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Pathogenicity of *Metarhizium* and *Cordyceps* isolates against larvae of different *Agriotes* species and populations in correlation with conidial size and germination

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ABSTRACT

Entomopathogenic fungi offers a promising approach to control *Agriotes* larvae, although virulence varies for different *Agriotes* species. Therefore, the first step was to look for a highly virulent fungal strain for different *Agriotes* species. To achieve this, six *Metarhizium* and two *Cordyceps* strains were studied. Several *Metarhizium* strains were highly efficient and achieved over 90% mortality over four months by dip treatment. The most promising strain to control *A. lineatus* and *A. obscurus* was the *M. brunneum* strain JKI-BI-1450, while *M. robertsi*i strain JKI-BI-1442 was most effective against *A. sputator*. The *Cordyceps* strains had no pathogenic effect. In the second step, we investigated whether fungal strain-specific characteristics such as conidial size and germination could be related to the effect of the strains against *Agriotes* larvae. A correlation could not be confirmed for *A. lineatus* and *A. obscurus*. In contrast, against larvae of *A. sputator* shorter and wider conidia as well as those that germinate later and show a lower germination rate after 96 h were more effective. In the third step, we investigated whether populations of the same *Agriotes* species, differ in their susceptibility to entomopathogenic fungi. Different populations of *A. obscurus* larvae showed variable susceptibility. Significant differences in the Restricted Mean Time Lost (RMTL), but not in the final mortality, were determined for different populations of *A. sputator* larvae. In contrast, the efficacy on *A. lineatus* was similar among the various populations tested. Our study indicates highly specific and complex interactions between the *Agriotes* species and the *Metarhizium* strains.

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Introduction

Wireworms are the polyphagous subterranean larvae of click beetles (Coleoptera: Elateridae) and one of the most widespread and detrimental soil pests. They feed on the roots and seedlings of many crops, such as potatoes, wheat and corn (Ansari et al., 2009; Horton, 2006; Johnson et al., 2008; Milosavljević et al., 2017; Reddy et al., 2014). In Germany and other European countries, larvae of the genus *Agriotes* cause major damage, especially the species *A. obscurus*, *A. lineatus* and *A. sputator* (Burghause & Schmitt, 2011; Lehmhus, 2012, 2017; Lehmhus & Niepold, 2013; Parker & Howard, 2001; Sufyan et al., 2007; Vidal & Petersen, 2011). Moreover, these three *Agriotes* species were accidentally introduced to North America and are becoming an increasing problem there (Vernon et al., 2001; Vernon et al., 2005; Wilkinson et al., 1976). The control of wireworms is difficult, due to their long and subterranean life cycle, overlapping generations, polyphagous nutrition and their occurrence in many different arable and grassland crops (Furlan, 1998, 2004; Ritter & Richter, 2013; Sonnemann et al., 2014; Sufyan et al., 2014; Traugott et al., 2013; Traugott et al., 2015; Vernon et al., 2013). Furthermore, many of the chemical insecticides formerly used for wireworm control are now banned in the European Union, especially the neonicotinoids. Today, only some pesticides are still authorised for wireworm control under §53 of Regulation (EC) 1107/2009. Other control strategies like the rotation of crops (Willis et al., 2010), weed control, mechanical tillage and the cultivation of selected potato varieties help reducing wireworm damage, but are not efficient in controlling wireworm populations. Therefore, studies on potential natural antagonists as biocontrol agents are of great interest. Several natural antagonists, e.g. bacteria, fungi and nematodes have been repeatedly isolated from wireworm populations collected in the field (Kleespies et al., 2013; Leclerque et al., 2013). Especially fungi of the genus *Metarhizium* have already been successfully used against wireworms (Eckard et al., 2017; Kabaluk et al., 2005; Kabaluk & Ericsson, 2007; Razinger et al., 2018). Through these studies, many *Metarhizium* strains are known to have good activity against larvae of the *Agriotes* genus, but the effectiveness of one fungus against different *Agriotes* species varies (Eckard et al., 2013; Eckard et al., 2014). For this reason, the first aim of this study was to find a fungus with good activity against the three main *Agriotes* species. We selected six *Metarhizium* spp. and two *Cordyceps fumosorosea* (formally *Paecilomyces* or *Isaria*) strains. Three of the six selected *Metarhizium* strains have already been tested against wireworms in previous studies, like the *M. brunneum* strain JKI-BI-1339 (well known as M.a. 43, BIPESCO 5 or F52) (Antwi et al., 2018; Eckard et al., 2014; Reddy et al., 2014). Kabaluk (2014) and Kabaluk et al. (2015) investigated the effect of *M. brunneum* strain LRC112 against *Agriotes* beetles both in laboratory and in field conditions. Furthermore, a soil granule based on *M. brunneum* strain JKI-BI-1450 was investigated for its ability to reduce wireworm damage to potatoes in the field (Paluch, 2021). The other three *Metarhizium* strains and the two *Cordyceps* strains were investigated, as they were isolated from *Agriotes*. In contrast to *Metarhizium*, the effect of *Cordyceps* on *Agriotes* larvae is much less studied although fungi of this species have previously been isolated from *Agriotes* larvae (Kleespies et al., 2013; Ritter & Richter, 2013).

Since such efficacy studies are very laborious and time-consuming, the second aim of this study was to identify strain-specific characteristics that influence the pathogenicity

against the target host, which would be an important pre-selection criterion for the development of a biopesticide. Germination is an important point in the infection of an insect with a fungus. The infection begins with the contact of the conidia with the cuticle (Boucias et al., 1988; Zimmermann, 2007a, 2007b). Thereupon the conidia attaches, germinates and forms a germ tube, which penetrates the cuticle through mechanical pressure and the secretion of hydrolytic enzymes (Jarrold et al., 2007; Schrank & Vainstein, 2010; Zimmermann, 2007a, 2007b). Rapid germination can benefit an infection, since the more time elapses between attachment and host penetration, the higher the risk that conidia may dry out, be inhibited by cuticular peptides or removed through moulting or other insect behaviours (Hassan et al., 1989; Samuels et al., 1989). Furthermore, a high germination rate is advantageous, as a positive dose–response relationship has already been described for *Metarhizium* by Cherry et al. (2005) and Ekesi et al. (2002). Jackson et al. (1985) investigated the correlation of the germination and spore length of 18 isolates of *Verticillium lecanii* and their virulence against *Macrosiphoniella sanborn*. Fast germination could be associated with virulence, but not spore length. In contrast, Altre et al. (1999) showed a correlation between the effect of *Cordyceps fumosorosea* against *Plutella xylostella* with the GT_{50} (time of 50% germination) as well as the spore length GT_{50} was strongly negatively and the length of spores positively correlated with a determined infection index ($100 \times$ (angular-transformed % mycosis/log $_{10}$ dosage)). In order to achieve the second aim, the germination and conidial size of the potent fungal strains from the efficacy experiment were determined.

The third aim of our study was to find out if pathogenicity of selected *Metarhizium* strains is specific only on host species level, or differs even intra-specifically, among different populations of a given host species. The latter would obviously impede successful commercialisation of a given strain, while a promising biocontrol agent would exhibit high efficacy across a wide range of target host populations. Although population-dependent host sensitivity to entomopathogenic fungi is very important, it has hardly been studied. We have set up this hypothesis because there are various studies in which the same fungal strain is pathogenic in different ways against the same *Agriotes* species. In the study of Eckard et al. (2014), *M. brunneum* V1002 achieved a mortality rate of about 40% after eight weeks against larvae of *A. lineatus*, whereas the same fungus achieved a mortality rate of about 90% after already three weeks in the study of Ansari et al. (2009). The experimental conditions in the two studies were very similar, as Eckard et al. (2014) followed the methods of Ansari et al. (2009). However, the larval stages as well as the amount and composition of the soil in which the larvae were incubated differed, which could influence the results. A difference in the effect of *M. brunneum* ART2825 on *A. lineatus* larvae was also reported by Eckard et al. (2014) with 70% after eight weeks and by Kölliker et al. (2011) with 50% after nine weeks, even if the difference is less clear than in the previous example. However, this could be explained by the lower spore concentration in the study by Kölliker et al. (2011) compared to Eckard et al. (2014). The effect of the fungus on larvae of *A. obscurus* with 80% and *A. sputator* with 40–45% were very similar in both studies. To investigate this hypothesis, the effect of two *M. brunneum* strains with proven efficacy against different populations of the three main *Agriotes* species was examined. These two fungal strains tested were the best from our

efficacy experiments and the strain ART2825. Eckard et al. (2014), Kölliker et al. (2011), Mayerhofer et al. (2017), Reinbacher et al. (2021) and Rogge et al. (2017) explored the impact of *M. brunneum* strain ART2825 on the mortality of *Agriotes* larvae, the decrease of their number in the field, damage on potatoes and the establishment of the fungus in the soil.

Material and methods

Click beetle collection and laboratory rearing

Adult *Agriotes* were collected from the following six locations in Germany and Switzerland: near Wohld (52°18′11.0″N, 10°41′11.6″E, 89 m asl (above sea level)), Beienrode (52°17′36.6″N, 10°49′53.7″E 92 m asl), Geinsheim am Rhein (49°53′16.8″N, 8°25′10.7″E, 84 m asl) and Mühlheim am Main (50°07′48.1″N, 8°49′41.3″E, 103 m asl) in Germany and Wallestalden (46°56′41.6″N, 7°50′17.4″E, 921 m asl) near Langnau in Emmental and Zurich (47°25′3.0″N, 8°31′26.9″E 453 m asl) in Switzerland. Adult click beetles were caught using plastic sheets that were spread out on the ground, covered with cut grass under which the beetles aggregated Kölliker et al. (2009). With these, laboratory rearings were set up following the protocol of Lehmhus and Niepold (2015) in Germany or Kölliker et al. (2009) in Switzerland.

Fungal strains

All suspensions and solutions are prepared with sterile deionised water, unless otherwise specified. The fungi investigated in this study are summarised in Table 1. They were cultured on Malt Peptone Agar (MPA) containing 3% (w/v) malt extract (Merck, Darmstadt, Germany), 0.5% (w/v) peptone from soybean (Merck) and 1.8% (w/v) agar-agar (Roth, Karlsruhe, Germany) for 14 days at 25°C in the dark. After incubation, the conidia were removed from the plates with sterile 0.5% (v/v) Tween® 80 (Merck) by using a Drigalski spatula. The suspension was filtered through four layers of gauze to remove mycelium. The filtrate was placed in an ultrasonic bath (Sonorex RK 52, Bandelin electronic GmbH & Co.; Berlin, Germany, KG 35 kHz.) for 15 min to separate the conidia from each other. Afterwards, the conidial concentration was determined using a haemocytometer and a 5 ml suspension with a concentration of 10^8 conidia ml^{-1} was prepared by diluting with 0.5% (v/v) Tween® 80 solution for each fungus.

Table 1. Origin of entomopathogenic fungi species, strains ID and origin.

Species	Strain ID	Host/origin	Geographic origin
Metarhizium brunneum	JKI-BI-1339	*Cydia pomonella*	Austria
Metarhizium robertsii	JKI-BI-1441	*Agriotes* spp.	Italy
Metarhizium robertsii	JKI-BI-1442	*Agriotes* spp.	Italy
Metarhizium robertsii	JKI-BI-1448	*Agriotes ustulatus*	Italy
Metarhizium brunneum	JKI-BI-1450	*Agriotes lineatus*	Germany
Metarhizium brunneum	LRC112*	*Agriotes obscurus*	Canada
Metarhizium brunneum	ART2825	*Agriotes obscurus*	Switzerland
Cordyceps fumosorosea	JKI-BI-1513	*Agriotes ustulatus*	Germany
Cordyceps fumosorosea	JKI-BI-1514	*Agriotes ustulatus*	Italy

*was provided by Todd Kabaluk from AAFC/AAC

Efficacy study on entomopathogenic fungi against Agriotes

The effect of the *M. brunneum* strains JKI-BI-1339, JKI-BI-1450, and LRC112, the *M. robertsii* strains JKI-BI-1441, JKI-BI-1442 and JKI-BI-1448 and the *C. fumosorosea* strains JKI-BI-1513 and JKI-BI-1514 were tested against the larvae of *A. lineatus*, *A. obscurus* and *A. sputator*, offspring from beetles collected at Wohld. Therefore, larvae of all three *Agriotes* species were dipped into suspensions of each of the nine fungal strains with a conidial concentration of 10^8 conidia ml^{-1} for 2–3 s. In addition to the fungal treatments, an untreated control and a control with 0.5% (v/v) Tween® 80 was carried out in the same way. Thereafter, the larvae were placed individually in plastic containers without prior drying (diameter = 7 cm; height = 2.5 cm, with lid, not perforated). The containers were filled two-thirds with commercially available potting soil (Fruhstorfer Erde Typ Nullerde, Archut GmbH u. Co. KG Industrie-Erdenwerk, Lauterbach, Germany) with the following nutritional content: Nitrogen (N) = 20–40 mg l^{-1}, Phosphate (P_2O_5) = 20–40 mg l^{-1}, Potassium (K_2O) = 40–60 mg l^{-1}, pH value = 5.9, Salt content KCl = 0.2 g l^{-1}. The soil was autoclaved beforehand at 121°C for 20 min. 500 g of the soil was mixed with 100 ml of autoclaved deionised water. Ten larvae of each *Agriotes* species were used per treatment and incubated at 25°C in the dark for four months. The soil was kept moist by spraying sterile deionised water on the surface of the soil. Weekly, the number of alive, dead or dead and mycosed larvae was monitored. For the *Metarhizium* strains JKI-BI-1339, JKI-BI-1441, JKI-BI-1442, JKI-BI-1448, JKI-BI-1450 and LRC112 the experiment was repeated five times. Since it became apparent after three replicates that the *Cordyceps* strains investigated had no mortal effect on the *Agriotes* larvae and the availability of the larvae was limited, only three replicates were performed for JKI-BI-1513 and JKI-BI-1514.

Determination of the conidial size

The conidial size of the *Metarhizium* strains effective against *Agriotes* larvae were determined. Therefore, suspensions of JKI-BI-1339, JKI-BI-1441, JKI-BI-1442, JKI-BI-1448, JKI-BI-1450 and LRC112 were prepared as previously described. Fifty conidia of each fungal strain were photographed, and the length and width were measured using the program cellSens Standard, Olympus. The conidia index was calculated by dividing the length by the width.

Determination of the germination over time

In addition to the conidial size, the germination of the *Metarhizium* strains was also investigated. JKI-BI-1339, JKI-BI-1441, JKI-BI-1442, JKI-BI-1448, JKI-BI-1450 and LRC112 were incubated for 14 days at 25°C in the dark. After the incubation a small amount of conidia of each fungus was transferred into an Eppendorf-tube filled with 0.5% (v/v) Tween® 80 using an inoculation loop. The Eppendorf-tubes were placed on a Vortexer for 10 s and afterwards into an ultrasonic bath (Sonorex RK 52, Bandelin electronic GmbH & Co., Berlin, Germany, KG 35 kHz.) for 15 min. The conidia concentration was determined using a haemocytometer. For each fungus, a 1 ml suspension with a concentration of 10^6 conidia ml^{-1} was prepared by diluting with 0.5% (v/v)

Tween® 80. Three drops (10 µl each) of each suspension were dropped onto eight MPA plates, corresponding to the number of evaluation time points. The plates were incubated at 25°C in the dark and the germination rate was determined after 9, 12, 15, 18, 21, 24, 27 and 33 h. For each drop 100 conidia were observed under a compound light microscope (× 400) and the percentage of germinated conidia was determined. Conidia were rated as germinated when the germ tube was longer than the width of the conidia. This experiment was repeated six times.

Determination of the germination rate after 96 h

For determination of the overall germination rate after 96 h, another experiment was set up as described above and 25 mg l^{-1} Benomyl (Sigma Aldrich, Buchs, Switzerland) was added to the MPA. The germination rate was only determined after 96 h. Benomyl does not affect germination but inhibits mycelial growth. The experiment was repeated six times.

Investigation of the efficacy of two M. brunneum *strains against* Agriotes *larvae from different populations*

To determine if *Agriotes* larvae susceptibility is population dependent, the efficacy of two *M. brunneum* strains against different populations of *A. lineatus*, *A. sputator*, and *A. obscurus* from Germany and Switzerland was investigated. The examined fungal strains were JKI-BI-1450 and ART2825. JKI-BI-1450 caused the highest mortality in two of three *Agriotes* species in our previous experiments and ART2825 has also been proven effective against *Agriotes* larvae in laboratory experiments (Eckard et al., 2014; Kölliker et al., 2011).

Just before the experiment was set up, the head capsules of the larvae were measured and the larval stage was determined according to Klausnitzer (1994). The program cellSens Standard, Olympus, Tokyo, Japan was used for the *Agriotes* larvae from Germany and VHX 6000, Keyence, Osaka, Japan for the larvae from Switzerland. The preparation of the fungal suspensions and the dipping of the larvae were carried out as previously described. In addition to the fungal treatments, controls with 0.5% (v/v) Tween® 80 were prepared. The containers in which the larvae were placed after treatment were filled with approx. 12 g of commercially available potting soil (Fruhstorfer Erde Typ Nullerde, Archut GmbH u. Co. KG Industrie-Erdenwerk) with the following nutritional content: Nitrogen (N) = 20–40 mg l^{-1}, Phosphate (P_2O_5) = 20–40 mg l^{-1}, Potassium (K_2O) = 40–60 mg l^{-1}, pH value = 5.9, Salt content KCl = 0.2 g l^{-1}. The soil was autoclaved beforehand at 121°C for 20 min. The residual moisture of the soil was 45%, which was determined using a moisture determination balance (Ma30, Sartorius, Göttingen, Germany). To maintain the moisture content during the experiment, control containers were filled with soil only and weighed at regular intervals throughout incubation. Water was added to every container according to the determined corresponding weight discrepancies observed from the control containers. The containers were incubated at 25°C in the dark and evaluated weekly for 70 days to determine the number of alive, dead, or dead and mycosed insects.

The efficacy of JKI-BI-1450 was tested against larvae of *A. lineatus* from Geinsheim and Wohld, *A. obscurus* from Beienrode, Mühlheim, Wallestalden and Wohld and *A. sputator* from Mühlheim, Zurich and Wohld. All these treatments were performed with 10 individuals in five independent repetitions, except for the population from Wallenstalden where 20 individuals were available per repetition. The fungal strain ART2825 were tested against larvae of *A. lineatus* from Zurich and Wohld, *A. obscurus* from Beienrode and *A. sputator* from Beienrode with 10 individuals in five independent repetitions, *A. obscurus* larvae from Wallestalden and *A. sputator* larvae from Zurich with 10 individuals in four independent repetitions, *A. obscurus* larvae from Zurich with five individuals in three independent repetitions, *A. sputator* larvae from Zurich with 10 individuals in three independent repetitions and *A. sputator* larvae from Mühlheim with 10 individuals in four independent repetitions and a fifth repetition with four individuals only. Results on the treatment with JKI-BI-1450 of larvae from the location Wohld were the same as in the study described above, evaluated 70 days after inoculation. All other treatments were set up exclusively for the population test.

Statistical analysis

Data were analysed with the software SAS Studio 3.8. The efficacy of fungi against *Agriotes* larvae was compared based on a survival analysis (Kaplan-Meier-Wilcoxon). Because the mortality was less than 50% for multiple treatments, the Restricted Mean Time Lost (RMTL) was calculated for all larvae that have died (RTML mortality) and for all larvae that have died and are mycosed (RTML mycoses). This is defined as the area above the Kaplan-Meier survival curve. RMTL is zero when no experimental animal dies within the experimental period. High and rapid mortality is indicated by a high RMTL. The final mortality and the final mycoses, as well as the larval stage, conidial length, width and index were compared by using a generalised linear models with Wald statistics for type 3 analysis and multiple comparison according to Tukey (GLMM, $p < 0.05$). For the experiment on the efficacy of *Metarhizium* and *Cordyceps* against three species of *Agriotes* larvae, all of the above comparisons were made for all treatments within one *Agriotes* species. To compare the germination over time of the different fungal strains, τ (time at which half of the maximum achieved germination rate was reached) and the slope at the inflection points were calculated for each replication of all repetitions by Seib et al. (2023) according to Dantigny et al. (2011). The germination rate after 96 h, τ and the slope at the inflection point of all fungi were investigated and compared using a generalised linear model with Wald statistics for type 3 analysis and multiple comparison according to Tukey (GLMM, $p < 0.05$). The correlation between final mortality and pathogenicity factors was determined using Pearsons's Correlation Test ($p < 0.05$). The mean value was always used for the pathogenicity factors. At the experiment on the efficacy of *Metarhizium* against three species of *Agriotes* larvae from different populations, all parameters were compared within one *Agriotes* species and one fungal treatment across all associated populations. Furthermore, we compared the RMTL mortality, the RMTL mycoses, the final mycoses and the final mortality of all populations from Switzerland with all populations from Germany by using a generalised linear model with Wald statistics for type 3 analysis and multiple comparison according to Tukey with underlying normal distribution (GLMM, $p < 0.05$).

Table 2. Efficacy of six *Metarhizium* strains (*M. brunneum*: JKI-BI-1339, JKI-BI-1450, LRC112; *M. robertsii*: JKI-BI-1441, JKI-BI-1442, JKI-BI-1448) and two *Cordyceps fumosorosea strains* (JKI-BI-1513, JKI-BI-1514) against *Agriotes* larvae over 4 months. Different letters represent significant differences within columns and *Agriotes* species. *Metarhizium*: $n = 5$ with 10 individuals, *Cordyceps*: $n = 3$ with 10 individuals.

	RMLT mortality*	**	RMLT mycoses *	**	Final mortality [%] ***	****	Final mycoses [%]***	****
A. lineatus								
JKI-BI-1339	17.8 ± 4.6	AB	12.7 ± 4.1	A	28.0 ± 10.7	BCD	18.0 ± 6.6	BC
JKI-BI-1441	20.0 ± 4.7	AB	11.3 ± 3.8	A	38.0 ± 3.7	ABC	18.0 ± 3.7	BC
JKI-BI-1442	27.6 ± 5.6	AB	24.5 ± 5.3	A	44.0 ± 6.8	AB	38.0 ± 7.3	AB
JKI-BI-1448	15.4 ± 4.4	ABC	13.2 ± 4.1	A	30.0 ± 8.9	ABCD	24.0 ± 7.5	ABC
JKI-BI-1450	31.1 ± 4.8	A	22.0 ± 4.1	A	58.0 ± 9.7	A	46.0 ± 6.8	A
LRC112	24.5 ± 5.4	AB	14.3 ± 4.3	A	34.0 ± 11.7	ABC	20.0 ± 12.6	ABC
JKI-BI-1513	14.2 ± 6.2	ABC	0.0 ± 0.0	B	16.7 ± 3.3	BCD	0.0 ± 0.0	C
JKI-BI-1514	9.6 ± 5.2	ABC	0.0 ± 0.0	B	10.0 ± 5.8	BCD	0.0 ± 0.0	C
untreated	8.5 ± 3.7	BC	n.m.		10.0 ± 3.2	CD	n.m.	
0.5% Tween® 80	1.5 ± 1.5	C	n.m.		4.0 ± 2.4	D	n.m.	
A. obscurus								
JKI-BI-1339	23.7 ± 4.6	B	18.2 ± 4.5	B	50.0 ± 7.1	BC	36.0 ± 5.1	BC
JKI-BI-1441	27.0 ± 4.2	B	24.8 ± 4.2	B	62.0 ± 5.8	B	56.0 ± 4.0	AB
JKI-BI-1442	27.6 ± 4.4	B	22.0 ± 3.9	B	66.0 ± 10.3	AB	58.0 ± 13.9	AB
JKI-BI-1448	23.7 ± 4.2	B	23.5 ± 4.2	B	50.0 ± 10.0	BC	46.0 ± 10.8	B
JKI-BI-1450	70.1 ± 4.4	A	66.9 ± 4.7	A	94.0 ± 4.0	A	90.0 ± 7.7	A
LRC112	55.6 ± 5.6	A	48.3 ± 5.9	A	76.0 ± 11.2	AB	64.0 ± 12.9	AB
JKI-BI-1513	0.0 ± 0.0	C	0.0 ± 0.0	C	0.0 ± 0.0	D	0.0 ± 0.0	C
JKI-BI-1514	2.8 ± 2.0	C	0.0 ± 0.0	C	6.7 ± 3.3	D	0.0 ± 0.0	C
untreated	10.6 ± 3.8	BC	n.m.		22.0 ± 8.0	CD	n.m.	
0.5% Tween® 80	2.1 ± 1.3	C	n.m.		8.0 ± 3.7	D	n.m.	
A. sputator								
JKI-BI-1339	40.1 ± 5.2	A	37.0 ± 5.1	A	70.0 ± 5.5	AB	66.0 ± 5.1	BC
JKI-BI-1441	53.8 ± 4.6	A	51.2 ± 4.7	A	90.0 ± 4.5	A	86.0 ± 4.0	AB
JKI-BI-1442	57.7 ± 4.6	A	55.4 ± 4.9	A	94.0 ± 2.4	A	88.0 ± 3.7	A
JKI-BI-1448	45.9 ± 4.6	A	44.4 ± 4.8	A	80.0 ± 6.3	AB	76.0 ± 8.1	AB
JKI-BI-1450	19.0 ± 3.7	B	17.2 ± 3.8	B	56.0 ± 5.1	BC	48.0 ± 5.8	C
LRC112	17.8 ± 4.3	B	11.0 ± 4.0	BC	34.0 ± 11.2	CD	16.0 ± 8.7	D
JKI-BI-1513	11.4 ± 5.4	B	0.0 ± 0.0	C	16.7 ± 3.3	D	0.0 ± 0.0	D
JKI-BI-1514	11.0 ± 4.2	B	0.0 ± 0.0	C	20.0 ± 15.3	D	0.0 ± 0.0	D
untreated	7.1 ± 3.0	B	n.m.		16.0 ± 6.8	D	n.m.	
0.5% Tween® 80	6.0 ± 3.0	B	n.m.		10.0 ± 3.2	D	n.m.	

*RMTL = Restricted mean time lost (area above the Kaplan-Meier survival curve in the interval 0 to t_{max}) Mean ± SE. ** Survival analysis (Kaplan Meier-Wilcoxon). *** Mean ± SE. **** GLMM, $p < 0.05$; n.m. = no mycoses.

Results

Efficacy study on entomopathogenic fungi against Agriotes larvae

RMTL mortality and final mortality of *Cordyceps* treatments were lower than *Metarhizium* treatments in all three *Agriotes* species and did not differ significantly from the controls (Table 2). Furthermore, the treatment with *Cordyceps* never led to fungal growth on the cadaver. In contrast, *Metarhizium* treatments had a mortal effect on all *Agriotes* species resulted in high mycoses rate of the insects. The final mortality caused by *Metarhizium* infection was generally much lower for *A. lineatus* larvae with an average of 39% than for larvae of *A. obscurus* with 66% and *A. sputator* with 71%. There were significant differences in the efficacy parameters of the various fungal strains investigated within the

species *A. lineatus* (RMTL mortality: $x^2 = 75.97$; df = 9; $p < 0.0001$; RMTL mycoses: $x^2 = 88.86$; df = 6; $p < 0.0001$; final mortality: $x^2 = 55.23$; df = 9; $p < 0.0001$; final mycoses: $x^2 = 34.86$; df = 7; $p < 0.0001$). *M. brunneum* strain JKI-BI-1450 showed the best effect with the highest final mortality with 58% followed by *M. robertsii* strain JKI-BI-1442 with 44%. JKI-BI-1442 had the same RMLT mortality on larvae of *A. lineatus* and *A. obscurus* with 27.6. However, the final mortality on *A. obscurus* larvae was higher with 66%, which indicates a reduced infection time for JKI-BI-1442 on *A. lineatus* larvae compared to *A. obscurus* larvae. As with *A. lineatus*, the efficacy parameters of the different fungal strains showed significant differences on *A. obscurus* larvae (RMTL mortality: $x^2 = 505.70$; df = 9; $p < 0.0001$; RMTL mycoses: $x^2 = 384.51$; df = 6; $p < 0.0001$; final mortality: $x^2 = 181.49$; df = 9; $p < 0.0001$; final mycoses: $x^2 = 79.32$; df = 7; $p < 0.0001$). The fungus JKI-BI-1450 had also the best effect on *A. obscurus* larvae with the highest final mortality (94%). The second-best effect was observed with *M. brunneum* strain LRC112 with a final mortality of 76%. Efficacy parameters of the different fungal strains also revealed significant differences in tests with *A. sputator* larvae (RMTL mortality: $x^2 = 211.81$; df = 9; $p < 0.0001$; RMTL mycoses: $x^2 = 410.40$; df = 6; $p < 0.0001$; final mortality: $x^2 = 276.58$; df = 9; $p < 0.0001$; final mycoses: $x^2 = 301.67$; df = 7; $p < 0.0001$). *M. robertsii* strain JKI-BI-1442 was the most effective with the highest final mortality of 94% closely followed by *M. robertsii* strain JKI-BI-1441 with 90%.

Determination of the conidial sizes

The length, width and index of the conidia differed significantly between the various *Metarhizium* strains (length: $x^2 = 343.44$; df = 5; $p < 0.0001$; width: $x^2 = 647.74$; df = 5; $p < 0.0001$; index: $x^2 = 480.37$; df = 5; $p < 0.0001$). *M. brunneum* strain LRC112 formed the significantly longest conidia with 8.62 µm (Table 3), whereas *M. robertsii* strain JKI-BI-1442 had the significantly widest conidia with 2.76 µm, except *M. robertsii* strain JKI-BI-1448. However, JKI-BI-1448 and JKI-BI-1442 had the significantly lowest conidial index, which becomes manifested in the most round-shaped conidia. A correlation between the size parameters of the conidia and their effect against larvae of *A. lineatus* (length: $r = -0.05077$, $p = 0.7899$; width: $r = -0.01980$, $p = 0.9173$; index: $r = -0.02584$, $p = 0.8922$) and *A. obscurus* (length: $r = 0.18677$, $p = 0.3230$; width: $r = -0.21762$, $p = 0.2480$; index: $r = 0.28743$, $p = 0.1235$) could not be established. The effect on larvae of *A. sputator* was negative correlated with the conidial length ($r =$

Table 3. Sizes of conidia produced by six strains of *Metarhizium* (*M. brunneum*: JKI-BI-1339, JKI-BI-1450, LRC112; *M. robertsii*: JKI-BI-1441, JKI-BI-1442, JKI-BI-1448) on MPA after 14 days at 25°C. Significant differences within columns are represented by different letters; $n = 50$.

Fungi	Conidial length [µm]*	**	Conidial width [µm]	**	Conidial index	**
JKI-BI-1339	6.80 ± 0.06	C	2.19 ± 0.02	D	3.13 ± 0.04	B
JKI-BI-1441	7.70 ± 0.06	B	2.65 ± 0.02	B	2.92 ± 0.03	C
JKI-BI-1442	7.39 ± 0.06	B	2.76 ± 0.02	A	2.68 ± 0.03	D
JKI-BI-1448	7.03 ± 0.05	C	2.68 ± 0.02	AB	2.63 ± 0.03	D
JKI-BI-1450	7.01 ± 0.06	C	2.27 ± 0.02	CD	3.11 ± 0.04	B
LRC112	8.62 ± 0.15	A	2.32 ± 0.03	C	3.74 ± 0.06	A

* Mean ± SE. ** GLMM, $p < 0.05$.

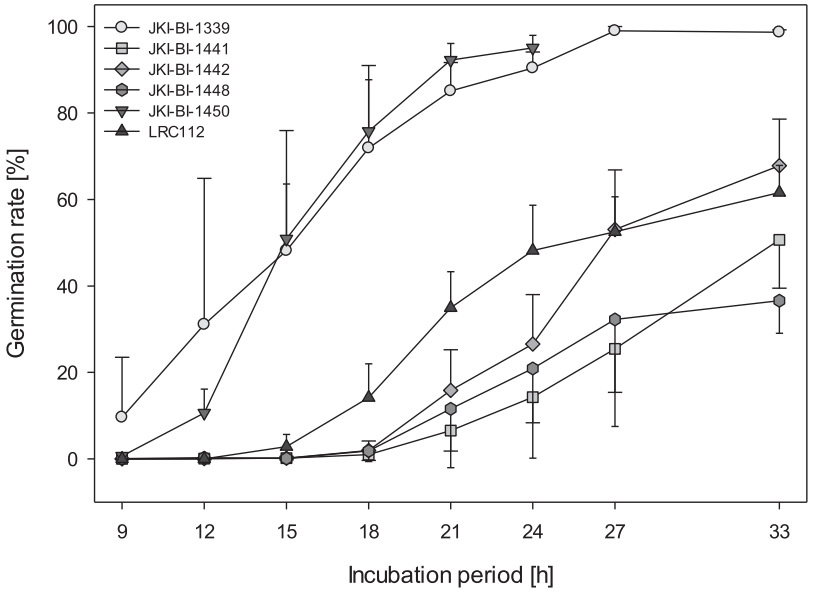

Figure 1. Germination rate over 33 h of conidia of the *M. brunneum* strain JKI-BI-1339, JKI-BI-1450 and LRC112 and of the *M. robertsii* strains JKI-BI-1441, JKI-BI-1442 and JKI-BI-1448. Means and standard deviation are shown. The error bars of JKI-BI-1339, JKI-BI-1441 and JKI-BI-1442 have large cap widths and for JKI-BI-1448, JKI-BI-1450 and LRC112 small cap widths. After incubation for more than 24 h, the conidia of JKI-BI-1450 formed such long germ tubes that the germination rate could not be determined.

−0.40944, $p = 0.0246$) and index ($r = -0.76672$, $p < 0.0001$) and positive correlated with the width ($r = 0.63655$, $p = 0.0002$).

Determination of the germination over time and germination rate after 96 h

The germination of the conidia differed between the various *Metarhizium* strains (Figure 1). *M. brunneum* strains JKI-BI-1339 and JKI-BI-1450 germinated faster and achieved a germination rate of over 90% after only 24 h. The germination of the *M. robertsii* strains occurred more slowly and was similar within the first 24 h. The conidia of *M. brunneum* strain LRC112 germinated slower compared to the other

Table 4. Germination rate after 96 h, τ (time point when 50% of the maximal germinated conidia are germinated) and the slope inflection (slope of the inflectional tangent at the inflection point) over 33 h. *M. brunneum*: JKI-BI-1339, JKI-BI-1450, LRC112; *M. robertsii*: JKI-BI-1441, JKI-BI-1442, JKI-BI-1448; Significant differences within columns are represented by different letters; $n = 18$.

Fungi	Germination after 96 h [%]*	**	τ [h]*	**	Slope inflection*	**
JKI-BI-1339	98.3 ± 0.41	A	14.5 ± 0.79	A	13.3 ± 0.80	A
JKI-BI-1441	92.7 ± 0.98	B	32.2 ± 0.66	C	4.8 ± 0.26	C
JKI-BI-1442	93.0 ± 1.36	B	26.6 ± 0.50	B	8.3 ± 0.65	B
JKI-BI-1448	90.7 ± 1.04	B	31.7 ± 1.49	C	5.6 ± 0.55	BC
JKI-BI-1450	96.8 ± 0.80	A	15.1 ± 0.17	A	16 ± 1.33	A
LRC112	98.5 ± 0.31	A	25.9 ± 0.37	B	4.1 ± 0.13	C

* Mean ± SE. ** GLMM, $p < 0.05$.

M. brunneum strains, but achieved a germination rate after 96 h that was just as high as the others (Table 4). The germination rate after 96 h and the two factors of the germination process (τ and the slope at the inflection point) differed significantly between the different fungal strains (germination rate after 96 h: $x^2 = 71.87$; df = 5; $p < 0.0001$; τ: $x^2 = 526.19$; df = 5; $p < 0.0001$; slope inflection: $x^2 = 238.74$; df = 5; $p < 0.0001$). The germination rate after 96 h of all three *M. brunneum* strains was over 96% and significantly higher compared to those of the three *M. robertsii* strains with a germination rate after 96 h of 90–93%. τ was reached fastest by *M. brunneum* strains JKI-BI-1339 and JKI-BI-1450 after 15 h, followed with significantly difference by LRC112 (*M. brunneum*) and JKI-BI-1442 (*M. robertsii*) after 26 h and the two *M. robertsii* strains JKI-BI-1441 and JKI-BI-1448 after 32 h. The strains JKI-BI-1339 and JKI-BI-1450 also show the significantly steepest slope at the inflection point. A correlation between the germination of the conidia and their effect against larvae of *A. lineatus* (τ: $r = -0.06943$, $p = 0.7154$; slope at the inflection point: $r = 0.12885$, $p = 0.4974$; germination rate after 96 h: $r = 0.02805$, $p = 0.8830$) and *A. obscurus* (τ: $r = -0.31957$, $p = 0.0852$; slope at the inflection point: $r = 0.18723$, $p = 0.3218$; germination rate after 96 h: $r = 0.26467$, $p = 0.1575$) could not be established. The effect on larvae of *A. sputator* was positive correlated with τ ($r = 0.70567$, $p < 0.0001$) and negative with the germination rate after 96 h ($r = -0.65536$, $p < 0.0001$). A correlation with the slop could not be proven ($r = -0.30013$, $p = 0.1071$).

Investigation of the efficacy of two M. brunneum *strains against* Agriotes larvae *from different populations*

There were no significant differences in susceptibility between the various populations of larvae of *A. lineatus* against the two strains of *M. brunneum* strains examined, only to the controls (Table 5) (JKI-BI-1450: RMTL mortality: $x^2 = 30.87$; df = 3; $p < 0.0001$; RMTL mycoses: $x^2 = 6.15$; df = 1; $p = 0.0132$; final mortality: $x^2 = 78.65$; df = 3; $p < 0.0001$; final mycoses: $x^2 = 1.82$; df = 1; $p = 0.1775$; ART2825: RMTL mortality: $x^2 = 44.15$; df = 3; $p < 0.0001$; RMTL mycoses: $x^2 = 0.09$; df = 1; $p = 0.7684$; final mortality: $x^2 = 79.37$; df = 3; $p < 0.0001$; final mycoses: $x^2 = 0.54$; df = 1; $p = 0.4611$). The final mortality of the larvae treated with JKI-BI-1450 was 38% at both populations and for ART2825 the final mortality approx. 50%. It is important to note that in treatments with ART2825, the average larval stage of 4.5 of larvae from Wohld was significant lower compared to the average larval stage of 6.2 of larvae from Zurich ($x^2 = 122.03$; df = 3; $p < 0.0001$). In contrast, the effect of both fungal treatments on the larvae of *A. obscurus* differed depending on the population (JKI-BI-1450: RMTL mortality: $x^2 = 476.29$; df = 7; $p < 0.0001$; RMTL mycoses: $x^2 = 34.93$; df = 3; $p < 0.0001$; final mortality: $x^2 = 507.94$; df = 7; $p < 0.0001$; final mycoses: $x^2 = 46.36$; df = 3; $p < 0.0001$; ART 2825: RMTL mortality: $x^2 = 1467.19$; df = 7; $p < 0.0001$; RMTL mycoses: $x^2 = 61.13$; df = 3; $p < 0.0001$; final mortality: $x^2 = 978.03$; df = 7; $p < 0.0001$; final mycoses: $x^2 = 58.63$; df = 3; $p < 0.0001$). Final mortality ranged by treatment with JKI-BI-1450 from 94% by larvae from Mühlheim to 44% by larvae from Wallestalden, whereby the larvae with the smallest larval stage were the most insensitive ($x^2 = 227.04$; df = 5; $p < 0.0001$). ART2825 treatments on *A. obscurus* larvae resulted in significantly lower final mortality of larval populations from Zurich with 30% compared to the other

Table 5. Efficacy of two *M. brunneum* strains against different populations of *Agriotes* larvae over 70 days. Different letters represent significant differences within columns and *Agriotes* species and one fungal treatment across all associated populations. JKI-BI-1450: $n = 5$ with 10 individuals expect Wallenstalden with 20 individuals; ART2825: *A. lineatus* Wohld and Zurich, *A. obscurus* Beienrode, *A. sputator* Beienrode: $n = 5$ with 10 individuals, *A. obscurus* Wallestalden and *A. sputator* Zurich: $n = 4$ with 10 individuals, *A. obscurus* Zurich: $n = 3$ with 5 individuals, *A. sputator* Zurich: $n = 3$ with 10 individuals, *A. sputator* Mühlheim: $n = 4$ with 10 individuals and 1 with 4 individuals.

Population	Treatment	RMTL mortality *	**	RMTL mycoses *	**	Final mortality [%] ***	****	Final mycoses [%] ***	****	Larval stage	****
A. lineatus											
Geinsheim	JKI-BI-1450	12.6 ± 2.6	A	12.6 ± 2.6	A	38.0 ± 5.8	A	38.0 ± 5.8	A	5.8 ± 0.2	A
Wohld	JKI-BI-1450	7.4 ± 2.1	A	4.9 ± 1.7	A	38.0 ± 5.8	A	30.0 ± 3.2	A	n.d.	
Geinsheim	Control	0.6 ± 0.4	B	n.m.		4.0 ± 2.4	B	n.m.		5.9 ± 0.2	A
Wohld	Control	0.6 ± 0.6	B	n.m.		2.0 ± 2.0	B	n.m.		n.d.	
Wohld	ART2825	23.1 ± 4.2	A	17.9 ± 4.1	A	53.5 ± 6.3	A	41.3 ± 3.7	A	4.5 ± 0.1	B
Zurich	ART2825	19.5 ± 3.3	A	19.5 ± 3.3	A	48.0 ± 8.6	A	48.0 ± 8.6	A	6.2 ± 0.2	A
Wohld	Control	1.8 ± 1.8	B	n.m.		3.6 ± 3.6	B	n.m.		4.3 ± 0.1	B
Zurich	Control	2.1 ± 1.5	B	n.m.		4.0 ± 2.4	B	n.m.		6.0 ± 0.2	A
A. obscurus											
Beienrode	JKI-BI-1450	27.4 ± 2.7	AB	26.5 ± 2.8	A	86.0 ± 6.8	AB	80.0 ± 7.7	A	6.3 ± 0.1	AB
Mühlheim	JKI-BI-1450	35.3 ± 2.4	A	33.1 ± 2.6	A	94.0 ± 4.0	A	84.0 ± 2.4	A	6.4 ± 0.1	AB
Wallestalden	JKI-BI-1450	17.0 ± 2.3	BC	13.7 ± 2.2	B	44.0 ± 2.4	C	33.0 ± 3.0	B	5.0 ± 0.1	C
Wohld	JKI-BI-1450	25.8 ± 2.8	AB	25.0 ± 2.7	A	74.0 ± 7.5	B	70.0 ± 10.0	A	n.d.	
Beienrode	Control	0.7 ± 0.7	D	n.m.		2.0 ± 2.0	D	n.m.		5.9 ± 0.1	B
Mühlheim	Control	0.4 ± 0.3	D	n.m.		4.0 ± 2.4	D	n.m.		6.5 ± 0.1	A
Wallestalden	Control	9.5 ± 2.1	C	n.m.		21.0 ± 8.6	D	n.m.		5.0 ± 0.1	C
Wohld	Control	0.1 ± 0.1	D	n.m.		2.0 ± 2.0	D	n.m.		n.d.	
Beienrode	ART2825	52.1 ± 2.2	A	51.6 ± 2.4	A	94.7 ± 3.1	A	92.2 ± 4.8	A	5.3 ± 0.2	AB
Wallestalden	ART2825	49.3 ± 2.2	A	47.3 ± 2.6	A	95.6 ± 4.4	A	86.7 ± 8.2	A	5.6 ± 0.2	AB
Wohld	ART2825	51.5 ± 2.5	A	51.5 ± 2.5	A	95.0 ± 5.0	A	95.0 ± 5.0	A	5.0 ± 0.2	B
Zurich	ART2825	8.2 ± 5.3	B	8.2 ± 5.3	B	30.0 ± 10.0	B	30.0 ± 10.0	B	6.2 ± 0.3	A
Beienrode	Control	3.2 ± 2.2	B	n.m.		5.6 ± 5.6	C	n.m.		5.3 ± 0.2	AB
Wallestalden	Control	1.2 ± 1.2	B	n.m.		2.9 ± 2.9	C	n.m.		5.6 ± 0.2	AB

(*Continued*)

Table 5. Continued.

Population	Treatment	RMTL mortality *	**	RMTL mycoses *	**	Final mortality [%] ***	****	Final mycoses [%] ***	****	Larval stage	****
Wohld	Control	0.2 ± 0.2	B	n.m.		2.5 ± 2.5	C	n.m.		4.9 ± 0.2	B
Zurich	Control	0 ± 0	B	n.m.		0 ± 0	C	n.m.		5.8 ± 0.3	AB
A. sputator											
Mühlheim	JKI-BI-1450	4.6 ± 1.4	B	4.1 ± 1.3	B	26.0 ± 8.1	AB	24.0 ± 8.7	A	6.0 ± 0.1	B
Wohld	JKI-BI-1450	3.6 ± 1.4	BC	3.6 ± 1.4	B	18.0 ± 5.8	AB	18.0 ± 5.8	A	n.d.	
Zurich	JKI-BI-1450	18.5 ± 3.8	A	16.7 ± 3.7	A	36.0 ± 11.7	A	32.0 ± 11.1	A	7.3 ± 0.1	A
Mühlheim	Control	0.3 ± 0.3	C	n.m.		4.0 ± 2.4	B	n.m.		5.7 ± 0.1	B
Wohld	Control	0.6 ± 0.6	BC	n.m.		4.0 ± 2.4	B	n.m.		n.d.	
Zurich	Control	7.7 ± 2.7	ABC	n.m.		20.0 ± 11.4	AB	n.m.		7.2 ± 0.1	A
Beienrode	ART2825	21.5 ± 3.9	A	19.3 ± 3.8	A	41.8 ± 12.3	A	37.6 ± 11.9	A	7.0 ± 0.1	AB
Mühlheim	ART2825	16.8 ± 3.9	AB	15.6 ± 3.8	AB	37.0 ± 10.4	AB	33.0 ± 7.7	A	7.2 ± 0.1	AB
Zurich	ART2825	6.0 ± 3.3	BC	6.0 ± 3.3	B	10.7 ± 6.4	ABC	10.7 ± 6.4	A	6.8 ± 0.2	B
Beienrode	Control	5.6 ± 2.2	BC	n.m.		14.4 ± 4.2	BC	n.m.		7.3 ± 0.1	A
Mühlheim	Control	1.5 ± 1.4	C	n.m.		2.0 ± 2.0	C	n.m.		7.2 ± 0.1	AB
Zurich	Control	0 ± 0	C	n.m.		0 ± 0	C	n.m.		7.3 ± 0.1	A

* RMTL = Restricted mean time lost (area above the Kaplan-Meier survival curve in the interval 0 to t_{max}) Mean ± SE. ** Survival analysis (Kaplan Meier-Wilcoxon). *** Mean ± SE. **** GLMM, $p < 0.05$; n.m. = no mycoses; n.d. = not determin.

three larval populations with approx. 95% final mortality. Contrary to the results from the JKI-BI-1450 treatments, on average older larvae exhibited the lowest final mortality ($x^2 = 24.33$; df = 7; $p = 0.0010$). Both fungal treatments on *A. sputator* larvae showed that the RMTL mortality and RMTL mycoses were significantly dependent on the populations (JKI-BI-1450: RMTL mortality: $x^2 = 43.66$; df = 5; $p < 0.0001$; RMTL mycoses: $x^2 = 11.35$; df = 2; $p = 0.0034$ ART2825: RMTL mortality: $x^2 = 60.53$; df = 5; $p < 0.0001$; RMTL mycoses: $x^2 = 7.68$; df = 2; $p = 0.0215$), but not the final mortality and final mycoses (JKI-BI-1450: final mortality: $x^2 = 15.56$; df = 5; $p = 0.0082$; final mycoses: $x^2 = 1.58$; df = 2; $p = 0.4536$; ART2825: final mortality: $x^2 = 31.11$; df = 5; $p < 0.0001$; final mycoses: $x^2 = 4.40.$; df = 2; $p = 0.1108$). When treated with JKI-BI-1450, the RMTL mortality was significantly higher on the significantly older larvae from Zurich ($x^2 = 134.52$; df = 3; $p < 0.0001$) with 18.5 compared to the other two populations with approx. 4.1. The RMTL mortality of the treatments with ART2825 was significantly higher on larvae from Beienrode with 21.5 compared to the larvae of Zurich with 6.0, although the larval stages of these populations were not significantly different with an average of 7 ($x^2 = 13.00$; df = 5; $p = 0.0234$).

Finally, RMTL mortality, the RMTL mycoses, the final mortality and the final mycoses of the fungal treatments were compared across all populations from Switzerland and Germany. However, there were no significant differences between the populations of the two countries in any of the parameters (RMTL mortality: $x^2 = 0.24$; df = 1; $p = 0.6235$; RMTL mycoses: $x^2 = 0.22$; df = 1; $p = 0.6366$;. final mortality: $x^2 = 1.06$; df = 1; $p = 0.3037$; final mycoses: $x^2 = 1.08$; df = 1; $p = 0.2985$).

Discussion

The first aim of this study was to find a fungal strain with a good efficacy against larvae of *A. lineatus*, *A. obscurus* and *A. sputator*. We could confirm that all investigated *Metarhizium* strains were pathogenic to the larvae of the three *Agriotes* species and led to mycoses on the cadavers regardless of the host from which it was isolated or origin. Similar results to the effectiveness from *Metarhizium* against *Agriotes* larvae were shown by Kabaluk et al. (2005). In their study, 14 *Metarhizium* isolates were tested against larvae of *A. obscurus* and *A. lineatus* and several showed promising biocontrol effects. On average, the *Metarhizium* strains in our study showed a weaker effect on *A. lineatus* larvae compared to the larvae of the other two *Agriotes* species. A lower susceptibility of *A. lineatus* larvae than of *A. obscurus* and *A. sputator* larvae was also reported by Eckard et al. (2014) for two of the three *M. brunneum* strains studied. Moreover, our study showed that the effect of one fungus strain can differ between *Agriotes* species. The different susceptibility of the larvae of various *Agriotes* species was also shown by Ansari et al. (2009) and Eckard et al. (2013, 2014). This is problematic for the development of a plant protection product as a mixed population of *Agriotes* larvae is often found in the field (Lehmhus & Niepold, 2015). Thus, a product based on a single fungal strain will, most likely, not be effective against all *Agriotes* species. However, we found a fungal strain with a broader host range that showed the best activity against two of the three *Agriotes* species studied. The *M. brunneum* strain JKI-BI-1450 showed the best efficacy against larvae of *A. lineatus* and *A. obscurus* and an intermediate efficacy against *A. sputator* larvae. Therefore, this strain is a very promising candidate for

the development of a biologic plant protection product. Furthermore, we have also identified a fungal strain that has a good effect against larvae of *A. sputator*, the *M. robertsii* strain JKI-BI-1442. Therefore, one possible solution would be the development of a biological pesticide based on two or more fungal strains. The two investigated *Cordyceps* strains proved to have no pathogenic effect against the larvae of *A. lineatus*, *A. obscurus* and *A. sputator*, although the strain were isolated from another *Agriotes* species (here: *A. ustulatus*) and many studies have shown that fungi are very effective against the host from which they were isolated (Altre et al., 1999; Chandler, 1992; Pilz et al., 2007). Possibly, the tested *Cordyceps* strains have species-specific virulence against *A. ustulatus*. Further investigations including *A. ustulatus* larvae are necessary to answer this question. Ansari et al. (2009) also reported that *Cordyceps* has no pathogenic effect against *Agriotes* larvae.

The second aim of this study was to identify strain-specific characteristics that influence pathogenicity of the fungal strains to the three major *Agriotes* species. Therefore, the conidial size and the germination of the fungal strains were examined. We could not determine a correlation between conidial size and their virulence for *A. lineatus* and *A. obscurus* but we did for *A. sputator*. The negative correlation showed that shorter and wider spores are more pathogenic against the larvae of *A. sputator*. In the study of the effect of *M. anisopliae* against *Nilaparvata lugens* by Samuels et al. (1989), isolates with small spores were also more pathogenic. In contrast, Altre et al. (1999) found that *Cordyceps fumosorosea* strains with longer conidia were more effective against *Plutella xylostella*. It should be noted that the most effective strains in both of the above-mentioned studies also showed a faster germination of the conidia. These results contrast with those in our study. Here, slower germinating fungal strains as well as those with lower germination rates after 96 h were more pathogenic to these larvae of *A. sputator*. Even though a positive correlation between rapid germination and high virulence of entomopathogenic fungi has often been described (Al-Aidroos & Seifert, 1980; Dillon & Charnley, 1985; Dillon & Charnley, 1990; Samuels et al., 1989), contradictory results were also observed. According to Boucias and Pendland (1984), a slow-germinating isolate of the entomopathogenic fungus *Nomuraea rileyi* showed higher virulence against noctuid caterpillars than faster-germinating isolates. A correlation between the speed of germination and the virulence of the fungal strains against *A. lineatus* and *A. obscurus* could not be determined. These results indicate that conidial size and germination are exclusive criteria for preselecting strains for the control of *A. sputator* larvae, but not for the other two species. This underlines the complexity of the infection process of entomopathogenic fungi. Jackson et al. (1985) reached a similar conclusion for the virulence of 18 isolates of the entomopathogenic fungi *Verticillium lecanii* to the aphid *Macrosiphoniella sanbori*. The relationship between virulence and fast germination, high sporulation rate, absence of extracellular amylase activity, high extracellular chitinase activity and spore size were examined; however, the virulence could not be attributed to any single virulence factor. Also Talaei-Hassanloui et al. (2006) could not establish a correlation between conidial size, germination rate and mycelial growth with virulence of 10 *Beauveria bassiana* isolates against *Plutella xylostella* and *Leptinotarsa decemlineata*. The identification of parameters that could help indicate the efficacy of an entomopathogenic fungus would be very helpful for the development of microbial-based

biocontrol agents. However, our research and the available literature do not reveal such general parameters of effectiveness.

The third aim was to determine whether there are variations in the efficacy of one fungal strain against different populations of one *Agriotes* species. For this purpose, the effect of two *M. brunneum* strains against different populations of the three *Agriotes* species was investigated. A population-dependent efficacy could be confirmed by our experiments for the species *A. obscurus*. RMTL mortality and RMTL mycoses as well as final mortality and final mycoses differed between the populations. For *A. sputator* this could only be confirmed for RTML mortality and RTML mycoses. This means that there is no difference in the final effect, but in the duration until death or mycoses of the larvae. No difference was found in the examination of *A. lineatus*, whereby only two populations could be examined here. Therefore, the hypothesis of a population-dependent effect can be confirmed only for *A. obscurus*, for the other two species, especially *A. lineatus*, further experiments are necessary. The population-dependent effect did not differ essentially between the two *M. brunneum* strains studied. The diverse effects of one entomopathogenic fungal strain against different populations of one pest were already noted by Keller et al. (1999) for the effects of *Beauveria brongniartii* against *Melolontha melolontha*. In this study, the insects from Italy were generally less susceptible compared to insects from Switzerland. A difference in susceptibility between the populations from the two countries studied could not be confirmed in our study. The susceptibility of the populations from Germany did not differ from the populations from Switzerland. Furthermore, younger larvae are often more susceptible to pathogens than older larvae (Butt et al., 2016). However, in our experiments no relationship between larval stage and susceptibility was found.

Our results show that fungi of the genus *Metarhizium* can be effective in controlling wireworms of different *Agriotes* species. Final mortality of the strains tested in this study varied considerably, and ranged from 30 to 94%. None of the strains tested was most effective against all tested species. However, the *M. brunneum* strain JKI-BI-1450 showed less species-specificity and may exhibit a broader host range. This strain showed a population-dependent effect only in the species *A. obscurus* just like the *M. brunneum* strain ART2825. We conclude that these strains are more promising as candidates for an area-wide control of wireworms, and recommend further testing of these strains, especially under field conditions in different areas in Europe or even worldwide.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Authors' contribution

T.S.: Coordinated the experiments, evaluated the data statistically, collected the data, and wrote the paper, Co-authors: L.R. and G.G.: Coordinated the experiment 'Investigation of the efficacy of two *M. brunneum* strains against *Agriotes* larvae from different populations', bred the wireworms and made them available for the experiments and collected the data from treatments with ART2825, M.P. and J.L.: bred the wireworms and made them available for the experiments, R.N.: Collected the data, D.S.: Supervised the study, involved in planning the experiments, involved in writing the paper.

Ethical approval

This research did not involve any studies with human participants or animals (vertebrates).

Consent for publication

All authors consent to publication.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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