Evaluation of Insect Associated and Plant Growth Promoting Fungi in the Control of Cabbage Root Flies

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ABSTRACT *Delia radicum* L. or cabbage maggot is an important pest for Brassicaceous crops. There are currently no registered chemical control agents for its control in Slovenia. Fungal control agents for cabbage maggot were therefore sought among nine rhizosphere-compatible and plant growth-promoting, soil-adapted, and entomopathogenic species to cabbage maggots and were assayed in in vitro and soil laboratory bioassays. In the in vitro tests, the conidial suspensions were applied directly to cabbage maggot eggs. The soil tests mimicked pathways of natural exposure of various insect life stages to the fungal strains. Conidial concentrations used in soil tests were comparable to economic rates for in-furrow application. The following fungi were tested: *Trichoderma atroviride* P. Karst. (2 isolates), *Trichoderma koningiopsis* Samuels, C. Suárez & H.C. Evans (1), *Trichoderma gamsii* Samuels & Druzhin. (3), *Beauveria brongniartii* (Saccardo) Petch (1), *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (2), *Metarhizium robertsii* J.F. Bisch., Rehner & Humber (1), *Metarhizium anisopliae* (Metschn.) Sorokin (4), *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (2), and *Clonostachys solani* f. *nigrovirens* (J.F.H. Beyma) Schroers (2). Abbott's corrected mortality in the in vitro tests ranged from 0.0 ± 18.9 to 47.6 ± 9.0% and in the soil test from 2.4 ± 13.0 to 68.2 ± 21.5%. Seven isolates (*B. bassiana* [isolate 1174], *C. solani* [1828], *M. anisopliae* [1154 and 1868], *T. atroviride* [1872], *T. koningiopsis* [1874], and *T. gamsii* [1876]) caused significant cabbage maggot mortality in either in vitro or soil tests. The importance of fungal ecology as a criterion during the screening of potential biological control agents is discussed.

KEY WORDS biological control, *Delia radicum*, Diptera, entomopathogenic fungi, rhizosphere competence

Brassicaceous crops are attacked by a wide range of pest insects (Klingen et al. 2002). In northern Europe, the cabbage maggots *Delia radicum* L. and *Delia floralis* Fallén are among the most important pests of many brassica crops (Vänninen et al. 1999). In addition to the use of chemical insecticides (Straub 1988, Yildrim and Hoy 2003), many other methods of *Delia* spp. control exist (Finch and Collier 1984, Städler and Schöni 1990, Dosdall et al. 1996, Nawrocka 1996, Dosdall 1999, Yildrim and Hoy 2003). No chemical control agents for cabbage maggot are registered in Slovenia (Phytopharmaceutical Substances 2013). The possibility of controlling *Delia* spp. larvae using entomopathogenic fungi has been reported (Vänninen et al. 1999, Klingen et al. 2002, Bruck et al. 2005), but there appear to have been no reports on biological control of cabbage maggot using fungi other than *Beauveria* spp. and *Metarhizium* spp.

Dipterous insects can be killed by fungi following their ingestion or by infection via external contact (Thomas and Read 2007, Lacey et al. 2009). Both routes require that, to be effective, a biological control agent should live in juxtaposition to either the crop or the insect pest. Because the maggot that mainly affects the host plants is root dwelling (Harris and Svec 1966, Vänninen et al. 1999, Bruck et al. 2005), any fungal biological control agents should be well-adapted to the soil or to the rhizosphere (Harman et al. 2004, Bruck 2005, Meyling and Eilenberg 2007, Verma et al. 2007, St Leger 2008, Pava-Ripoll et al. 2011, Abdul-Wahid and Elbanna 2012). Rhizosphere competence, i.e., the ability to colonize and grow in association with plant roots, is a frequently encountered trait of fungi that can enhance plant growth. Fungal rhizosphere competence is also associated in some cases with saprotrophism and the ability of the plant compatible fungus to parasitize animal pests or other fungi (Harman et al. 2004, Verma et al. 2007).

The aim of the current study was therefore to determine the effects of entomopathogenic fungi and also possible plant growth promoters or soil saprotrophs on soil-dwelling cabbage maggots. We hypothesized that the different ecological preferences of the various fungal species would affect their ability to

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Table 1. List of fungal isolates used in the study

Isolate no.	Genus and species	Host organism or isolated from
1174	*B. bassiana*	Soil
1878	*B. bassiana*	*Melolontha melolontha* L.
1877	*B. brongniartii*	*Me. melolontha*
1828	*C. solani* f. *nigrovirens*	Potato tuber
1860	*C. solani* f. *nigrovirens*	Unidentified insect larva
1704	*M. anisopliae*	*Agriotes* sp. imago
1858	*M. anisopliae*	Soil
1154	*M. anisopliae*	Soil
1868	*M. anisopliae*	*Agriotes* sp. imago
1880	*M. robertsii*	Unknown
1796	*P. lilacinum*	Soil
1797	*P. lilacinum*	Soil
1872	*T. atroviride*	Maize kernels infected by *Fusarium* spp.
1873	*T. atroviride*	Maize kernels infected by *Fusarium* spp.
1874	*T. koningiopsis*	Maize kernels infected by *Fusarium* spp.
1875	*T. gamsii*	Maize kernels infected by *Fusarium* spp.
1876	*T. gamsii*	Maize kernels infected by *Fusarium* spp.
1879	*T. gamsii*	Maize kernels infected by *Fusarium* spp.

All isolates originated from Slovenia.

attack cabbage maggots in different experimental systems.

Materials and Methods

Rearing *D. radicum*. Cabbage maggots were reared according to the protocols described by Harris and Svec (1966). The flies were kept in an environmental chamber at 20 and 18°C at day- and nighttime, respectively, 77 ± 3% relative humidity (RH), and a photoperiod of 18:6 (L:D) h. The flies were fed dry (10 g of glucose, 1 g of soy meal, 10 g of powdered milk, and 1 g of dry baker's yeast) and wet (5 g of honey, 5 g of soy meal, 1 g of fresh brewers' yeast, and 10 ml of water) feed. Fresh tap water was provided in a separate vessel. Larvae were fed rutabaga. Eggs were regularly removed from the egg deposition vessels by flotation, enabling the use of fresh, up to 2-d-old eggs for experiments.

Collection and Growth of Fungi. Eighteen strains of nine entomopathogenic or potentially plant growth promoting fungal species were used for testing. The fungal strains had been isolated from various substrata in Slovenia and were kept in the mycological collection of the Agricultural Institute of Slovenia (Table 1).

All fungal isolates were grown on potato dextrose agar (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), except for *Trichoderma* spp. that were grown on malt extract agar (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to retain more sporulation. Fungal cultures were incubated at 24 ± 1°C in darkness for 14 d. Colony surfaces were scraped with a sterile microscope slide and aerial fungal material, mainly consisting of conidia, was transferred to 50-ml centrifuge tubes in a laminar chamber. Ten milliliters of 0.05% Tween 80 (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was added. The tubes were then vortexed vigorously for 30 s and shaken on an orbital shaker at 650 rpm for 30 min. Suspensions comprised $\approx 10^8$ conidia per milliliter. Conidial viability was assessed by plating 100 μl of 10-fold diluted original suspensions onto malt extract agar. The plates were incubated at 24 ± 1°C. After 24 h, the agar surface was covered with cover slips (20 by 40 mm) and conidial germination rate assessed under a microscope at a magnification of 200×. Conidia with germination tubes at least twice as long as the diameter of the conidium were considered viable. Test suspensions at the desired concentrations were prepared following viability assessment (Vänninen et al. 1999).

In Vitro Tests. In vitro tests were designed to screen the effect of fungal treatments on the survival of cabbage maggot larvae and pupae. Sterile filter paper (9 cm in diameter) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was placed in a petri dish, moistened with 1.5 ml of sterile demineralized water and a 4-mm-thick slice of rutabaga placed on it. Ten cabbage maggot eggs were transferred on top of the rutabaga, using a small piece of sterile filter paper. Fifty microliter of conidium suspension (1×10^8 viable conidia per milliliter) was applied directly to the cabbage maggot eggs. Petri dishes were sealed with two layers of parafilm and incubated at 20°C and 80% RH, without illumination. The insecticide "Marshal 25 CS" (Maag Agro, Dielsdorf, Switzerland), based on carbosulfan (24.5% active ingredient), was used as a positive control, at the recommended concentration of 0.1% (vol:vol). In addition, Naturalis (Andermatt biocontrol AG, Grossdietwil, Switzerland, based on *Beauveria bassiana* ATCC 74040) and Delfin (Andermatt biocontrol AG, Grossdietwil, Switzerland, based on *Bacillus thuringiensis* variety *kurstaki*) were used as reference biocontrol agents, at recommended concentrations of 0.1 and 0.05% (wt:vol), respectively. Tween 80 (0.05%, vol:vol) was used as the negative control. To check the possible impact of decaying rutabaga on the experiment, a "zero" control was made that lacked cabbage maggot eggs. Five replicates per treatment with 10 eggs per individual test vessel were made. The experiment was checked after 7 d to estimate the hatching of eggs, after 14 d to count the living

larvae and to replace rutabaga, and after 35 d to count pupae.

Soil Tests. The soil tests mimicked the natural exposure pathways of the various insect life stages to the fungal isolates. The concentrations used in soil tests were comparable with the economic rates for in-furrow application (Bruck et al. 2005). To 100 g of nonsterile air-dried soil rich in organic matter (60% white peat, 20% garden compost, 15% woody pieces, 5% sand [all percentages vol:vol], and horn shavings; Floragard, Germany), 50 ml of diluted conidial suspension was added to give a concentration of 3.85×10^6 viable conidia per gram of air-dried soil. Fifty microliter of sterile demineralized water was also added to the soil. The soil was thoroughly mixed in a sterile plastic bag to ensure homogenous dispersion of the conidia. Twenty grams of this moist soil and conidia was transferred to each of 300-ml plastic dishes, followed by eggs and rutabaga as described in section In Vitro Tests above. The filter paper was placed upside down, so the eggs were in contact with the soil. The distance between cabbage maggot eggs and the rutabaga piece was ≈4 cm, allowing the hatched first-instar larvae to crawl through the soil to the food (Mukerji 1971). Each dish was closed with a finely perforated lid and incubated in a chamber at 20°C and 80% RH (in the dark). Sterile demineralized water was used as a negative control and 0.1% Marshal 25 CS as a positive control. Four replicates of each fungal or control treatment with 10 eggs per individual test dish were performed. The rutabaga was replaced after 14 d. After 35 d, the experiment was terminated. To determine the number of pupae, each dish was flooded with 200 ml of tap water. The dish contents were retrieved and thoroughly mixed with a spatula; floating pupae were collected with tweezers.

Statistical Evaluation of Data. The numbers of insects identified as healthy at the end of the experiments were incorporated in the data analyses (Vänninen et al. 1999, Davidson and Chandler 2005). The spontaneous or random deaths were accounted for by using Abbott's formula "$100(X - Y)/X$," where X is the percentage of living larvae in the control sample and Y is samples inoculated with fungi (Abbott 1925). Mortality data were corrected throughout by Abbott's formula, unless stated otherwise. The numbers of mycotic cabbage maggot cadavers were not analyzed because it has been shown that the death of *D. radicum* (Vänninen et al. 1999) or wireworms (Furlan et al. 2010) caused by entomopathogenic fungi is not always followed by fungal colonization of the cadaver. Data presented are mean values ±SE. All experiments were repeated at least twice. Statistical analyses were performed with GraphPad Prism 5.00. Data were examined for normality of distribution by the D'Agostino-Pearson omnibus K2 test. The significance of the difference between fungi-treated and control groups was tested by one-way analysis of variance and Bonferonni's multiple comparison post tests (Motulsky 1995). The difference was considered significant at P levels <0.05 and is denoted in Table 2 by an asterisk (*).

Results

Cabbage Maggot Development in the Experimental Systems. The rutabaga pieces in the "zero" control did not rot in the absence of eggs. The degree of hatching of cabbage maggot eggs in the negative control in in vitro tests was 66.5 ± 4.9%. The negative control egg hatching rate in soil tests was not evaluated because the eggs were directly exposed to the soil and could not be recovered. The overall negative control mortality of the in vitro tests without Abbott's correction was 33.9 ± 4.1% after 14 d and 54.1 ± 4.2% after 35 d, not taking into account the egg hatching rate. Thus, 0.4 and 20.6% of hatched larvae died of unknown causes or disappeared in the negative control group before the inspection points at days 14 and 35. The overall negative control mortality of the soil tests without Abbott's correction evaluated at day 35 was 37.4 ± 4.9%.

Effect of the Fungal Isolates on Cabbage Maggot Survival. Mortality in in vitro tests evaluated at day 14 ranged from 8.7 ± 5.0 to −47.6 ± 9.0% and at day 35 from 0.0 ± 18.9 to 43.8 ± 13.6%. The mortality in soil tests ranged from 2.4 ± 13.0 to 68.2 ± 21.5%. One-way analysis of variance showed a significant effect of fungal treatment on cabbage maggot mortality in both in vitro and soil tests ($P < 0.001$). The two fungal isolates causing highest mortality in in vitro tests evaluated after 14 d were *Metarhizium anisopliae* (Metchnikoff) Sorokin (1154) and *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (1174). After 35 d, the best two isolates were *M. anisopliae* (1868 and 1154). In soil tests, the best two isolates were *Clonostachys solani* f. *nigrovirens* (J.F.H. Beyma) Schroers (1828) and *Trichoderma koningiopsis* Samuels, C. Suárez & H.C. Evans (1874). The following fungal isolates caused no significant change in cabbage maggot mortality in in vitro or in soil tests compared with the negative control: *Metarhizium robertsii* J.F. Bisch., Rehner & Humber (1880), *Metarhizium anisopliae* (1704, 1858), *Clonostachys solani* (1860), *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (1796, 1797), *Trichoderma gamsii* Samuels & Druzhin. (1875, 1879), *Trichoderma atroviride* P. Karst. (1873), *B. bassiana* (1878), and *Beauveria brongniartii* (Saccardo) Petch (1877). The chemical insecticide based on Carbosulfan (Marshal 25 CS) achieved 100% mortality in in vitro and soil tests (Table 2). All *Metarhizium* and *Beauveria* isolates sporulated on the cadavers at one or more cabbage maggot life stages (eggs, larvae, pupae, or imago). The highest incidence of sporulating mycelium was observed on cabbage maggot treated with fungal isolates from the genus *Metarhizium.* Isolates 1154, 1868 (both *M. anisopliae*), and 1880 (*M. robertsii*) sporulated on eggs, larvae, pupae, and imagos. No sporulation on imagos was observed in cabbage maggot treated with *M. anisopliae* (1858) and no sporulation on imagos or pupae was observed in isolate 1704 (Table 2).

Comparison of Fungal Performance in Different Experimental Systems. *Trichoderma* spp. gave contrasting results. *T. atroviride* (1872) and *T. gamsii*

Table 2. Mortality of cabbage root fly (*Delia radicum*) treated with different fungal taxa in in vitro tests after 14 or 35 d and in soil tests after 35 d

Taxon and isolate	In vitro tests		Soil test	Sporulation[b]			
	Mortality[a] larvae (%)	Mortality[a] pupae (%)	Mortality[a] pupae (%)	E	L	P	I
B. bassiana 1174	39.68 ± 8.26*	22.87 ± 11.78	31.82 ± 25.03	x	x		
B. bassiana 1878	12.84 ± 9.42	nd	27.19 ± 14.27	x	x		
B. brongniartii 1877	31.23 ± 13.15	nd	12.07 ± 12.73	x		x	
C. solani 1828	15.55 ± 11.76	40.09 ± 15.51	56.27 ± 10.64*				
C. solani 1860	18.68 ± 11.24	14.29 ± 32.73	22.73 ± 23.91				
M. anisopliae 1704	21.00 ± 9.72	37.50 ± 12.50	54.55 ± 21.64	x	x		
M. anisopliae 1858	20.39 ± 7.74	31.25 ± 27.24	36.37 ± 21.64	x	x	x	
M. anisopliae 1154	47.60 ± 9.03*	42.60 ± 15.16*	35.37 ± 11.77*	x	x	x	x
M. anisopliae 1868	31.23 ± 10.32	43.78 ± 13.55*	27.07 ± 11.54	x	x	x	x
M. robertsii 1880	12.84 ± 20.83	nd	24.42 ± 17.20	x	x	x	x
P. lilacinum 1796	14.06 ± 10.60	0.00 ± 18.90	4.55 ± 13.64				
P. lilacinum 1797	24.45 ± 11.76	nd	2.40 ± 12.97				
T. atroviride 1872	38.08 ± 9.94*	0.00 ± 31.25	4.55 ± 26.11				
T. atroviride 1873	8.71 ± 4.98	30.90 ± 7.66	31.21 ± 9.52				
T. koningiopsis 1874	22.12 ± 5.69	35.47 ± 10.84	68.18 ± 21.48*				
T. gamsii 1875	11.47 ± 5.85	19.46 ± 4.28	23.87 ± 11.52				
T. gamsii 1876	32.07 ± 8.51*	25.62 ± 11.08	30.60 ± 10.49				
T. gamsii 1879	20.09 ± 8.34	31.88 ± 11.16	34.74 ± 14.46				
Control	0.00 ± 4.81	0.00 ± 10.72	0.00 ± 6.49				
Naturalis[c]	38.54 ± 7.30*	39.37 ± 12.31*	nd	x			
Delfin[d]	27.07 ± 9.77*	41.77 ± 10.04*	nd				
Marshal[e]	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*				
Statistical parameters	$F_{21, 206} = 10.9$; $P < 0.0001$	$F_{17, 184} = 3.3$; $P < 0.0001$	$F_{19, 161} = 6.7$; $P < 0.0001$				

The data presented are means ± SE in percent (%). nd, not determined.
[a] Abbott's corrected mortality.
[b] Sporulation indicates that sporulating mycelium was observed on eggs (*E*), larvae (*L*), pupae (*P*), or imago (*I*).
[c] Commercial product based on *B. bassiana* strain ATCC 74040, used at a recommended concentration of 0.1% (vol:vol).
[d] Commercial product based on *B. t. kurstaki*, used at a recommended concentration of 0.05% (w/V).
[e] Commercial insecticide based on Carbosulfan, used at a recommended concentration of 0.1% (vol:vol).
* Asterisk denotes a significant difference from negative control samples ($P < 0.05$).

(1876) caused significant mortality in in vitro tests, but not in soil tests, whereas the opposite was true for *T. koningiopsis* (1874). Similarly, contrasting results were also observed for *B. bassiana* (1174): significant mortality was observed in in vitro tests, but not in soil tests. *M. anisopliae* (1154) caused significant cabbage maggot mortality in both kinds of tests. *C. solani* (1828) and *T. koningiopsis* (1874) caused significant mortality only in soil tests (Table 2).

Discussion

The experimental platform developed here enabled us to perform an initial screening (Köhl et al. 2011) of the effects of various fungal isolates on cabbage maggot survival under artificial and seminatural conditions. Five isolates caused significant mortality under in vitro conditions and three in soil tests. The isolate *M. anisopliae* 1154 caused significant mortality in both types of test (Table 2).

The isolates causing highest mortality in in vitro tests caused higher mortality of cabbage maggot than the best performing agents in some previously reported studies. Vänninen et al. (1999) reported highest pathogenicity against cabbage maggot in petri dish tests for *M. anisopliae* and *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm., the former causing 35.5% and the latter 13.3% mortality. The best performing *M. anisopliae* of the current study, strain 1154, caused mortality of 47.6 ± 9.0% (Table 2). Bruck et al. (2005) reported comparable mortalities for two isolates of *M. brunneum* Petch (cited as *M. anisopliae*); however, another isolate, F52, outperformed the isolates tested in our study. The mortality reported for their isolate of *B. bassiana* was comparable with that for our isolate 1174. However, their results are not directly comparable with ours as they used lower concentrations of conidia and a sterile substrate in their bioassays. Chandler and Davidson (2005) achieved up to 92% reduction of emergence of adult cabbage maggot flies in glasshouse experiments. However, they used very high conidial application rates in their drenching experiments (4 by 40 ml of 1 by 10^8 conidia per milliliter per plant). Bruck (2005) also tested *M. anisopliae* strain F52 against the black vine weevil *Otiorhynchus sulcatus* F. and recorded virulence rates of >90%.

The entomopathogenic potential of *C. solani* has not been reported; however, a mortality of 82.5% for *Oncometopia tucumana* Schroder and 45.5% for *Sonesimia grossa* Signoret was caused by *Clonostachys rosea* (Link: Fries) Schroers, Samuels, Seifert, & Gams (Toledo et al. 2006). Our *C. solani* isolate 1828, isolated from a potato tuber, caused significant mortality in soil tests and, remarkably, showed a higher mortality rate than *C. solani* isolate 1860 that originated from an insect host (Table 2). Our results provide evidence for the first time that the soil fungus *C. solani* is pathogenic to cabbage maggot. Also, no data on the direct entomopathogenicity of the *Trichoderma* species used in our study could be found, although *Trichoderma har-*

zianum has been tested against *Oncopeltus fasciatus* Dallas, showing mortality of up to 100% (Santamarina et al. 2002) against aphids (Ganassi et al. 2000; mortality >60%), and cockroaches (Abdul-Wahid and Elbanna 2012; mortality 10–100%). *P. lilacinum* has been reported to be a good candidate for the biological control of pests of stored maize. Ahmed (2010) reported its significant pathogenicity against the maize weevil *Sitophilus zeamais* Motschulsky. Barra et al. (2012) tested 20 strains of *P. lilacinum* against *Tribolium confusum* Jacquelin du Val and *Rhyzopertha dominica* F. The results of our study indicate that isolates 1796 and 1797, however, do not have a high potential as biological control agents for cabbage maggot.

The most effective *M. anisopliae* isolate (1154) performed better in soil tests than our best *B. bassiana* isolate (1174). The former caused significant mortality in in vitro and soil tests. The latter, however, caused significant mortality only in in vitro tests (Table 2). *Beauveria* spp. and *Metarhizium* spp. are poor competitors for organic resources compared with the opportunistic saprophytic fungi that are common in soils. It was postulated that they probably lie in wait in the soil as conidia, waiting to infect a new host (Meyling and Eilenberg 2007). The natural background levels of *M. anisopliae* and *B. bassiana* in soil have been reported to be 1,040 and 830 CFU/g soil, respectively (Scheepmaker and Butt 2010). While these concentrations are in the same range, *M. anisopliae* may become activated through root exudates (Bruck 2005, Meyling and Eilenberg 2007, St Leger 2008, Pava-Ripoll et al. 2011), may persist on decaying organic matter in the soil (Hu and St Leger 2002) and is not adversely affected by soil cultivation (Vänninen 1996). Our results are in accordance with the hypothesis proposed by Meyling and Eilenberg (2007) that *Metarhizium* sp. is better adapted to a soil environment than *B. bassiana.*

The genus *Clonostachys* comprises ≈50 species, but only one, *C. rosea,* has been cited as being active against insects (Toledo et al. 2006). Little is known about *C. solani,* although it has been isolated from a broad diversity of substrata including soil, potato, bark and twigs, galleries of the ambrosia beetle *Platypus cylindrus* F., and fungal sclerotia (Schroers 2001). In accordance with our hypothesis, *C. solani* isolate 1828 caused significant mortality of cabbage maggot in soil test but not in an in vitro test. Moreover, the only other fungus to cause significant mortality in soil tests was *T. koningiopsis* (1874; Table 2), which also occurs in soils, mainly in tropical but also in temperate regions (Samuels et al. 2006).

The protocols developed in the in vitro and soil tests were based on those of Vänninen et al. (1999) and Bruck et al. (2005). The former used laboratory-bred second- and third-stage larvae and the latter field-collected larvae. We aimed at improving them by using laboratory-bred cabbage maggot eggs and by infecting the eggs directly or by exposing the eggs or neonate larvae indirectly to fungal conidia incorporated in the test substrate. This approach has several advantages. The organisms used are of similar age, are in the same developmental stage, and comprise single species only. Assuming that the cabbage maggot eggs are the first target of a fungal biological control agent, we included this development stage in the experiments. However, a drawback of our approach is that not all larvae could be recovered, probably because of death of eggs or early larval stages and their decomposition before the inspection point. This was especially evident in the soil tests. The fungal insect-damaging effects were therefore assessed indirectly by counting the number of living individuals (larvae or pupae) and correcting the mortalities obtained by Abbott's formula (Abbott 1925) that addresses natural or unexplained mortality in the control groups. In addition, Abbott's corrected mortality can be readily compared with the results from further repetitions or other experiments. The formula has also been used in other, similar studies (Vänninen et al. 1999, Davidson and Chandler 2005, Furlan et al. 2010).

The negative control mortalities without Abbott's correction were lower than those reported by Vänninen et al. (1999). Also, negative control mortalities without Abbott's correction were lower in the soil tests (37.4 ± 4.9%) than in the in vitro tests (54.1 ± 4.2%), evaluated after 35 d, as was expected from a soil-dwelling maggot. When the cabbage maggot egg hatching rate was taken into consideration for calculating the negative control mortality in the in vitro experiments, 0.4 and 20.6% of the successfully hatched larvae died of unknown causes or disappeared before the inspections at days 14 and 35, respectively. This indicates that the soil tests would represent the preferred environment for assessing cabbage maggot pupal mortality. The percentage of unexplained mortality could not be determined in soil tests because the eggs or egg shells were not recoverable at the time of observation.

In conclusion, we have shown that select fungi not generally known as entomopathogenic species, may be novel biological control candidates against cabbage maggot. Our hypothesis that soil saprotrophs or rhizosphere-competent fungi could be promising biological control agents against cabbage maggot is supported by experimental results. *C. solani* (isolate 1828) and *T. koningiopsis* (isolate 1874) were more effective in soil than in in vitro systems. Similarly, most of our *Metarhizium* isolates outperformed the *Beauveria* isolates in soil tests, providing additional evidence that the former are better adapted to the soil environment. This emphasizes the importance of adopting meaningful criteria for selecting biological control agents. Interestingly, most programs using *M. anisopliae* for biocontrol have ignored habitat preferences and survival outside the host (Hu and St Leger 2002). Our results suggest that rhizosphere competence and soil dwelling traits, rather than direct entomopathogenicity, are more important factors in determining the success of a fungal biological control agent against soil pests.

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