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Occurrence of entomopathogenic fungi in soils in Southern Italy*

ABSTRACT

The natural occurrence of entomopathogenic fungi was investigated in Southern Italian soils using larvae of *Galleria mellonella* L. (Lepidoptera: Galleriidae) as "bait insect". Since September (1996) to March (1997) 188 samples of soil were collected from different habitats (woodland, orchard, field, seacoast, grassland, uncultivated land and salt pan). Parasitic fungi were obtained from 14.9% of the soil samples. There were 3 entomopathogenic species: *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Paecilomyces lilacinus* (Thom) Samson and *Beauveria bassiana* (Balsamo) Vuillemin. The most common fungal pathogen was *B. bassiana.* Both *M. anisopliae* and *P. lilacinus* were isolated only once. The occurrence of *B. bassiana* seems to be affected by the soil type and the habitat.

Key words: survey, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus.*

INTRODUCTION

Entomopathogenic fungi, mainly Hyphomycetes and Ascomycetes, were regularly found infecting insects in soil. The Hyphomycete, *Metarhizium anisopliae* (Metchnikoff) Sorokin, is probably the best known of these species. However, practically nothing is known about the occurrence and importance of these pathogens in soils in Southern Europe. Especially, no data are available as regards to the soil inhabiting entomopathogenic fungi in Southern Italy.

In the framework of a research program originally undertaken, at the University of Bari, on soil-inhabiting entomopathogenic nematodes (TARASCO & TRIGGIANI, 1997) an extensive survey in five regions in Southern Italy was carried out. Soil samples were taken from various locations and nematodes

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* Supported by Murst 60%

were isolated using *Galleria mellonella* L. (Lepidoptera: Galleriidae) as "bait insect". This method also allowed us to isolate entomopathogenic fungi. The aim of this paper is to present the first corresponding results.

MATERIAL AND METHODS

A total of 188 soil samples taken during September 1996 - March 1997 from 63 sites in Southern Italy (in the vicinity of Apulia, Molise, Basilicata, Campania and Calabria Regions) were tested for the presence of insect pathogenic fungi (fig. 1).

Fig. 1 - Sampling locations for soil inhabiting entomopathogenic fungi in Southern Italy. 1) Campania; 2) Molise; 3) Apulia; 4) Basilicata; 5) Calabria.

In order to sample ecologically different habitats such as fields, orchards, sea coasts, salt pans, uncultivated lands, woodlands and grasslands, random soil samples were taken.

Final instars of *G. mellonella* were used as bait insects for the surveys. Approximately 2 kg soil were collected for each sample by pooling 3-4 subsamples taken at depths of 10-20 cm from an area of about 50 m^2.

Isolation of entomopathogenic fungi was attemped using the "*Galleria* bait method", initially developed by BEDDING and AKHURST (1975) for trapping insect parasitic nematodes and later adapted by ZIMMERMANN (1986), slightly modified. The procedure was as follows: the soil was transported in sterile plastic bags to the laboratory and six *G. mellonella* larvae were placed in a

long-handled tea infuser in the middle of each sample. Afterwards the samples were moistened and incubated at 25°C for 7-10 days; if the larvae did not die after 2 weeks, the sample was tested again with *G. mellonella* larvae. Infected wax moth larvae from each sample were surface-sterilized by keeping them for 3 min. in 1% sodium hypochlorite and rinsing them in distilled water. After this, the larvae were incubated at 25°C in Petri dishes with moistened filter paper till the presence of pathogens could be assessed.

When sporulating structures appeared on the cadaver, attempts to isolate the fungus were made by transferring spores to potato dextrose agar in Petri dishes. Inoculated Petri dishes were then checked every day and the tubes with pure culture were subcultured in potato dextrose agar medium. Cultures were then stored at 8°C.

For each sampling location, texture, pH, content of organic matter and organic carbon of the soil were recorded. The % organic carbon and % organic content of soil were tested by the Walkley and Black method. The soil structure was determined by texture. The soils with *Beauveria bassiana* (Balsamo) Vuillemin were tested for % NaCl.

RESULTS

Fungi were isolated from 28 soil samples (14.9% occurrence), on a total of 188 (fig. 2); more than 10 species were isolated belonging to 7 genera (tab. 1).

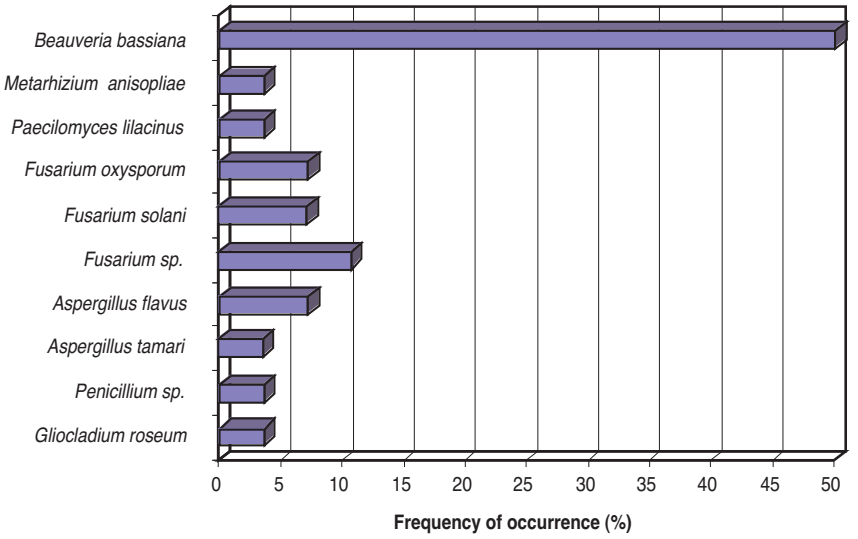

Fig. 2 - Frequency of occurrence of fungal species isolated from Southern Italian soils.

Tab. 1 - Entomopathogenic fungi isolated from various Southern Italian soils: characteristics of the sampled places, time, characteristics of the soil tested.

Fungi	Locality	Altitude (m a.s.l.)	Time	Habitat	Soil texture	pH	Org. cont.%	Org. carb.%
Beauveria bassiana	Gravina (BA)	350	September 1996	Uncultivated land	Clay	8.0	2.83	1.64
Beauveria bassiana	Rapolla (PZ)	540	October 1996	Orchard	Loamy sand	7.6	2.01	1.17
Beauveria bassiana	Mariotto (BA)	120	September 1996	Orchard	Silt loam	7.7	3.46	1.43
Beauveria bassiana	Turi (BA)	270	October 1996	Uncultivated land	Loamy sand	7.5	1.39	0.81
Beauveria bassiana	Cerignola (FG)	120	October 1996	Field	Silt loam	7.4	2.54	1.48
Beauveria bassiana	Grassano (MT)	300	October 1996	Woodland	Clay	7.5	1.40	0.81
Beauveria bassiana	Matera (MT)	400	September 1996	Woodland	Clay	8.0	1.43	0.83
Beauveria bassiana	Salandra (MT)	300	October 1996	Uncultivated land	Silt loam	7.7	1.32	0.77
Beauveria bassiana	Margherita di Savoia (FG)	30	October 1996	Salt pan	Silt loam	7.8	1.98	1.15
Beauveria bassiana	Acquaviva delle Fonti (BA)	350	October 1996	Field	Silt loam	7.1	3.79	2.20
Beauveria bassiana	Lucera (FG)	70	October 1996	Uncultivated land	Clay	7.9	2.94	1.71
Beauveria bassiana	Altamura (BA)	475	October 1996	Uncultivated land	Silt loam	8.0	4.94	2.87
Beauveria bassiana	Margherita di Savoia (FG)	30	October 1996	Uncultivated land	Sand	7.9	2.30	1.34
Beauveria bassiana	Margherita di Savoia (FG)	30	October 1996	Uncultivated land	Sand	7.9	2.30	1.34
Metarhizium anisopliae	Castellaneta (TA)	50	September 1996	Field	Clay	7.4	2.03	1.18
Paecilomyces lilacinus	Minervino Murge (BA)	450	October 1996	Field	Silt loam	7.8	3.85	2.24
Aspergillus flavus	Bitonto (BA)	120	September 1996	Orchard	Sand	7.5	1.81	1.05
Aspergillus flavus	Margherita di Savoia (FG)	30	October 1996	Uncultivated land	Sand	7.9	2.30	1.34
Aspergillus tamari	Castellaneta (TA)	0	September 1996	Sea coast	Sand	7.4	3.47	2.02
Fusarium solani	Matera (MT)	400	September 1996	Orchard	Loamy sand	7.9	2.12	1.23
Fusarium solani	Matera (MT)	400	September 1996	Orchard	Loamy sand	7.9	2.12	1.23
Fusarium oxysporum	Gravina (BA)	350	September 1996	Field	Clay	7.6	1.33	0.77
Fusarium oxysporum	Gravina (BA)	350	September 1996	Field	Clay	7.6	1.33	0.77
Fusarium sp.	Rapolla (PZ)	540	October 1996	Orchard	Loamy sand	7.6	2.01	1.17
Fusarium sp.	Picciano (MT)	400	September 1996	Woodland	Loamy sand	7.6	1.58	0.92
Fusarium sp.	Altamura (BA)	475	October 1996	Uncultivated land	Silt loam	8.0	4.94	2.87
Penicillium sp.	Margherita di Savoia (FG)	30	October 1996	Uncultivated land	Sand	7.9	2.30	1.34
Gliocladium roseum	Minervino Murge (BA)	450	October 1996	Field	Silt loam	7.8	3.85	2.24

Fungi were collected from 16 of 63 locations (25.9%, fig. 3) and the isolation succeeded in September-October 1996 while soil samples were taken from September 1996 to March 1997.

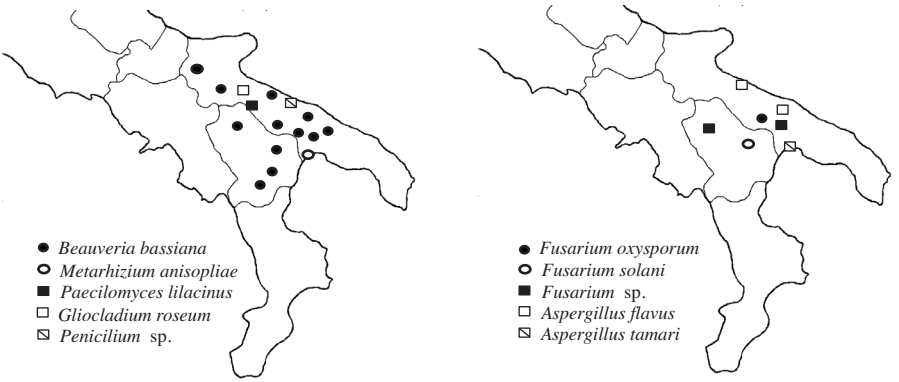

Fig. 3 - Occurrence of fungal species isolated in Southern Italy.

Fungi were found to be distributed from 0 to 540 m a.s.l. and were collected in all habitats. The pH ranged between 7.1 and 8.0 and organic content between 1.32 and 4.94%.

The frequency for the occurrence of fungal species in the different biotopes was as follows: 35.7% in uncultivated land, 21.4% orchard, 25% field, 10.7% woodland, 3.5% sea coast and 3.5% salt pan. No fungi were found in grasslands (fig. 4).

Three entomopathogenic species, *B. bassiana, M. anisopliae* and *Paecilomyces lilacinus* (Thom) Samson were isolated in little more than the half of positive samples, *B. bassiana* being the most numerous (isolated from 14 sites, 7.5%, the half of positive samples). Other fungi were isolated, among them several *Fusarium* strains - *F. oxysporum* Schlechtend, *F. solani* (Mart.) Saccardo and *Fusarium* sp., 3 *Aspergillus* strains (2 *A. flavus* Link and 1 *A. tamari* Kita), 1 *Penicillium* sp. and 1 *Gliocladium roseum* Bainier. They are common in soils and have no entomopathogenic potential. A few of the strains were morphologically atypic, which explains why in some cases the identification at the species level was not achieved (sp).

B. bassiana was isolated from soil under orchards, uncultivated lands, fields, woodlands and salt pans. This entomopathogen was most commonly isolated from loam soils (loamy sand and silt loam soils) rather than from

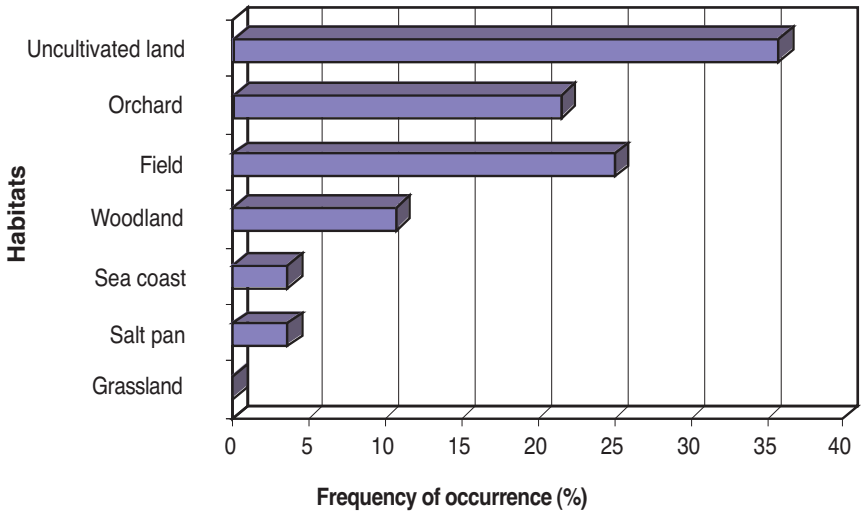

Fig. 4 - Frequency of occurrence of fungi in different habitats in Southern Italy.

sandy or clay soils. Furthermore *B. bassiana* occurred in soils with different pH (ranging from 7.1 to 8.0) and organic content (ranging from 1.32 to 4.94). *B. bassiana* was isolated once in a soil of salt pan border where the presence of Cl^- was 3,550 mg/100 g while it was 0.25 mg/g in uncultivated land 0.04 mg/g in woodland and 0.09 mg/g in sandy beach.

M. anisopliae was only found once in a maize field with clay soil, as the less known *P. lilacinus,* found in a wheat field with silt loam soil.

Simultaneous presence of a fungus and an insect parasitic nematode was observed in 2 cases, in different larvae of the same sample: from salt pan border, near Margherita di Savoia, we collected *B. bassiana* and *Steinernema anomali* (Kozodoi, 1984), and from maize field, near Castellaneta Marina, we collected *M. anisopliae* and *Heterorhabditis bacteriophora* Poinar, 1976. In both cases there was no antagonism between the pathogens but each one developed on different larvae.

No simultaneous presence of 2 fungal species was observed.

DISCUSSION

Owing to the “insect bait method”, the occurrence of entomopathogenic fungi in soils in Southern Italy was demonstrated for the first time. Our results

showed clearly the ability of entomopathogenic fungi to survive in conditions (high temperatures, dryness) which are not generally considered as suitable for fungi.

Entomopathogenic fungi are known in Italy only from observations on cadavers or insects showing obvious signs of disease (ARZONE & OZINO-MARLETTO, 1984; OZINO, 1989; NANNI *et al*., 1988; PELAGATTI *et al*., 1988, 1993; TRIGGIANI, 1984; TRIGGIANI, 1986; TRIGGIANI & LIPA, 1989; TRIGGIANI, 1992/93; TRIGGIANI *et al*., 1992/93) except for one report about the occurrence of *Beauveria brongniartii* (Sacc.) Petch, an efficient natural control agent of *Melolontha melolontha* L. (Coleoptera: Scarabaeidae), in the soil of Valle d'Aosta, Northwest Italy (CRAVANZOLA *et al*., 1996).

In Southern Italian soils, *B. bassiana* appeared as the most common species, suggesting that it is favoured by soil and, especially, climatic conditions. *M. anisopliae* and *P. lilacinus* were found more rarely. This is the first time that *P. lilacinus* was isolated from soils using "insect bait method". The situation in soils from Southern Italy appeared therefore different from that observed in Northern Europe. In Germany *M. anisopliae* appeared more abundant (KLEESPIES *et al*., 1989). This species, togheter with *B. bassiana* and *Paecilomyces farinosus* (Dickson & Fries) Brown & Smith, were isolated at about the same frequency in Finland (VÄNNINEN *et al*., 1989). *M. anisopliae* and *Paecilomyces fumosoroseus* (Wize) Brown & Smith were the dominant species in Polish soils (MIĘTKIEWSKI *et al*., 1991, 1991/92, 1995, 1996).

The three entomopathogenic species found in soils in Southern Italy, especially *B. bassiana*, are potential natural enemies of local soil insects or local insects which have in soil at least a developmental stage. They, therefore, deserve further investigations.

In our study we observed that *B. bassiana* was most commonly isolated from loam soils than sandy or clay soils and that the soil type may affect the distribution of the fungal species within a particular locality, but occurrence was not correlated to soil pH or organic content. These data agree with the results obtained in Finland by VÄNNINEN *et al.* (1989): *B. bassiana* and *P. farinosus* were most common from loam soils while *M. anisopliae* was most commonly isolated from clay soils, although occurrence was not correlated to soil pH, conductivity or organic content. According to Polish studies, *M. anisopliae* as well as *P. fumosoroseus* seemed to dominate in sandy soils (MIĘTKIEWSKI *et al*., 1991, 1991/92; MIĘTKIEWSKI & MIĘTKIEWSKA, 1993; MIĘTKIEWSKI & KOLCZAREK, 1995; TKACZUK & MIĘTKIEWSKI, 1996).

In Tasmania, RATH *et al.* (1992) observed that *M. anisopliae* was more common from loam soils than from clay but occurrence was not related to pH,

conductivity, moisture, rainfall, altitude or tempererture.

As for the possible influence of the type of crop, further studies are needed. However, in a locality of Bulgaria, Mięłkiewski and Ignatowicz (1995) demonstrated that *M. anisopliae* was the dominant entomopathogenic species in sugar beet soil, whereas *P. fumosoroseus* dominated in corn soil.

In Germany Kleespies *et al.* (1989) investigated on the natural occurrence of entomopathogenic fungi and nematodes in Darmstadt area, and observed clear differences among different biotopes; they isolated more fungi and nematodes in the soil samples of organic fields and orchards. Regarding the association between habitat and *B. bassiana* occurrence, we found this pathogen most commonly in uncultivated land (wild vegetation) than in the other biotopes.

Extremely interesting is the presence of *B. bassiana* in the soil of salt pan borders with 3,550 mg of Cl^- /100 g of soil.

Survival characteristics, temperature requirements, etc. of the strains isolated in Southern Italy should be compared to strains originated from ecoclimatically different regions. More precisely, the strains of *B. bassiana* should be compared to strains which, in northern part of the country, are studied due to their potential in the control of larvae of *Parectopa robiniella* Clemens (Lepidoptera: Gracillariidae) (Ozino *et al.*, 1990).

More generally, these strains could be compared to strains of other species studied in Italian conditions, i.e. *Verticillium lecanii* (Zimmermann) Viégas isolated from cadavers of *Ziginydia pullula* Boh. (Homoptera: Auchenorrhyncha: Typhlocybinae) (Ozino & Zeppa, 1989) and *B. brongnartii* isolated from dead larvae of *M. melolontha* (Piatti *et al.*, 1995; Cravanzola *et al.*, 1996).

RIASSUNTO

Funghi entomopatogeni nei terreni dell'Italia meridionale

È stata effettuata un'indagine per valutare la presenza e la distribuzione di funghi entomopatogeni nei terreni dell'Italia meridionale utilizzando larve di *Galleria mellonella* L. (Lepidoptera: Galleriidae) come insetto esca. Da settembre 1996 a marzo 1997, sono stati esaminati 188 campioni di terreno provenienti da diversi biotopi. Sono state rinvenute larve infettate da funghi in 28 campioni (14,9%) e sono state identificate 10 specie di funghi parassiti di cui 3 propriamente entomopatogeni: *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Paecilomyces lilacinus* (Thom) Samson e *Beauveria bassiana* (Balsamo) Vuillemin. Il fungo patogeno rinvenuto con maggior frequenza è risultato essere *B. bassiana*. La sua presenza sembra correlata alle caratteristiche del suolo e dell'habitat.

Parole chiave: indagine, *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus*.

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