RESEARCH ARTICLE

A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest

Emma L. Ormond[1], Alison P.M. Thomas[1], Philip J.A. Pugh[1], Judith K. Pell[2] & Helen E. Roy[3]

[1]Department of Life Sciences, Anglia Ruskin University, Cambridge, UK; [2]Plant and Invertebrate Ecology Department, Rothamsted Research, Hertfordshire, UK; and [3]NERC Centre for Ecology and Hydrology, Wallingford, Crowmarsh Gifford, Oxfordshire, UK

Correspondence: Helen E. Roy, NERC Centre for Ecology and Hydrology, Wallingford, Crowmarsh Gifford, Oxfordshire OX10 8BB, UK. Tel.: +44 1491 692 252; fax: +44 1491 692424; e-mail: hele@ceh.ac.uk

Received 2 December 2009; revised 9 June 2010; accepted 10 June 2010.
Final version published online 20 July 2010.

DOI:10.1111/j.1574-6941.2010.00939.x

Editor: Philippe Lemanceau

Keywords
Beauveria bassiana; fungal entomopathogen; ISSR-PCR; population ecology; *Hypocreales*; conifer forest.

Abstract

The fungal entomopathogen *Beauveria bassiana* is ubiquitous in below-ground systems; however, there is a dearth of information on the above-ground diversity, temporal and spatial distribution of this fungus. Therefore, we assessed its occurrence in a conifer forest (*Pseudotsuga monziesii* and *Pinus nigra* var. *maritima*) using selective media to isolate *B. bassiana* from soil, branch and bark samples collected in October 2005, March and June 2006. Fungal density was the highest at all locations in October, declining in March and June, and absent from conifer branches in June. This above-ground decline most likely resulted from more extreme environmental conditions compared with those below ground. Molecular analyses (ISSR-PCR) indicated that *B. bassiana* is genetically diverse, comprising both distinct microhabitat-specific and seasonal isolates. The occurrence of dissimilar above- and below-ground isolates suggests that *B. bassiana* occupies various overlapping niches in these systems.

Introduction

The globally ubiquitous anamorphic genus *Beauveria* (*Ascomycota*: *Hypocreales*) is thought to have a broad host range and has received considerable attention as a potential biological control agent of pest insects. However, the wealth of literature on the role of such fungal entomopathogens in controlling pest insects is countered by a lack of basic ecological understanding on aspects such as their distribution in natural habitats (Meyling *et al.*, 2009; Roy *et al.*, 2009, 2010; Vega *et al.*, 2009; Hesketh *et al.*, 2010). Despite the potential economic importance of *Beauveria*, research into its fundamental ecology is scarce and its phylogeny and taxonomy remain debatable (Rehner & Buckley, 2005; Rehner *et al.*, 2006). Indeed, it is appropriate to consider *Beauveria bassiana* in the broadest sense as '*B. bassiana sensu lato*' (Rehner & Buckley, 2005; Rehner *et al.*, 2006); here, we will refer to *B. bassiana* as a taxonomical entity (morphospecies), but with the understanding that *Beauveria* comprises two distinct clades that cannot be separated morphologically.

It is generally assumed that genetic groups of *B. bassiana* have coevolved with particular host taxa (Couteaudier & Viaud, 1997; Berretta *et al.*, 1998; Gaitan *et al.*, 2002); yet recent studies suggest that *B. bassiana* also includes generalist entomopathogens (Wang *et al.*, 2003; Rehner & Buckley, 2005). Environmental factors are speculated to determine the patterns of distribution of *B. bassiana* because particular phylogenetic associations with the insect host appear to be singularly absent (Bidochka *et al.*, 2002; Meyling *et al.*, 2009). Little is known about the community ecology of *B. bassiana* in natural (or semi-natural) habitats, which would be invaluable in further developing our understanding of the ecology of this fungus. If *B. bassiana* is widely distributed above ground, then we might expect that there are separate genetic groups of isolates adapted to the different environmental conditions associated with foliage and bark compared with soil. Genotypes could, however, also vary across foliage types as well as between foliage and soil environments. There is a precedence for genotype selection in relation to habitat type, for example, Bidochka *et al.* (2002) identified distinct genetic groups associated with the soil from three different (forest, agricultural and arctic) Canadian habitats. Genetic groups isolated from Arctic and forested habitats grew at lower temperatures relative to those from the agricultural habitat, which also proved tolerant of UV exposure (Bidochka *et al.*, 2002).

A number of studies have demonstrated the prevalence of *B. bassiana* on above-ground vegetation (Doberski & Tribe,

1980; Meyling & Eilenberg, 2006; Reay *et al.*, 2008), with elm bark representing the first report of *B. bassiana* on vegetation (Doberski & Tribe, 1980). Posada & Vega (2005) isolated *B. bassiana* as an endophyte of cocoa seedlings (*Theobrama cacao*), while Meyling & Eilenberg (2006) isolated the pathogen from hedgerow vegetation. More recently, Reay *et al.* (2008) surveyed entomopathogenic fungi from soil, bark and insect frass in New Zealand *Pinus radiata* forest sites and reported that the density of *Beauveria* species varied both within and between sites and substrates. These authors identified three *Beauveria* species using molecular analyses: *B. bassiana*, *Beauveria malawiensis* and *Beauveria caledonica*; the latter was uniquely isolated from several substrates including mycosed bark beetles, soils, bark and insect frass.

The application of modern molecular techniques has identified genetic clades of *B. bassiana*, and indeed, many other fungal species (Castrillo *et al.*, 2003; Atkins & Clark, 2004; Rehner & Buckley, 2005). DNA-based methods provide insights into fungal ecology that are not possible via morphological characters; indeed, some DNA-based techniques can (1) distinguish individual isolates, (2) monitor and track isolates during laboratory and field studies and (3) assess their distribution, persistence and potential for genetic exchange. Castrillo & Brooks (1998) used RAPD-PCR to demonstrate the genetic proximity between *B. bassiana* isolates from similar habitats (habitat selection), detecting a variation both between different regions and among isolates collected from the same insect host. Further isolate correlation with the host and geographical origin has been demonstrated using both AFLP-PCR (Aquino de Muro *et al.*, 2005) and inter simple sequence repeat (ISSR)-PCR (Wang *et al.*, 2005; Estrada *et al.*, 2007).

The primary objective of this work was to assess the population ecology of *B. bassiana* on temporal and spatial scales. We describe a field study conducted between October 2005 and June 2006 to investigate the distribution, density and genetic diversity of *B. bassiana* from the soil, conifer bark and branches in a forest in south-east England. In addition to providing insights into the spatial and temporal dynamics of *B. bassiana*, this study tests the sensitivity of the ISSR-PCR technique in identifying genetic groups. Previous works on the genetic diversity of *B. bassiana* have been conducted on a broad geographical scale comparing isolates from different areas within and between countries (Bidochka *et al.*, 2002; Aquino de Muro *et al.*, 2005; Estrada *et al.*, 2007); thus, a key aim of this study was to focus on the differences between *B. bassiana* isolates over restricted spatial and temporal scales.

Materials and methods

Sampling and isolation

The 20 × 20 m field site comprised a stand of small (2–3 m high) conifers, in a large mixed age/species conifer forest at Kings Forest, Thetford, Norfolk, England (OS grid reference: TL 815 752; latitude: 52°20′43″N and longitude: 0°39′50″E), the two dominant species being Douglas fir (*Pseudotsuga monziesii*) and Corsican pine (*Pinus nigra* var. *maritima*).

Samples were taken from Douglas fir and Corsican pine on three dates: 15 October 2005, 18 March 2006 and 24 June 2006. The dates were chosen to represent three seasons within Britain: autumn, spring and summer. Twelve samples were taken at each sampling date from four locations (soil, bark, low branch and mid branch) from three trees of a similar size (*c.* 3 m high), yielding a total of 48 samples from each species at each sampling date and 288 samples across the entire study. Trees were not marked for resampling, but randomly sampled on each visit, and so it is unlikely that the same trees were sampled more than once.

Individual soil samples (approximately 50 g) were collected, using a stainless-steel trowel (alcohol washed between samples), from four randomly selected positions around the base of each tree beneath the moss and humus layer at a depth of 2 cm. The soil was placed in 180 × 229-mm polyethylene food bags for transport back to the laboratory.

The terminal 5 cm of a branch, with needles, was removed from a lower (< 1 m) and a mid section (< 1.5 m) of the tree using surgical scissors, which were alcohol washed between samples. The terminal section was selected as the most convenient section to remove. 3 × 2 × 0.2-cm sections of bark were collected from the trunk 1 m above the soil.

All samples were placed in individual 50-mL disposable plastic tubes (29 × 115 mm) and stored in the shade for no more than 3 h until return from the field to the laboratory, where they were stored for no more than 6 h at 5 °C before processing.

To isolate *B. bassiana* from the soil samples, a subsample of 1 g of soil was taken from the field sample and suspended in 9 mL of sterile 0.03% Tween 80 (Fisher Scientific, Loughborough, UK) and vortexed for 1 min. Triplicate 100-μL aliquots of this dilution were streaked out onto oatmeal dodine agar (ODA; Chase *et al.*, 1986). The plates were placed in a dark (nonilluminated) incubator at 22 °C and the number of CFUs was recorded after 1 week. To isolate *B. bassiana* from the tree samples, the above process was repeated with a 2-cm section of distal branch or 0.3 g of bark (approximately 1/4th of the field sample). Bark weight was used as opposed to a surface area because the bark sample comprised both the inner and the outer bark.

Molecular characterization

The isolates for molecular characterization were obtained by randomly selecting CFUs (one per plate) from 144 of the initial 288 ODA plates (ensuring, where possible, equal numbers of each sample type, although CFUs were not present in all samples). Each CFU was streaked out onto a second

Table 1. ISSR primer sequences, with details of the number and the size of amplified fragments

Primer	Sequence	Total number of polymorphic bands	Amplicon size range (bp)
6	5′-GATATCGTCCGACGACGACGA-3′	43	180–1500
7	5′-CTATCCTGTGTGTGTGTG-3′	41	180–1600
I	5′-GCCTCCTCCTCCTC-3′	34	220–1500
D	5′-GTGTGTGTGTGTGTGTG-3′	40	130–1600

ODA plate and placed in a dark incubator at 22 °C for 8 days. A CFU (assumed to be single conidium isolate), again randomly selected from each plate, was placed on a fresh ODA plate and maintained in a dark incubator at 22 °C for 2–4 weeks. This incubation regime yielded sufficient fungal growth for DNA extraction from a total of 144 isolates.

DNA was extracted by suspending fungal material scraped with a sterilized microbiological loop from a sporulating single conidium isolate in 100 μL of 0.03% Tween 80. The DNA was then extracted using a DNEasy Tissue Kit (Qiagen, West Sussex, UK) according to the manufacturer's instructions.

Screening for molecular diversity among the isolates was via ISSR-PCR using one of four primers (Table 1) in each reaction. Each 20 μL PCR comprised 2 μL buffer [750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM $(NH_4)_2SO_4$], 2 μL 100 mM dNTPs (ABgene, UK), 4.7 μL sterile-distilled water, 4 μL primer, 4 μL Q solution (Qiagen), 0.1 μL Taq polymerase (Qiagen) and 2 μL DNA. PCR was performed on a TC-412 thermocycler (Techne). The PCR programme for primer 6 was composed of an initial 3 min at 94 °C, followed by 35 cycles at 94 °C for 1 min, 63 °C for 2 min and 72 °C for 6 min, and finalized by 7 min at 72 °C. The PCR programmes for primers 7, I and D were identical, except for the annealing temperatures, which were 52, 47.8 and 58 °C, respectively. The PCR programmes were run using a manual hot start: 1.2 μL of $MgCl_2$ (25 mM) was added to each PCR when the reaction temperature reached 94 °C. Amplified PCR products were run on a 2% agarose gel, containing 1 μL of ethidium bromide (25 mg mL^{-1}), and photographed in UV light using a Canon digital camera. Reproducibility was established for each primer using a sample of 20 isolates before full screening was undertaken.

Data analysis

The statistical software R (version 2.8.1) (www.r-project.org) was used for the generalized linear models (GLM). GLMs were used to analyse (1) the frequency of *B. bassiana* across all positions (soil, bark, low and mid branches), (2) the CFU density data obtained for soil and bark samples and (3) the CFU count data for branch samples (mid and low positions). The values for bark and soil were compared by converting the number of CFUs to density per gram of substrate. Samples from branches at low and mid locations were directly comparable as number of CFUs per 2 cm of branch. Full models with all interactions were fitted for both data sets and nonsignificant factors were eliminated successively until a best-fit model was achieved. The quasi-binomial function was used for the frequency of occurrence of *B. bassiana* and the quasi-Poisson function was used for the CFU density and count data. GLM-derived coefficients were compared using ANOVA.

Gels were scored manually for band size and the resulting binary (presence/absence of amplified bands) data matrices were analysed using the MULTIVARIATE STATISTICAL PACKAGE (MVSP) (Kovach, 1999). Our original primary MS 'EXCEL' spreadsheet defined the data, in terms of gel bands, via the five discrete parameters of 'tree' (Corsican pine/Douglas fir), 'date' (October/March/June), 'substrate' (bark/low foliage/mid foliage/soil), 'aspect' (north/east/south/west) and 'replicate' (1–6). The primary dataset was totalled by 'band', and all '0'-scoring bands were removed. The residual band data were analysed in MVSP via principal components analysis (PCA), which applied Kaiser's rule (Legendre & Legendre, 1983) to transposed and centred data under default 'advanced settings' with scatter plots shown as variable loadings.

Cluster analysis

Gel band matrices were further analysed using FREE TREE (Hampl *et al.*, 2001), where 10 000 bootstrap replicates of trees generated via the Jaccard coefficient and neighbour-joining algorithms were produced for each of the four ISSR primers, and then pooled to produce a tree using the programme TREEVIEW (Page, 1996).

Results

Spatiotemporal distribution of *B. bassiana*

Beauveria bassiana was isolated from the soil and foliage of both conifer species (Table 2). There was no difference in the frequency of the occurrence of *B. bassiana* on the Corsican pine compared with the Douglas fir ($F_{1,286} = 0.0143$; $P = 0.904$). The occurrence of *B. bassiana* was lower in June compared with March and October ($F_{2,282} = 53.0$; $P < 0.001$) and on the branches compared with the soil and bark ($F_{3,284} = 44.1$; $P < 0.001$). The only significant interaction was between date and position ($F_{6,276} = 31.8$; $P < 0.001$), whereby the frequency of occurrence was high

Table 2. The percentage of samples from each tree species (Corisican pine and Douglas fir) at each sample point (soil, low branch, mid branch, bark) and collection date (15 October 2005, 18 March and 24 June 2006) that yielded *Beauveria bassiana* when plated out on ODA ($n = 12$ for each location–tree combination)

	Sampling date			
	Location	15 October 2005	18 March 2006	24 June 2006
Corsican pine	Soil	100.0	100.0	83.3
	Bark	91.6	16.6	0.0
	Low branch	91.6	16.6	0.0
	Mid branch	91.6	33.3	0.8
Douglas fir	Soil	100.0	100.0	100.0
	Bark	100.0	0.0	0.0
	Low branch	75.0	8.3	0.0
	Mid branch	91.6	50.0	66.6

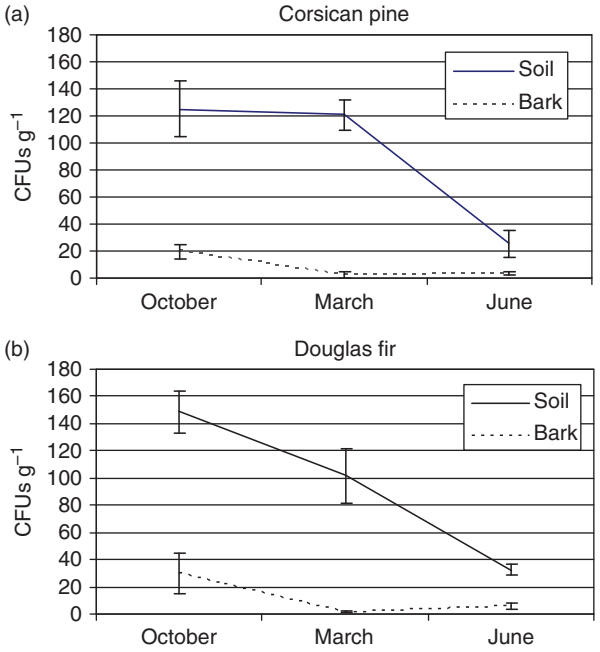

Fig. 1. Density (CFU g^{-1}) of *Beauveria bassiana* isolated from samples of soil and bark taken from Corsican pine trees (a) and Douglas fir trees (b) in October, March and June 2006 (error bars = SE).

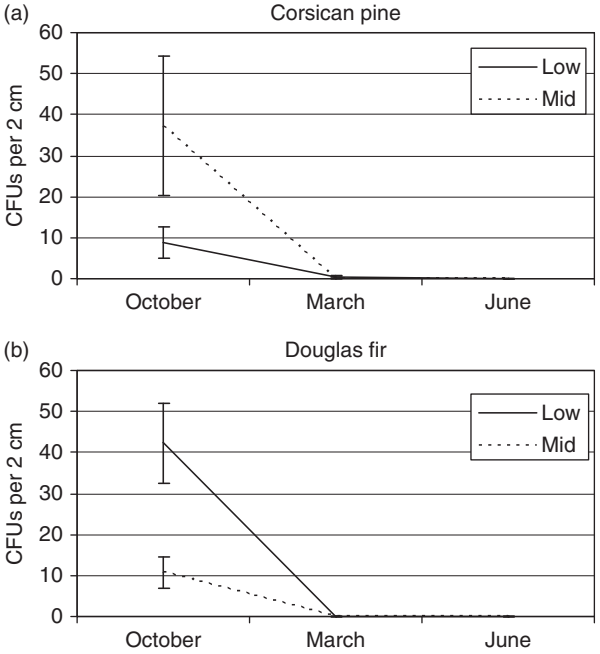

Fig. 2. Number (CFU per 2 cm) of *Beauveria bassiana* isolated from branch samples (mid and low positions on the tree) taken from Corsican pine trees (a) and Douglas fir trees (b) in October, March and June 2006 (error bars = SE).

in October for all samples, but declined for samples from conifer branches in March and June (none isolated from branches in June), while remaining consistently high for soil and bark samples throughout the year.

Corsican Pine mean (±SE) *B. bassiana* CFU densities ranged from 26.0 (±7.2) to 125.0 (±17.0) per gram of soil and from 2.0 (±8.0) to 20.0 (±2.0) per gram of bark. Corsican pine mean (±SE) CFUs ranged from 0.0 (±0.0) to 8.8 (±3.8) per 2 cm low branch and from 0.0 (±0.0) to 37.3 (±16.9) per 2 cm mid branch.

Douglas fir mean (±SE) *B. bassiana* CFU densities ranged from 32.6 (±4.5) to 148.9 (±15.5) per gram of soil and 1.7 (±0.8) to 28.8 (±14.8) per gram of bark. Douglas fir mean (±SE) CFU number ranged from 0.0 (±0.0) to 42.3 (±9.9) per 2 cm low branch and from 0.0 (±0.0) to 10.8 (±3.8) per 2 cm mid branch.

The density of *B. bassiana* on the two tree species was similar throughout the study (Fig. 1a and b). There was, however, a significant difference between sampling periods, with a pronounced decline in density in June compared with the previous months ($F_{2,140} = 26.0$; $P < 0.001$; Fig. 1a and b). The fungal density was higher in the soil compared with the bark ($F_{1,142} = 33.2$; $P < 0.001$; Fig. 1a and b). There was a significant interaction between tree species and sampling date ($F_{2,138} = 10.0$; $P < 0.001$; Fig. 1a and b), which relates to the more rapid decline in all March samples, with the exception of the soil samples taken from the base of the Corsican pine in which the fungal density was similar in October and March samples.

There was a significant difference in the number of CFUs of *B. bassiana* per 2 cm of branch on the different sampling dates ($F_{1,141} = 83.6$; $P < 0.05$; Fig. 2a and b). There was also a significant interaction between tree species and branch position on the tree (low or mid) ($F_{1,138} = 30.2$; $P < 0.001$; Fig. 2a and b). The fungus could only be isolated from

branches in October and on the Corsican pine, the mid branch had a higher number of CFUs than the low branch, and on the Douglas fir, the pattern was reversed.

Molecular characterization

One hundred and fifty-eight polymorphic ISSR bands were scored for presence/absence across the 144 isolates. Initial PCA analysis of the complete data extracted 78% of potential variance from 32 axes. This, together with the random scatter plot derived from the 19.1% variance extracted by the first two axes, suggests a substantial interaction between the five sample parameters of tree species, sample date, substrate, aspect and replicate. To overcome this problem of parameter interaction, we sorted the data into the 10 paired-parameter combination data sets and again removed all '0'-scoring bands. PCAs of the residual scoring bands identified four significant groupings that extracted substantially more variance from fewer axes (Table 3). One implies that tree species is significant and yet three (e.g. Figs 3 and 4) identify sample month as significant in terms of consistent 'March', 'June', 'October' PCA groupings, but no clearly defined complementary signal in either 'aspect' (Fig. 3) or 'tree' (Fig. 4). This suggests the presence of potentially genetically distinct isolates on different trees, although more convincingly in summer (June) compared with autumn (October) and winter (March). The cluster analysis indicated variability among the 144 isolates (Fig. 5) that were habitat specific (38% soil, 31% foliage and 31% bark isolated). Each habitat type included isolates from both tree species.

Discussion

Our isolation of *B. bassiana* from conifer branches and bark samples confirms the few records of this fungus on foliar material (Doberski & Tribe, 1980; Meyling & Eilenberg, 2006; Reay *et al.*, 2008; Meyling *et al.*, 2009). Despite the frequent records of *B. bassiana* from both soil (Bidochka *et al.*, 1998; Glare & Inwood, 1998; Sun & Liu, 2008) and insect hosts (Lipa *et al.*, 1975; Gaitan *et al.*, 2002), little is known about its wider occurrence in terrestrial ecosystems and especially on foliage (Reay *et al.*, 2008). All locations sampled (above and below ground) yielded *B. bassiana*, but the density of this fungus was greater in the soil than any of the tree samples and the greatest in October. Meyling & Eilenberg (2006) also found that *B. bassiana* was more abundant in September (autumn) compared with May and July (summer) samples.

Table 3. Congruent cluster analysis/PCA groupings

	Substrate	Aspect	Replicate	Month
Tree	None	**Fir vs. pine**	None	**June vs. March vs. October**
	60.3%	55.0%	46.0%	58.4% (Fig. 3)
Substrate		None	None	None
		40.8%	30.1%	44.4%
		(60.8% from 3)	(59.9% from 7)	(57.5% from 3)
Aspect			None	**June vs. March and October**
			35.4%	44.2% (Fig. 4)
			(59.5% from 7)	(53.3% from 3)
Replicate				**June vs. March and October**
				40.8%
				(53.9% from 4)

Data show major groupings identified (bold) in terms of variance extracted from both axes 1 and 2, and (in parentheses) total variance if more than extracted from total ($n \geq 3$) axes.

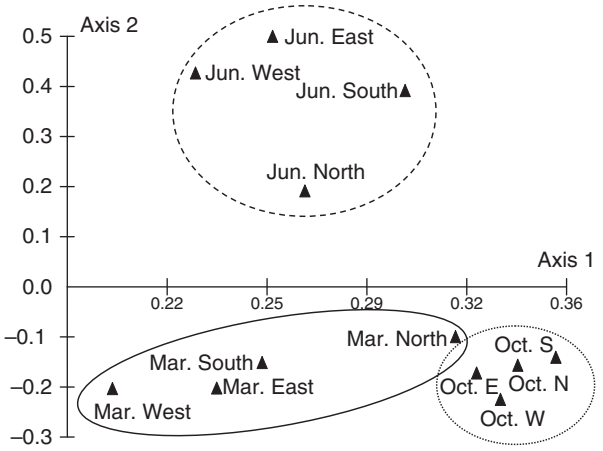

Fig. 3. PCA scatterplot of 'month' vs. 'aspect' sample groups. March (solid oval) is separated from October (dotted) by axis 1 and from June (dashed) by axis 2. PCA extracts 44.2% of variance from axes 1 (32.2%) and 2 (12.0%).

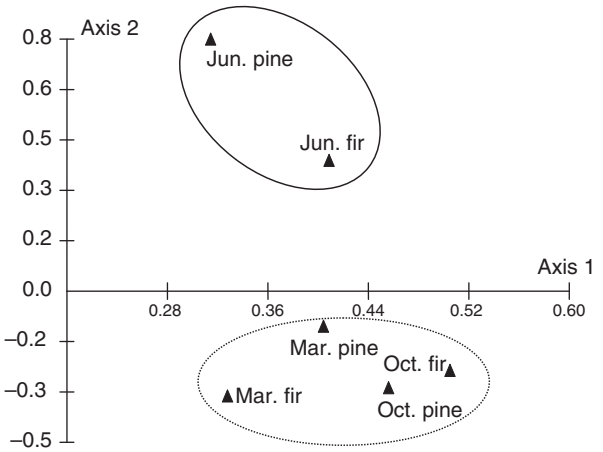

Fig. 4. PCA scatterplot of 'month' vs. 'tree' sample groups. The combined March and October (dotted oval) is clearly separated from June along PCA axis 2. PCA extracts 58.4% of variance from axes 1 (37.7%) and 2 (20.7%).

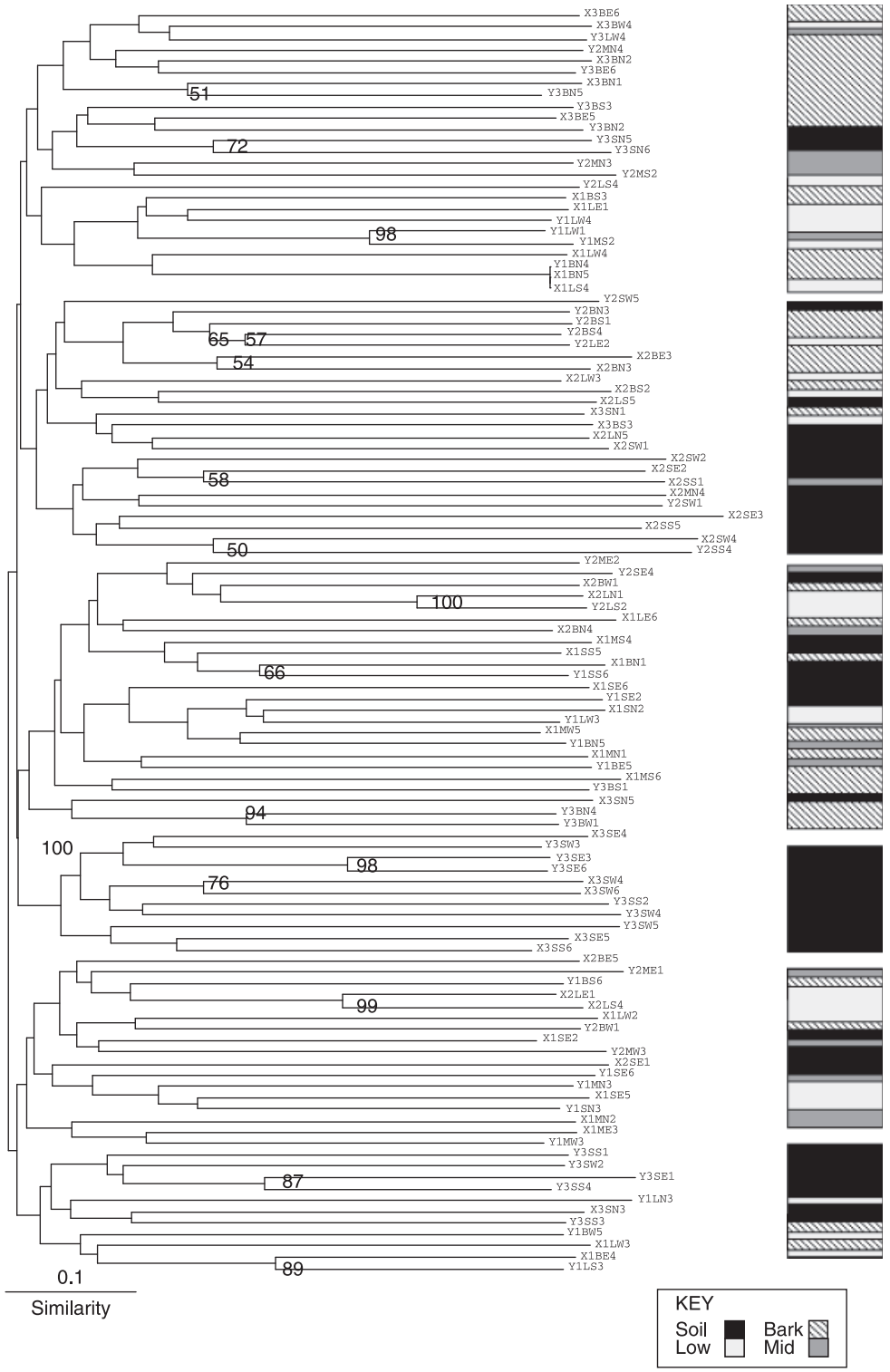

Fig. 5. Spatial relationships inferred from neighbour-joining analysis of the similarity matrix created using Jaccard's coefficient. Only bootstrap values above 50% are shown (combined ISSR-PCR results).

The spatial and temporal dynamics of *B. bassiana* within an ecosystem are dependent on both abiotic and biotic factors. Furthermore, the movement of *B. bassiana* inoculum could follow a number of ecosystem pathways (Meyling & Eilenberg, 2006; Meyling *et al.*, 2006; Hesketh *et al.*, 2010) including dispersal via air currents and insect hosts (Hajek, 1997) or rain splash from the soil to above-ground vegetation (Bruck & Lewis, 2002a). The latter is possibly confirmed by the observed decrease in conidia density on branches at high locations. However, the movement of host insects can be important in the dispersal of this fungus. Meyling *et al.* (2006) demonstrated the ability of nettle insects (*Anthocoris nemorum*) to disperse the conidia of *B. bassiana* from soil or sporulating cadavers into the nettle canopy. The ability of sap beetles (*Coleoptera*: *Nitidulidae*) to carry a specific strain of *B. bassiana* to overwintering sites has also been demonstrated (Bruck & Lewis, 2002b; Dowd & Vega, 2003). Host insects could also play a role in dispersing the fungus from the soil to the trees, and indeed between trees, within this conifer forest. Many insects are present in this conifer system throughout the year, with ladybirds (*Coleoptera*: *Coccinellidae*) being particularly abundant in this system, and *B. bassiana* is a major mortality factor of the seven-spot ladybird, *Coccinella septempunctata* L. (Majerus, 1994; Cottrell & Shapiro-Ilan, 2003; Roy *et al.*, 2008). Seven-spot ladybirds, *C. septempunctata*, overwinter in the soil, moving into the trees in the spring (Majerus, 1994). Seven-spot ladybirds have been shown to move an obligate aphid fungal pathogen between aphid colonies (Roy *et al.*, 2001). It is feasible that the dispersal of *B. bassiana* is facilitated through the movement of this and other insect hosts, although this requires further study.

We observed clear temporal trends in both tree and soil data with decreasing conidial density (more so in trees than in soil) from October to June. Our molecular data show separate clustering of October, March and June isolates, suggesting seasonal genetic differentiation. The fungal communities of plant surfaces are exposed to different, and more variable, environmental factors, such as UV flux, desiccation and thermal regime, than those in the soil. The detrimental effects of UV light on the conidial survival of entomopathogenic fungi are well established (Moore *et al.*, 1996; Morley-Davies *et al.*, 1996; Fargues *et al.*, 1997), and moisture content is thought to be the principal underlying cause of decreasing *B. bassiana* density in the soil (Sandhu *et al.*, 1993) because *B. bassiana* conidia require high levels of humidity to germinate. Although summer temperatures are favourable for conidium production, it is likely that conidium germination and hyphal growth are limited by suboptimal humidity.

This study has shown that the DNA profiling technique ISSR-PCR is a powerful tool for differentiating between *B. bassiana* isolates collected from a single ecosystem. Previous work has shown that *B. bassiana* isolates cluster at the geographic scale of a country (Wang *et al.*, 2003; Aquino de Muro *et al.*, 2005); here, we report on isolates clustering within a single ecosystem. This confirms that *B. bassiana* isolates correlate with spatial and temporal factors, with some being ubiquitous within the habitat throughout the year and others restricted to certain locations or time periods. This could reflect the facultative nature of *B. bassiana* as an insect pathogen, with survival driven primarily by adaptation to habitat factors. Furthermore, the simultaneous occurrence of some isolates in the soil and on trees suggests that the pathogen is moving between above- and below-ground environments. Meyling *et al.* (2009) found similar results in a study on an arable field/hedgerow agroecosystem and, as here, noted (1) considerable genetic diversity among isolates and (2) both ubiquitous and habitat (field vs. hedgerow)-specific isolates. The results presented in this paper indicate that fluctuating environmental factors within a single habitat can drive genetic differentiation from both a spatial (above and below ground) and a temporal perspective as suggested by Bidochka *et al.* (2002).

The results of this investigation contradict the conventional opinion that *B. bassiana* is principally a soil fungus; identification of isolates above and below ground suggests that this taxon occupies various overlapping niches. Meyling *et al.* (2009) propose a metapopulation structure for fungi occupying an agroecosystem and we envisage that the *B. bassiana* conifer forest discussed here represents a metapopulation in which local extinction and colonization are driven by the presence of suitable hosts, favourable abiotic conditions and dispersal events.

Acknowledgements

The authors would like to thank the British Mycological Society for a grant that supported the molecular studies and Nicolai Meyling for discussions on this work. E.L.O. was funded by the Environmental Sciences Research Centre (Anglia Ruskin University) for her PhD studies through which this work was undertaken. A.P.M.T. and P.J.A.P. are funded by the Department of Life Sciences (Anglia Ruskin University). J.K.P. is funded by the Department for Environment, Food and Rural Affairs (Defra) of the United Kingdom. Rothamsted Research is an institute of the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. H.E.R. is funded by the Environmental Change Integrating Fund through the Centre for Ecology & Hydrology (Natural Environmental Research Council (NERC) and the Joint Nature Conservation Committee (JNCC). The experiments comply with the current laws of the country in which they were performed.

References

Aquino De Muro M, Elliott S, Moore D, Parker BL, Skinner M, Reid W & El Bouhssini M (2005) Molecular characterisation of *Beauveria bassiana* isolates obtained from overwintering sites of Sunn Pests (*Eurygaster* and *Aelia* species). *Mycol Res* **109**: 294–306.

Atkins SD & Clark IM (2004) Fungal molecular diagnostics: a mini review. *J Appl Genetics* **45**: 3–15.

Berretta MF, Lecuona RE, Zandomeni RO & Grau O (1998) Genotyping isolates of the entomopathogenic fungus *Beauveria bassiana* by RAPD with fluorescent labels. *J Invertebr Pathol* **71**: 145–150.

Bidochka MJ, Kasperski JE & Wild GAM (1998) Occurrence of the entomopathogenic fungi *Metarhizium anispoliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. *Can J Botany* **76**: 1198–1204.

Bidochka MJ, Menzies FV & Kamp AM (2002) Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. *Arch Microbiol* **178**: 531–537.

Bruck DJ & Lewis LC (2002a) Rainfall and crop residue effects on soil dispersion and *Beauveria bassiana* spread to corn. *Appl Soil Ecol* **20**: 183–190.

Bruck DJ & Lewis LC (2002b) *Carpophilus freemani* (Coleoptera: Nitidulidae) as a vector of *Beauveria bassiana*. *J Invertebr Pathol* **80**: 188–190.

Castrillo LA & Brooks WM (1998) Differentiation of *Beauveria bassiana* isolates from the Darkling Beetle, *Alphitobius diaperinus*, using isozyme and RAPD analyses. *J Invertebr Pathol* **72**: 190–196.

Castrillo LA, Vandenberg JD & Wraight SP (2003) Strain-specific detection of introduced *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers. *J Invertebr Pathol* **82**: 75–83.

Chase AR, Osborne LN & Ferguson VM (1986) Selective isolation of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* from an artificial potting medium. *Fla Entomol* **69**: 285–292.

Cottrell TE & Shapiro-Ilan DI (2003) Susceptibility of a native and an exotic lady beetle (Coleoptera: Coccinellidae) to *Beauveria bassiana*. *J Invertebr Pathol* **84**: 137–144.

Couteaudier Y & Viaud M (1997) New insights into the population structure of *Beauveria bassiana* with regard to vegetative compatibility groups and telomeric restriction fragment length polymorphisms. *FEMS Microbiol Ecol* **22**: 175–182.

Doberski JW & Tribe HT (1980) Isolation of entomogenous fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. *T Brit Mycol Soc* **74**: 95–100.

Dowd PF & Vega FE (2003) Autodissemination of *Beauveria bassiana* by sap beetles (Coleoptera: Nitidulidae). *Biocontrol Sci Techn* **13**: 69–79.

Estrada ME, Camacho MV & Benito C (2007) The molecular diversity of different isolates of *Beauveria bassiana* (Bals.) Vuill. as assessed using intermicrosatellites (ISSRs). *Cell Mol Biol Lett* **12**: 240–252.

Fargues J, Goettel MS, Smits N, Ouedraogo A & Rougier M (1997) Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. *Mycologia* **89**: 383–392.

Gaitan A, Valderrama AM, Saldarriaga G, Velez P & Bustillo A (2002) Genetic variability of *Beauveria bassiana* associated with the coffee berry borer, *Hypothenemus hampei* and other insects. *Mycol Res* **106**: 1307–1314.

Hajek AE (1997) Ecology of terrestrial fungal entomopathogens. *Adv Microb Ecol* **15**: 193–249.

Hampl V, Pavlícek A & Flegr J (2001) Construction and bootstrap analysis of DNA fingerprinting-based phylogenetic trees with a freeware program FreeTree: application to trichomonad parasites. *Int J Syst Evol Micr* **51**: 731–735.

Hesketh H, Roy HE, Eilenberg J, Pell JK & Hails RS (2010) Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. *BioControl* **55**: 55–73.

Glare TR & Inwood AJ (1998) Morphological and genetic characterisation of *Beauveria* spp. from New Zealand. *Mycol Res* **102**: 250–256.

Kovach WL (1999) *MVSP – A Multivariate Statistical Package for Windows, Version 3.1*. Kovach Computing Services, Pentraeth, UK.

Legendre L & Legendre P (1983) *Numerical Ecology*. Elsevier Scientific Publishing Company, UK.

Lipa JJ, Pruszynski S & Bartkowski J (1975) The parasites and survival of the lady bird beetles (Coccinellidae) during winter. *Acta Parasit Pol* **23**: 453–461.

Majerus MEN (1994) *Ladybirds*. Harper Collins Publisher, Somerset, UK.

Meyling NV & Eilenberg J (2006) Isolation and characterisation of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation. *Mycol Res* **110**: 188–195.

Meyling NV, Pell JK & Eilenberg J (2006) Dispersal of *Beauveria bassiana* by the activity of nettle insects. *J Invertebr Pathol* **93**: 121–126.

Meyling NV, Lübeck M, Buckley EP, Eilenberg J & Rehner SA (2009) Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. *Mol Ecol* **18**: 1282–1293.

Moore D, Higgins PM & Lomer CJ (1996) Effects of simulated and natural sunlight on the germination of conidia of *Metarhizium flavoviride* Gams and Rozsypal and interactions with temperature. *Biocontrol Sci Techn* **6**: 63–76.

Morley-Davies J, Moore D & Prior C (1996) Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated sunlight and a range of temperatures. *Mycol Res* **100**: 31–38.

Page RDM (1996) Tree view: an application to display phylogenetic trees on personal computers. *Bioinformatics* **12**: 357–358.

Posada F & Vega FE (2005) Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota:

Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacoa*). *Mycologia* **97**: 1195–1200.

Reay SD, Brownbridge M, Cummings NJ, Nelson TL, Souffre B, Lignon C & Glare TR (2008) Isolation and characterization of *Beauveria* spp. associated with exotic bark beetles in New Zealand *Pinus radiate* plantation forests. *Biol Control* **46**: 484–494.

Rehner SA & Buckley EP (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **97**: 84–98.

Rehner SA, Posada F & Buckley EP (2006) Phylogenetic origins of African and Neotropical *Beauveria bassiana s.l.* pathogens of the coffee berry borer, *Hypothenemus hampei*. *J Invertebr Pathol* **93**: 11–21.

Roy HE, Pell JK & Alderson PG (2001) Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the aphid predator *Coccinella septempunctata*. *Biocontrol Sci Techn* **11**: 101–112.

Roy HE, Brown PMJ, Rothery P, Ware RL & Majerus MEN (2008) Interactions between the fungal pathogen *Beauveria bassiana* and three species of ladybird: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *BioControl* **53**: 265–276.

Roy HE, Hails RS, Hesketh H, Roy DB & Pell JK (2009) Beyond biological control: non-pest insects and their pathogens in a changing world. *Insect Conserv Biodivers* **2**: 65–72.

Roy HE, Brodie EL, Chandler D, Goettel MS, Pell JK, Wajnberg E & Vega F (2010) Deep space and hidden depths: understanding the evolution and ecology of fungal entomopathogens. *BioControl* **55**: 1–6.

Sandhu SS, Rajak RC & Agarwal GP (1993) Studies on prolonged storage of *Beauveria bassiana* conidia: effects of temperature and relative humidity on conidial viability and virulence against chickpea borer, *Helicoverpa armigera*. *Biocontrol Sci Techn* **3**: 47–53.

Sun B-D & Liu X-Z (2008) Occurrence and diversity of insect-associated fungi in natural soils in China. *Appl Soil Ecol* **39**: 100–108.

Vega FE, Goettel MS, Blackwell M *et al.* (2009) Fungal entomopathogens: new insights on their ecology. *Fungal Ecol* **2**: 149–159.

Wang CS, Shah FA, Patel N, Li Z-Z & Butt TM (2003) Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. *Environ Microbiol* **5**: 908–915.

Wang S, Miao X, Zhao W, Huang B, Fan M, Li Z & Huang Y (2005) Genetic diversity and population structure among strains of the entomopathogenic fungus, *Beauveria bassiana*, as revealed by inter-simple sequence repeats (ISSR). *Mycol Res* **109**: 1364–1372.