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Insect-associated fungi in soils of field crops and orchards

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ABSTRACT

Insect-pathogenic fungi are important natural enemies of insect pests but their dynamics in the soils of different agroecosystems are incompletely understood. In the present study, the seasonal occurrence and species diversity of insect-associated fungi in soil were investigated by baiting soil samples with larvae of *Galleria mellonella*. The survey included insect-pathogenic fungi, opportunistic insect pathogens, and secondary colonizers of insect cadavers. Soil samples from two habitat types (field crops and orchards) were collected from 2004 to 2005 at 2-month intervals except during wintertime. A total of 29 species were isolated and identified, with 25 species in field crop soils and 20 species in orchard soils. Although the common insect-pathogenic species, such as *Beauveria bassiana*, *Metarhizium anisopliae* var. *anisopliae*, and *Paecilomyces fumosoroseus* (Ascomycota: Hypocreales), were detected in both field crop and orchard soils, their frequency in the two agroecosystems differed significantly: *B. bassiana* and *P. fumosoroseus* were more frequently detected in orchard soils, while *M. anisopliae* var. *anisopliae* was more frequently detected in field crop soils. The diversity of insect-pathogenic fungi was greater in field crop soil than in orchard soil. The number of *Galleria* larvae that died from fungal infection was larger in orchard soil than in field crop soil. *B. bassiana* was the most abundant species in orchard soil, and the number of larvae killed by *B. bassiana* was larger in June and December than in April and August. However, *Mucor* spp. (opportunistic pathogens) were the most abundant species in field crop soils, and the number of larvae killed by them was larger in August than at any other time of the year.

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1. Introduction

Fungal diseases of insects are common and widespread and often contribute to the natural regulation of insect populations (Samson et al., 1988; Hajek and St. Leger, 1994). Soil plays an important role as a reservoir of insect-pathogenic fungi, and several species of insect-pathogenic fungi are frequently recorded in cultivated soil worldwide. Of these, *Beauveria* spp., *Metarhizium anisopliae* var. *anisopliae* (Metschn.) Sorokīn, and *Paecilomyces* spp. are the most common (Keller and Zimmerman, 1989; Klingen and Haukeland, 2006). However, other fungal species, including opportunistic pathogens as well as secondary colonizers, can also greatly affect insect population dynamics (Gunde-Cimerman et al., 1998; Ali-Shtayeh et al., 2002). Our previous study showed that some of these other species were highly pathogenic to insects (Sun and Liu, 2008). The fungi, which were isolated from bait insects exposed to soil, were grouped into three categories according to their life history and association with insects. These three groups were: insect-pathogenic fungi, opportunistic pathogenic fungi, and secondary colonizers. Our previous study also showed that both insect-pathogenic fungi and opportunistic pathogenic fungi frequently occurred in unmanaged soils in China (Sun and Liu, 2008).

Human activities and cultivation regimes influence the occurrence and abundance of soil-borne natural enemies of insects in agroecosystems (Klingen et al., 2002; Klingen and Haukeland, 2006). Knowledge of the species composition and distribution of indigenous insect-pathogenic fungi is essential for assessing the biocontrol potential of these fungi in a specific agroecosystem. Much work has been done on the occurrence of soil-dwelling insect-pathogenic fungi in different countries (Mietkiewski et al., 1991; Chandler et al., 1997; Ali-Shtayeh et al., 2002; Klingen et al., 2002; Keller et al., 2003; Meyling and Eilenberg, 2006). Most previous studies concerning the occurrence and diversity of insect-pathogenic fungi in soil have focused on the differences in species composition between areas defined by habitat types (e.g., cultivated soils, natural soils, etc.) at one point in time. However, Meyling and Eilenberg (2006) compared the occurrence and abundance of insect-pathogenic fungi between two consecutive years in a single organically farmed field and found that high and low densities of the fungi occurred within specific areas. Although research has demonstrated that insect-pathogenic nematodes (Campbell et al., 1995; Glazer et al., 1996; Půža and Mráček, 2005) and nematode-trapping fungi (Miao and Liu, 2003) have clear seasonal population

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fluctuations in soil, to our knowledge no study has focused on the fluctuation of insect-pathogenic fungi in soil among different seasons.

In the present study, the diversity and temporal distribution of insect-associated fungi in agricultural soils from China were investigated for the first time. The study included soil from both heavily cultivated crop fields as well as less cultivated orchards. The occurrence and diversity were determined using the '*Galleria* bait method' (Zimmermann, 1986).

2. Materials and methods

2.1. Collection of soil samples

Sampling was conducted in four districts within the Beijing suburb: Changping (CP), Daxing (DX), Fangshan (FS), and Haidian (HD). Beijing is located on north longitude 39°28′ to 41°5′ and east latitude 115°25′ to 117°30′ and has a half-humid monsoonal type climate in the North Temperate Zone. In each district, four to nine sampling sites (about 50 m^2 per site) were chosen, and soils were collected from April to December at two-month intervals (Table 1).

At each sampling time, at least 20 soil cores from each site were collected with a cylindrical soil core borer (2.0 cm diameter, 5–20 cm deep) along two zigzag paths. The cores were mixed to form one sample, which was placed in a plastic bag and stored at 4 °C before further processing.

Table 1
Description of sampling sites

Area	Sampling plot code	Description	Habitat type
Changping	CP-1	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	CP-2	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	CP-3	Soybean field, with inorganic fertilization	Crop
	CP-4	Corn fields, with inorganic fertilization	Crop
	CP-5	Corn fields, with inorganic fertilization	Crop
	CP-6	Corn fields, with inorganic fertilization	Crop
	CP-7	Peach orchard, with organic and inorganic fertilization	Orchard
	CP-8	Apple orchard, with organic and inorganic fertilization	Orchard
	CP-9	Apple orchard, with organic and inorganic fertilization	Orchard
Daxing	DX-1	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	DX-2	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	DX-3	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	DX-4	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
Fangshan	FS-1	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	FS-2	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	FS-3	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	FS-4	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
	FS-5	Peach orchard, with organic and inorganic fertilization, intercropped with peanut	Orchard
	FS-6	Winter wheat/summer maize rotation, with inorganic fertilization	Crop
Haidian	HD-1	Peach orchard, with organic and inorganic fertilization	Orchard
	HD-2	Peach orchard, with organic and inorganic fertilization	Orchard
	HD-3	Peach orchard, with organic and inorganic fertilization	Orchard
	HD-4	Peach orchard, with organic and inorganic fertilization	Orchard

2.2. Isolation and identification of fungi

Insect-associated fungi were isolated from soil samples using the '*Galleria* bait method' within 1 week. Each soil sample was mixed thoroughly after it was passed through 2-mm pore sieve to remove plant tissues and molding gravels or blocks. If dry, the soil samples were moistened with sterile-distilled water. The soil sample from each site and date, which was moistened with sterile-distilled water if dry, was added to three autoclaved 50-ml centrifuge tubes, leaving 1 cm of free air at the top.

The wax moth, *Galleria mellonella* L. (Lepidopteria: Pyralidae), was reared in constant darkness at 28 °C. Third or fourth instar larvae (approximately 30 days after egg hatching) were used as baits. Fifteen larvae were placed on the soil surface in each tube, and the tubes were incubated at room temperature (20–25 °C) for 2 weeks. During the first 4 days, the tubes were upended twice each day to keep the larvae moving in the soil. The larvae were examined 7 and 14 days after placement in the tubes. Dead larvae were counted, surface sterilized with 3% sodium hypochlorite for 3 min, and then rinsed twice with sterile-distilled water. After free water was removed from the surfaces of the dead larvae, they were placed on potato dextrose agar plates containing 0.1 g/L streptomycin and 0.05 g/L tetracycline. The plates were maintained in the dark at 25 °C.

After the fungi formed colonies on the plates, they were identified mainly based on morphological characteristics with the aid of several taxonomic keys (Domsch et al., 1980; Nelson et al., 1983; Samson et al., 1988). For those isolates that sporulated poorly, the internal transcribed spacer (ITS) region of ribosomal DNA was amplified and sequenced following the procedure of White et al. (1990). ITS sequences were then subjected to BLAST searches against the GenBank database. Results from BLAST searches were used to guide further morphological examination and identification.

2.3. Data analysis

The frequency of occurrence of fungi was determined as the percentage of tubes (samples) from which a particular fungus was isolated. The population density of the fungi was inferred from the number of dead larvae (of 15 total larvae per tube) from which a particular fungus was isolated. Total larval mortality was simply the number of dead larvae per tube, and mortality was assumed to be caused by the fungus that was isolated from the dead larva. Mortality was always considered to be caused by fungi because fungi were always isolated from the dead larvae; only one species of fungus was isolated from each dead larva.

Frequency of occurrence of insect-associated fungi was compared in field crop soil and orchard soil by standard χ^2 tests. One-way analysis of variance (ANOVA) was used to test the differences in population density of the common species in different seasons; the data were square-root transformed before analysis. Student–Newman–Keuls test was used to compare all pairs of means following one-way ANOVA, and a $P < 0.05$ was considered statistically significant. The statistical software SPSS 15.0 was used for the analysis (SPSS Inc., Chiago, IL, USA).

3. Results

3.1. Occurrence of insect-associated fungi in field crop soils and orchard soils

In total, 2567 *G. mellonella* larvae died (out of 10,350) during the experiment, and from them 29 insect-associated fungal species

belonging to 18 genera were identified (Table 2). Among them, 25 species occurred in field crop soils and 20 species in orchard soils (Table 2). Species that are not well-known insect-pathogenic fungi but that have been reported on insects were considered as opportunistic pathogenic fungi. Species for which there was no information about their pathogenicity to insects were grouped into secondary colonizers. Thus, the fungi isolated from the two kinds of agricultural soils were grouped into insect-pathogenic fungi, opportunistic pathogenic fungi, and secondary colonizers (Table 2). While isolation of a fungus from a dead insect is not proof that the fungus killed the insect, we have assumed that the isolated fungi were responsible for the mortality.

The most commonly found insect-pathogenic fungi in both habitat types were *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* (Zimm.) Zare & W. Gams, *M. anisopliae* var. *anisopliae*, and *Paecilomyces fumosoroseus* (Wize) Brown & Smith. The frequencies of occurrence of those fungi (except for *L. lecanii*) were significantly different in field crop soil vs. orchard soil (Table 2). *B. bassiana* and *P. fumosoroseus* were more common in orchard soil than in field crop soil, while *M. anisopliae* var. *anisopliae* was more common in field crop soil than in orchard soil. *Nomuraea rileyi* (Farl.) Samson was infrequently detected and only in field crop soil, and *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm. was also infrequently detected and only in orchard soil.

Species of *Aspergillus*, *Fusarium*, *Mucor*, and *Penicillium* were the most common opportunistic pathogenic fungi (Table 2). When the species were analyzed individually, *Fusarium solani* (Mart.) Sacc., *Fusarium oxysporum* Schltdl., and *Mucor* spp. were the most common species encountered. The frequencies of these opportunistic species did not significantly differ between agroecosystems (Table 2). Other opportunistic pathogens with low frequency were *Absidia* sp., *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, and *Mortierella* sp.

Most species of secondary colonizers were isolated with low frequency from the soil collected from the two habitat types (Table 2). The common species of secondary colonizers were *Chaetomium globosum* Kunze, *Clonostachys rosea f. rosea* (Link) Schroers, Samuels, Seifert & W. Gams, *Rhizopus oryzae* Went & Prins. Geerl, *Talaromyces flavus* (Klöcker) Stolk & Samson, and *Trichoderma harzianum* Rifai. The frequencies of these species were not significantly different between agroecosystems, except that *T. flavus* occurred more frequently in field crop soils than in orchard soils. Four species detected in field crop soils were not detected in orchard soils. All species of secondary colonizers detected in orchard soil were also detected in field crop soil except an ascomycetous yeast, *Williopsis satumus*, which did not occur in field crop soil but occurred in orchard soil with low frequency.

3.2. *Number of* G. mellonella *larvae that died of fungal infection in field crop soil vs. orchard soil (averaged over all dates)*

Significantly more larvae died of fungal infections in soil from orchards than from field crops ($\chi^2 = 51.07$; d.f. $= 1$; $P < 0.001$), indicating higher number of insect-associated fungi in orchard soil. The average number of *G. mellonella* that died of infection in each sample of 15 larvae was 2.56 ± 0.88 in field crop soil and 5.53 ± 2.02 in orchard soil.

Table 2
Occurrence of insect-associated fungi in crop field and orchard soils

Fungal species	Occurrence[a] (%)		χ^2	P[b]	Reported on insect by other authors
	Crop field	Orchard			
Insect-pathogenic fungi					
Beauveria bassiana	27.4	86.3	61.88	**<0.001**	Yes, several
Lecanicillium lecanii	0.7	2.8	0.31	0.577	Yes, several
Metarhizium anisopliae var. *anisopliae*	60.0	26.4	21.24	**<0.001**	Yes, several
Nomuraea rileyi	0.7	–	–	–	Yes, several
Paecilomyces farinosus	–	2.8	–	–	Yes, several
Paecilomyces fumosoroseus	15.6	37.5	12.70	**<0.001**	Yes, several
Opportunistic pathogenic fungi					
Absidia sp.	0.7	–	–	–	Yes, Ali-Shtayeh et al., 2002
Aspergillus flavus	5.2	5.6	0.05	0.832	Yes, Domsch et al., 1980; Gunde-Cimerman et al., 1998
Cladosporium cladosporioides	–	1.4	–	–	Yes, Gunde-Cimerman et al., 1998
Clonostachys rosea f. rosea	9.6	13.9	0.86	0.353	Yes, Toledo et al., 2006
Fusarium avenaceum	0.7	4.2	1.38	0.240	Yes, Li, 1988
Fusarium chlamydosporum	4.4	2.8	0.05	0.831	Yes, Bai and Chen, 1991
Fusarium oxysporum	33.3	40.3	0.99	0.321	Yes, Ali-Shtayeh et al., 2002
Fusarium solani	38.5	33.3	0.88	0.349	Yes, Ali-Shtayeh et al., 2002
Mortierella sp.	0.7	–	–	–	Yes, Gunde-Cimerman et al., 1998
Mucor spp.	70.4	59.7	2.40	0.122	Yes, Gunde-Cimerman et al., 1998; Ali-Shtayeh et al., 2002
Penicillium chrysogenum	1.5	–	–	–	Yes, Gunde-Cimerman et al., 1998
Penicillium thomii	0.7	–	–	–	Yes, Gunde-Cimerman et al., 1998
Penicillium spp.	3.0	9.7	3.03	0.082	Yes, Gunde-Cimerman et al., 1998
Secondary colonizer					
Chaetomium globosum	2.2	6.9	0.74	0.390	No, only present study
Fusarium aqueductum	0.7	–	–	–	No, only present study
Fusarium proliferatum	–	1.4	–	–	No, only present study
Fusarium subglutinans	0.7	–	–	–	No, only present study
Phialophora phaeophora	0.7	–	–	–	No, only present study
Rhizopus oryzae	3.7	1.4	0.26	0.610	No, only present study
Talaromyces flavus	14.8	5.6	3.93	**0.047**	No, only present study
Trichoderma album	0.7	–	–	–	No, only present study
Trichoderma harzianum	4.4	6.9	0.19	0.661	No, only present study
Williopsis satumus	–	1.4	–	–	No, only present study

[a] Occurrence (%) = number of samples with a specific fungal species × 100/total number of soil samples (for crop field, total number of soil samples = 135; for orchard, total number of soil samples = 72).
[b] Bold values indicate that the occurrences the species were significantly different between the two kinds of soils.

3.2.1. Fluctuation of the number of larvae that died of fungal infection in field crop soil

Mucor spp. caused the most larval mortality in field crop soil (Table 3). Other species commonly isolated from dead larvae in the field crop soils were *B. bassiana*, *M. anisopliae* var. *anisopliae*, *P. fumosoroseus*, *F. oxysporum*, *F. solani*, and *T. flavus* (Table 3). More larvae were killed by all fungi in October and December and than in April. More larvae were killed by *Mucor* spp. in June and August and than in April and December, indicating an obvious effect of temperature. Larval mortality caused by *M. anisopliae* var. *anisopliae* and *F. solani* did not significantly differ among sampling times. Fluctuations in mortality caused by *B. bassiana*, *P. fumosoroseus*, *T. flavus*, and other species were not analyzed because their occurrences were too low for a reliable analysis. Larval mortality caused by *F. oxysporum* in field crop soil fluctuated greatly between seasons and years (Table 3).

3.2.2. Number of G. mellonella larvae that died of fungal infection in orchard soil

Species that caused the most larval mortality in orchard soil were *B. bassiana*, *M. anisopliae* var. *anisopliae*, *P. fumosoroseus*, *F. oxysporum*, *F. solani*, *Mucor* spp., and *C. rosea f. rosea* (Table 4). Larval mortality was greater in June and December than in April and August. Larval mortality caused by *B. bassiana* in orchard soil significantly differed among sampling times (Table 4). As was the case for mortality caused by all fungi, mortality caused by *B. bassiana* was higher in June and December than in April and August. Mortality caused by *F. oxysporum*, *F. solani*, and *Mucor* spp. did not significantly differ among sampling times. Changes in mortality caused by *M. anisopliae* var. *anisopliae*, *P. fumosoroseus*, *C. rosea f. rosea*, and other species were not analyzed because of their low occurrence.

4. Discussion

Most insect-pathogenic fungi found in China in the present study have been reported from other parts of the world (Vänninen et al., 1989; Vänninen, 1995; Bidochka et al., 1998; Meyling and Eilenberg, 2006). The predominance of *M. anisopliae* var. *anisopliae* in cultivated habitats and *B. bassiana* in natural habitats has been reported in Europe (Mietkiewski et al., 1991; Stenzel, 1992; Vänninen, 1995; Klingen et al., 2002), America (Bidochka et al., 1998), and Australia (Rath et al., 1992). The result of high frequency of *M. anisopliae* var. *anisopliae* in crop field soil and *B. bassiana* in orchard soil in present study provided more evidence for the ecological characteristics of these two fungi.

P. fumosoroseus has been detected most commonly in hedgerow soils and rarely in the agricultural soil (Vänninen, 1995; Chandler et al., 1997; Meyling and Eilenberg, 2006), although Mietkiewski et al. (1991) found the fungus frequently in soils of rye fields in Poland. In the present study, *P. fumosoroseus* was found in both soils of field and orchard but more frequent in orchard soil. The higher frequency of occurrence in orchard soil again suggests that this fungus is adapted to more natural, less-disturbed habitats. *P. farinosus*, which was reported to be common in agricultural soils in the earlier studies (Vänninen, 1995; Chandler et al., 1997; Meyling and Eilenberg, 2006), was seldom isolated in this study. Considering that *P. farinosus* was among the most frequently isolated species in natural soils in China (Sun and Liu, 2008), we infer that this fungus is sensitive to the influence of human activities. *N. rileyi*, a common species on soybean field pests (Ferron, 1978), was isolated from field crop soil by bait insects for the first time in our study.

Vänninen (1995) found that insect-pathogenic fungi were more abundant in forest soil than in intensively cultivated soil in Finland. The present study similarly demonstrated that insect-associated

Table 3
Mean number of Galleria larvae that died from infections of fungi during the baiting of soil samples collected in crop fields (test larvae number = 15)

Fungal species	2004				2005				2006			
	June[a]	August	October	December	April	June	August	October	December	April	F	P
All fungi	–	–	2.64 ± 0.71 abc[c]	2.98 ± 0.82 abc	1.58 ± 0.40 ab	2.38 ± 0.44 abc	2.89 ± 0.41 bc	3.80 ± 0.45 c	3.16 ± 0.42 bc	1.04 ± 0.20 a	3.667	**0.001**
Beauveria bassiana	–	0.20 ± 0.12	0.13 ± 0.07	1.47 ± 0.91	0.04 ± 0.03	0.29 ± 0.16	0.000	1.51 ± 0.41	0.36 ± 0.18	0.000	–	–
Metarhizium anisopliae var. *anisopliae*	0.87 ± 0.31	2.42 ± 0.56[b]	0.51 ± 0.24	0.51 ± 0.17	0.31 ± 0.09	0.64 ± 0.24	0.49 ± 0.12	0.40 ± 0.13	0.78 ± 0.36	0.29 ± 0.08	0.707	0.685
Paecilomyces fumosoroseus	–	0.11 ± 0.05	0.000	0.04 ± 0.03	0.000	0.09 ± 0.05	0.07 ± 0.05	0.11 ± 0.05	0.09 ± 0.05	0.07 ± 0.04	–	–
Fusarium oxysporum	–	0.62 ± 0.22	0.24 ± 0.12 ab	0.11 ± 0.07 a	0.22 ± 0.09 ab	0.04 ± 0.03 a	0.09 ± 0.06 a	0.53 ± 0.11 bc	0.71 ± 0.19 c	0.02 ± 0.02 a	6.039	**<0.001**
Fusarium solani	–	0.69 ± 0.22	0.42 ± 0.18	0.18 ± 0.12	0.24 ± 0.14	0.20 ± 0.10	0.47 ± 0.20	0.22 ± 0.11	0.42 ± 0.12	0.18 ± 0.07	0.865	0.537
Mucor spp.	–	2.98 ± 0.71	1.22 ± 0.35 ab	0.49 ± 0.13 ab	0.47 ± 0.11 ab	0.87 ± 0.27 ab	1.42 ± 0.37 b	0.71 ± 0.21 ab	0.60 ± 0.15 ab	0.29 ± 0.08 a	2.603	**0.016**
Talaromyces flavus	–	0.000	0.04 ± 0.04	0.16 ± 0.07	0.11 ± 0.06	0.11 ± 0.06	0.07 ± 0.05	0.20 ± 0.16	0.07 ± 0.05	0.07 ± 0.04	–	–

[a] During the baiting and isolation of samples collected in June 2004, only the *Metarhizium anisopliae* var. *anisopliae* was recorded.
[b] When baiting the samples collected in August 2004, the larvae that produced webbing were peeled out and placed back to the tubes, and the data were not included in the ANOVA.
[c] Values within the same row followed by different letters are significantly different at the $\alpha = 0.05$ level.

Table 4
Mean number of Galleria larvae that died from infections of fungi during the baiting of soil samples collected in orchards (test larvae number = 15)

Fungal species	2004				2005				2006		F	P
	June[a]	August	October	December	April	June	August	October	December	April		
All fungi	–	4.25 ± 1.25 ab	5.08 ± 1.18 ab	6.63 ± 1.38 ab	2.29 ± 0.74 a	6.46 ± 1.26 ab	3.46 ± 0.56 ab	6.92 ± 0.72 b	8.54 ± 1.23 b	4.83 ± 1.59 ab	2.909	**0.008**
Beauveria bassiana	4.17 ± 1.03 ab[b]	1.58 ± 0.68 ab	2.96 ± 1.19 ab	3.79 ± 1.32 ab	0.88 ± 0.40 a	2.88 ± 0.70 ab	1.50 ± 0.53 ab	4.21 ± 0.82 ab	5.75 ± 1.47 b	3.17 ± 1.56 ab	2.189	**0.033**
Metarhizium anisopliae var. anisopliae	–	0.38 ± 0.33	0.46 ± 0.46	0.33 ± 0.24	0.21 ± 0.13	1.83 ± 1.60	0.17 ± 0.17	0.83 ± 0.83	0.42 ± 0.42	0.17 ± 0.17	–	–
Paecilomyces fumosoroseus	–	0.17 ± 0.09	0.21 ± 0.11	0.33 ± 0.21	0.08 ± 0.05	0.13 ± 0.09	0.38 ± 0.17	0.38 ± 0.13	0.42 ± 0.25	0.08 ± 0.05	–	–
Fusarium oxysporum	–	0.58 ± 0.29	0.17 ± 0.13	0.33 ± 0.22	0.50 ± 0.35	0.42 ± 0.28	0.25 ± 0.12	0.29 ± 0.13	0.71 ± 0.27	0.17 ± 0.17	0.660	0.724
Fusarium solani	–	0.25 ± 0.14	0.71 ± 0.47	0.17 ± 0.13	0.000	0.71 ± 0.36	0.42 ± 0.21	0.13 ± 0.09	0.08 ± 0.08	0.71 ± 0.22	1.625	0.136
Mucor spp.	–	0.71 ± 0.25	0.46 ± 0.23	1.17 ± 0.42	0.42 ± 0.21	0.42 ± 0.20	0.54 ± 0.27	0.83 ± 0.27	0.67 ± 0.26	0.25 ± 0.18	1.101	0.374
Clonostachys rosea f. rosea	–	0.04 ± 0.04	0.000	0.08 ± 0.08	0.04 ± 0.04	0.000	0.08 ± 0.05	0.04 ± 0.04	0.04 ± 0.04	0.13 ± 0.06	–	–

[a] During the baiting and isolation of samples collected in June 2004, only the *Beauveria bassiana* was recorded.
[b] Values within the same row followed by different letters are significantly different at the $\alpha = 0.05$ level.

fungi were more abundant in the less-disturbed orchard soil than in frequently cultivated field crop soil. In our study, however, the diversity of insect-associated fungi, especially opportunistic pathogens and secondary colonizers, was greater in field crop soil than in orchard soil. The greater diversity but lower abundance of insect-associated fungi in field crop soil may be due to the tillage regimes in arable soil, which result in disturbance of soil fungal communities. Such disturbance may disrupt community structure, as well as change substrate availability and the physical environment (White and Pickett, 1985). Repeated ploughing, reseeding, and fertilizing may increase the diversity of insect-associated fungi by increasing environmental patchiness and niche availability. Such agricultural practices may prevent the build-up of high populations of insect-pathogenic fungi by disrupting infection foci, exposing pathogens to adverse environmental conditions on the surface of soil, or burying them away from potential hosts (Chandler et al., 1997). With repeated disturbance, the fast growing species, and especially opportunistic species and secondary colonizers, might increase more quickly than pathogenic species.

M. anisopliae var. *anisopliae* was considered to have a wide tolerance to agricultural chemicals and mechanical disturbance (Vänninen, 1995). Latch and Fallon (1976) reported that the conidia of *M. anisopliae* var. *anisopliae* could persist for a long time in soil in the absence of host insects. However, Keller and Zimmerman (1989) found that *M. anisopliae* var. *anisopliae* was a poor competitive saprophyte in soil. In our study, a stable but low frequency of larvae infected by *M. anisopliae* var. *anisopliae* was detected. This finding is consistent with the idea that the fungus is persistent but was less competitive in soil. Although *M. anisopliae* var. *anisopliae* occurred less frequently in orchard soil than in field crop soil, its frequency was similar in intercropped orchard soil (e.g., FS-5) and in field crop soil (data not shown). This indicates that this fungus could be enhanced in the orchard by intercrop.

Temperature is one of the most important environmental factors affecting density of fungi, such as *B. bassiana* in soil (Lingg and Donaldson, 1981; Keller and Zimmerman, 1989; Bing and Lewis, 1993). The optimum temperature for development and activity of the fungus was about 24 °C (Studdert and Kaya, 1990); high temperature (above 27 °C) inhibited mycelial growth and killed the spores (Aregger-Zavadil, 1992; Kessler et al., 2003). Data in the present study were consistent with these earlier findings in that mortality caused by *B. bassiana* increased from April to June as the soil temperature was warming but decreased in August when the temperature was apparently too high for growth and infection. After August, soil temperatures declined and the number of larvae killed by *B. bassiana* increased again. However, there was an abrupt decrease in mortality caused by *B. bassiana* between December and the next April. This indicated that the low soil temperatures (<0 °C) in December could have damaged the fungus or at least greatly inhibited its activity.

The colonization, multiplication, and activity of insect-associated fungi in different ecosystems are incompletely understood. The present study demonstrated that the abundance of insect-associated fungi in soil differs greatly in two agroecosystems. This was especially true for two fungi with great potential for providing biocontrol of insect pests: *M. anisopliae* and *B. bassiana*, and the results provide essential information for effectively using *M. anisopliae* and *B. bassiana* for management of insect pests. We suggest that *M. anisopliae* should be applied to cultivated agricultural fields rather than to less-disturbed habitats and that frequent supplemental application of *M. anisopliae* may be needed. In contrast, we suggest that *B. bassiana* be applied to relatively undisturbed habitats such as orchards and forests, and that application in June will maximize the survival the fungus and improve the biological control effect.

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