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A biobased superabsorbent formulation for above-ground application of a new entomophthoralean fungus for biological psyllid pest control

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Abstract Entomophthoralean fungi have long been recognized as promising candidates for biological insect pest control. However, due to technical challenges, no preparation based on these fungi has been established for practical use so far. Low water availability is a key limiting factor of conidial discharge and germination. In the present study, sporulation of psyllid-pathogenic entomophthoralean fungus *Pandora cacopsyllae* Eilenberg, Keller and Humber (Entomophthorales Entomophthoraceae) was not observable under reduced water activity ($a_W \leq 0.97$). To support sporulation of encapsulated *P. cacopsyllae* hyphae from submerged culture under low humidity conditions in above-ground applications in field, we developed a novel paste-type formulation containing biobased superabsorbents, which retained water for a prolonged time period. In co-application with the superabsorbent formulation, the otherwise fast-drying capsules were kept sufficiently moist for sporulation for at least six days in laboratory trials at low humidity below 40%. Using the new formulation, we measured conidial discharge by *P. cacopsyllae* from the capsules under dry semi-field conditions in summertime by trapping conidia at a vertical distance of up to 40 cm from the sporulation source. By considering the cardinal directions, fewer conidia were discharged on the sun-facing side in the east than on the sun-averted side in the west. The developed formulation improved the sporulation efficacy significantly. Since water availability is a limiting factor for many fungal biocontrol agents, the developed formulation has the potential to also improve their efficacy.

Keywords Entomophthorales · Entomopathogenic fungi · Water activity · Sporulation · *Pandora* sp. nov. inedit. (ARSEF 13372) · *Pandora cacopsyllae* Eilenberg, (Entomophthorales Entomophthoraceae)

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Introduction

Entomophthoralean fungi have a high potential for insect pest control due to their high host specificity, their fast speed-to-kill and the ability to cause epizootics (Jaques and Patterson 1962; Pell et al. 2001; Keller 2007; Vega et al. 2009; Eilenberg et al. 2019). Despite their potential, no Entomophtorales-based biocontrol product has been established and commercialized for practical use so far.

The entomophthoralean fungus *Pandora cacopsyllae* Eilenberg, Keller and Humber (Entomophthorales: Entomophthoraceae) was isolated from an infected psyllid collected in a Danish pear orchard (Jensen et al. 2018) and was recently described as a new species (Eilenberg et al. 2023). Psyllids (Hemiptera: Psyllidae) are phloem-feeding insects and serve as vector insects of phytoplasmas, such as '*Candidatus* Phytoplasma pyri', which is vectored by the pear psyllid *C. pyri* and '*Candidatus* Phytoplasma mali' by the summer apple psyllid *Cacopsylla picta* Foerster 1848 Phytoplasmas cause high economic losses in European fruit production, and phytoplasma-infected plants cannot be cured. Preventive management strategies are restricted to vector control with chemical insecticides to minimize the spread of phytoplasma diseases (Jarausch et al. 2019).

In our previous studies, we documented the pathogenicity of *Pandora cacopsyllae* against several psyllid species: *Cacopsylla pyri*, *C. pyricola*, *C. picta*, *C. mali*, *C. pruni,* and *Trioza apicalis,* under laboratory conditions (Jensen et al. 2017; Herren 2018; Jensen et al. 2018; Görg et al. 2021). Subsequent research aimed to convert *P. cacopsyllae* into a biocontrol agent for application in biological psyllid pest control strategies (Muskat 2022). Therefore, a suitable fermentation medium and process with potential for mass production of *P. cacopsyllae* was established by Muskat et al. (2022a). Further, the transfer of *P. cacopsyllae* into an easily applicable form was realized by encapsulation of hyphal biomass in calcium alginate capsules, additionally providing nutrients for improved sporulation capacity. Ca-alginate capsules are biocompatible and biodegradable and have gained much attention in encapsulation of living cells, including entomopathogenic fungi (Nussinovitch 2010; Vemmer and Patel 2013). With this formulation, the two target psyllid species *C. picta* and *C. pyri* were successfully infected by *P. cacopsyllae* and showed a mortality of up to 89% (Muskat et al. 2022b). Nonetheless, experiments were carried out under optimal humidity conditions (> 97% RH) in the laboratory.

In biological control strategies in the field, efficient sporulation of the entomopathogenic fungus is required simultaneously with the target insect's abundancy. Moreover, very short viability and infectivity of the discharged conidia are suggested (Yendol 1968; Brobyn et al. 1985, 1987; Carruthers et al. 1988; Hajek et al. 1990; Uziel and Kenneth 1991; Griggs et al. 1999). Previous studies have shown that water availability is a main limiting factor of conidial discharge and germination of entomophthoralean species, and inadequate water availability under field conditions is one of the main reasons for the lack of success in field trials (Hall and Papierok 1982; Hajek et al. 1990; Delalibera et al. 2006).

An important parameter for water availability is the water activity value (a_W), which can be defined as the biologically available water and, thus, water available to microorganisms to grow. To be more precise, the definition of a_W is $a_W = p_{sample}/p_{water}$, where p_{sample} is the partial vapor pressure in equilibrium with the tested material or solution and p_{water} is the partial vapor pressure of pure water at the same temperature. The relative humidity of air in equilibrium with a material or solution is also called the Equilibrium Relative Humidity (ERH) expressed in %. It is equal to water activity according to $ERH = a_W \times 100\%$.

For some species, sporulation and conidia germination only occurs in presence of free water, which corresponds to an a_W value of 1.0 or a RH of 100%, e.g., in *Entomophthora delphacis* Hori (Entomophthorales: Entomophthoraceae) or *Entomophaga maimaiga* Humber, Shimazu and R.S. Soper (Entomophthorales: Entomophthoraceae) (Shimazu 1977; Hajek et al. 1990) or at least under between 90 and 100% RH, as described for *Zoophthora phalloides* Batko (Entomophthorales: Entomophthoraceae) or *Entomophthora gammae* (Weiser) D.M. MacLeod and Müller-Kögler (Entomophthorales: Entomophthoraceae) (Newman and Carner 1975; Glare et al. 1986). This can be explained by the active mode of conidial discharge of *Pandora* spp. and other Entomophthorales. Page and Humber (1973) discussed that medium osmotic pressure of the environment directly affects the conidiophore's and the spore's turgor pressure, which is needed, as described

for the case of *Conidiobolus coronatus* and other fungi, for spore discharge and germination (Inglis et al. 2001; Webster and Weber 2007). Thus, in some species from the genera *Conidiobolus*, *Zoophthora* and *Pandora*, free water is needed for the generation of a high turgor pressure in the conidiophores, which is required for the ballistic spread of the conidia (Latgé et al. 1989).

Few semi-field and field attempts have been made addressing the humidity challenges of the Entomophthorales. Pell et al. (1993) designed a trap for co-application of *Zoophthora radicans* Brefeld (Entomophthorales: Entomophthoraceae) with attracting semiochemicals in order to develop an attract-and-kill strategy for the control of the diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). Sufficient moisture remained high within the trap in order to enable sporulation of the fungus by a wick connected to a water reservoir placed in the central arena of the trap. Another more practical option for technical application is the use of formulated entomopathogenic fungi: Zhou and Feng (2009) developed a granular broomcorn millet formulation supplemented with synthetic polyacrylate superabsorbent polymers for improved sporulation capacity of *Pandora nouryi* (Remaud. and Hennebert) Humber (Entomophthorales: Entomophthoraceae) under non-saturated humidity conditions. Unfortunately, solid-state fermentation was required and polyacrylic derivatives of synthetic origin were used, which are manufactured in a toxic preparation process. As this is hardly biodegradable and hence not an environmentally friendly solution, it would not be approved for organic agriculture, for example. However, efficient sporulation is obligatory for successful application of an entomophthoralean fungus in pest control strategies. Therefore, the overall aim of this study was to develop and evaluate a biobased and biodegradable formulation that compensates for the specific moisture requirements of the encapsulated entomopathogenic fungus *P. cacopsyllae* to support sporulation under low humidity conditions, such as those faced in above-ground applications. The specific objectives of this study were (1) to identify the water activity values necessary for growth and sporulation of *P. cacopsyllae*, (2) to demonstrate a formulation that maintains a sufficiently high water activity under low humidity conditions that allows for sporulation of *P. cacopsyllae*, and (3) to validate the potential of the formulation for improved sporulation under semi-field conditions.

Materials and methods

Chemicals

Chemicals were acquired either from Carl Roth GmbH (Karlsruhe, Germany) or VWR International GmbH (Darmstadt, Germany). Carboxymethylcellulose was purchased from Dow (Dow Chemical Company, Midland, MI, USA) and Xanthan gum from DuPont (DuPont de Nemours GmbH, Neu-Isenburg, Germany). Concentrations are given as (w/w), unless otherwise stated.

Fungal isolate

The *Pandora cacopsyllae* isolate originated from an infected *Cacopsylla pyri* specimen collected in a Danish pear orchard (55°50′24.3″N, 12°33′46.5″E) and was named KVL 16–44. The strain is deposited in the USDA Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (Ithaca, NY, USA) as ARSEF 13372. As the fungus was recently described, former studies referred to the fungus as *Pandora* sp. nov. inedit. (ARSEF 13372).

Cultivation of *Pandora cacopsyllae*

P. cacopsyllae was grown on solid medium adapted from Hajek et al. (2012), composed of 4.0% glucose, 2.0% casein, and 2.0% agar [Sabouraud Dextrose Agar (SDA)], supplemented with 20% of a mixture of 60% egg yolk and 40% fresh skimmed milk (SDAME) on Petri dishes (Ø = 90 mm), sealed with Parafilm®, and incubated at 18 °C in the dark. To prevent loss of virulence, the fungus was reisolated after infecting the host insect *C. pyri* frequently, at least after every fourth subculture.

Submerged cultures of *P. cacopsyllae* were grown in liquid medium following the method described by Muskat et al. (2022a). Briefly, the pre-culture was composed of fresh skimmed milk (1.5% fat content; 10%; w/w), in which the fungus grew for 48 h. The main culture was inoculated with 10% (v/v) of the pre-culture and was composed of glucose (2.66%), sodium chloride (NaCl; 1%), yeast extract (0.33%),

skimmed milk powder (Heirler Cenovis GmbH, Radolfzell, Germany; 0.33%) and a low-cost protein hydrolysate from animal by-products (ANiPept, ANiMOX GmbH, Berlin, Germany, batch No. 1176; 0.33%), and was grown for a further 48 h under the same conditions as the pre-culture. All media components were dissolved in ultrapure water and separately autoclaved for 6 min at 121 °C and 2 bar.

Preparation of capsules

All experimental steps were carried out under sterile conditions, and all solutions and components were autoclaved for 6 min at 121 °C and 2 bar, unless otherwise stated. Composition and preparation of capsules were adapted from Muskat et al. (2022b). Briefly, finely dispersed hyphae of *P. cacopsyllae* were collected from liquid culture by centrifugation (4700 g; 15 min; 18 °C). Sodium alginate (Manugel GMB, FMC Corporation, PA, USA, batch No. G7708901) was dissolved in ultrapure water to a final concentration of 3% and autoclaved for 6 min at 121 °C. The encapsulation suspension was composed of sodium alginate, heat-sterilized native corn starch (Maisita, Agrana Beteiligungs-AG, Vienna, Austria; 10%), pre-dissolved skimmed milk powder (Heirler Cenovis GmbH, Radolfzell, Germany; 4%) and hyphal material of *P. cacopsyllae* (10%).

For capsule formation, the solution was dripped into a stirred (250 rpm) calcium chloride solution (0.1 M) by using a syringe with a cannula (2.1 × 0.8 mm). Capsules were kept in the solution under stirring for 20 min and were subsequently washed with ultrapure water for 1 min. The mean size of the capsules was 2.84 mm (± 0.08; n = 15).

Preparation of the water-retaining paste formulation

Candelilla wax (CLW; Kahlwax GmbH & Co. KG, Trittau, Germany; batch code: 10–1185; 1.5% w/w) was mixed with sesame oil (Rapunzel Naturkost GmbH, Memmingen, Germany) and maintained under stirring at 100 °C for 20 min for sterilization. Carboxymethylcellulose (WALOCEL CRT 60000 GA 07, Dow Chemicals, Batch code: F294H89011, MW: 60000; CMC) was heat-sterilized at 100 °C overnight in an oven. CMC was added to the hot wax-oil mixture at a ratio of 1:1 and dispersed by a spatula while cooling the mixture to room temperature for solidification. Xanthan gum (GRINDSTED® Xanthan 80, A45100, DuPont, Neu-Isenburg, Germany; batch code: 4453438167; 2%; w/w) was dispersed in ultrapure water and autoclaved at 121 °C and 2 bar for 21 min. About 0.5 g of the oil-wax-CMC mixture was spread over the bottom of a Petri dish (Ø = 35 mm) by aid of a spatula and overlayered by 7.5 g of the xanthan gum gel. The Petri dishes were closed with the Petri dish lid, sealed with Parafilm®, maintained at 5 °C for at least 24 h, and stored there until use.

Effect of water activity on mycelial growth and sporulation of *Pandora cacopsyllae*

A basic solid culture medium, composed of 4.0% glucose, 2.0% casein and 2.0% agar (SDA) supplemented with 10% fresh skimmed milk (SDAM) and water agar (2%; w/w) was modified by addition of glycerol following the method of Hallsworth and Magan (1999). Glycerol was selected as a_W modifier, as it is known for its a_W stability at different temperatures (Hallsworth and Magan 1999). For media preparation, 0%/10%/20%/30% (w/w) of the water content of the medium was replaced by glycerol. The water activity (a_W) of the modified media was determined using a water activity meter (LabMASTER-aw, Novasina AG, Lachen, Switzerland) at 25 °C.

The a_W value of the medium or the water agar was 0.98/0.99 (0%), 0.96/0.97 (10%), 0.93/0.95 (20%), 0.89/0.91 (30%) (medium/water agar). Pieces of agar with mycelium (0.5 cm^2 surface) were placed in the middle of the culture medium (SDAM) and radial mycelium growth was determined daily in two perpendicular directions.

For determination of the water activity effect on sporulation, one capsule per dish, prepared as described above, was placed in the center of glycerol-modified water-agar plates. The discharged conidia were collected according to the method described in Muskat et al. (2022b). Briefly, the plates with the capsules were inverted and placed above a smaller Petri dish (Ø = 35 mm) filled with 3 ml SDS (0.5%) and incubated at 18 °C in the darkness for 12 days, as sporulation of *P. cacopsyllae* is then almost complete (Muskat et al. 2022b). Conidia were counted in a Fuchs–Rosenthal hemocytometer with six repetitions per sample.

Sporulation experiments under low humidity conditions in the laboratory

One *Pandora cacopsyllae* capsule per dish was placed on top of the paste formulation filled in Petri dishes on top of the paste formulation. Capsules without the paste were fixed with a drop of pure xanthan gel (2%) on the Petri dish bottom. The Petri dishes were fixed upside down at a height of 3 cm above Petri dishes (Ø = 35 mm) filled with SDS solution (0.5%) for collection of conidia in a desiccator with the lid opened throughout the experiment to maintain low humidity conditions (Fig. 1). The formulations were incubated at < 40% room humidity (Fig. 1b) and 21–24 °C for 12 days. Petri dishes containing the formulations and placed in the same experimental set-up in a desiccator with the bottom filled with water and the lid closed to maintain saturated humidity conditions (100% RH) within (Fig. 1a) served as a control (data not shown). Room humidity and temperature were recorded with a datalogger (EBI 20-TH 1, Ebro Electronic™, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany) throughout the experiment. The datalogger was placed next to SDS-filled Petri dishes. The SDS was replaced every 24 h. The collected conidia were counted in a Fuchs-Rosenthal hemocytometer with six repetitions per sample.

Semi-field sporulation trial considering cardinal directions

Net cages, 175 × 175 × 175 cm each (Aerarium Nets, Bern, Switzerland) with sun and rain shelters were built on an experimental field at the Julius Kühn-Institut (Dossenheim, Germany) (Supplementary figure S1a). One potted pear tree (*Pyrus communis* L. cv. 'Williams Christ' grown on cv. *P. communis* 'Kirchensaller Mostbirne' rootstock) was placed in each net cage (Supplementary figure S1b). Before starting the experiment, the capsules were pre-incubated on the water-retaining paste formulation in Petri dishes (Ø = 35 mm) for three days. The dishes containing the paste formulation and 20 pre-incubated capsules were fixed to the top of the potted pear tree with the dish opening facing downwards (Supplementary figure S1c). Four Petri dishes were fixed to each tree, considering in each case the different cardinal directions. Sporulation was determined by collection of conidia on glass slides (Thermo Scientific, Braunschweig, Germany) fixed at regular intervals of 5, 20 and 40 cm of vertical distance from the sporulation source. The experiment was conducted from 14 to 17 September 2020. Humidity and temperature were recorded with data loggers (DS1923-F5, Hygrochron Temp/Luftfeuchte, Elektronik Fuchs, Weingarten, Germany) throughout the experiment. After 72 h, the glass slides were collected. The conidia attached to the slides were scanned and counted with a digital microscope (VHX 7000, Keyence ® Deutschland, Neu-Isenburg, Germany).

Statistical analysis

Statistical analysis of mycelial growth and sporulation of *P. cacopsyllae* was carried out using the software SPSS Statistics V25.0 (SPSS, Chicago, IL, USA). All data are given as mean values ± SD. Data for conidial discharge were checked for normality and homogeneity of variance using Shapiro–Wilk and Levene tests. Mean numbers of discharged conidia were tested for significant differences by one-way ANOVA followed by Bonferroni post-hoc tests. If the criteria for variance homogeneity and normal distribution were not met, data were calculated by Kruskal–Wallis tests

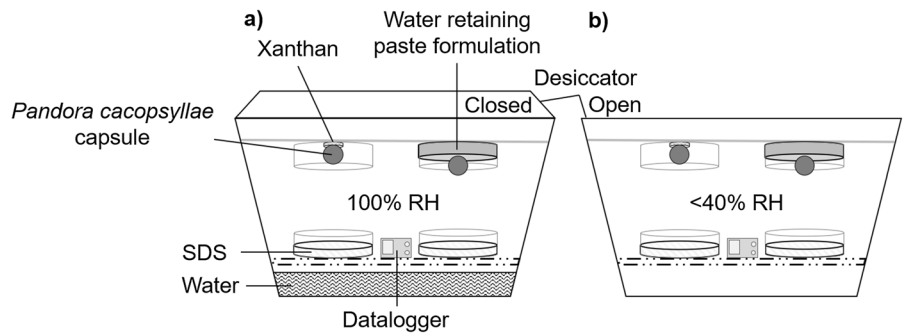

Fig. 1 Experimental set-up of the sporulation experiment. The formulations were incubated in a closed desiccator for maintenance of high humidity conditions (**a**) or in an exsiccator with the lid opened to maintain low humidity conditions of < 40% (**b**)

followed by Bonferroni tests for multiple comparisons with one treatment. The number of discharged conidia over time was compared with repeated-measures ANOVA (RM-ANOVA), with time and treatment as independent variables. The sphericity of the matrix assumption was assessed with the Mauchly sphericity test. If the outcome of the test was significant, the Greenhouse–Geisser adjustment was used to correct for violations of sphericity. As the criteria for variance homogeny were not met, a Games-Howell post-hoc test was used to determine the effect of time and treatment on conidial discharge. The effect of the distance from the sporulation source and the sun -exposure on the sporulation in the semi-field trial was analyzed by a one-way ANOVA followed by a Tukey post-hoc test. The level of significance was set to $p < 0.05$. All experiments were carried out with at least five repetitions, unless otherwise stated.

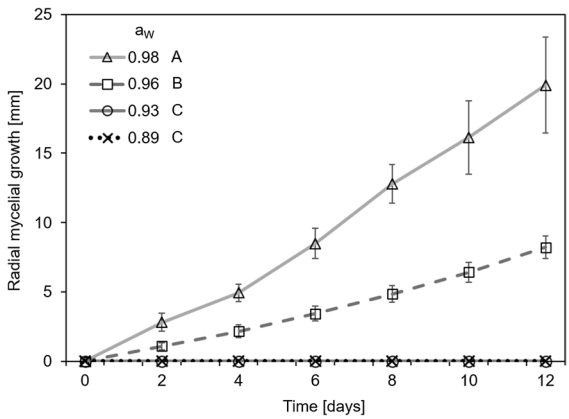

Fig. 2 Effect of water activity (a_W) on radial mycelial growth of *Pandora cacopsyllae*. Different letters in the legend indicate significant differences according to RM-ANOVA followed by post-hoc tests ($p < 0.05$). Means ± SD, n = 5

Results

Effect of water activity on mycelial growth and sporulation of *Pandora cacopsyllae*

Water activity of the medium had a significant effect on the growth speed of *P. cacopsyllae* over time ($F_{5,15} = 303.13$; $p < 0.001$), as shown in Fig. 2. Water activity itself had a significant effect on mycelial growth ($F_{3,76} = 933.05$; $p < 0.001$). Mycelial growth was slower at a_W 0.96 compared to a_W 0.98, and no more observable at $a_W \leq 0.93$.

As shown in Fig. 3a, sporulation from capsules was also significantly affected by water activity ($\chi^2 = 102.79$; df = 3; $p < 0.05$). At a_W 0.99, 4.36×10^6 ($\pm 9.35 \times 10^5$; n = 5) conidia per capsule were discharged, and thereby significantly more than in all other water activity regimes during the 12 days of experiment duration. Under these conditions, growth and sporulation of *P. cacopsyllae* from the capsules was clearly visible (Fig. 3b). At a_W 0.97, dense mycelial growth from the capsules was observable (Fig. 3c), while only 1.25×10^4 ($\pm 2.4 \times 10^4$; n = 5)

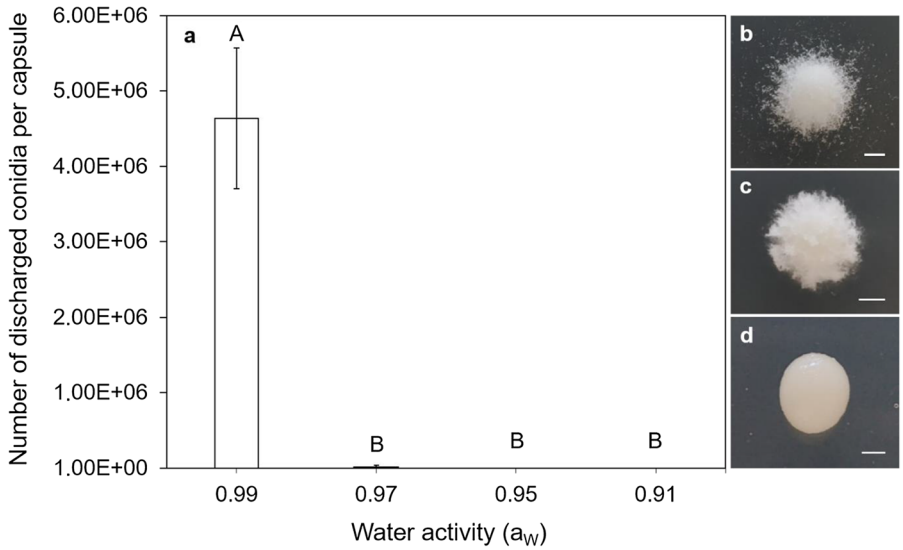

Fig. 3 Effect of water activity on total conidia numbers released by encapsulated *Pandora cacopsyllae* (**a**). Different letters above bars indicate significant differences according to Kruskall-Wallis test followed by Bonferroni post-hoc tests at $p < 0.05$. Means ± SD, n = 5. Scale bar = 1 mm. Pictures of the capsules after four days of incubation at a_W 0.99 (**b**), a_W 0.97 (**c**) and $a_W \leq 0.95$ (**d**)

conidia per capsule were discharged. At $a_W \leq 0.95$, neither mycelial growth (Fig. 3d) nor conidial discharge (Fig. 3a) was noticeable.

Sporulation of *Pandora cacopsyllae* under low humidity conditions in a laboratory experiment

In the laboratory experiment, the mean temperature during the experiment was 21.6 °C (min 21.2 °C; max 24.6 °C). The mean humidity set for low and 'non-saturated' conditions was 39.3% (min 31.0%; max 49.1%) and constant 100% for the control (saturated conditions). Compared to raw capsules, which dried under the same experimental conditions, encapsulated *P. cacopsyllae* was able to discharge conidia for seven days at < 40% RH when co-applied with the paste formulation ($F_{1,58} = 734.95$; $p < 0.001$; Fig. 4).

A peak sporulation event was observable on day 4 with 6.96×10^5 ($\pm 3.08 \times 10^5$) conidia per capsule. The total number of conidia released from the capsules during the seven days of sporulation was 1.79×10^7 ($\pm 3.21 \times 10^6$) conidia per capsule.

Sporulation under semi-field conditions

In the semi-field trial, the mean temperature during the experiment was 19.5 °C (min 11 °C; max 28 °C) and mean humidity was 64% (min 32%; max 100%). Formulated *P. cacopsyllae* sporulated under these application conditions and conidia were observable on all glass slides fixed at different vertical distances below the sporulation source. At the lowest distance of 5 cm, 2730 (±566) conidia per cm^2, at 20 cm distance 362 (±85) conidia per cm^2, and at 40 cm distance 90 (±16) conidia per cm^2 were counted after the 72 h of experiment duration (Fig. 5). The number of conidia was significantly different between the lowest distance of 5 cm when compared with 20 and 40 cm ($F_{2,9} = 4.54$; $p < 0.05$), but not between 20 and 40 cm ($F_{1,6} = 3.74$; $p = 0.10$).

When comparing sporulation rates at the closest distance of 5 cm from the sporulation source, considering cardinal directions, fewer conidia were found on the sun-facing side in the east than on the sun-averted side in the west, but this difference was not significant ($F_{1,2} = 4.53$; $p = 0.06$ Fig. 5).

Discussion

To date, the effect of reduced water activity on mycelial growth on solid media or on sporulation from Ca-alginate capsules has not been reported for any entomophthoralean fungus. In the present study, we found that mycelial growth by *P. cacopsyllae* was possible under reduced a_W of 0.97 from capsules, but not observable at a_W 0.94. Similarly, Glare et al. (1986) found that *Z. phalloides* was able to grow from *Myzus persicae* cadavers at 98% RH, but not at 94%, and sporulation was only possible at saturated humidity conditions (100% RH). Hence, there is a difference in humidity or water activity requirement for entomophthoralean mycelial growth and the ability to sporulate, which might be connected to the turgor built-up for the ballistic spread of the conidia (Latgé et al. 1989).

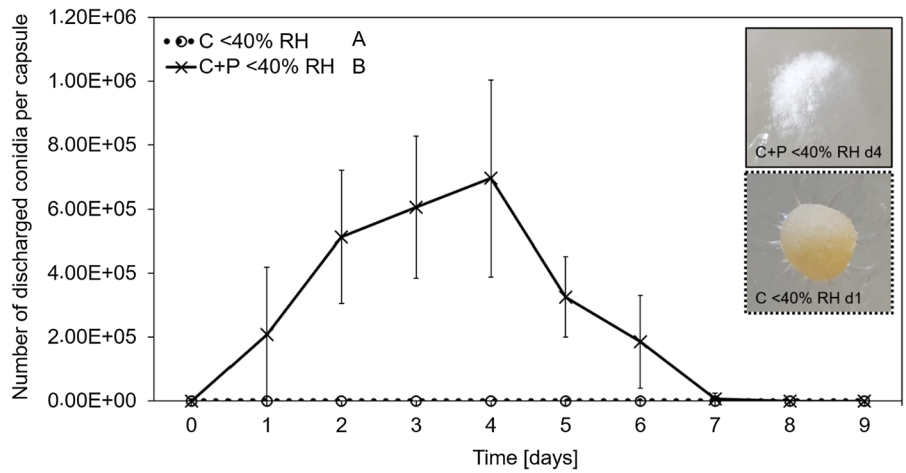

Fig. 4 Sporulation of *Pandora cacopsyllae* from capsules (C) and from capsules co-applied with a biobased superabsorbent past-type formulation (C + P) under low humidity conditions (< 40% RH) within nine days. Different letters in the legend indicate significant differences according to RM-ANOVA and Games-Howell post-hoc tests at $p < 0.01$. Means ± SD, n = 5

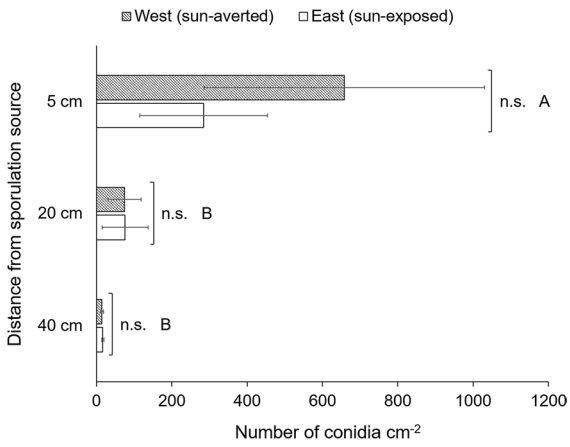

Fig. 5 Sporulation of *Pandora cacopsyllae* in a semi-field trial. The semi-field trial was carried out twice in two tents. The Petri dishes containing the paste formulation and the *Pandora cacopsyllae* capsules were fixed to the top of potted pear trees with the dish opening facing downward, considering the sun-exposed (East) and the sun-averted (West) side. Glass slides were placed below the sporulation source at 5, 20 and 40 cm vertical distance to the sporulation source for conidia collection. Different letters indicate significant differences between the condia numbers at different distances from the sporulation source according to one way-ANOVA followed by Tukey post-hoc tests ($p < 0.05$). No significant difference was found when comparing the effect of sun-exposure for each distance (n.s. = not significant; $p < 0.05$). Means ± SD, n = 2

Pandora cacopsyllae sporulation was only observable when a_W was 0.99. This is in line with several other studies on entomophthoralean sporulation from infected cadavers, which have reported that sporulation was associated with high humidity conditions and often occurred during night time when RH and leaf wetness was high enough (Milner and Bourne, 1983; Yu et al. 1995; Hemmati et al. 2001a, b; 2002; Nielsen and Hajek 2006). Most tested entomophthoralean species have shown conidial discharge from infected cadavers or mycelial mats only when RH was higher than 90% (Glare et al. 1986). The individual humidity needed for growth and sporulation depends on the entomophthoralean genus and species: sporulation by *Entomophthora aphidis* [= *Pandora neoaphidis* (Remaud. and Hennebert) Humber (Entomophthorales: Entomophthoraceae)] and *E. thaxteriana* [= *Conidiobolus obscurus* (I.M. Hall and P.H. Dunn) Remaudiere and S. Keller (Entomophthorales: Entomophthoraceae)] was only possible at > 90% RH (Wilding 1969), by *Neozygites tanajoae* Delalibera Jr, Humber and Hajek (Entomophthorales: Entomophthoraceae) at > 96% RH (Delalibera Jr al., 2006), and for *Erynia* sp. (Entomophthorales: Entomophthoraceae) at > 91% RH (Millstein et al. 1982). Only a few Entomophthorales were found to be capable of sporulating at humidities below 90%, such as *Entomophaga maimaiga* (Hajek et al. 1990) and *E. muscae* (Cohn) Fresenius (Entomophthorales: Entomophthoraceae) (Kramer 1980).

In any case, persistence and sustainability of sporulation are required to consistently deliver viable conidia in the presence of the target insect, and this requires consistent water supply. One option to provide water for a prolonged period is the use of superabsorbents. Superabsorbents are polymers or composites that can absorb and retain extremely large amounts of a liquid relative to its own mass (Horie et al. 2004). A more environmentally friendly alternative to classical non-biodegradable polyacrylic-based superabsorbents are biobased polymers with a high water absorption capacity (Chen et al. 2022). Derivatives of starch or cellulose have gained attention for different agricultural applications, especially for improved water capacity of soil (Demitri et al. 2013). The paste-type formulation tested in the present study contains cellulose and xanthan as polymers of high water absorption capacity, which are known to be biodegradable. Moreover, these biopolymers are registered for biopesticide applications even in organic agriculture (Speiser et al. 2022). The biopolymer-based paste formulation provides a water activity of 0.99–1.0 to the co-applied capsules, which was found to be sufficiently high for sporulation of *P. cacopsyllae*. By co-application, sporulation under low humidity conditions was enabled for at least six days. The low RH values of < 40% were selected as the test conditions for practical reasons, as it is the present room humidity in our laboratories and does not need additional equipment or modification by salt solutions. Additionally, as recorded in the semi-field trial, 32% RH was also present in the field and, thus, < 40% are humidity conditions that might be overcome by the water retaining paste formulation.

The total number of discharged conidia under humidity conditions below 40% RH (1.79×10^7) during the seven days of conidial discharge was as high as under saturated conditions, where 9.57×10^6 ($\pm 8.34 \times 10^5$) conidia per capsule were reported during the 12 days of conidia release by the same fungus from capsules of the same composition (Muskat

et al. 2022). Thus, the paste formulation is capable of compensating for unsuitable environmental humidity conditions by providing a satisfactory water activity to the encapsulated fungus and thereby enabling the release of conidia at an amount similar to optimal room humidity conditions. However, the sporulation duration under low humidity conditions was shorter compared to the previous experiment, possibly due to the drying of the paste formulation. Experiments were carried out at a constant humidity regime of < 40% RH, while conditions in the field and alternating humidity conditions in the place of application will ensure that the formulation will be rewetted for a prolonged period.

In addition to alternating moisture conditions, direct sunlight and elevated temperatures also need to be considered when determining the success rate of sporulation (Kalsbeek et al. 2001). The effect of temperature on sporulation of *Pandora cacopsyllae* was investigated by Olsen et al. (2019) who found that the higher the temperature, the shorter the duration of conidial discharge. This supports the assumption that the lower amount of discharged conidia in the present semi-field trial was due to the increased temperature in the field. Nevertheless, sporulation occurs, which is an essential information when the fungus is developed for biological control.

Another aspect is that the exposure to direct sunlight, high temperatures, and drying environmental conditions are known to cause mortality of the fungus and the discharged conidia of the Entomophthorales (Yendol 1968; Roberts and Campbell 1977; Brobyn et al. 1985; Carruthers and Haynes 1986). Carruthers et al. (1988) showed defined interactions between the duration and intensity of exposure to simulated and solar radiation in the field on *Entomophaga grylli* conidia viability. They developed a model based on combining these two factors in a single predictive variable. After exposure to cumulative solar radiation given in Langley they predicted > 95% mortality in the most open habitats in Alpine Arizona, USA after exposure to approx. 50 Langleys (≈ 0.58 kWh). As a sun shelter was used in the present study, the solar radiation should have been lower compared to open habitats in Arizona, but obvious differences were noted in the total amount of conidia discharged on the different cardinal directions and indicate a negative effect on the encapsulated fungus by the direct and cumulative solar radiation on the eastern side. Besides using these data as a base to determine abiotic factors affecting the amount of discharged conidia as well as survival of the fungus, the subsequent epizootic development in the field is especially dependent upon successful contact of the conidia with the target insect. However, it needs to be investigated whether the released conidia, once they have landed on the cuticle of the target insect, would germinate and cause infection of the insect under low humidity conditions, as this also requires high humidity conditions. With the present study, a formulation is presented that allows for sporulation under dry environmental conditions. This solved the first problematic step of conidia discharge under non-optimal humidity conditions, but there is no guarantee of activity of the fungus under these conditions. This is an important issue due to the very short viability and infectivity of the discharged conidia and requires further investigation. Studies on the distance of conidia discharged are rare: Six and Mullens (1997) found that conidia of *Entomophthora musca* and *E. schizophorae* from *Musca domestica* cadavers are only discharged over a distance of 0.00–8.75 cm in a chamber under still air. Carruthers (1982) found a wider range of up to 34 cm when fly cadavers were fixed at a height of 50 cm in the field. The observations of Carruthers (1982) are similar to the present study, as conidia were found even at a vertical distance of 40 cm from the sporulation source. In view of the planned application of the fungus *P. cacopsyllae* in psyllid pest control strategies in pear, apple, and other fruit trees in Central Europe, the results of our studies are promising. In commercial apple and pear plantations, the trees that need to be protected are of a mean height of 2–4 m. Thus, when the fungus is able to release its conidia over a distance of at least 40 cm, the application of only a few *P. cacopsyllae* capsule/paste formulations per tree could be sufficient to cause infections in target insects, but more field trials are needed to prove this. In nature, even very short sporulation durations from cadavers of less than three days were sufficient for other entomophthoralean fungi to cause epizootics in insect populations (Aoki 1981; Kalsbeek et al. 2001; Wraight et al. 2003; Li et al. 2006). Hence, the sporulation duration of six days we observed for *P. cacopsyllae*

even under constantly low humidity conditions could be effective in initiation of an epizootic.

Due to the exposed position of killed hosts, the actively discharged conidia of entomophthoralean fungi can spread over a large area. The airborne conidia can be transported over long distances by wind (Weseloh and Andreadis 1992; Dwyer et al. 1998; Hemmati et al. 2001a, b; Keller 2007). In addition to the humid microenvironment, the paste also has the benefit of a high adhesive performance and thus allows for overhead application. Thereby the formulation enabled fixation of the sporulation source at a high and exposed place in the plant in order to simulate the natural death orientation, providing for a sporulation of the fungus over a wide area by simulating the naturally occurring situation. To improve the effectiveness of the formulation, it should be combined with an attractant in order to lure the target insect close to the formulation (attract-and-kill strategy), as more conidia were counted at a short distance.

To conclude, in this study we demonstrated that sporulation by the entomophthoralean fungus *P. cacopsyllae* can be enabled under low humidity conditions by co-application with a specific water-absorbing formulation. For the first time, our findings present a strategy of using biobased polymers that are known for their huge water absorption capacity and water retention to enable sporulation of an entomophthoralean fungus under field conditions as demonstrated in the semi-field trial. These promising results will pave the way for further developments. What should be addressed in the future is the scale-up of the formulation and an appropriate application method, as well as the approval of the formulation under application conditions with the target insect.

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Author contributions LCM conceptualized, designed and conducted the lab experiments, analyzed the data, visualized the graphics and wrote the first draft of the manuscript. BK designed and conducted the semi-field trial analyzed the data, and complemented the manuscript. JG conceptualized the experiments from the semi-field trial. AP conceptualized the experiments in the laboratory. LCM, JG, BK, JE (fungal biology) and AP (formulation) edited the manuscript. AP and JG acquired funding of the project. All authors read, edited and approved the manuscript.

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References

Aoki J (1981) Pattern of conidial discharge of an *Entomophthora* species ("grylli" type) (Entomophthorales: Entomophthoraceae) from infected cadavers of *Mamestra brassicae* L. (Lepidoptera: Noctuidae). Appl Entomol Zool 16(3):216–224

Brobyn PJ, Wilding N, Clark SJ (1985) The persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis* on leaves in the field. Ann Appl Biol 107(3):365–376

Brobyn PJ, Wilding N, Clark SJ (1987) Laboratory observations on the effect of humidity on the persistence of infectivity of conidia of the aphid pathogen *Erynia neoaphidis*. Ann Appl Biol 110(3):579–584

Carruthers RI (1982) The biology and ecology of *Entomophthora muscae* in the onion agroecosystem. PhD dissertation, Michigan State University, East Lansing, MI

Carruthers RI, Haynes DL (1986) Temperature, moisture, and habitat effects on *Entomophthora muscae* conidial germination and survival in the onion agroecosystem. Environ Entomol 15(6):1154–1160

Carruthers RI, Feng Z, Ramos ME, Soper RS (1988) The effect of solar radiation on the survival of *Entomophaga grylli* (Entomophthorales: Entomophthoraceae) conidia. J Invertebr Pathol 52(1):154–162

Chen J, Wu J, Raffa P, Picchioni F, Koning CE (2022) Superabsorbent polymers: from long-established, microplastics generating systems, to sustainable, biodegradable and future proof alternatives. Prog Polym Sci 125:101475

Delalibera I Jr, Demétrio CG, Manly BF, Hajek AE (2006) Effect of relative humidity and origin of isolates of *Neozygites tanajoae* (Zygomycetes: Entomophthorales) on production of conidia from cassava green mite, *Mononychellus tanajoa* (Acari: Tetranychidae), cadavers. Biol Control 39(3):489–496

Demitri C, Scalera F, Madaghiele M, Sannino A, Maffezzoli A (2013) Potential of cellulose-based superabsorbent hydrogels as water reservoir in agriculture. Int J Polym Sci 13:435073

Dwyer G, Elkinton JS, Hajek AE (1998) Spatial scale and the spread of a fungal pathogen of gypsy moth. Am Nat 152(3):485–494

Eilenberg J, Saussure S, Fekih IB, Jensen AB, Klingen I (2019) Factors driving susceptibility and resistance in aphids that share specialist fungal pathogens. Curr Opin Insect Sci 33:91–98

Eilenberg J, Keller S, Humber RA, Jensen AH, Jensen AB, Görg LM, Muskat LC, Kais B, Gross J, Patel AV (2023) Pandora cacopsyllae Eilenberg, Keller & Humber (Entomophthorales Entomophthoraceae), a new species infecting Cacopsylla pyri L (Hemiptera: Psyllidae). J Invertebr Pathol 200:107954

Glare TR, Milner RJ, Chilvers GA (1986) The effect of environmental factors on the production, discharge, and germination of primary conidia of *Zoophthora phalloides* Batko. J Invertebr Pathol 48(3):275–283

Görg L, Eilenberg J, Jensen AB, Jensen AH, Gross J (2021) Pathogenicity against hemipteran vector insects of a novel insect pathogenic fungus from Entomophthorales (*Pandora* sp. nov. inedit.) with potential for biological control. J Invertebr Pathol 183(3):107621

Griggs MH, Vandenberg JD, Sawyer AJ (1999) Effect of relative humidity on viability of primary conidia of *Zoophthora radicans*. J Invertebr Pathol 73(3):315–320

Hajek AE, Carruthers RI, Soper RS (1990) Temperature and moisture relations of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymantriidae). Environ Entomol 19(1):85–90

Hajek AE, Papierok B, Eilenberg J (2012) Methods for study of the Entomophthorales. In: Lacey LA (ed) Manual of techniques in invertebrate pathology, 2nd edn. Academic Press, Amsterdam, pp 285–316

Hall R, Papierok B (1982) Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology 84(4):205–240

Hallsworth JE, Magan N (1999) Water and temperature relations of growth of the entomogenous fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. J Invertebr Pathol 74(3):261–266

Hemmati F, McCartney HA, Clark SJ, Deadman ML (2001a) Conidial discharge in the aphid pathogen *Erynia neoaphidis*. Mycol Res 105(6):715–722

Hemmati F, Pell J, Mccartney H, Deadman M (2001b) Airborne concentrations of conidia of *Erynia neoaphidis* above cereal fields. Mycol Res 105(4):485–489

Hemmati F, Pell J, Mccartney H, Deadman M (2002) Aerodynamic diameter of conidia of *Erynia neoaphidis* and other entomophthoralean fungi. Mycol Res 106(2):233–238

Herren P (2018) Conidial discharge of *Pandora* sp., a potential biocontrol agent against *Cacopsylla* spp. in apple and pear orchards. MSc thesis, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Horie K, Barón M, Fox RB, He J, Hess M, Kahovec J, Kitayama T, Kubisa P, Maréchal E, Mormann W, Stepto RFT, Tabak D, Vohlídal J, Wilks ES, Work WJ (2004) Definitions of terms relating to reactions of polymers and to functional polymeric materials (IUPAC Recommendations 2003). Pure Appl Chem 76(4):889–906

Inglis GD, Goettel MS, Butt TM, Strasser H (2001) Use of hyphomycetous fungi for managing insect pests. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents: progress problems and potential. CABI Publishing, Wallingford, pp 23–69

Jaques RP, Patterson NA (1962) Control of the apple sucker, *Psylla mali* Schmidb., by the fungus *Entomophthora sphaerosperma* (Fresenius). Can Entomol 94(8):818–825

Jarausch B, Tedeschi R, Sauvion N, Gross J, Jarausch W (2019) Psyllid vectors. In: Bertaccini A, Weintraub PG, Rao GP, Mori N (eds) Phytoplasmas: plant pathogenic bacteria, vol 2. Transmission and management of phytoplasma-associated diseases. Springer, Singapore, pp 53–78

Jensen AH (2017) A new insect pathogenic fungus from Entomophthorales with potential for psyllid control. MSc thesis, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

Jensen AH, Gross J, Jensen AB, Gallinger J, Eilenberg J (2018) A new insect pathogenic fungus from Entomophthorales with potential for psyllid control. Mitt Dtsch Ges Allg Angew Entomol 21:283–286

Kalsbeek V, Pell JK, Steenberg T (2001) Sporulation by *Entomophthora schizophorae* (Zygomycetes: Entomophthorales) from housefly cadavers and the persistence of primary conidia at constant temperatures and relative humidities. J Invertebr Pathol 77(3):149–157

Keller S (2007) Arthropod-pathogenic Entomophthorales: biology, ecology, identification. Office for Official Publications of the European Communities, Luxembourg

Kramer JP (1980) The house-fly mycosis caused by *Entomophthora muscae*: influence of relative humidity on infectivity and conidial germination. J NY Entomol Soc 88(4):236–240

Latgé JP, Perry DF, Prévost MC, Samson RA (1989) Ultrastructural studies of primary spores of *Conidiobolus,*

Erynia and related Entomophthorales. Canad J Bot 67(9):2576–2589

Li W, Xu WA, Sheng CF, Wang HT, Xuan WJ (2006) Factors affecting sporulation and germination of *Pandora nouryi* (Entomophthorales: Entomophthoraceae), a pathogen of *Myzus persicae* (Homoptera: Aphididae). Biocontrol Sci Technol 16(6):647–652

Millstein JA, Brown GC, Nordin GL (1982) Microclimatic humidity influence on conidial discharge in Erynia sp.(Entomophthorales: Entomophthoraceae), an entomopathogenic fungus of the alfalfa weevil (Coleoptera: Curculionidae). Environ Entomol 11(6):1166–1169

Milner RJ, Bourne J (1983) Influence of temperature and duration of leaf wetness on infection of *Acyrthosiphon kondoi* with *Erynia neoaphidis*. Ann Appl Biol 102(1):19–27

Muskat LC (2022) Development of attract and kill formulations for biological psyllid pest control. PhD Dissertation, University ofGöttingen, Germany

Muskat LC, Przyklenk M, Humbert P, Eilenberg J, Patel AV (2022a) Fermentation of the psyllid-pathogenic fungus *Pandora* sp. nov. inedit. (Entomophthorales: Entomophthoraceae). Biocontrol Sci Technol 32(5):564–585

Muskat LC, Görg LM, Humbert P, Gross J, Eilenberg J, Patel AV (2022b) Encapsulation of the psyllid-pathogenic fungus *Pandora* sp. nov. inedit. and experimental infection of target insects. Pest Manag Sci 78(3):991–999

Newman GG, Carner GR (1975) Environmental factors affecting conidial sporulation and germination of *Entomophthora gammae*. Environ Entomol 4(4):615–618

Nielsen C, Hajek AE (2006) Diurnal pattern of death and sporulation in *Entomophaga maimaiga*-infected *Lymantria dispar*. Entomol Exp Appl 118(3):237–243

Nussinovitch A (2010) Polymer macro- and micro-gel beads: fundamentals and applications. Springer, New York

Olsen NL, Herren P, Markussen B, Jensen AB, Eilenberg J (2019) Statistical modelling of conidial discharge of entomophthoralean fungi using a newly discovered *Pandora* species. PLoS ONE 14(5):e0215914

Page RM, Humber RA (1973) Phototropism in *Conidiobolus coronatus*. Mycologia 65(2):335–354

Pell JK, Eilenberg J, Hajek AE, Steinkraus DC (2001) Biology, ecology and pest management potential of Entomophthorales. In: Butt TM, Jackson C, Magan N (eds) Fungi as biocontrol agents: progress problems and potential. CABI Publishing, Wallingford, pp 71–154

Pell JK, Macaulay EDM, Wilding N (1993) A pheromone trap for dispersal of the pathogen, *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) amongst populations of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). Biocontrol Sci Technol 3(3):315–320

Roberts DW, Campbell AS (1977) Stability of entomopathogenic fungi. Misc Publ Entomol Soc Amer 10(3):19–76

Shimazu M (1977) Factors affecting conidial germination of *Entomophthora delphacis* Hori (Entomophthorales: Entomophthoraceae). Appl Entomol Zool 12(3):260–264

Six DL, Mullens BA (1997) Distance of conidial discharge of *Entomophthora muscae* and *Entomophthora schizophorae*. J Invertebr Pathol 67(3):253–258

Speiser B, Tamm L, Leschenne V, Kretzschmar U, Brändle I, Maurer V, Schneider C, Dittmann M, Berger T (2022) Betriebsmittelliste 2022 für den ökologischen Landbau in der Schweiz. Forschungsinstitut für biologischen Landbau FiBL, Frick

Uziel A, Kenneth RG (1991) Survival of primary conidia and capilliconidia at different humidities in Erynia (subgen. *Zoophthora*) spp. and in *Neozygites fresenii* (Zygomycotina: Entomophthorales), with special emphasis on *Erynia radicans*. J Invertebr Pathol 58(1):118–126

Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzón A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009) Fungal entomopathogens: new insights on their ecology. Fungal Ecol 2(4):149–159

Vemmer M, Patel AV (2013) Review of encapsulation methods suitable for microbial biological control agents. Biol Control 67(3):380–389

Webster J, Weber R (2007) Introduction to fungi. Cambridge University Press, Cambridge

Weseloh RM, Andreadis TG (1992) Epizootiology of the fungus *Entomophaga maimaiga*, and its impact on gypsy moth populations. J Invertebr Pathol 59(2):133–141

Wilding N (1969) Effect of humidity on the sporulation of *Entomophthora aphidis* and *E. thaxteriana*. Trans Br Mycol Soc 53(1):126–130

Wraight SP, Galaini-Wraight S, Carruthers RI, Roberts DW (2003) *Zoophthora radicans* (Zygomycetes: Entomophthorales) conidia production from naturally infected *Empoasca kraemeri* and dry-formulated mycelium under laboratory and field conditions. Biol Control 28(1):60–77

Yendol WG (1968) Factors affecting germination of *Entomophthora* conidia. J Invertebr Pathol 10(1):116–121

Yu Z, Nordin GL, Brown GC, Jackson DM (1995) Studies on *Pandora neoaphidis* (Entomophthorales: Entomophthoraceae) infectious to the red morph of tobacco aphid (Homoptera: Aphididae). Environ Entomol 24(4):962–966

Zhou X, Feng MG (2009) Sporulation, storage and infectivity of obligate aphid pathogen *Pandora nouryi* grown on novel granules of broomcorn millet and polymer gel. J Appl Microbiol 107(6):1847–1856

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