiana* HpA-5 isolated from *Hypera postica* (Gyllenhal) was determined as $2.37 \times 10^4$ conidia $ml^{-1}$ against its host larvae (Yucel et al., 2018). In a study using *B. pseudobassiana* isolated from *Dendroctonus micans* Erichson against its host larvae, the $LC_{50}$ was $2.79 \times 10^5$ conidia $ml^{-1}$ (Kocacevik et al., 2015). Trudel et al. (2007) also calculated the $LC_{50}$ of two *B. bassiana* isolates as $5.87 \times 10^7$ and $7.36 \times 10^7$ conidia $ml^{-1}$ against *Pissodes strobi* Peck.

Several studies have been conducted on the horizontal transmission of conidia of EPF from treated to untreated insects (Arthurs et al., 2001; Scholte et al., 2004; Toledo et al., 2007; Kocacevik et al., 2015; Sonmez et al., 2017). A study of *Ceratitis capitata* (Wiedemann) adults showed that fungal conidia were horizontally transmitted among pest populations during mating (Quesada-Moraga et al., 2008). Kocacevik et al. (2016) reported that fungal spores were effectively transmitted between populations of the bark beetles *Ips sexdentatus* (Boerner) and *Ips typographus* (L.). Another study showed that *B. bassiana* conidia were horizontally transmitted from treated to untreated larvae in populations of *Thaumetopoea pityocampa* Schiffermüller (Sonmez et al., 2017). This study shows that fungal spores can also be transmitted horizontally, between *P. abietina* larvae. *Beauveria bassiana* Pa4 caused 73% mortality at the lowest larval treatment rate (25%) 5 days after

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application and all larvae died because of fungal infection 15 days after treatment. However, when 25% of the larvae were treated with isolate Pa6, 55% died by day 5 and 77% died 15 days after treatment. Even when 100% of the larvae were treated with isolate Pa6, only 89% mortality occurred, probably due to lower virulence than isolates Pa4 and Pa5. An increase in the number of larvae that was initially infested led to increased mortality on day 5 and higher mortality overall. These results indicated that spores of three *B. bassiana* isolates (Pa4, Pa5, and Pa6) could be horizontally transmitted to untreated *P. abietina* larvae in the population. The horizontal transmission rate of *B. bassiana* Pa4 was found to be higher than for the other two *B. bassiana* isolates.

The efficacy of three *B. bassiana* isolates (Pa4, Pa5, and Pa6) that showed promising results under laboratory conditions was tested under semi-field conditions against *P. abietina* larvae in spruce. Low mortality was observed at the larval stage, whereas mortality in response to all three isolates exceeded 80% at the pupal stage. Previous bioassays using *B. bassiana*, *Isaria farinosa* (Holmsk.) Fr., and *M. anisopliae*, as well as histological analyses, indicated that the fungal infection in larvae is due to spore contamination occurring before pupae formation and that penetration of fungi from outside the cocoon wall appears very unlikely (Führer et al., 2001). However, Ohnesorge (1961) reported that fungal diseases are secondary infections of weakened larvae because of limited nutrients and abiotic stress. None of the isolates in the current study had an insecticidal effect on adults. Whereas *B. bassiana* Pa5 and Pa6 caused 100% mortality at a concentration of $10^9$ spores $ml^{-1}$ under laboratory conditions, the same dose resulted in 85% mortality under semi-field conditions. This difference probably arose from the adverse effects of environmental factors such as relative humidity, UV rays, and temperature on fungal spores (Jaronski, 2010).

In conclusion, biodiversity and pathogenicity of EPF associated with *P. abietina* were determined in this study. One *B. bassiana* isolate, Pa4, was highly pathogenic and virulent on the pest under laboratory and semi-field conditions. Fungal spores could be horizontally transmitted in a *P. abietina* population. Based on the results of the current study, *B. bassiana* isolate Pa4 could prove to be a suitable microbial control agent that could be used as part of an IPM strategy of *P. abietina*. Future studies could investigate techniques for mass production of *B. bassiana* isolate Pa4 as a potential mycoinsecticide.

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## References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265–266.

Aksu Y & Göktürk CB (2008) *Picea orientalis* ormanlarda zarar yapan *Pristiphora abietina* (Christ) (Hymenoptera: Tenthredinidae) 'nin biyolojisi, morfolojisi ve mücadele üzerine yapılan araştırma. Orman Mühendisliği Dergisi 45: 35–39.

Ali-Shtayeh M, Mara'i ABB & Jamous RM (2002) Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. Mycopathologia 156: 235–244.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.

Anderbrant O (1993) Pheromone biology of sawflies. Sawfly Life History Adaptations to Woody Plants (ed. by MR Wagner & KF Raffa), pp. 119–154. Academic Press, London, UK.

Ansari MA, Brownbridge M, Shah FA & Butt TM (2008) Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis*, in plant-growing media. Entomologia Experimentalis et Applicata 127: 80–87.

Arthurs SP, Thomas MB & Lawton JL (2001) Seasonal patterns of persistence and infectivity of *Metarhizium anisopliae* var. *acridum* in grasshopper cadavers in the Sahel. Entomologia Experimentalis et Applicata 100: 69–76.

Askary H, Kohestani K, Zarabi M & Baghdadi A (2014) Pathogenicity of *Beauveria bassiana* and *Lecanicillium muscarium* on cotton whitefly (*Bemisia tabaci* (Homoptera: Aleyrodidae)) in simultaneously use. Biological Control of Pests and Plant Disease 3: 31–40.

Avery P, Queeley G, Faull J & Simmonds M (2010) Effect of photoperiod and host distribution on the horizontal transmission of *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) in greenhouse whitefly assessed using a novel model bioassay. Biocontrol Science and Technology 20: 1097–1111.

Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J & Sayers EW (2012) GenBank. Nucleic Acids Research 40: D48–D53.

Bischoff JF, Rehner SA & Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101: 512–530.

Boevé JL (2008) Sawflies (Hymenoptera: Tenthredinidae). Encyclopedia of Entomology (ed. by JL Capinera), pp. 315–322. Springer-Verlag, Heidelberg, Germany.

Brudea V & Pei G (2006) Bioecology and control researches concerning the little spruce sawfly *Pristiphora abietina* (Christ.) (Hymenoptera: Tenthredinidae). Analele Ştiinţifice ale Universităţii 'AL. I. CUZA' Iaşi, s. Biologie Animală 52: 131–136.

15707458, 2021, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/eea.13035 by Bilecik Seyh Edebali, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Davari B, Limoee M, Khodavaisy S, Zamini G & Izadi S (2015) Toxicity of entomopathogenic fungi, *Beauveria bassiana* and *Lecanicillium muscarium* against a field-collected strain of the German cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae). Tropical Biomedicine 32: 463–470.

Dhawan M & Joshi N (2017) Enzymatic comparison and mortality of *Beauveria bassiana* against cabbage caterpillar *Pieris brassicae* LINN. Brazilian Journal of Microbiology 48: 522–529.

Egger A (1989a) The effect and persistence of chemical and biological control measures against the spruce sawfly (*Pristiphora abietina*). Centralblatt für das Gesamte Forstwesen 106: 67–77.

Egger A (1989b) On the outbreak and aviochemic control of *Pristiphora abietina* (Christ) (Hymenoptera, Tenthredinidae) 1981–1987 in spruce forests in upper Austria. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz 62: 127–132.

Finney DJ (1971) Probit Analysis. Cambridge University Press, Cambridge, UK.

Führer E, Rosner S, Schmied A & Wegensteiner R (2001) Studies on the significance of pathogenic fungi in the population dynamics of the lesser spruce sawfly, *Pristiphora abietina* Christ (Hym., Tenthredinidae). Journal of Applied Entomology 125: 235–242.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Holuša J & Drápela K (2003) Integrated management of little spruce sawfly (*Pristiphora abietina*): design pattern. Proceedings: Ecology, Survey and Management of Forest Insects (ed. by ML McManus & AM Liebhold), pp. 16–24.USDA, Forest Service, Northeastern Research Station, Newtown Square, PA, USA.

Holuša J & Holuša O (2002) Monitoring of sawfly (Hymenoptera: Tenthredinidae) infestation on spruce. Journal of Forest Science 48: 219–224.

Humber RA (1997) Identification of entomopathogenic fungi. Manual of Techniques in Insect Pathology (ed. by LA Lacey), pp. 151–187.Academic Press, London, UK.

Iskender NA, Ortucu S, Aksu Y & Saral A (2017) Isolation, characterization and pathogenicity of fungi from *Pristiphora abietina* (Hymenoptera: Tenthredinidae). Fresenius Environmental Bulletin 26: 628–633.

Jaronski ST (2010) Ecological factors in the inundative use of fungal entomopathogens. BioControl 55: 159–185.

Kaplan EL & Meier P (1958) Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53: 457–481.

Kocacevik S, Sevim A, Eroglu M, Demirbag Z & Demir I (2015) Molecular characterization, virulence and horizontal transmission of *Beauveria pseudobassiana* from *Dendroctonus micans* (Kug.) (Coleoptera: Curculionidae). Journal of Applied Entomology 139: 381–389.

Kocacevik S, Sevim A, Eroglu M, Demirbag Z & Demir I (2016) Virulence and horizontal transmission of *Beauveria pseudobassiana* SA Rehner & Humber in *Ips sexdentatus* and *Ips typographus* (Coleoptera: Curculionidae). Turkish Journal of Agriculture and Forestry 40: 241–248.

Kolubajiv S (1958) On the bionomics, ecology and epidemiology of spruce sawflies of the group Nematini. Ceskoslovensku Akademie Zemedelskych Ved Sbornik 4: 123–150.

Lobinger G & Skatulla U (1989) Reaction of *Pristiphora abietina* (Christ) on a virus strain (CPV). Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz 62: 91–92.

Muirhead-Thomson, RC (1991) Trap responses of flying insects: The influence of trap design on capture efficiency, 1st edn. Academic Press, London, UK.

Ohnesorge B (1961) Untersuchungen über die Populationsdynamik der Kleinen Fichtenblattwespe, Pristiphora abietina (Christ) (Hym. Tenthr.). Zeitschrift für Angewandte Entomologie 49: 113–162.

Olenici N & Olenici V (2005) *Pristiphora abietina* (Christ) (Hymenoptera, Tenthredinidae) un daunator important al molidului din afara arealului natural de vegetatie. Revista Padurilor 120: 3–13.

Porcar M, Gómez F, Gruppe A, Gómez-Pajuelo A, Segura I & Schrödere R (2008) Hymenopteran specificity of *Bacillus thuringiensis* strain PS86Q3. Biological Control 45: 427–432.

Quesada-Moraga E, Martin-Carballo I, Garrido-Jurado I & Santiago-Alvarez C (2008) Horizontal transmission of *Metarhizium anisopliae* among laboratory populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Biological Control 47: 115–124.

Romón P, Hatting H, Goldarazena A & Iturrondobeitia JC (2017) Variation in virulence of *Beauveria bassiana* and *B. pseudobassiana* to the pine weevil *Pissodes nemorensis* in relation to mycelium characteristics and virulence genes. Fungal Biology 121: 189–197.

Saitou N & Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406–425.

Schedl K (1953) Die Kleine Fichtenblattwespe (*Lygaeonematus pini* Retz.). Forstlichen Bundesversuchsanstalt Mariabrunn, Vienna, Austria.

Schmied A & Führer E (1996) The importance of Ichneumonflies in damage areas of *Pristiphora abietina* Christ. Journal of Plant Diseases and Protection 103: 644–657.

Scholte EJ, Knols BGJ, Samson RA & Takken W (2004) Entomopathogenic fungi for mosquito control: a review. Journal of Insect Science 4: 19.

Sevim A, Demir I, Höfte M, Humber RA & Demirbag Z (2010) Isolation and characterization of entomopathogenic fungi from hazelnut-growing region of Turkey. BioControl 55: 279–297.

Sevim A, Demir I, Sonmez E, Kocacevik S & Demirbag Z (2013) Evaluation of entomopathogenic fungi against the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae). Turkish Journal of Agriculture and Forestry 37: 595–603.

Sonmez E, Demir I, Bull JC, Butt TM & Demirbag Z (2017) Pine processionary moth (*Thaumetopoea pityocampa*, Lepidoptera: Thaumetopoeidae) larvae are highly susceptible to the entomopathogenic fungi *Metarhizium brunneum* and *Beauveria bassiana*. Biocontrol Science and Technology 27: 1168–1179.

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Sonmez E, Sevim A, Demirbag Z & Demir I (2016) Isolation, characterization and virulence of entomopathogenic fungi from *Gryllotalpa gryllotalpa* (Orthoptera: Gryllotalpidae). Applied Entomology and Zoology 51: 213–223.

Svestka M & Holuša J (2000) Effect of aerial treatment of insecticides Dimilin 48 SC, Mimic 240 LV and Trebon 10 F on the little spruce sawfly (*Pristiphora abietina*) larvae. Zprávy Lesnického Výzkumu 45: 15–29.

Tamura K, Stecher G, Peterson D & Filipski A (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Taut I, Simonca V & Holonec L (2011) Detection and prognosis defoliators *Tortrix viridana* and *Pristiphora abietina* in the Northwestern Transylvania Forest. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Horticulture 68: 469–473.

Toledo J, Campos SE, Flores S, Liedo PF, Barrera J et al. (2007) Horizontal transmission of *Beauveria bassiana* in *Anastrepha ludens* (Diptera: Tephritidae) under laboratory and field cage conditions. Journal of Economic Entomology 100: 291–297.

Toledo J, Flores S, Campos S, Villaseñor A, Enkerlin W et al. (2017) Pathogenicity of three formulations of *Beauveria bassiana* and efficacy of autoinoculation devices and sterile fruit fly males for dissemination of conidia for the control of *Ceratitis capitata*. Entomologia Experimentalis et Applicata 164: 340–349.

Trudel R, Lavallée R, Guertin C, Côté C, Todorova SI et al. (2007) Potential of *Beauveria bassiana* (Hyphomycetes: Moniliales) for controlling the white pine weevil, *Pissodes strobi* (Col., Curculionidae). Journal of Applied Entomology 131: 90–97.

White TJ, Bruns T, Lee S & Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols, a Guide to Methods and Applications (ed. by MA Innis, DH Gelfand, JJ Sninsky & TJ White), pp. 315–322.Academic Press, London, UK.

Wiener L (1993) Forstschutz-Behandlungskonzept gegen die Kleine Fichtenblattwespe. Landesforstdirektion, Salzburg, Austria.

Yucel B, Gozuacik C, Gencer D, Demir I & Demirbag Z (2018) Determination of fungal pathogens of *Hypera postica* (Gyllenhall) (Coleoptera: Curculionidae): isolation, characterization, and susceptibility. Egyptian Journal of Biological Pest Control 28: 39

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Microscopy of fungi associated with the lesser spruce sawfly, *Pristiphora abietina*. Conidia of Pa4, Pa5, Pa6, Pa13 isolates (*Beauveria bassiana*) and Pa1, Pa2, Pa7, Pa10, Pa12 isolates (*Beauveria pseudobassiana*) were round in shape. Conidia of Pa3, Pa8, Pa9 and Pa11 (*Lecanicillium muscarium*) were cylindrical, ellipsoid in shape and relatively uniform in size.

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