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Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils

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ABSTRACT

Factors affecting the occurrence and distribution of entomopathogenic fungi in 244 soil samples collected from natural and cultivated areas in Spain were studied using an integrated approach based on univariate and multivariate analyses. Entomopathogenic fungi were isolated from 175 of the 244 (71.7 %) soil samples, with only two species found, *Beauveria bassiana* and *Metarhizium anisopliae*. Of the 244 soil samples, 104 yielded *B. bassiana* (42.6 %), 18 yielded *M. anisopliae* (7.3 %), and 53 soil samples (21.7 %) harboured both fungi. Log-linear models indicated no significant effect of habitat on the occurrence of *B. bassiana*, but a strong association between *M. anisopliae* and soils from cultivated habitats, particularly field crops. Also, irrespective of habitat type, *B. bassiana* predominated over *M. anisopliae* in soils with a higher clay content, higher pH, and lower organic matter content. Logistic regression analyses showed that pH and clay content were predictive variables for the occurrence of *B. bassiana*, whereas organic matter content was the predictive variable for *M. anisopliae*. Also, latitude and longitude predicted the occurrence of these same species, but in opposite directions. Altitude was found to be predictive for the occurrence of *B. bassiana*. Using principal component analysis, four factors (1 to 4) accounted for 86 % of the total variance; 32.8, 22.9, 19.6 and 10.4 % of the cumulative variance explained, respectively. Factor 1 was associated with high positive weights for soil clay and silt content and high negative weights for soil sand content. Factor 2 was associated with high positive weights for soil organic matter content and high negative weights for soil pH. Factor 3 was associated with high positive weights for latitude and longitude of the sampled localities and factor 4, had high positive weights only for altitude. Bi-plot displays representing soil samples were developed for different factor combinations and indicated that, irrespective of geographical location, absence of both fungal species was determined by alkaline sandy soils with low organic matter content, whereas heaviness of soil texture, acidity and increasing organic matter content led to progressively higher percentages of samples harbouring entomopathogenic fungi. These results could aid decision-making as to whether or not a particular cultivated or natural soil is suitable for using entomopathogenic fungi as a pest control measure and for selecting the fungal species best suited to a particular soil.

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Introduction

Soil-inhabiting entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems and play a key role in regulating insect populations, particularly soil-dwelling insect pests (Keller & Zimmerman 1989; Jackson *et al.* 2000). Many species belonging to the *Hypocreales* (*Ascomycota*) inhabit the soil for a significant part of their life cycle when they are outside of their insect host. Among them, *Beauveria* spp., *Metarhizium anisopliae*, and *Paecilomyces* spp. are especially common (Keller & Zimmerman 1989). Isolation of indigenous entomopathogenic fungi is essential to provide an insight into naturally occurring fungal biodiversity and to provide a pool of potential biological control agents to be conserved or inundatively released into the agroecosystem for pest-control purposes. Currently, fungal biological control agents frequently perform inconsistently in the soil due to a lack of environmental competence (Jackson *et al.* 2000). An understanding of the parameters that determine the diversity and distribution of entomopathogenic fungal species in the soil would help to identify those species best suited to a particular environment and improve biological control efficacy. The effects of factors such as geographical location, climatic conditions, habitat type, cropping system, and soil properties on the occurrence and distribution of insect pathogenic fungi have been studied by several authors (e.g. Rath *et al.* 1992; Steenberg 1995; Tkaczuk & Mietkiewski 1996; Vänninen 1996; Chandler *et al.* 1997; Tarasco *et al.* 1997; Bidochka *et al.* 1998; Klingen *et al.* 2002; Asensio *et al.* 2003; Keller *et al.* 2003; Bruck 2004; Meyling & Eilenberg 2006). However, these studies evaluated the effects of only one or a few of the variables listed above. Although a description of the effect of a single variable on the occurrence of entomopathogenic fungi in the soil can give significant and useful ecological and agronomical information (Maranhao & Santiago-Álvarez 2003; Santiago-Álvarez *et al.* 2005), there may be relationships among the different variables that have to be elucidated to adequately understand the ecology of soil-inhabiting entomopathogenic fungi. Methods for the analysis of multivariate data in ecology are becoming increasingly important as ecologists often need to test hypotheses concerning the effects of experimental treatments on whole assemblages of species at once. Multivariate analyses provide the statistical methods to describe the complex relationships amongst variables. Because several variables can be considered simultaneously, interpretations can be made that are not possible with univariate statistics (James & McCulloch 1990). Here we use both univariate and multivariate analyses in an integrated approach to evaluate several variables affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils in Spain, and by using logistic regression to predict the occurrence of a particular fungus for given site characteristics. These factors include geographical location and altitude, habitat type (natural or cultivated), sub-habitat type (cropping system in cultivated soils or type of pristine natural habitat) and soil composition.

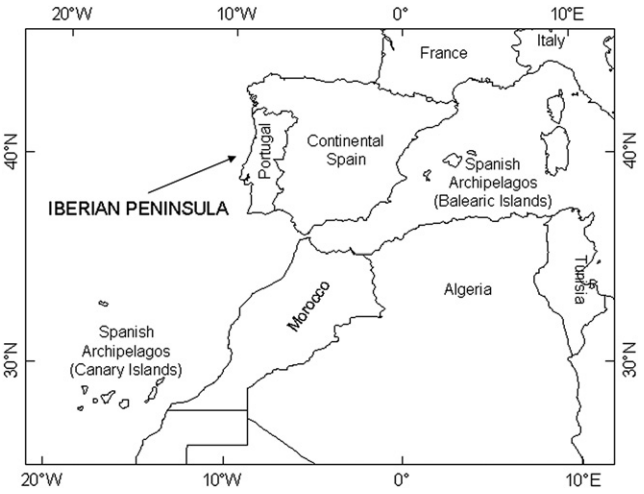

Fig 1 – Map of the sampling area: the Spanish territory, Iberian Peninsula and the Canary and Balearic archipelagos.

Materials and methods

Soil samples

Two hundred and forty-four soil samples were collected from different geographical sites distributed throughout the Continental area of Spain (Iberian Peninsula) and the Canary and the Balearic Archipelagos from March 2001 to March 2006 (Fig 1). The locations and altitudes of the sampled soils were recorded using global positioning system (GPS) equipment (Trimble, Sunnyvale, CA; Table 1). There were 127 samples from cultivated habitats [42 samples from fruit crops (olive and stone-fruit crops)] and 85 from field crops (horticultural crops, cereals crops, leguminous crops and sunflower) and 117 samples from pristine natural habitats [76 samples from natural forests, 28 from pastures and 13 from other habitats (such as river banks and desert areas)] (Table 1). Soil samples were collected with a garden spade to a depth of 20 cm after removal of surface litter. At every site, five 500 g soil samples were collected from five randomly selected points from an area of 50 m^2, placed in clear plastic bags (35 × 25 cm), sealed with a rubber band, and returned to the laboratory. The five samples were combined to form a single sample for each site, mixed thoroughly, sieved through a 2 mm mesh and stored at 4 °C for no longer than 5 d before further processing. Soils were then spread on a tray and kept open until moisture was equilibrated with that of the laboratory in order to avoid entomopathogenic nematode infestation. For each sample, soil pH was measured in water at a 1:2.5 solution ratio (Thoma 1989). Organic matter was determined by dichromate oxidation (Walkley & Black 1934) and particle soil distribution (sand, silt, and clay content) was determined using the pipette method (Gee & Bauder 1986).

Isolation of entomopathogenic fungi

Entomopathogenic fungi were isolated from soil using the *Galleria* bait method (Zimmerman 1986). *Galleria mellonella* were

Table 1 – Geographical location of soil sampling sites, habitat type, and soils properties

Sample	Geographical location					Habitat		Soil factors				
	Locality	Province	Latitude	Longitude	Altitude (m)	Habitat	Subhabitat	pH (in h20)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
1	Cazorla	Jaén	37.9105	−3.0017	768	Natural	Forest	8.06	2.62	27.4	33.5	39.1
2	Andújar	Jaén	38.0367	−4.0544	212	Cultivated	Field crop	7.88	1.22	16.9	32.1	51.0
3	Úbeda	Jaén	38.0081	−3.3685	735	Natural	Forest	8.16	1.08	19.2	37.4	43.4
4	Jaén	Jaén	37.7657	−3.7895	572	Cultivated	Fruit crop	8.26	1.15	27.5	40	32.5
5	Guadix	Granada	37.3004	−3.1346	949	Natural	Forest	8.08	1.87	59.0	30.4	10.6
6	Purchena	Almería	37.3465	−2.3602	555	Natural	Forest	8.05	2.66	77.6	16.8	5.6
7	Abla	Almería	37.1417	−2.7792	861	Cultivated	Fruit crop	7.75	3.84	56.5	34.1	9.4
8	Sierra de los Filabres	Almería	37.1841	−2.4394	960	Natural	Others	8.72	0.72	70.2	14.7	15.1
9	Carboneras	Almería	36.9966	−1.8948	10	Natural	Others	8.68	2.55	52.9	32.5	14.6
10	Cuevas del Amanzora	Almería	37.2971	−1.8815	88	Natural	Forest	8.04	0.68	15.0	57.7	27.3
11	Montilla	Córdoba	37.5896	−4.6383	371	Cultivated	Fruit crop	8.14	1.87	48.3	24.4	27.3
12	San Andrés (El Hierro)	Tenerife	27.7667	−17.9500	1047	Natural	Pasture	5.45	2.23	73.2	25.7	1.1
13	Jinama (El Hierro)	Tenerife	27.7667	−18.0000	79	Natural	Pasture	5.44	3.35	66.0	32.9	1.1
14	Castuera - La Serena	Badajoz	38.7226	−5.5455	478	Natural	Pasture	5.48	2.26	55.8	32.3	11.9
15	Cabeza del Buey	Badajoz	38.7214	−5.2199	515	Natural	Pasture	5.82	5.48	60.9	28.8	10.3
16	Aguamansa (Tenerife)	Tenerife	28.3639	−16.5012	1120	Natural	Pasture	5.44	18.18	61.5	36.1	2.4
17	La Laguneta (Tenerife)	Tenerife	28.4156	−16.4032	1419	Natural	Pasture	4.94	11.17	48.8	45	6.2
18	Córdoba (CIFA)	Córdoba	37.8863	−4.7769	110	Cultivated	Fruit crop	8.15	2.18	42.4	41.7	15.9
19	Baena	Córdoba	37.6143	−4.3265	405	Cultivated	Fruit crop	8.00	2.11	50.5	35.5	14.0
20	Alcalá la Real	Jaén	37.4636	−3.9251	918	Cultivated	Fruit crop	8.23	2.00	60.2	28.7	11.1
21	Granada	Granada	37.1764	−3.5980	738	Natural	Forest	7.80	3.67	64.1	25.5	10.4
22	Mezquitilla	Málaga	36.7446	−4.0402	97	Natural	Forest	8.12	3.13	78.9	14.7	6.4
23	Herrera (a)	Sevilla	37.3617	−4.8500	254	Cultivated	Field crop	8.39	2.29	30.1	33.8	36.1
24	Herrera (b)	Sevilla	37.3617	−4.8500	254	Cultivated	Fruit crop	8.38	1.27	59.6	15.1	25.3
25	Pozoblanco	Córdoba	38.3774	−4.8484	654	Natural	Pasture	7.97	2.33	60.8	18.2	21.0
26	Espiel	Córdoba	38.1886	−5.0188	548	Natural	Forest	5.80	0.62	16.9	40	43.1
27	Antequera	Málaga	37.0194	−4.5629	511	Cultivated	Field crop	8.18	1.67	26.0	37.8	36.2
28	Los Villares	Córdoba	37.9404	−4.8165	562	Natural	Forest	6.57	9.45	68.5	24.1	7.4
29	Sevilla	Sevilla	37.3905	−5.9980	7	Natural	Forest	7.94	1.20	90.2	1.9	7.9
30	Cerro Perea	Sevilla	37.5893	−4.9826	230	Cultivated	Field crop	8.04	1.31	27.8	40.9	31.3
31	Tabernas	Almería	37.0528	−2.3871	400	Natural	Others	8.45	1.42	73.1	22.8	4.1
32	Níjar	Almería	36.9627	−2.2068	356	Cultivated	Field crop	8.33	3.13	63.8	26	10.2
33	Veléz Rubio	Almería	37.6478	−2.0743	847	Cultivated	Field crop	8.73	0.36	84.1	11.5	4.4
34	El Ejido	Almería	36.775	−2.8127	80	Cultivated	Field crop	8.47	1.64	77.9	13.5	8.6
35	Priego de Córdoba	Córdoba	37.4389	−4.1948	652	Cultivated	Field crop	8.12	1.30	48.2	27.6	24.2
36	Pinos Puente	Granada	37.2515	−3.7493	576	Cultivated	Field crop	8.17	1.66	16.0	43	41.0
37	San Roque	Cádiz	36.2097	−5.3846	109	Natural	Pasture	7.07	4.84	82.8	13.2	4.0
38	La Victoria	Córdoba	37.6812	−4.8529	262	Cultivated	Field crop	8.39	1.16	31.8	34.8	33.4
39	Carcabuey - Subética	Córdoba	37.4436	−4.2735	642	Natural	Forest	8.26	0.94	54.9	28.3	16.8
40	Puente Genil	Córdoba	37.3905	−4.7705	216	Cultivated	Fruit crop	8.22	1.66	44.9	35	20.1
41	Cabra - Subética	Córdoba	37.4744	−4.4259	452	Natural	Forest	9.31	2.06	40.7	41	18.3

(*continued on next page*)

Table 1 – (*continued*)

Sample	Geographical location					Habitat		Soil factors				
	Locality	Province	Latitude	Longitude	Altitude (m)	Habitat	Subhabitat	pH (in h20)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
42	Monturque	Córdoba	37.4733	−4.5817	395	Cultivated	Fruit crop	8.23	2.02	26.5	36.3	37.2
43	Fernan Nuñez	Córdoba	37.6719	−4.7239	322	Cultivated	Field crop	8.03	1.95	45.1	36.4	18.5
44	Santaella	Córdoba	37.5663	−4.8448	238	Natural	Forest	8.25	1.37	30.5	41.7	27.8
45	Rute	Córdoba	37.3259	−4.3713	635	Natural	Forest	8.11	2.96	33.5	45.2	21.3
46	Campillos	Málaga	37.045	−4.8614	496	Cultivated	Field crop	8.29	1.16	34.6	28	37.4
47	Ronda	Málaga	36.742	−5.1664	723	Natural	Forest	6.31	3.65	60.9	18.6	20.5
48	El Bosque	Cádiz	36.7474	−5.5070	298	Cultivated	Fruit crop	7.97	7.73	49.6	33.1	17.3
49	Villavueva de Algaidas	Málaga	37.1855	−4.4501	536	Cultivated	Fruit crop	8.42	0.87	83.0	7.2	9.8
50	Colmenar	Málaga	36.9066	−4.3356	671	Cultivated	Fruit crop	7.95	5.4	54.3	28.3	17.4
51	Velez Málaga	Málaga	36.7786	−4.1007	60	Natural	Forest	8.40	0.76	17.6	41.9	40.5
52	Torremolinos	Málaga	36.6219	−4.5000	49	Natural	Pasture	8.22	2.11	43.3	39	17.7
53	Ojen	Málaga	36.5642	−4.8565	335	Natural	Forest	8.16	0.52	84.3	15.6	0.1
54	Tolox	Málaga	36.6875	−4.9047	285	Cultivated	Fruit crop	8.15	1.97	13.4	35.9	50.7
55	Coín	Málaga	36.6598	−4.7522	202	Cultivated	Fruit crop	8.15	0.59	56.2	23.1	20.7
56	Ardales	Málaga	36.878	−4.8465	445	Cultivated	Fruit crop	8.18	1.63	6.4	42.1	51.5
57	Alora	Málaga	36.8248	−4.7027	222	Natural	Forest	8.24	0.31	30.0	28.2	41.8
58	Montoro	Córdoba	38.0262	−4.3819	195	Natural	Forest	7.88	1.90	58.9	35.3	5.8
59	El Carpio	Córdoba	37.9405	−4.4988	138	Cultivated	Field crop	8.02	2.25	45.5	33.7	20.8
60	Martos	Jaén	37.7228	−3.9663	740	Cultivated	Field crop	8.25	1.32	25.3	44.9	29.8
61	Santisteban del Puerto	Jaén	38.2473	−3.2063	675	Cultivated	Fruit crop	8.21	3.53	26.5	38.5	35.0
62	Jodar	Jaén	37.844	−3.3526	647	Natural	Forest	8.12	2.66	31.5	41.3	27.2
63	Jabalquinto	Jaén	38.0193	−3.7240	496	Natural	Pasture	6.97	5.06	66.2	23.1	10.7
64	Cabra de Santo Cristo	Jaén	37.7051	−3.2860	942	Cultivated	Fruit crop	7.66	1.23	30.8	28.7	40.5
65	La Carolina	Jaén	38.3742	−3.3600	595	Natural	Pasture	6.01	3.15	71.6	20.7	7.7
66	Blanquillo	Jaén	38.0412	−2.4727	1608	Natural	Forest	8.04	1.57	42.9	28	29.1
67	Santo Tomé	Jaén	38.0282	−3.1019	454	Cultivated	Fruit crop	7.99	1.30	9.4	41.1	49.5
68	Arroyo del Ojanco	Jaén	38.3209	−2.8950	540	Cultivated	Fruit crop	8.17	1.74	11.7	36.8	51.5
69	Genave	Jaén	38.4301	−2.7328	823	Natural	Forest	8.04	0.58	64.6	25.1	10.3
70	Quesada	Jaén	37.8451	−3.0676	676	Natural	Forest	8.10	1.68	28.6	35.1	36.3
71	Virgen de la Cabeza	Jaén	38.1781	−4.0381	628	Natural	Forest	7.85	3.35	49.7	26.9	23.4
72	Lopera	Jaén	37.9436	−4.2149	276	Cultivated	Fruit crop	8.17	1.92	42.8	26.8	30.4
73	Carchelejo	Jaén	37.6338	−3.6408	810	Natural	Forest	8.21	1.95	39.1	34.8	26.1
74	Belalcazar	Córdoba	38.5784	−5.1671	488	Natural	Pasture	5.29	1.27	41.7	34.5	23.8
75	Hinojosa del Duque	Córdoba	38.501	−5.1483	542	Cultivated	Field crop	7.31	2.36	59.9	15.4	24.7
76	Villanueva del Duque	Córdoba	38.3914	−5.0000	585	Cultivated	Field crop	5.93	9.16	55.6	32.9	11.5
77	Villaharta	Córdoba	38.1395	−4.9031	580	Natural	Forest	6.92	0.62	78.4	9.7	11.9
78	Castro del Río	Córdoba	37.6903	−4.4810	227	Natural	Forest	8.27	1.43	18.4	39.3	42.3
79	Huéscar	Granada	37.8097	−2.5404	953	Natural	Forest	7.99	3.84	57.2	38.2	4.6
80	Montefrío	Granada	37.3208	−4.0114	834	Natural	Forest	8.12	0.74	12.9	37.9	49.2
81	Zújar	Granada	37.5401	−2.8428	775	Cultivated	Field crop	8.31	1.34	46.6	32.4	21.0
82	Otura	Granada	37.0943	−3.6351	813	Cultivated	Field crop	8.29	0.66	31.7	41.7	26.6
83	Ventas de Huelma	Granada	37.0685	−3.8221	854	Natural	Forest	7.96	2.38	53.0	43.6	3.4
84	Huétor Santillán	Granada	37.2182	−3.5174	1015	Natural	Forest	8.07	2.56	45.9	32	22.1
85	Gor	Granada	37.3695	−2.9695	1238	Cultivated	Fruit crop	7.97	1.76	27.2	41.7	31.1

86	Cúllar	Granada	37.5844	−2.5984	897	Natural	Forest	8.12	1.70	21.3	38.7	40.0
87	Villanueva de las Torres	Granada	37.5566	−3.0902	633	Cultivated	Field crop	8.12	3.19	44.5	33.3	22.2
88	Darro	Granada	37.3492	−3.2924	1120	Cultivated	Field crop	8.15	2.09	47.2	32.4	20.4
89	Zagra	Granada	37.253	−4.1681	682	Cultivated	Fruit crop	8.13	3.04	40.4	37.8	21.8
90	Campotíjar	Granada	37.4813	−3.6165	920	Natural	Forest	8.28	1.61	26.9	31.6	41.5
91	Torre Cardela	Granada	37.5447	−3.3558	1214	Natural	Forest	8.16	2.06	34.3	39.5	26.2
92	Puebla D. Fradique	Granada	37.9621	−2.4388	1164	Natural	Forest	8.38	1.13	29.6	35.9	34.5
93	Castril	Granada	37.7944	−2.7797	890	Natural	Forest	8.19	2.09	29.7	34	36.3
94	Alhama de Granada	Granada	37.0023	−3.9881	895	Natural	Forest	8.09	2.59	41.3	36	22.7
95	Castillejar	Granada	37.7149	−2.6435	792	Cultivated	Field crop	8.25	2.82	44.5	30.3	25.2
96	Aguadulce	Almería	36.8144	−2.5719	60	Natural	Others	8.78	0.15	28.7	52.7	18.6
97	Albuñol	Granada	36.7918	−3.2059	250	Natural	Forest	8.04	1.07	57.1	31.5	11.4
98	Motril	Granada	36.7447	−3.5167	45	Cultivated	Field crop	8.02	2.60	33.5	51.6	14.9
99	Albuñuelas	Granada	36.9288	−3.6300	730	Cultivated	Fruit crop	8.26	0.93	65.9	24.5	9.6
100	Valor	Granada	36.9959	−3.0831	909	Cultivated	Field crop	7.91	2.10	49.9	40	10.1
101	Pitres (S. Nevada)	Granada	36.9354	−3.3263	1295	Cultivated	Field crop	7.83	4.86	48.6	39.8	11.6
102	Salteras	Sevilla	37.4182	−6.1116	152	Natural	Forest	7.84	2.99	46.7	37	16.3
103	Carmona	Sevilla	37.4706	−5.6426	235	Cultivated	Field crop	8.32	2.13	13.7	45.1	41.2
104	Punta Umbría	Huelva	37.1809	−6.9677	7	Natural	Others	6.21	0.89	95.5	3.5	1.0
105	Villamanrique de la Condesa	Sevilla	37.2475	−6.3070	33	Cultivated	Fruit crop	8.56	1.17	65.5	22.2	12.3
106	Mazagón	Huelva	37.1128	−6.7624	6	Natural	Others	8.69	0.38	98.0	0.8	1.2
107	Palos de la Frontera	Huelva	37.2309	−6.8925	23	Natural	Forest	5.91	1.80	76.7	5.7	17.6
108	Cartaya	Huelva	37.2833	−7.1552	26	Natural	Forest	6.02	1.27	91.2	6.1	2.7
109	El Rocío	Huelva	37.1307	−6.4849	75	Natural	Forest	7.22	0.41	89.8	7.9	2.3
110	La Campana	Sevilla	37.5694	−5.4267	134	Cultivated	Field crop	7.70	1.73	44.0	38	18.0
111	La Gomera	Tenerife	28.0922	−17.1119	5	Cultivated	Field crop	5.39	3.46	66.9	22.5	10.6
112	Alcacer do Sal	Portugal	38.3711	−8.5195	64	Natural	Forest	7.23	5.99	84.2	6.5	9.3
113	Setubal	Portugal	38.5245	−8.8931	34	Natural	Others	7.05	2.82	94.5	2.5	3.0
114	Torrão	Portugal	38.293	−8.2263	86	Natural	Forest	6.79	0.96	97.7	0.8	1.5
115	Beja	Portugal	38.0156	−7.8652	285	Cultivated	Field crop	7.00	1.46	37.8	18.1	44.1
116	Vila Verde de Ficalho	Portugal	37.9488	−7.2995	182	Cultivated	Field crop	7.52	2.85	38.9	22.6	38.5
117	Villanueva del Rey	Córdoba	38.1996	−5.1515	555	Cultivated	Field crop	6.81	1.88	56.0	28.9	15.1
118	Peñarroya Pueblonuevo	Córdoba	38.303	−5.2729	537	Cultivated	Field crop	8.27	1.67	43.8	37.7	18.5
119	Los Blázquez	Córdoba	38.4064	−5.4393	508	Cultivated	Field crop	8.28	1.64	52.2	26.6	21.2
120	Fuente Obejuna	Córdoba	38.267	−5.4202	625	Cultivated	Field crop	7.73	2.40	61.5	22.2	16.3
121	Lora del Río	Sevilla	37.6592	−5.5263	38	Cultivated	Field crop	8.15	1.39	33.3	35.6	31.1
122	Navas de la Concepción	Sevilla	37.9335	−5.4648	436	Cultivated	Field crop	6.68	2.44	43.4	44.7	11.9
123	Guadalcanal	Sevilla	38.0922	−5.8207	662	Cultivated	Fruit crop	8.00	3.03	48.0	40.1	11.9
124	Constantina	Sevilla	37.8723	−5.6191	555	Natural	Forest	7.55	7.98	39.6	50.4	10.0
125	Alanís	Sevilla	38.0376	−5.7148	660	Natural	Forest	7.06	8.94	24.7	67.1	8.2
126	Fuente Palmera	Córdoba	37.7033	−5.1042	158	Cultivated	Field crop	7.85	2.2	21.3	42.8	35.9
127	Palma del Rio	Córdoba	37.7016	−5.2838	55	Cultivated	Fruit crop	8.06	1.93	37.2	32.6	30.2
128	Villanueva del Río Minas	Sevilla	37.6525	−5.7129	72	Cultivated	Field crop	7.58	1.48	29.3	48.1	22.6
129	Almaden de la Plata	Sevilla	37.87	−6.0800	450	Natural	Forest	7.76	3.58	57.9	33.7	8.4
130	Puebla de los Infantes	Sevilla	37.7785	−5.3890	230	Cultivated	Field crop	7.85	4.06	21.9	54.6	23.5
131	Castiblanco de los Arroyos	Sevilla	37.6749	−5.9893	313	Cultivated	Field crop	7.94	1.72	36.8	31.1	32.1
132	Cañada Rosal	Sevilla	37.5976	−5.2098	168	Cultivated	Field crop	7.90	1.17	32.5	32	35.5
133	El Pedroso	Sevilla	37.8422	−5.7635	414	Natural	Forest	7.29	10.46	33.3	52.5	14.2

(*continued on next page*)

Table 1 – (*continued*)

Sample	Geographical location					Habitat		Soil factors				
	Locality	Province	Latitude	Longitude	Altitude (m)	Habitat	Subhabitat	pH (in h20)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
134	Cazalla de la Sierra	Sevilla	37.9296	−5.7605	595	Natural	Forest	7.28	1.69	31.5	32.6	35.9
135	Brenes	Sevilla	37.5506	−5.8731	18	Cultivated	Field crop	7.62	2.58	22.4	31.7	45.9
136	El Garrobo	Sevilla	37.6254	−6.1724	275	Cultivated	Field crop	6.46	9.08	44.4	48.1	7.5
137	El Castillo de las Guardas	Sevilla	37.6917	−6.3143	347	Cultivated	Field crop	7.49	3.65	34.2	37	28.8
138	La Puebla del Río	Sevilla	37.2675	−6.0626	22	Cultivated	Fruit crop	6.58	6.21	43.9	50.8	5.3
139	Conquista	Córdoba	38.4084	−4.5010	596	Natural	Forest	7.52	4.58	29.4	42.2	28.4
140	Marchena	Sevilla	37.3297	−5.4165	131	Cultivated	Field crop	8.07	1.96	17.7	29.7	52.6
141	Mairena del Alcor	Sevilla	37.3731	−5.7476	135	Cultivated	Field crop	7.86	2.65	31.3	32	36.7
142	El Palmar de Troya	Sevilla	37.0628	−5.8044	40	Cultivated	Field crop	8.21	1.51	65.0	13.2	21.8
143	El Arahal	Sevilla	37.2641	−5.5428	117	Cultivated	Fruit crop	7.21	1.00	85.4	7.6	7.0
144	Arcos de la Frontera	Cádiz	36.7508	−5.8124	185	Cultivated	Field crop	7.99	2.15	36.4	32.5	31.1
145	Bornos	Cádiz	36.8146	−5.7434	182	Cultivated	Fruit crop	8.15	1.48	20.2	25.6	54.2
146	Nueva Jarilla	Cádiz	36.76	−6.0327	56	Cultivated	Fruit crop	8.13	2.36	25.6	36.5	37.9
147	Los Arenales	Sevilla	37.119	−5.4496	265	Cultivated	Field crop	7.94	2.01	24.2	43.6	32.2
148	Espera	Cádiz	36.8705	−5.8067	164	Cultivated	Field crop	7.98	1.06	50.1	20.7	29.2
149	La Barca de la Florida	Cádiz	36.6492	−5.9339	27	Natural	Forest	7.95	5.25	50.7	19.6	29.7
150	Trebujena	Cádiz	36.8695	−6.1767	69	Cultivated	Field crop	8.12	1.97	62.5	23.2	14.3
151	Algodonales	Cádiz	36.6809	−5.4046	370	Cultivated	Field crop	8.03	1.80	34.9	28.2	36.9
152	La Puebla de Cazalla	Sevilla	37.2244	−5.3123	177	Cultivated	Field crop	8.03	1.20	68.7	11.7	19.6
153	Olvera	Cádiz	36.9355	−5.2675	643	Cultivated	Field crop	8.33	1.37	37.7	37.7	24.6
154	Écija	Sevilla	37.5436	−5.0808	100	Cultivated	Field crop	7.80	1.48	27.9	29.1	43.0
155	Algar	Cádiz	36.6553	−5.6574	212	Cultivated	Field crop	7.67	0.99	59.3	18.7	22.0
156	Ubrique	Cádiz	36.6777	−5.4466	330	Cultivated	Field crop	8.06	1.83	15.3	15.9	68.8
157	Las Cabezas de San Juan	Sevilla	36.9813	−5.9409	76	Cultivated	Field crop	8.11	2.22	59.0	17.2	23.8
158	Villanueva de San Juan	Sevilla	37.0507	−5.1766	466	Cultivated	Field crop	7.93	2.82	12.7	28.2	59.1
159	Osuna	Sevilla	37.237	−5.1028	282	Cultivated	Field crop	7.71	1.73	22.4	46.7	30.9
160	El Rubio	Sevilla	37.3557	−4.9889	209	Cultivated	Field crop	8.09	2.15	24.5	27.3	48.2
161	Martin de la Jarra	Sevilla	37.1065	−4.9619	405	Cultivated	Fruit crop	8.13	1.27	61.3	13.2	25.5
162	Moron de la Frontera	Sevilla	37.1223	−5.4519	297	Cultivated	Fruit crop	8.41	0.88	34.7	42.2	23.1
163	Utrera	Sevilla	37.1814	−5.7815	49	Cultivated	Fruit crop	7.82	1.27	25.0	30.1	44.9
164	Montellano	Sevilla	36.9956	−5.5709	250	Cultivated	Field crop	8.30	2.12	21.9	42	36.1
165	Los Palacios y Villafranca	Sevilla	37.1586	−5.9242	8	Cultivated	Field crop	8.05	1.51	64.4	17.1	18.5
166	Fuente de Pedra	Málaga	37.1356	−4.7299	459	Cultivated	Field crop	7.77	1.69	28.4	45.4	26.2
167	La Palma del Condado	Huelva	37.3878	−6.5530	93	Natural	Forest	7.07	2.78	51.2	29.1	19.7
168	Santa Barbara de Casa	Huelva	37.7965	−7.1886	316	Natural	Forest	7.25	7.75	65.9	24.5	9.6
169	Aroche	Huelva	37.9443	−6.9542	420	Natural	Forest	6.13	10.53	52.2	35.3	12.5
170	Valverde del Camino	Huelva	37.5723	−6.7538	273	Natural	Forest	5.33	11.56	85.9	11.5	2.6
171	Almonte	Huelva	37.2825	−6.5172	75	Natural	Forest	6.24	6.00	56.5	29	14.5
172	Beas	Huelva	37.4294	−6.7936	117	Natural	Forest	6.04	0.51	72.9	7.7	19.4
173	Nerva	Huelva	37.6951	−6.5513	332	Natural	Forest	7.18	1.13	76.7	18.3	5.0
174	Rosal de la Frontera	Huelva	37.9676	−7.2207	216	Cultivated	Field crop	5.77	2.3	49.6	39.1	11.3
175	Valdelamusa	Huelva	37.7882	−6.8799	352	Natural	Forest	5.63	4.32	55.4	32.8	11.8
176	Cortegana	Huelva	37.9099	−6.8208	673	Natural	Forest	5.38	2.85	45.2	44.7	10.1
177	Aracena	Huelva	37.8924	−6.5596	682	Natural	Forest	5.25	2.00	35.0	50.6	14.4

178	Cabezas Rubias	Huelva	37.7266	−7.0866	222	Natural	Forest	5.09	1.74	59.1	28.5	12.4
179	Encinasola	Huelva	38.1353	−6.8726	432	Cultivated	Field crop	4.99	4.51	40.8	48.1	11.1
180	Villanuev. De los Castillejos	Huelva	37.5012	−7.27	224	Natural	Forest	7.64	1.78	45.1	27.1	27.8
181	Chiclana de la Frontera	Cádiz	36.4191	−6.1494	21	Natural	Forest	8.06	2.84	50.9	23.7	25.4
182	Cortadura	Cádiz	36.4956	−6.2715	6	Natural	Others	8.82	0.22	96.6	2.2	1.2
183	Jerez de la Frontera	Cádiz	36.6886	−6.1372	56	Cultivated	Field crop	8.23	1.96	11.1	31.8	57.1
184	Chipiona	Cádiz	36.7406	−6.4363	4	Natural	Others	9.10	0.15	94.0	3.3	2.7
185	Conil de la Frontera	Cádiz	36.2767	−6.0884	41	Natural	Others	9.31	0.22	93.9	3.3	2.8
186	Tarifa	Cádiz	36.0127	−5.603	7	Natural	Others	8.64	0.15	92.9	4.4	2.7
187	Paterna de Rivera	Cádiz	36.5223	−5.8661	127	Cultivated	Field crop	8.58	1.89	6.3	35.8	57.9
188	Alcalá de los Gazules	Cádiz	36.4623	−5.7214	165	Natural	Forest	8.44	2.33	20.9	39.4	39.7
189	Malcocinado	Cádiz	36.3586	−5.8679	80	Cultivated	Field crop	8.65	1.05	18.1	47.7	34.2
190	Jimena de la Frontera	Cádiz	36.434	−5.4535	99	Natural	Forest	8.31	1.96	67.2	21.7	11.1
191	Bolonia	Cádiz	36.0805	−5.7955	33	Cultivated	Field crop	8.35	2.40	24.8	35.9	39.3
192	Algatocín	Málaga	36.5729	−5.2757	725	Cultivated	Field crop	8.06	2.54	61.9	21.5	16.6
193	Punta de la Doncella	Málaga	36.4124	−5.157	17	Natural	Others	9.05	0.15	91.6	6.7	1.7
194	Palma-posadas	Córdoba	37.7016	−5.2838	55	Cultivated	Fruit crop	8.17	2.64	49.8	31.2	19
195	Palma-posadas	Córdoba	37.7016	−5.2838	55	Cultivated	Fruit crop	8.65	1.96	22.1	33.4	44.5
196	Palma-posadas	Córdoba	37.7016	−5.2838	55	Cultivated	Fruit crop	8.6	1.95	21.7	34.6	43.7
197	Puerto Águilas Grazalema	Cádiz	36.7871	−5.3759	1177	Natural	Forest	8.1	7.83	26.7	35.6	37.7
198	El Saucejo	Sevilla	37.0701	−5.0965	527	Cultivated	Field crop	8.4	1.77	49.1	21.7	29.2
199	Ecija-Palma	Sevilla	37.5436	−5.0808	100	Cultivated	Field crop	8.48	1.94	11.4	36.7	51.9
200	Grazalema	Cádiz	36.7584	−5.3661	812	Natural	Forest	6.96	4.06	8.6	58.6	32.8
201	Écija-palma	Sevilla	37.5436	−5.0808	100	Cultivated	Field crop	8.84	1.63	12.7	36.9	50.4
202	Grazalema	Cádiz	36.7584	−5.3661	812	Natural	Forest	5.62	3.67	22.9	27.6	49.5
203	Puerto Palombera	Cantabria	43.0617	−4.2319	1284	Natural	Pasture	5.54	13.65	48.8	41.9	9.3
204	Valle. Cabuérniga	Cantabria	43.2037	−4.3038	248	Natural	Pasture	5.33	7.18	57.7	35.7	6.6
205	San Vicente de Toranzo	Cantabria	43.2088	−3.9389	150	Cultivated	Field crop	7.62	5.86	63.1	32	4.9
206	Nacimiento Ebro	Cantabria	43.0176	−4.1896	903	Natural	Others	6.36	11.39	40.7	40.6	18.7
207	Páramo de Masa	Burgos	42.5994	−3.727	1034	Natural	Pasture	9.04	4.11	39.4	38.8	21.8
208	Sedano	Burgos	42.7163	−3.75	850	Natural	Forest	6.07	2.85	77.4	15.6	7
209	Nacimiento Ebro	Cantabria	43.0176	−4.1896	903	Natural	Forest	6.83	13.81	40.1	45.6	14.3
210	San Felices de Buelna	Cantabria	43.2661	−4.0352	219	Natural	Pasture	7.07	8.22	42.4	46.4	11.2
211	Ruente.Borde de la fuentona	Cantabria	43.2596	−4.2657	208	Natural	Pasture	6.71	15.21	38.9	49.9	11.2
212	Sta Maria Trassierra	Córdoba	37.9264	−4.8967	360	Natural	Forest	7.17	5.38	24.5	66.2	9.3
213	Sta Maria Trassierra	Córdoba	37.9264	−4.8967	360	Natural	Forest	6.59	4.83	49.8	39.4	10.8
214	Marañon	Navarra	42.6296	−2.4393	624	Cultivated	Field crop	8.32	2.73	35.5	44	20.5
215	Boceguillas	Segovia	41.3368	−3.6381	958	Cultivated	Field crop	7.33	1.98	45.2	24.4	30.4
216	Markinez	Alava	42.7025	−2.33	690	Cultivated	Field crop	8.04	1.23	59.9	31.7	8.4
217	Km. 40	Madrid	40.7922	−3.6175	863	Natural	Pasture	7.43	3.48	62.7	18.5	18.8
218	Aranda de Duero	Burgos	41.6717	−3.6886	793	Cultivated	Field crop	8.56	1.76	39.2	32.5	28.3
219	Haro	La Rioja	42.5772	−2.8463	447	Cultivated	Fruit crop	8.64	0.98	37.9	44.5	17.6
220	Km. 233	Burgos	42.2606	−3.6957	859	Cultivated	Field crop	8.57	1.52	53.8	28.4	17.8
221	Zambrana	Alava	42.6	−2.8794	512	Cultivated	Field crop	8.61	1.66	46.5	33.9	19.6
222	Peñarroya Pueblonuevo	Córdoba	38.303	−5.2729	537	Cultivated	Fruit crop	6.89	1.9	71.2	25.6	3.2
223	Figueres	Gerona	42.2675	2.9608	42	Cultivated	Fruit crop	7.89	3.11	49.9	31.5	18.6
224	Gometxa	Vitoria	42.8274	−2.7323	557	Cultivated	Field crop	8.4	2.17	82.6	12.7	4.7
225	Cortijo El Ceacejo	Jaén	37.8334	−3.4635	660	Cultivated	Fruit crop	8.68	2.09	18.7	52.6	28.7
226	Mareny	Valencia	39.246	−0.2647	2	Cultivated	Fruit crop	8.2	0.77	86.3	9.9	3.8

(*continued on next page*)

Table 1 – (continued)

Sample	Locality	Geographical location				Habitat		Soil factors				
		Province	Latitude	Longitude	Altitude (m)	Habitat	Subhabitat	pH (in h20)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)
227	Busot (Cabezón)	Alicante	38.4848	−0.4172	329	Natural	Forest	8.72	1.21	31.6	46.2	22.2
228	Xixona	Alicante	38.5387	−0.509	484	Cultivated	Fruit crop	8.08	2.01	29.1	46.8	24.1
229	Busot (Cabezón)	Alicante	38.4848	−0.4172	329	Natural	Forest	8.45	3.95	23.3	49.7	27
230	Cangas de Onis	Asturias	43.35	−5.1198	94	Cultivated	Field crop	6.25	1.36	64.5	26.3	9.2
231	Dunas del Cornalejo	Fuerteventura (Las Palmas)	28.7165	−13.8394	6	Natural	Pasture	8.99	0.3	95	2.1	2.9
232	Playa blanca	Lanzarote (Las Palmas)	28.8667	−13.8333	78	Natural	Pasture	9.39	1.5	66.5	25.9	7.6
233	Costa Caleta FV Km 12	Fuerteventura (Las Palmas)	28.497	−13.862	16	Natural	Pasture	8.96	1.45	52.9	39.5	7.6
234	Villaverde	Fuerteventura (Las Palmas)	28.6343	−13.8948	206	Natural	Pasture	9.14	1.06	27.4	58.1	14.5
235	Litoral FV Km 12	Fuerteventura (Las Palmas)	28.2197	−14.0232	50	Natural	Pasture	8.69	0.54	66.4	23.4	10.2
236	Timanfage Sunt	Lanzarote (Las Palmas)	28.986	−13.814	96	Natural	Pasture	9	0.4	99.6	0.1	0.3
237	Tahiche	Lanzarote (Las Palmas)	29.0134	−13.5458	138	Natural	Pasture	9.08	1.56	71.5	22.5	6
238	Picón	Lanzarote (Las Palmas)	29.0151	−13.6147	270	Natural	Pasture	9.26	0.58	98.1	1.1	0.8
239	Tinajo	Lanzarote (Las Palmas)	29.0614	−13.6709	212	Natural	Pasture	9.06	1.42	50.7	44	5.3
240	Tuineje	Fuerteventura (Las Palmas)	28.324	−14.0446	184	Cultivated	Field crop	8.42	1.26	11.1	56.9	32
241	Estación de Obejo	Córdoba	38.1319	−4.8004	707	Natural	Pasture	8.06	2.04	54.1	35.8	10.1
242	San Carlos	Ibiza	39.0333	1.5655	77	Cultivated	Field crop	8.41	1.79	13.7	44.9	41.4
243	San Carlos	Ibiza	39.0333	1.5655	77	Natural	Forest	8.26	4	15.6	31.6	52.8
244	San Carlos	Ibiza	39.0333	1.5655	77	Cultivated	Field crop	8.52	2.38	33.4	37.3	29.3

reared following the method of Dutky *et al.* (1962). From each combined soil sample, five 50 g sub-samples were taken and placed into five 90 mm diam, plastic, non-vented Petri dishes. Ten fifth instar *G. mellonella* larvae were placed on the surface of each dish and the dishes were sealed with parafilm (Pechiney, Chicago, USA) and inverted and incubated at 25 °C for 7 d. The dishes were inverted daily to ensure the larvae moved through the soil regularly. Soil was kept moist (approximately field capacity) throughout. After the incubation period the soil was examined for dead larvae, which were removed immediately and surface sterilised in 1 % sodium hypochlorite for 3 min followed by three washes in sterile, distilled water. Surface-sterilised larvae were placed on sterile wet filter paper in sterile, plastic, non-vented Petri dishes sealed with parafilm incubated at room temperature, and inspected daily for the presence of fungal mycelium. All potential mycopathogens were identified microscopically based on morphological characteristics using taxonomic keys (Barnett & Hunter 1987; Humber 1997). A soil sample was considered to harbour a given entomopathogenic fungal species if this species was present in at least one of the five replicates. All fungal isolates obtained in this study were deposited in the culture collection of the Department of Agricultural and Forestry Sciences, ETSIAM, University of Cordoba, Spain.

Data analysis

Each of the 244 soil samples were characterized according to nine variables: (1) presence and identity of entomopathogenic fungi; (2) organic matter content; (3) clay content; (4) sand content; (5) silt content; (6) pH; (7) site latitude; (8) site longitude; and (10) site altitude.

The dependent variable (occurrence of entomopathogenic fungi), was multinomial with four possible components (presence or absence for each of two species of fungus). We denoted each component as: (a) *Beauveria bassiana* only present; (b) *Metarhizium anisopliae* only present; (c) both species present; (d) neither species present. Consequently, presence of *B. bassiana* was represented by a + c, and similarly presence of *M. anisopliae* was represented by b + c.

Log-linear analyses. Log-linear models were used to analyse contingency tables (Agresti 1990). These models enabled us to compare how the observed data of the occurrence of entomopathogenic fungi (dependent variable) was affected by habitat type and its interaction with the fungal species (independent variables). Because of the unbalanced design of sub-habitat types within the two main habitat types, we performed a separate analysis for data for each habitat type to determine the occurrence of entomopathogenic fungi within sub-habitat types. Similarly, the relationships between the occurrence of entomopathogenic fungi and soil and geographic variables were analysed using the same procedure. For this, soil and geographic variables were categorized into groups as follows: pH: 1: <7, 2: ≥7–7.5, 3: ≥7.5–8, 4: ≥8 to 8.5, and 5: ≥8.5; organic matter content: 1: 0–1 %, 2: ≥1–2 %, 3: ≥2–3 %, and 4: ≥3 %; clay content: 1: 0–10 %, 2: ≥10–20 %, 3: ≥20–30 %, 4: ≥30–40, and 5: ≥40 %; latitude: 1: <36°, 2: ≥36–38°, 3: ≥38–40°, 4: ≥40–42°, and 5: ≥42°; longitude: 1: <−8°, 2: ≥−8 to −6°, 3: ≥−6 to −4°, 4: ≥−4 to −2°, and 5: ≥−2°; Altitude: 1:<10 m, 2: ≥10–50 m, 3: ≥50–400 m, 4: ≥400–1000 m, and 5: ≥1000 m. The CATMOD

procedure in SAS software (Statistical Analysis System, version 8.2; SAS Institute, Cary, NC) with a Poisson error and log link was used.

Logistic regression analyses. Logistic regression (Hosmer & Lemeshow 1989) was used to assess the effects of the independent variables associated with soil properties and geographic location variables on the occurrence of entomopathogenic fungi and identify those variables significantly associated with them that could be used to predict the occurrence of a particular fungus for given site characteristics. The dependent variable was absence or presence of entomopathogenic fungi characterized into the four possible components indicated above. In logistic regression, if Y represents fungal presence in a sample and only takes on values 0 and 1 (absence or presence), the probability of fungal occurrence can be modelled as follows:

$$P(Y=1) = \frac{\exp(\sum b_i X_i)}{(1+\exp[\sum b_i X_i])} \quad (1)$$

Where b_i are parameters to be estimated and X_i are the covariates or predictors. A separate logistic regression model was fitted to each of the four possible components of entomopathogenic fungi as dependent variables and to either soil or geographic location components as independent predictor variables. The GENMOD procedure in SAS software, with a binomial distribution and logit link functions, was used. To select the best set of predictors, only hierarchical models were considered and the maximum term order was limited to two. A step-up variable selection with switching for model search was used. Starting with no terms in the model, the procedure searched for the term that, when added to the model, achieved the largest value of the log likelihood, and continued adding terms until the target value of the log-likelihood was achieved. At each step when a term was added, all terms in the model were switched one at a time with all candidate terms not in the model to determine whether they increased the value of the log likelihood. In the selected model, all predictors were significantly associated ($P < 0.05$) with the occurrence of entomopathogenic fungi. Among the statistics obtained in the logistic regression, the odds ratio (natural logarithm raised to the power of the coefficient value) is the most useful to interpret the effect of each independent variable included in the model. The odds ratio for a predictor (independent variable) is defined as the relative amount by which the odds of the outcome (occurrence of a given entomopathogenic species) increase (odds ratio > 1) or decrease (odds ratio < 1) when the value of the predictor variable is increased by 1 unit.

Multivariate analyses. In addition, a multivariate factor analysis was performed. Factor analysis is a multivariate procedure to reduce complex relationships in observed data into simpler forms through a reduction of an original set of correlated variables to a small number of uncorrelated variables. The assumption is that the observable variables X_i ($i = 1, \ldots, p$, i.e., organic matter, clay, sand, silt content, pH, latitude, longitude and altitude) are linear function non-observable variables, called factors F_j ($j = 1, \ldots, q$ with $q \leq p$). Each variable X_i can be written as: $X_i = b_{i1}F_1 + b_{i2}F_2 + \ldots + b_{iq}F_q + E_i$. The coefficients b_{ij} are called factor loadings and represent the correlation of the variable i with the factor j. The term E_i denotes a residual component that is specific to variable I and is not related to any of the other factors. The factor loadings are a measure of the variance accounting for each factor. Hence, low factor loadings may contribute little to the explained variance and only q out of the possible p factor loadings will account for most of the variance, which reflects the reduction in dimensionality. Graphically the factor loading corresponds to the projection of points in a multidimensional space into fewer dimensions. In a two-dimensional space the procedure to construct orthogonal components can be displayed by moving the origin of the original axes followed by a rotation to maximize the variance along the axes (Seal 1964). Among the several rotation procedures available, we selected the Varimax rotation because it is an efficient method to produce factors with few large loadings and as many loadings as possible that are nearly zero. In factor analysis, the first factor accounts for as much of the variability in the data as possible. Each succeeding factor accounts for as much of the remaining variability as possible. Factors were extracted using the principal components analysis method (Seal 1964). After the initial factor extraction, the Varimax rotation was used to estimate the factor loadings (Seal 1964). A factor loading was considered significant when it was >0.7. The analysis was performed using the FACTOR procedure of SAS software.

Results

Entomopathogenic fungi were isolated from 175 of the 244 (71.7 %) soil samples. In these samples only *Beauveria bassiana* and *Metarhizium anisopliae* were isolated and *B. bassiana* was the most common. Of the 244 soil samples, 104 yielded *B. bassiana* (42.6 %), 18 yielded *M. anisopliae* (7.3 %), 53 soil samples (21.7 %) harboured both species, and no entomogenous fungi were isolated from 69 of the soil samples (28.3 %). Only one species was recorded from any individual infected larva, except for three infected larvae from which both species were recovered.

Log-linear analyses indicated that the occurrence of entomopathogenic fungi was strongly influenced by both fungal species and main habitat type (Table 2). When all fungal species were pooled together, the occurrence of entomopathogenic fungi was not influenced by main habitat type ($P = 0.522$; i.e. cultivated *versus* natural habitats). However, the significant interaction ($P = 0.004$) between fungal species and habitat type indicates that, at the species level, except for the single occurrence of *B. bassiana* that showed a greater occurrence in natural habitats, all other species categories with at least one isolate species occurred with a greater frequency in cultivated soils (Table 2). In fact, although *M. anisopliae* alone or together with *B. bassiana* occurred 2.4 and 1.9 times more frequently in cultivated than in natural habitats, *B. bassiana* alone occurred 1.5 times more frequently in natural habitats. In both habitats, *M. anisopliae* showed the lowest frequency, and soils with neither species present occurred at nearly equal frequency (Fig 2A).

Within cultivated habitats (i.e. field and fruit crops subhabitats), both main factors ($P < 0.05$) and their interactions were significant ($P = 0.0002$) in the log-linear model (Table 2). Overall, the frequency of samples harbouring entomopathogenic fungi was significantly greater ($P < 0.05$) in the field crops

Table 2 – Maximum likelihood analysis of variance from log-linear analyses for the effects of habitat type on the occurrence of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in soils from Spain

Independent variable	Habitat (factor B)								
	Main habitat type			Cultivated subhabitat			Natural subhabitat		
	DF	Chi^2	P	DF	Chi^2	P	DF	Chi^2	P
Fungi (A)[a]	3	53.61	<0.0001	3	14.53	0.0023	3	45.64	<0.0001
Habitat (B)[b]	1	0.41	0.5222	1	63.48	<0.0001	2	45.99	<0.0001
A*B	3	13.38	0.0039	3	36.71	0.0002	6	42.01	<0.0001

a Factor A: the occurrence of entomopathogenic fungi had four possible components (presence or absence for each of two species of fungus).
b Factor B: main habitat type was binomial with two possible components, cultivated and natural habitats. Similarly cultivated sub-habitat with two components: field and fruit crops, and natural sub-habitat with three components forest: pastures and other habitats.

sub-habitat (70.7 % occurrence) than in the fruit crops sub-habitat (29.3 % occurrence; Table 2). However, important differences existed in the relative frequency at which a given fungal species occurred in each sub-habitat type. Thus, in the field crops sub-habitat *B. bassiana* was isolated with nearly the same frequency alone (30.6 % occurrence) or co-occurring with *M. anisopliae* (31.8 % occurrence), being 0.8 times less frequent but 1.5 times more frequent in field than in fruit crops, respectively. Conversely, in the fruit crops sub-habitat, the frequency of finding *B. bassiana* alone was nearly twice (40.5 % occurrence) the frequency of finding *B. bassiana* co-occurring with *M. anisopliae* (21.4 % occurrence). Interestingly, *M. anisopliae* alone was isolated in 14.1 % of samples of the field crops sub-habitat, but this species was present in just one sample in the fruit crops sub-habitat (2.4 % occurrence). As expected, frequency of samples with neither fungal species present was greater in the fruit crops sub-habitat (Fig 2B).

Within natural habitats (i.e. forest, pastures and other sub-habitats), all three factors of the log-linear model, the two main factors and their interaction were significant ($P < 0.0001$) (Table 2). Pooling all fungal species, the occurrence of entomopathogenic fungi was greater in the forest sub-habitat (68.7 % occurrence), being 3.6 and 5.7 times more abundant than in the pasture (19.37 % occurrence) or in the other sub-habitats (12 % occurrence), respectively. At species level, in the three sub-habitats, *B. bassiana* alone was the most frequent fungal species isolated (52.1 % occurrence), followed by it co-occurrence with *M. anisopliae* (14.5 % occurrence), and finally *M. anisopliae* that showed the smallest frequency (4.3 % occurrence) and was present alone only in the pasture sub-habitat. Samples with no fungal species present represented 60 % of samples from the other sub-habitat, but only 25 % of samples from the forest sub-habitat, and interestingly all soil samples from the pasture sub-habitat harboured at least one fungal species (Fig 2C).

The effect of soil factors (organic matter, clay, sand, silt content, and pH) and geographical location (latitude, longitude, and altitude) on the occurrence of entomopathogenic fungi was analysed by log-linear models and logistic regression (Tables 3 and 4).

Overall, the two main factors in the log-linear analysis (i.e. fungal species and the corresponding soil variable) and its interaction were significant ($P < 0.05$; Table 3). Irrespective of fungal species, soil samples with a pH value that ranged from 8–8.5 and those >8.5 harboured the greatest and smallest percentage of fungal isolation, respectively, except for those samples with neither species present that was greatest at pH >8.5 (37.7 % occurrence, that represented 76.5 % of samples in this pH group; Fig 3A). At the species level, *B. bassiana* seemed to have the narrowest pH optimum, as 52.9 % of samples harbouring this species were located in only one of the pH groups that ranged from 8–8.5, being occurrence in the remaining pH groups in the range of 6.7 to 18.3%. Conversely, soil samples harbouring *M. anisopliae* were located mainly in two pH groups, <7 (27.8 % occurrence) and 8–8.5 (38.9 % occurrence; Fig 3A). Organic matter also had an important influence in the occurrence of entomopathogenic fungi. Soils with moderate organic matter content seemed to be richer in *B. bassiana*, as 66.3 % of samples harbouring this species had an organic matter content that ranged from 1–3 % (41.3 % occurrence) or 2–3 % (25 % occurrence). Soil samples harbouring *M. anisopliae* alone or co-occurring with *B. bassiana* were characterised by a greater organic matter content, with the greatest recovery rates in soils with organic matter content higher than 3 % (38.9 and 37.7 % occurrence, respectively). Most of soil samples with neither species present (69.5 % occurrence) had an organic matter content <2 % (Fig 3B). Clay content showed no differential effect on the relative occurrence of entomopathogenic fungi, as indicated by the non-significant interaction between these two main factors ($P = 0.117$). Overall, moderate clay content favoured the occurrence of fungal species, as 49.1 % of samples harbouring at least one fungal species had a clay content that ranged from 10–30 %. However, the relative frequency of fungal species varied among clay content groups ($P = 0.004$). *B. bassiana* was well adapted to a wider range of clay content soils, showing its greatest frequency at soils with a clay content of 10–20 %, but also showing high occurrence at the remaining clay content groups, being the only species that showed a high frequency (19.2 % occurrence) at the highest clay content group (≥40 % clay content). Conversely, *M. anisopliae* showed a preference for those soils with low or moderate clay content, showing its greater occurrence at the smallest clay content group (<10 % clay content) with just one sample harbouring this species alone at the greatest clay content group. Interestingly, as expected, the co-occurrence of both fungi was greatest in soils with moderate clay content (34 % occurrence at soils with 20–30 % clay content). Low clay content clearly favoured the absence of

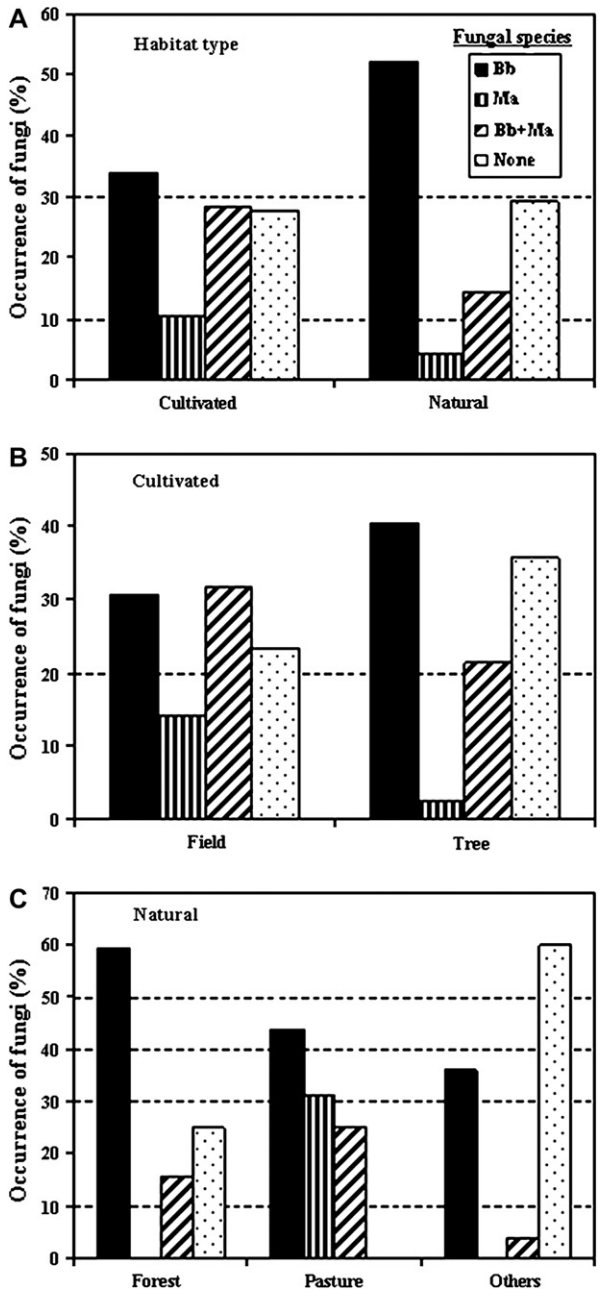

Fig 2 – Effect of habitat and subhabitat on the occurrence of entomopathogenic fungi in Spanish soils. (A) Main habitat type; (B) cultivated subhabitats and (C) natural subhabitats. Data are relative frequencies of isolation of each entomopathogenic species from 244 soil samples.

entomopathogenic fungi, as 60.8 % of samples harbouring no fungal species had a clay content <20 % (Fig 3C).

Concerning geographic variables, similarly to the soil variables described above, the two main factors in the log-linear analysis (i.e. fungal species and the corresponding geographic variable) and its interaction were significant ($P < 0.05$), except for the non-significant interaction ($P = 0.099$) between fungal species and altitude (Table 4). Both, latitude and longitude had a significant influence in fungal occurrence ($P < 0.05$). In the Canary Islands (latitude <36°N and longitude >−8°W groups) both fungal species could be isolated, although 53.3 % of samples harboured no fungi (Fig 3D–E). In the Balearic Islands (latitude 38 to 40°N and longitude <1°E groups), although the only fungal species isolated was *B. bassiana*, the low number of samples taken in this area, make it difficult to make conclusions. In the Iberian Peninsula, *B. bassiana* and *M. anisopliae* alone or co-occurring with *B. bassiana* were isolated from soils over a wide range of locations and altitudes. *B. bassiana* was frequently isolated from soils sampled at any location except from northern latitudes (latitude >40°N), where, from the 20 samples taken in this area, only five harboured this species (Fig 3D). The geographic distribution of *M. anisopliae* was more restricted, being located only in two areas at south (latitude 36 to 38°N) and north (latitude >42°N) central Spain (longitude −6 to −2°W). Soil samples harbouring both entomopathogenic fungi were located preferentially in the south west of Spain (latitude 36 to 38°N, longitude −8 to −4°W) (Fig 3D,E). Concerning the influence of altitude, the non-significant interaction ($P = 0.099$) between fungal species and altitude (Table 4) indicated that altitude had a similar effect on the distribution of all fungal species. Most samples harbouring entomopathogenic fungi were taken at moderate altitudes. In fact, 83.4 % were taken between 50–1000 m and only 2.3 and 6.3 % of samples harbouring fungi were sampled at the lowest (0–10 m) and highest (>1000 m) altitude groups, respectively. Except for *B. bassiana* alone that showed its greatest occurrence at the 400–1000 m altitude interval, all other fungal combinations showed greatest occurrence at the 50–400 m altitude interval (Fig 3F).

Logistic regression analyses. Overall, the only main effects were found to be predictive variables ($P < 0.05$) for the occurrence of entomopathogenic fungi, with rare significant interactions ($P < 0.05$) between those factors. However, significant main factors and the direction of the effects (i.e. positive or negative regression coefficients) varied between fungal species. Among soil factors, the occurrence of *B. bassiana* could be predicted ($P < 0.05$) based on soil pH (odds ratio = 0.59) and clay content (odds ratio = 1.03). For *M. anisopliae*, organic matter content was the only predictive variable ($P < 0.05$; odds ratio = 1.22). As expected, soil pH (odds ratio = 0.81) and organic matter content (odds ratio = 1.11) were predictive variables ($P < 0.05$) for the co-occurrence of both fungi. Finally, pH (odds ratio = 4.01), clay content (odds ratio = 1.34) and its interaction (odds ratio = 0.96) could be used to predict ($P < 0.05$) the absence of both fungal species (Table 5).

Among geographic variables, irrespective of the fungal species, longitude and latitude were predictive variables ($P < 0.05$) for the occurrence of entomopathogenic fungi, but in different directions. Thus, although latitude was negatively associated ($P < 0.05$) with the presence of *B. bassiana* (odds ratio = 0.61) and its co-occurrence with *M. anisopliae* (odds ratio = 0.93), it was positively associated ($P < 0.05$) with the single presence of this latter species (odds ratio = 1.25), but the opposite was true for longitude (odds ratio = 5.33, 1.83 and 0.81, respectively; Table 5). A significant interaction ($P < 0.05$) was also found between these two main factors when *B. bassiana* was present in the soil singly (odds ratio = 0.95) or co-occurring

Table 3 – Maximum likelihood analysis of variance from log-linear analyses for the effects of soil components variables on the occurrence of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in soils from Spain

Independent variable	Soil component variable (factor B)								
	Soil pH			Organic matter content			Clay content		
	DF	Chi^2	P	DF	Chi^2	P	DF	Chi^2	P
Fungi (A)[a]	3	53.61	<0.0001	3	41.22	<0.0001	3	53.61	<0.0001
Factor (B)[b]	4	78.52	<0.0001	3	28.58	<0.0001	4	15.63	0.0036
A*B	12	53.71	<0.0001	9	43.07	<0.0001	12	17.95	0.1172

a Factor A: the occurrence of entomopathogenic fungi had four possible components (presence or absence for each of two species of fungus).
b Factor B: values of the dependent variable were grouped at intervals: pH values: 1: <7, 2: ≥7 to 7.5, 3: ≥7.5 to 8, 4: ≥8 to 8.5, and 5: ≥8.5; organic matter content: 1: 0 to 1 %, 2: ≥1 to 2 %, 3: ≥2 to 3 %, and 4: ≥3 %; clay content: 1: 0 to 10 %, 2: ≥10 to 20 %, 3: ≥20 to 30 %, 4: ≥30 to 40 %, and 4: ≥40 %.

with *M. anisopliae* (odds ratio = 0.98). Altitude was significantly and positively associated ($P < 0.05$) with the single presence of *B. bassiana* (odds ratio = 1.00) but negatively associated with the absence of both species (odds ratio = 0.98). No significant effect ($P \geq 0.05$) of altitude was found on the presence of *M. anisopliae* or the co-occurrence of both species, except for the interaction between altitude and latitude for the later species (odds ratio = 1; Table 5).

The absence of entomopathogenic fungi could also be predicted using the same logistic models presented in Table 5, but with the opposite direction for the coefficient and therefore inverse odds ratio values. For example, the absence of *B. bassiana* could be predicted by pH and clay content with coefficients of 0.528 (odds ratio = 1.70) and −0.029 (odds ratio = 0.97), respectively (data not shown). Similarly, absence of *M. anisopliae* was predicted by latitude and longitude with coefficients of −0.220 (odds ratio = 0.80) and 0.210 (odds ratio = 1.23), respectively (data not shown).

In the principle components analysis, the first four factors identified accounted for 86 % of the total variance (Table 6). Variation accounted for by factors 5–8 was marginal. Therefore, only the first four factors were selected from the data. As a result, the dimensionality of the variables associated with soil samples was effectively reduced to four descriptive factors. Table 6 includes the eigenvalues for the factors extracted. Factors were a combination of all soil parameters in the analysis, and the corresponding values in the eigenvectors for each soil sample were used to interpret the weight of the factors. Factor 1 accounted for the largest explained variance and was associated with high positive factor loadings for soil clay (0.8) and silt (0.8), and high negative factor loadings for soil sand content (−0.99). Factor 2 was associated with high positive factor loadings for soil organic matter content (0.84) and high negative factor loadings for soil pH (−0.85). Factor 3 was associated with high positive factor loadings for latitude (0.96) and longitude (0.91) for the locality at which soil was sampled. Factor 4 accounted for the lowest percentage of the cumulative explained variance (10.4 %), being altitude the only dependent variable with a significant factor loading (0.95) in this factor.

Results of principal component analyses were represented graphically in Cartesian plots representing all soil samples projected on the plane of *x* and *y* axes, respectively, as follows: factors 1 and 2, factors 1 and 3 and factors 2 and 4 (Figs 4A, 5A and 6A). In addition similar plots on the plane of factor combinations indicated above were produced for soil samples taken from cultivated and natural soils (Figs 4B, 5B and 6B), separately (Figs 4C, 5C and 6C).

Factor 1 was positively correlated with clay and silt content and negatively correlated with sand content. Factor 2 was positively correlated with organic matter content and negatively correlated with soil pH, respectively. When soil samples were projected on the plane of factor 1 (*x* axis) and 2 (*y* axis), the clay and silt content increased and the sand

Table 4 – Maximum likelihood analysis of variance from log-linear analyses for the effects of geographic location variables on the occurrence of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in soils from Spain

Independent variable	Geographic location (factor B)								
	Latitude			Longitude			Altitude		
	DF	Chi^2	P	DF	Chi^2	P	DF	Chi^2	P
Fungi (A)[a]	3	48.37	<0.0001	3	48.37	<0.0001	3	50.46	<0.0001
Factor (B)[b]	4	223.17	<0.0001	4	203.99	<0.0001	4	134.15	<0.0001
A*B	12	50.44	<0.0001	12	28.46	0.0047	12	18.61	0.0985

a Factor A: the occurrence of entomopathogenic fungi had four possible components (presence or absence for each of two species of fungus).
b Factor B: values of the dependent variable were grouped at intervals: latitude: 1: <36°, 2: ≥36 to 38°, 3: ≥38 to 40°, 4: ≥40 to 42°, and 5: ≥42°; longitude: 1: >−8°, 2: ≤−8 to −6°, 3: ≤−6 to −4°, 4: ≤−4 to −2°, and 5: ≤−2°; altitude: 1: <10 m, 2: ≥10 to 50 m, 3: ≥50 to 400 m, 4: ≥400 to 1000 m, and 5: ≥1000 m.

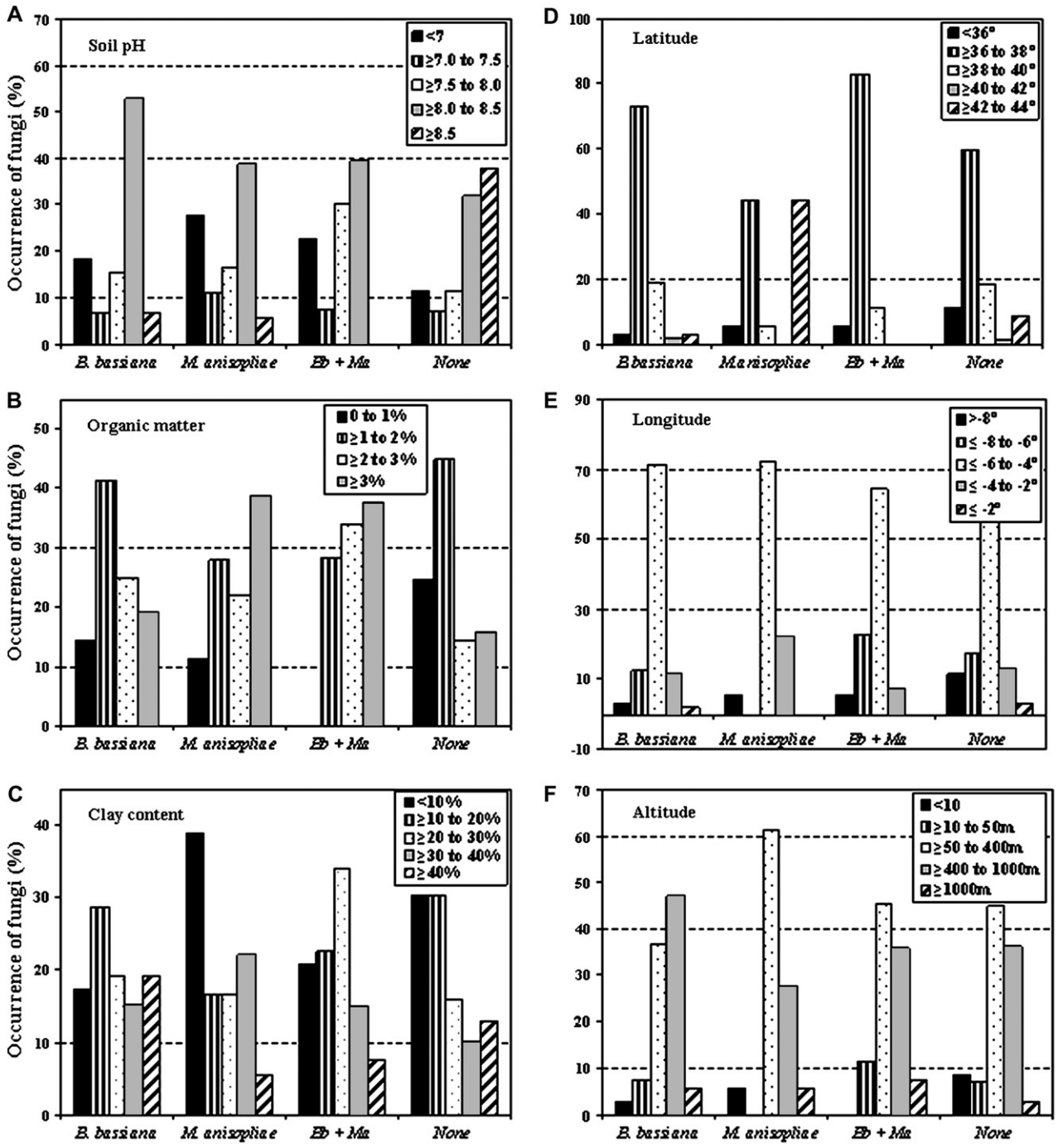

Fig 3 – Effect of soil factors (A, B and C) and geographical location (D, E, and F) on the occurrence of entomopathogenic fungi in Spanish soils. Data are relative frequencies of isolation of each entomopathogenic species from 244 soil samples. Soil and geographic variables were categorized into groups indicated in the corresponding plot legend.

content decreased, respectively, from left to right along the x axis. Along the y axis, pH decreases and organic matter content increases from bottom to top. Consequently, samples of light-structured, acid soils with the greatest organic matter content were grouped at the top right quadrant (I), whereas those samples with alkaline, heavy-structured soils with the smallest organic matter content were located at the bottom left quadrant (IV; Fig 4).

Factor 3 was associated with geographic position of soil samples. When soil samples were projected on the plane of factor 1 (x axis) and 3 (y axis) sample site location moved from southern to northern latitudes and from western to eastern longitudes along the y axis from bottom to top. In consequence, the top right quadrant (I) includes the lightest-structured soils sampled at more northern latitudes and more eastern longitudes, i.e. localities at the north-eastern

Table 5 – Logistic regression analysis of the relationship between the occurrence of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in soils from Spain as a function of soil characteristics and geographic location

Independent variable	Dependent variable															
	Beauveria bassiana				*Metarhizium anisopliae*				*B. bassiana* + *M. anisopliae*				None			
	Coef.	S.E.	Wald *P*	Odds ratio[a]	Coef.	S.E.	Wald *P*	Odds ratio	Coef.	S.E.	Wald *P*	Odds ratio	Coef.	S.E.	Wald *P*	Odds ratio
Soil characteristics																
Intercept	4.080	1.268	0.001	–	−1.454	0.217	<0.001	–	ns[b]	ns	ns	ns	−11.338	2.664	<0.001	–
pH (A)	−0.528	0.165	0.001	0.590	ns	ns	ns	ns	−0.207	0.028	<0.001	0.813	1.388	0.332	<0.001	4.005
Organic matter (B)	ns	ns	ns	ns	0.195	0.056	<0.001	1.216	0.101	0.048	0.037	1.107	ns	ns	ns	ns
Sand (C)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Clay (D)	0.029	0.010	0.004	1.030	ns	ns	ns	ns	ns	ns	ns	ns	0.291	0.120	0.015	1.338
A*D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	−0.040	0.015	0.008	0.961
Geographic location																
Intercept	18.197	4.919	<0.001	–	−10.251	3.409	0.003	–	ns	ns	ns	ns	5.896	2.733	0.031	–
Latitude (A)	−0.492	0.131	<0.001	0.611	0.220	0.082	0.007	1.246	−0.071	0.016	<0.001	0.932	−0.178	0.074	0.017	0.837
Longitude (B)	1.673	0.427	<0.001	5.329	−0.210	0.078	0.007	0.810	0.606	0.284	0.033	1.832	ns	ns	ns	ns
Altitude (C)	0.001	<0.001	0.007	1.001	ns	ns	ns	ns	ns	ns	ns	ns	−0.016	0.007	0.014	0.984
A*B	−0.048	0.013	<0.001	0.953	ns	ns	ns	ns	−0.024	0.010	0.021	0.977	ns	ns	ns	ns
A*C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.001	<0.001	0.017	1.000

a The odds ratio for a predictor (independent variable) is defined as the relative amount by which the odds of the outcome (occurrence of a given entomopathogenic species) increase (odds ratio > 1) or decrease (odds ratio < 1) when the value of the predictor variable is increased by 1 unit.

b ns = Not significant at $P \geq 0.05$.

Table 6 – Eigenvectors and eigenvalues[a] of factor analysis derived from soil parameters and geographical location used to characterize soil samples from Spain

Parameter	Factor[b]			
	F1	F2	F3	F4
Soil parameter				
pH	0.1211	**−0.8504**	−0.0189	0.0404
Organic matter (%)	0.0695	**0.8445**	−0.0072	0.1971
Sand	**−0.9915**	0.0521	−0.0992	−0.0473
Silt	**0.7998**	0.2588	0.0074	0.2486
Clay	**0.7975**	−0.3117	0.1449	−0.1507
Geographic location				
Latitude	0.0450	0.1720	**0.9564**	−0.0087
Longitude	0.1540	−0.2151	**0.9112**	0.1696
Altitude	0.0674	0.1085	0.1300	**0.9529**
Eigenvalues	2.6256	1.8298	1.5654	0.8357
Explained variance	2.3084	1.6908	1.7931	1.0642
Cumulative explained variance (%)	32.82	55.69	75.26	85.71

a Soil parameters and geographic location based on values obtained from 244 soil samples.
b Bold values indicate soil parameters dominating principal components, F1, F2, F3 and F4.

part of the Iberian peninsula and the Balearic islands. In contrast, the bottom left quadrant (IV) includes the heaviest-structured soils taken from more northern latitudes and more western longitudes, i.e. localities at the south-western part of the Iberian Peninsula and the Canary islands (Fig 5).

Factor 4 was associated with altitude. Consequently, when soil samples were projected on the plane of factors 2 (*x* axis) and 4 (*y* axis) samples of acid soils with the greatest organic matter content and sampled at the higher altitudes were grouped at the top right quadrant (I), whereas those samples with alkaline soils, lower organic matter content and sampled at the lower altitudes were located at the bottom left quadrant (IV; Fig 6).

Factor 1, representing the physical properties of the soil had the strongest influence on both the occurrence and distribution of entomopathogenic fungi. Both fungus species were absent in alkaline sandy soils with low organic matter content (Fig 4, quadrants IV) whereas heaviness of soil texture, acidity, and increasing organic matter content led to progressively higher percentages of samples harbouring entomopathogenic fungi (Fig 4, quadrants III, II, I, in this sequence). In general, the occurrence of entomopathogenic fungi was more common in samples from cultivated soils (Fig 4A), with lower sand content than that from natural habitats. In addition, co-occurrence of *B. bassiana* and *M. anisopliae* was more common in samples from cultivated soils (Fig 4A). The occurrence of *M. anisopliae* was more common in the field crops sub-habitat than in the fruit crops sub-habitat (Fig 4B). In natural habitats, there was a negative effect of greater sand content in soil on the occurrence of entomopathogenic fungi, as no fungi were isolated from these soil types (Fig 4C, quadrants III and IV). Overall, the occurrence of entomopathogenic fungi was greater in forest and pasture sub-habitats than in the remaining natural

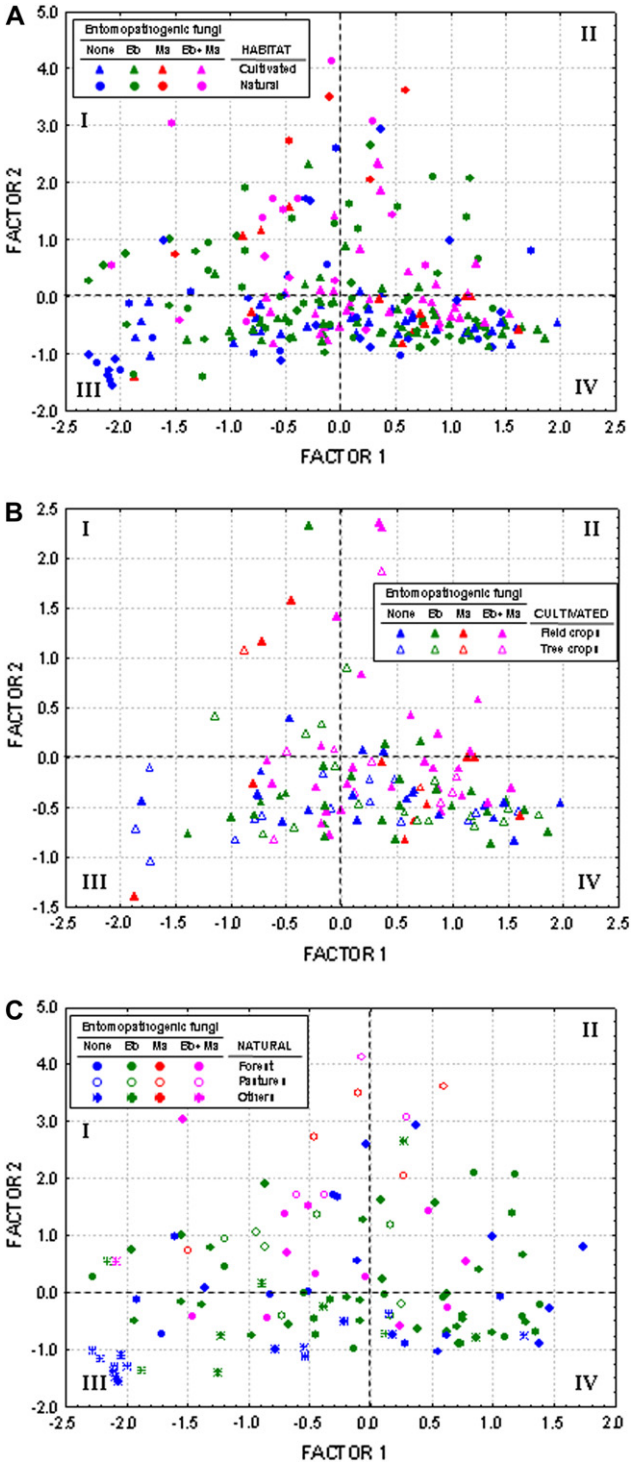

Fig 4 – Projection of factor scores on the plane of factors 1 and 2 from principal component analysis of soil samples from 244 localities in Spain for the effect of habitat type (A), crop type in cultivated habitats (B), and ecosystem in natural habitats (C). According to the position of the projected soil samples along the *x* axis, clay and silt content increase and sand content decrease, respectively, from left to right. Similarly, along the *y* axis, pH decrease and organic matter content increase from bottom to top.

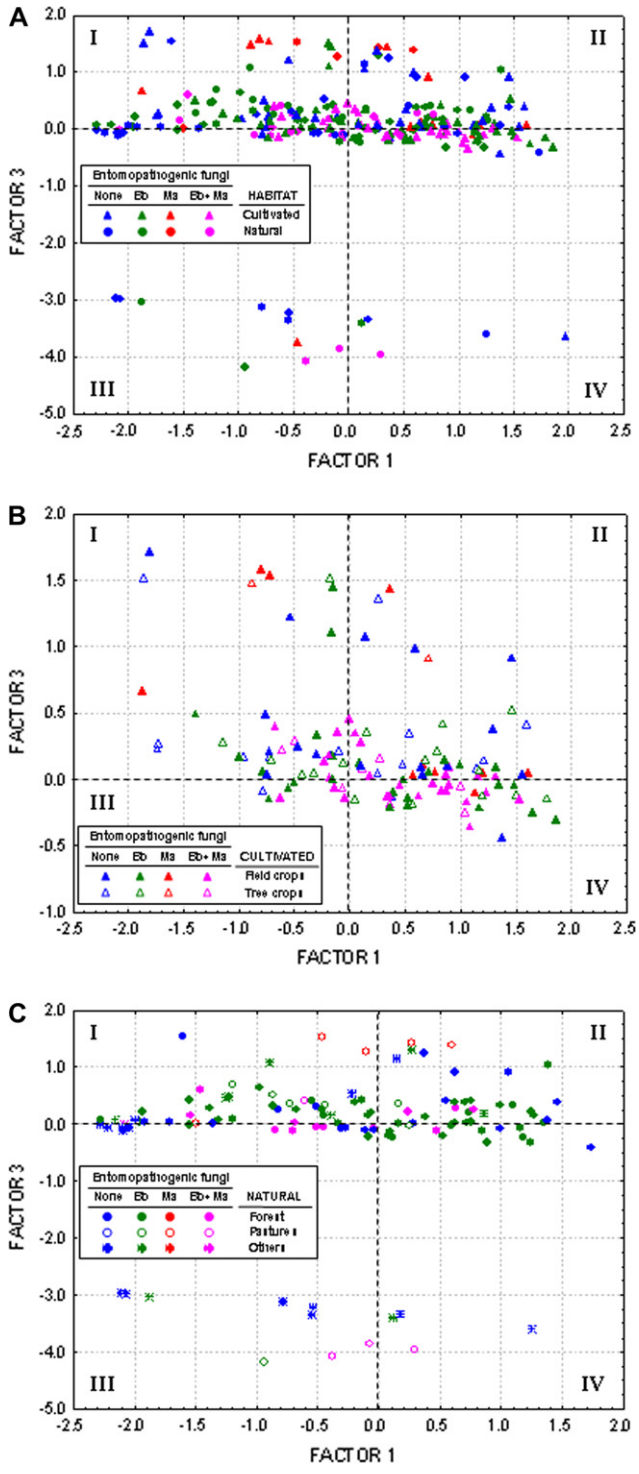

Fig 5 – Projection of factor scores on the plane of factors 1 and 3 from principal component analysis of soil samples from 244 localities in Spain for the effect of habitat type (A), crop type in cultivated habitats (B), and ecosystem in natural habitats (C). According to the position of the projected soil samples along the *x* axis, clay and silt content increase and sand content decrease, respectively, from left to right. Similarly, along the *y* axis, sample site move from south to north latitudes and from western to eastern longitudes from bottom to top.

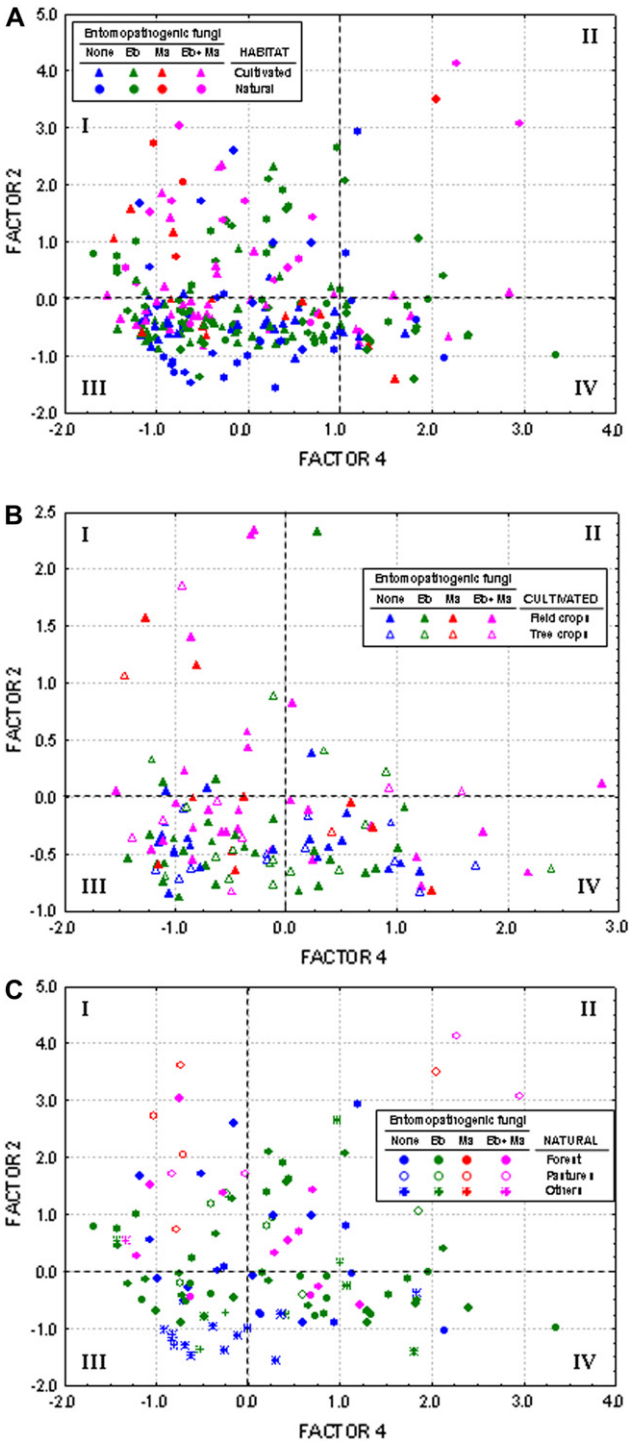

Fig 6 – Projection of factor scores on the plane of factors 2 and 4 from principal component analysis of soil samples from 244 localities in Spain for the effect of habitat type (A), crop type in cultivated habitats (B), and ecosystem in natural habitats (C). According to the position of the projected soil samples along the *x* axis, sample site altitude increase from left to right. Similarly, along the *y* axis, pH decrease and organic matter content increase from bottom to top.

sub-habitats, except for *M. anisopliae* that was equally distributed in all natural sub-habitats, although it was less abundant in pastures where *B. bassiana* was predominant. The preference of *M. anisopliae* for acidic, high organic matter content soils could determine this distribution.

Geographic location also influenced the occurrence of entomopathogenic fungi. Irrespective of soil texture, soils sampled in the Canary Islands (more western longitude and southern latitude) were associated with low incidence of entomopathogenic fungi and alkaline sandy soils (Fig 5, quadrants III). Those soils sampled at the most northern latitudes in the study (central-north eastern part of the Iberian Peninsula) were associated with more frequent occurrence of entomopathogenic fungi and acidic soils with moderate to large organic matter content (Fig 5, quadrants II). *M. anisopliae* occurred singly only in the latter soil types and locations. Altitude, as indicated by the factor loadings (Table 6), had the least influence on the occurrence of entomopathogenic fungi. Although entomopathogenic fungi were present in soils sampled from 5 to 1608 m altitude, 10.3 % of the isolates were obtained from soil sampled at 5–50 m, 52 % below 400 m, and only 6.3 % were sampled at localities above 1000 m. The presence of *M. anisopliae* was particularly favoured by lower altitudes (Fig 6).

Discussion

This work has five major outcomes. First, two important entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* were frequently isolated from natural and cultivated soils in continental Spain and the Archipelagos. Second, *B. bassiana* was equally common in both natural and cultivated soils, whereas *M. anisopliae* was more common in cultivated soils, particularly field crops. Third, log-linear analyses indicated that the occurrence of entomopathogenic fungi was strongly influenced by both fungal species and main habitat type. Fourth, both logistic regression and log-linear models indicated that the occurrence and distribution of both fungal species was related to soil factors (pH, organic matter content, and texture) and geographical location (latitude, longitude, and altitude), some of which may be predictive variables. Fifth, using principal component analysis complex interactions among soil and geographic variables could be described by four factors that accounted for 86 % of the total variance.

Entomopathogenic fungi were recovered from 71.7 % of the 244 sampled fields, which is similar to reports from other countries. Typical recovery rates were 17.5 % in the UK (Chandler *et al.* 1998), 32 % in Tasmania (Rath *et al.* 1992), 44.6 % in Finland (Vänninen *et al.* 1989), 52 % in the Pacific Northwest (Bruck 2004), 91 % in Ontario (Bidochka *et al.* 1998) and 96 % in Switzerland (Keller *et al.* 2003). However, comparisons must be made carefully because different assay protocols were used. In most cases fewer bait larvae were used per sample than in our study [e.g. Chandler *et al.* (1997) only used one larva per sample and Bidochka *et al.* (1998) used three larvae per sample].

Although entomopathogenic fungi were common in Spanish soils, the diversity of these fungi was low with only two species occurring, *B. bassiana* and *M. anisopliae*, with *B. bassiana* the most frequently isolated species. *B. bassiana* was also the most common entomopathogenic fungal species in other Mediterranean countries, such as Southern Italy (Tarasco *et al.* 1997), whereas *M. anisopliae* was more common in soils in northern countries with more humid and cooler climates such as Finland (Vänninen 1996), Norway (Klingen *et al.* 2002), Switzerland (Keller *et al.* 2003), Poland (Tkaczuk & Mietkiewski 1996), Canada (Bidochka *et al.* 1998) and the Pacific Northwest in USA (Bruck 2004). Exceptions occur in UK and Denmark where *B. bassiana* was more common than *M. anisopliae* (Chandler *et al.* 1997; 1998; Meyling & Eilenberg 2006). In our study, neither *Paecilomyces* spp. nor *Lecanicillium* spp. were recorded, although these are cosmopolitan in the soil elsewhere (Chandler *et al.* 1997; Bidochka *et al.* 1998; Keller *et al.* 2003). To our knowledge, entomopathogenic species from the genus *Paecilomyces* have never been recorded in Spain, although Asensio *et al.* (2003) did record *Lecanicillium* spp. (as *Verticillium lecanii*) in 4.8 % of soils in Alicante (south-eastern Spain). We only took three samples in this area which could explain its absence in our survey. *Paecilomyces fumosoroseus* has been recorded commonly in natural habitats elsewhere, particularly in hedges and forest soils (Vänninen 1996; Chandler *et al.* 1997), which according to Klingen & Haukeland (2006), may be due to the ability of this fungal species to thrive in more acid forest soil or because of its higher tolerance to aluminium compared with *B. bassiana* and *M. anisopliae*. The absence of *P. fumosoroseus* from forest soils in our study could be due to differences in the physicochemical properties of Spanish and northern European and American forests, where this species is common.

In our study, there was a significant effect of the main habitat type on the occurrence of entomopathogenic fungi at species level. Soil samples harbouring only *B. bassiana* were more commonly from natural habitats, but both presence of *M. anisopliae* alone or presence of both fungal species were more strongly associated with soils from cultivated habitats, particularly from field crops. In general this agrees with other published work (Bidochka *et al.* 1998; Vänninen 1996; Mietkiewski *et al.* 1991), although *M. anisopliae* was not common in cultivated soils in Denmark (Meyling and Eilenberg 2006). It has been suggested that this is because *M. anisopliae* conidia can persist longer without repeated infection of hosts than *B. bassiana* (Fargues & Robert 1985; Vänninen 1996). The lack of susceptible hosts in heavily cultivated soils could also reduce the persistence of *B. bassiana* in these soils. This hypothesis is supported by Klingen *et al.* (2002), who demonstrated that entomopathogenic fungi occurred more commonly in soils from organically managed arable fields compared with conventionally managed, arable fields, in which synthetic insecticides had greatly reduced the availability of suitable hosts. Apart form their effect on insect hosts, pesticides may also have deleterious effects on the entomopathogenic fungi in the soil, although results from laboratory experiments and field conditions may differ (Mietkiewski *et al.* 1997). Several studies suggest that some fungal species are more tolerant to pesticides than others, and *M. anisopliae* is considered to be more tolerant to pesticides than *B. bassiana*, which could also explain why the former is more common in cultivated habitats. This, together with a low competitive ability suggested for *B. bassiana* (Bidochka *et al.* 1998), could account for the greater

occurrence of *M. anisopliae* in cultivated soils, but all these hypotheses require further experimental evaluation.

Independent of habitat type, we have also shown using log-linear models and logistic regression that the occurrence and distribution of *B. bassiana* and *M. anisopliae* was also related to soil and geographic factors. In log-linear analyses we found that the occurrence of *B. bassiana* was correlated with the pH of the soil, showing a narrow pH range at which this species occurred more frequently (52.9 % occurrence at the 8–8.5 pH level). Also, although *B. bassiana* was present in some samples with pH values higher than 8.5 (6.7 % occurrence), this pH level appears to be detrimental for its occurrence. Moreover, the greater frequency of soil samples with neither species present occurred at pH >8.5 (37.7 % occurrence). This finding is also supported by the odds ratio, which for pH was 0.59 for *B. bassiana* alone and 4.01 for neither species being present. The fact that pH values higher than 8.5 did not favour the isolation of either fungal species is in agreement with the premise that fungi in general are more tolerant to acidity than to alkalinity (Foth 1984). Studies on the optimum pH range for *in vitro* growth of both species indicate that, despite intraspecific variability, *M. anisopliae* is better adapted than *B. bassiana* to slightly acidic soils (Padmavathi *et al.* 2003; Issaly *et al.* 2005), which could explain why in our study *M. anisopliae* predominated over *B. bassiana* in soils with pH lower than 7. Moreover, although Rath *et al.* (1992) found that one specific isolate of *M. anisopliae* was able to grow across a wide pH range (4 to 7.8), the upper pH threshold for *M. anisopliae* growth was much lower than that of the pH value measured in several soil samples in our study, and therefore could account for its less frequent occurrence compared with *B. bassiana* in more acidic soils.

The occurrence of entomopathogenic fungi was frequently associated with soils with a large organic matter content in our study, as previously reported (Milner 1989; Mietkiewski *et al.* 1997). This may be because higher cation exchange capacities in soil with greater organic matter enhance adsorption of fungal conidia or because soils with greater organic matter also have greater diversity and density of arthropod hosts in which the fungi can multiply (Ignoffo *et al.* 1977; Inglis *et al.* 2001; Klingen & Haukeland 2006). *B. bassiana* was more abundant in low organic matter soils (within the defined ranges), which may relate to the fungistatic compounds found in organic matter that have previously been shown to affect *B. bassiana* more than *M. anisopliae* (Lockwood 1977; Studdert *et al.* 1990; Kessler *et al.* 2003).

Low clay content clearly favoured the absence of entomopathogenic fungi, whereas the presence of entomopathogenic fungi was positively correlated with clay content, with *B. bassiana* more abundant at the highest clay content group (>40 %). It is well known that leaching of inoculum is correlated with the water infiltration value of soils and greater losses occur in sandy soils than finer-textured soils (Storey & Gardner 1987, 1988). It has also been suggested that high clay content in soil enhances the abundance and persistence of many insect pathogenic fungi because conidia are adsorbed onto clay particles (Studdert *et al.* 1990; Inglis *et al.* 2001). This may be particularly apparent for species with small conidia (Ignoffo *et al.* 1977), which is confirmed by our observation that *B. bassiana* was more abundant in clay soils than *M. anisopliae*, which has larger conidia. Similar results were obtained by Rath *et al.* (1992) in Tasmania, but contrasting results were found by Vänninen *et al.* (1989), who observed that *M. anisopliae* was more abundant than *B. bassiana* in clay soils in Finland. Clay may also protect against biodeterioration (Fargues *et al.* 1983; Keller and Zimmerman 1989). However, the mechanism behind the relationship between clay content and fungal occurrence needs to be evaluated experimentally.

Principal component analysis carried out in this study, allowed clear interpretations because bi-plot displays representing all soil samples in a single plot were developed for different factor combinations. Particularly relevant was the projection on the plane of factors 1 and 2 that enabled interpretation of the interactions between texture (factor 1), pH, and organic matter content (factor 2). Interestingly, absence of both fungal species was associated with alkaline sandy soils with low organic matter content whereas increasing clay content (within the range of 10–40 %), decreasing pH, and increasing organic matter content led to a progressively higher percentages of samples harbouring entomopathogenic fungi. In general, cultivated habitats provided soils more suitable for the occurrence of entomopathogenic fungi than soils from natural habitats. In cultivated soils, *M. anisopliae* occurred more frequently in field crops than fruit crops but the mechanisms responsible for this are unknown.

Among geographic variables latitude and longitude had a significant influence on the occurrence of entomopathogenic fungi. The most outstanding result is probably the greater occurrence of *M. anisopliae* at northern (>40°) latitudes. Our principal component analysis also supported the influence of latitude and longitude of the locality on distribution of entomopathogenic fungi. *Beauveria bassiana* occurred more commonly in southern latitudes (below 37°N) and *M. anisopliae* in northern latitudes (above 39°N). The relative importance of geographical location on the occurrence of entomopathogenic fungi has been evaluated previously by Vänninen (1996), who found geographical location the strongest factor determining the occurrence of *M. anisopliae* in Finland, which was a southern species compared with *B. bassiana*, which became more common northwards. However, *M. anisopliae* is more frequently reported prevailing in northern latitudes than *B. bassiana* (Tkaczuk & Mietkiewski 1996; Chandler *et al.* 1997; Bidochka *et al.* 1998; Klingen *et al.* 2002; Keller *et al.* 2003; Bruck 2004; Meyling & Eilenberg 2006). Concerning altitude, entomopathogenic fungi were present in a very wide range of altitudes in our study (from 5 m up to 1608 m). Our results slightly differ from that obtained by Keller *et al.* (2003) in Switzerland where no fungi were isolated from samples taken above 1000 m. However, similarly to this study most of our isolates were obtained from soils sampled at altitudes below 700 m.

Logistic regression analyses showed that pH and clay content were predictive variables for the occurrence of *B. bassiana* whereas organic matter content was predictive for *M. anisopliae*. Interestingly, the absence of both fungal species was also predicted by pH and clay content, with very low recovery rates in soils with pH higher than 8.5 and clay content higher than 40 %. A good example was the low incidence of entomopathogenic fungi in soils from the Canary Archipelago, probably due to their low clay content (high sand content) and high

pH values (very often above 8). This predictive potential of soil pH for fungal presence or absence seems to disagree with those reports indicating the minor effect of pH on abundance of entomopathogenic fungi, particularly in cultivated soils (Rath *et al.* 1992; Vänninen *et al.* 1989); however these differences could be due to the different pH range in these studies.

Our study provides information useful for deciding whether or not a particular cultivated or natural soil is suitable the application of entomopathogenic fungi as a pest control measure and for selecting the fungal species best suited to the prevailing conditions. Alkaline, sandy soils with a low organic matter content may greatly reduce the efficacy of fungal treatments, whereas heavy, moderate to slightly acidic soils with an organic matter content within the range of 2–4 % are the best suited for the use of entomopathogenic fungi. Both fungal species may be used in cultivated soils, although *B. bassiana* seems to be more well adapted than *M. anisopliae* to natural habitats. Our results also indicate that biological control of soil-dwelling pests by resident entomopathogenic fungi is likely to be more effective in annual field crops than in fruit crops. Furthermore, within the range of soil factors favouring fungal occurrence, *B. bassiana* is best suited to soils with a higher pH and clay content but lower organic matter content. Within the particular geographical situation of Spain, *M. anisopliae* seems to be more suited to northern latitudes and *B. bassiana* to the southern ones.

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