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Isolation and characterisation of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation

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ARTICLE INFO

Article history:
Received 5 May 2005
Accepted 30 September 2005
Corresponding Editor: Judith K. Pell

Keywords:
Biocontrol
Entomopathogenic fungi
Leaf imprinting
Population biology
Universally Primed PCR

ABSTRACT

A leaf imprinting technique combined with a selective medium was used to document the natural occurrence of *Beauveria bassiana* on phylloplanes of typical hedgerow plants (grasses, stinging nettle and hawthorn) in May, July and September in a hedgerow in Denmark. The density of *B. bassiana* (as measured by numbers of colony forming units) was greatest in September and on lower nettle leaves. *B. bassiana* was isolated from phylloplanes in a different hedgerow the following year and a similar picture of occurrence was found. Genetic diversity of selected *in vitro* isolates were characterised by Universally Primed (UP) PCR, and 13 distinguishable banding patterns were found at the two localities. Of these, four were shared between the field sites and all plant species harboured isolates of *B. bassiana* with at least two different banding patterns. The isolation method described represents a valuable tool for studying naturally occurring *B. bassiana* and for rapid isolation of indigenous strains of the fungus for future development of biocontrol agents. The significance of the findings for the life-cycle of *B. bassiana* is discussed.

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Introduction

The cosmopolitan anamorphic fungus *Beauveria bassiana* (*Ascomycota: Hypocreales*) is a well recognised entomopathogen known to infect hundreds of host species belonging to most insect orders. This ability has prompted extensive research on the potential of *B. bassiana* for biological control of pests and several commercial products have been developed (Inglis *et al.* 2001). *B. bassiana* is a facultative pathogen and the fungus can survive saprotrophically in the soil environment for extended time periods (Keller & Zimmerman 1989; Hajek 1997). Apart from the documentation of *B. bassiana* as natural infections in insects and its natural occurrence in soils, there is limited evidence for the distribution of the fungus outside the host in terrestrial ecosystems. Furthermore, there is limited knowledge of the spatial distribution and population dynamics of the species, especially above ground, which is essential to understand pathogen ecology and to improve insect pest management (Dwyer 1992).

It is generally believed that *B. bassiana*, as most entomopathogenic fungi in the *Hypocreales*, can disperse from sporulating cadavers (Gottwald & Tedders 1982; Long *et al.* 2000; Shah & Pell 2003), as infections in migrating insect hosts (Feng *et al.* 2004) or by infectious conidia on wind currents (Hajek 1997). Indeed, conidia of *B. bassiana* have been recovered from air samples (Airaudi & Marchisio 1996; Shimazu *et al.* 2002; Ulevicius *et al.* 2004). However, the passively dispersed conidia must eventually be deposited and one likely sink for these propagules would be aerial plant surfaces, such as phylloplanes. One report of isolation from elm bark demonstrates that *B. bassiana* can be present naturally on plant surfaces (Doberski & Tribe 1980). *B. bassiana* is also known to form an endophytic association with corn (Bing & Lewis 1991, 1992; Arnold & Lewis 2005) and Bruck & Lewis (2002b) isolated

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doi:10.1016/j.mycres.2005.09.008

B. bassiana from the surfaces of field collected corn plants. Studies of leaf-associated fungi have usually focused on plant pathogens (e.g. Kinkel 1997; Andrews & Harris 2000), and previous studies of the natural occurrence of phylloplane fungi have not identified *B. bassiana* among the species recovered (Dickinson 1976; Parbery *et al.* 1981; Newsham *et al.* 1997; Inacio *et al.* 2002; Pereira *et al.* 2002).

We conducted a field study in 2003 to investigate the natural occurrence, over time, of *B. bassiana* conidia on phylloplanes of common hedgerow plants in a field margin in Denmark. The method used was a leaf imprinting technique onto a selective medium. Leaf impressions onto water agar were previously used by Fransen (1995) to assess germination of *Aschersonia aleyrodis* (*Ascomycota*: *Hypocreales*) on cucumber leaves. The following year collections were made at a separate but comparable field site. Selected isolates of the fungus were subjected to Universally Primed (UP) PCR fingerprinting methods. UP-PCR primers are relatively long and target non-specific regions of the genome, and the method generates multiple bands (Bulat *et al.* 1998). High reproducibility of UP-PCR has been demonstrated between laboratories (Lübeck *et al.* 1999) and the method is suitable for screening for genetic diversity amongst fungus isolates.

Materials and methods

Field sites and collection of leaves

The hedgerow for collections in 2003 bordered the southeast side of a cereal field located on an experimental farm at Taastrup, 20 km west of Copenhagen, Denmark (55°40′N, 12°18′E). In the hedgerow, four sampling points of similar plant structure were selected 70-80 m apart. Each sampling point was characterised by the presence of hawthorn (*Crataegus monogyna*), stinging nettle (*Urtica dioica*) and various grasses (*Poaceae*), particularly orchard grass *Dactylis glomerata*, and common couch *Elytrigia repens*.

Leaves were collected on three different dates during the 2003 growing season: on 7 May, 7 July and 12 Sept. The vegetation in the hedgerow was divided into four plant groups: (1) hawthorn; (2) stinging nettle, upper leaves; (3) stinging nettle, lower leaves; and (4) grasses. At every collection date the heights of 50 nettle plants were measured.

In each of the four sampling points within the hedgerow, 25 arbitrarily chosen leaves from each plant group were collected. Hawthorn leaves were selected 10-15 cm from the tip of branches 1.5-2 m above the ground. Upper nettle leaves were defined as the highest leaves that were totally unfolded on nettle plants, while lower nettle leaves were the lowest leaves on nettle stems. Grass leaves were selected arbitrarily at each sampling point. All leaves were detached at the petiole with forceps except for the grass tillers which were held with forceps and cut at the stem using a pair of scissors. Between collections at each sampling point, forceps and scissors were rinsed in 70 % ethanol and water. Each leaf was placed in a separate polyethylene bag and kept shaded in an insulated box at 15-20 °C until transported to the laboratory. All leaves were collected between 9 am and noon on all sampling dates. In the laboratory, bags with leaves were stored in a refrigerator at 5 °C until processing and no longer than 4-5 h.

Additional sampling was conducted on 7 Sept. 2004 at another locality, at the village Østrup, North Zealand, Denmark (55°43′N, 12°13′E). This site was again a hedgerow hosting a plant community with a diversity and physical structure similar to the site sampled in 2003. The hedgerow was oriented southwest-northeast bordering a country road to the southeast and a cereal field to the northwest. Four sampling points were identified as described above. Leaves were collected in a similar manner and from the same plant groups as described above and processed after returning to the laboratory on the day of collection.

Isolation of fungi from leaf surfaces

Each leaf was taken from the respective polyethylene bag with sterile forceps and pressed onto a solid selective agar medium in 9 cm or 14 cm triple vented Petri dishes. The medium consisted of 5 g peptone (Becton Dickinson, Sparks, MD), 10 g glucose (Merck, Darmstadt) and 6 g agar (no. 1, Oxoid, Basingstoke) dissolved in 500 ml demineralised water and subsequently autoclaved for 20 min at 120 °C. When the medium had cooled to 55-60 °C the pH was adjusted to 6.3 using 1 M KOH. Aliquots of 0.5 ml of 0.6 g/ml^{-1} streptomycin (ICN Biomedicals, Aurora, OH), 0.5 ml of 0.05 g ml^{-1} tetracycline (CN Biosciences, La Jolla, CA), 0.5 ml of 0.1 g ml^{-1} dodine (Sigma-Aldrich, Steinheim) and 2.5 ml of 0.05 g ml^{-1} cyclohexamide (Sigma-Aldrich) were subsequently added. This medium was modified from that described by Strasser *et al.* (1996) developed for isolation of *Beauveria brongniartii*. An equivalent medium was recently used to study occurrence of *B. brongniartii* in soil (Kessler *et al.* 2003).

The upper (adaxial) surface of the leaf was first firmly pressed onto one side of the Petri dish, then the leaf was turned over and the lower (abaxial) surface was pressed onto the other side of the Petri dish. All leaves were processed between 13.00 h and 18.00 h on the day of collection. Six control dishes were left open for 4.5–5 h. on the benches in the laboratory to test for the presence of *B. bassiana* inoculum in the air. Only on one dish in Sept. 2003 was a single colony forming unit (CFU) of *B. bassiana* observed, which was considered to be negligible and thus unlikely to bias the data. All Petri dishes were incubated in darkness at 23 °C and CFUs were counted after two weeks. The identities of sporulating colonies resembling *B. bassiana* were verified under a light microscope (400× magnification).

After imprinting the leaves were mounted on paper sheets and photocopied. The surface area of each leaf was determined from these copies using Adobe Photoshop 5.5 (Adobe Systems).

Isolates are maintained in the collections of KVL (The Royal Veterinary and Agricultural University, Copenhagen), with representative strains being submitted to ARSEF (USDA, Ithaca, NY).

Molecular identification

Fifty single conidium isolates were prepared from 50 individual CFUs imprinted from different plant species from the

September collections at the two sites (25 from each site). Each single conidium isolate was arbitrarily selected from a cohort of conidia in dilution plating series of suspensions made from one CFU. These isolates were grown on Sabouraud Dextrose Agar (SDA) and incubated at 23 °C in darkness until sporulation. Flasks containing sterile liquid medium consisting of 2 % peptone, 3 % sucrose and 0.2 % yeast extract were individually inoculated with a loopful of conidia scraped from a sporulating single conidium isolate. Flasks were incubated for 3 d at room temperature (approx. 22 °C) on a shaker (170 rpm). The resulting fungal material was filtered through filter paper (Macherey-Nagel, Düren) under suction and lyophilised overnight on a HETOSICC CD 53-1 (HETO Lab Equipment, Birkerød). DNA was extracted from the fungal material using a Nucleon Phytopure kit (Amersham Biosciences, Little Chalfont) according to the manufacturers instructions.

Screening for diversity amongst these isolates was conducted by Universally Primed PCR (UP-PCR) using a single-primer, L15/AS19 (Lübeck *et al.* 1999). From earlier work this primer was known to distinguish between isolates of *B. bassiana* (N.V.M. unpubl.). The primer sequence was 5′-GAGGGTGGCGGCTAG-3′. For each 20 µl PCR reaction, 1 µl of DNA dilution, 1 µl primer (4 OD units) and 0.3 µl Dynazyme Polymerase were mixed in 18 µl of 1× Dynazyme buffer. The latter was prepared by mixing 100 µl 10× Dynazyme buffer, 20 µl 100 mM $MgCl_2$, 20 µl 10 mM dNTP and 860 µl sterile H_2O. PCR was performed on a Perkin Elmer GeneAmp PCR System 9600 thermo cycler. The PCR programme was composed of an initial cycle of 94 °C for 2 min, followed by 30 cycles at 92 °C for 20 s, 53 °C for 40 s and 72 °C for 30 s, and finalised by one cycle at 72 °C for 2 min. Amplified PCR products were run on a 1.5 % agarose gel, stained with ethidium bromide and photographed in UV light by a Canon PowerShot G5 digital camera. PCR was performed twice and reproducible banding patterns for each isolate were identified.

Data analysis

Data on the proportion of leaves from the different plant groups from which CFUs were obtained were analysed using mixed linear regression models (PROC MIXED) in the statistical package SAS (SAS Institute 1999). Full models with all interactions were initially fitted and non-significant factors eliminated successively until a best-fit model was found. Frequencies of occurrence of leaves from which CFUs were obtained in 2003 were arcsine transformed and analysed for each plant group at each sampling point for each of the three dates of collection, testing for effects of plant group, collection date and their interaction. In the model, degrees of freedom were adjusted by Satterthwaite formulae (Littell *et al.* 1996) and sampling point was applied as a random effect. Using a similar model, frequencies of occurrence of leaves from which CFUs were obtained in 2004 for each plant group at each sampling point were analysed after arcsine transformation. When significant effects were found differences were identified ($\alpha = 0.05$) by the LS MEANS option using the Tukey-Kramer adjustment.

The frequencies of occurrence of abaxial and adaxial leaf surfaces from which *B. bassiana* CFUs were obtained were compared by standard χ^2-tests. This was done separately for each plant group on each date of collection.

The numbers of CFUs (+1) obtained per leaf from the phylloplanes of the different plant groups were added for each sampling point and log transformed before analyses for effects of plant group, sampling date (in 2003) and leaf surface area as well as their interactions in a mixed linear regression model. Sampling point was applied as a random effect and adjustment of degrees of freedom was made with Satterthwaite formulae. When significant effects were found differences between groups were identified as described above.

Results

Natural occurrence of B. bassiana *on phylloplanes*

CFUs of *B. bassiana* were obtained from phylloplanes of grasses, nettles (both strata) and hawthorn in May, July and September in the hedgerow at Taastrup (Fig 1). Analysis by mixed linear regression of the frequencies of occurrence of leaves from which CFUs were obtained revealed significant effects of plant group ($F_{3,\ 32.2} = 22.02$; $P < 0.0001$) and sampling date ($F_{2,\ 32.2} = 19.07$; $P < 0.0001$), but no interaction ($F_{6,\ 32.2} = 1.05$; $P = 0.4101$). Significantly higher frequencies were observed in September compared to May or July ($P < 0.0001$). Comparisons amongst the different plant groups showed that there were significantly higher frequencies of lower nettle leaves from which CFUs were obtained than upper nettle leaves, grasses or hawthorn ($P < 0.001$). At Taastrup the mean height (SE) of nettle plants increased from 24.0 cm (0.5 cm) on 7 May, to 85.4 cm (2.1 cm) on 7 July and 91.0 cm (2.9 cm) on 12 Sept.

When examining leaves from the same plant species at Østrup in September 2004, *B. bassiana* CFUs were also obtained from all plant groups (Fig 2). Analysis revealed that there was a significant effect of plant group on the frequencies of occurrence of leaves from which CFUs were obtained ($F_{3,\ 9} = 21.23$; $P = 0.0002$), with significantly higher frequencies for lower nettle leaves compared to upper nettle leaves, grasses and hawthorn ($P < 0.01$), respectively.

Frequencies of occurrence of abaxial and adaxial leaf surfaces from which CFUs were obtained were mostly similar at Taastrup (Table 1). In September, however, there were significantly more abaxial leaf surfaces from which CFUs were obtained compared to adaxial leaf surfaces of hawthorn and upper nettle leaves. At Østrup CFUs were obtained mainly from adaxial leaf surfaces of both nettle leaf groups (Table 1).

Mean densities (SE) of CFUs of *B. bassiana* ranged between 0.02 (0.02) per 10 cm^2 and 0.58 (0.06) per 10 cm^2 in 2003 (Fig 3) and between 0.27 (0.13) per 10 cm^2 and 0.75 (0.12) per 10 cm^2 in 2004 (Fig 4). The numbers of CFUs recovered per leaf ranged between 0-20 in May 2003, 0-31 in July 2003, 0-19 in September 2003 and 0-43 in September 2004. Analyses of the numbers of CFUs per plant group at Taastrup in 2003 revealed no significant interactions for any factor combinations and these were thus successively omitted from the model. Of the individual factors, significant effects were found for sampling date ($F_{2,\ 39.4} = 5.62$; $P = 0.0074$) and plant group ($F_{3,\ 37.3} = 8.71$; $P = 0.0002$). Leaf area had no significant effect ($F_{1,\ 38.7} = 2.86$; $P = 0.0988$) on the numbers of CFUs recovered. Significantly

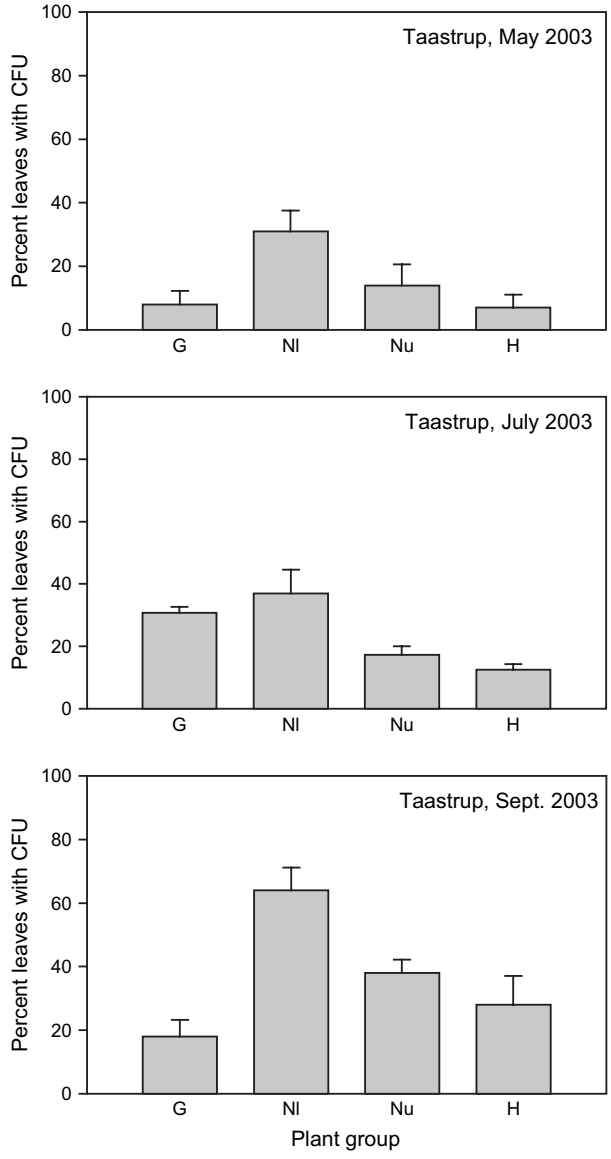

Fig 1 – Mean observed frequencies (+S.E.) of leaves from which CFUs of *Beauveria bassiana* were obtained at Taastrup, Denmark, in May, July and Sept. 2003. Plant groups on x-axis: G, grasses; Nl, nettle, lower leaves; Nu, nettle, upper leaves; H, hawthorn. The frequencies of occurrence were analysed by a mixed linear regression model. The test statistics are provided in the text.

more CFUs were found in Sept. 2003 compared to May or July ($P < 0.05$). Significantly more CFUs were recovered from lower nettle leaves compared to grasses or hawthorn ($P < 0.05$), respectively. Significantly more CFUs of *B. bassiana* were found on upper nettle leaves compared to grasses ($P < 0.005$), but not when compared to hawthorn ($P > 0.05$).

At Østrup in 2004, no effect of the interaction between plant group and leaf area ($F_{3,\ 5.67} = 0.21$; $P = 0.8838$) was found and thus this factor was omitted and analysis performed for main factor effects only. Neither plant group ($F_{3,\ 8.17} = 2.99$; $P = 0.0944$) nor leaf area ($F_{3,\ 8.54} = 0.06$; $P = 0.8151$) were found to have significant effects on the numbers of CFUs recovered.

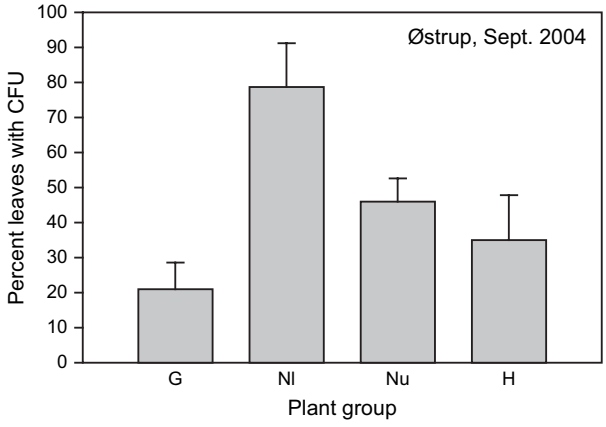

Fig 2 – Mean observed frequencies (+S.E.) of leaves from which CFUs of *Beauveria bassiana* were obtained at Østrup, Denmark, in Sept. 2003. Plant groups as in Fig 1. The frequencies of occurrence were analysed by a mixed linear regression model. The test statistics are provided in the text.

Genetic diversity of selected isolates

Large numbers of *Beauveria bassiana* isolates could be obtained using the isolation technique. At Østrup, for example, 95 single conidium isolates from individual CFUs were recovered on SDA. Screening for genetic diversity among selected single conidium isolates by UP-PCR with the primer L15/AS19 yielded 32 reproducible bands ranging in size between 1500 bp and 200 bp (Fig 5). Six distinct banding patterns were found for isolates from Taastrup and seven different banding patterns were found for isolates from Østrup (Fig 5, Table 2). Of the 13 banding patterns, four were shared between the localities and pattern 'A' was the most common at both sites (Table 2). At both localities, the four plant groups harboured isolates with at least two different banding patterns and all plant groups hosted the 'A' genotype. At Taastrup, grasses harboured four different banding patterns while the six selected single conidium isolates from upper nettle leaves at Østrup separated in five different banding patterns (Table 2).

Discussion

The isolation of naturally occurring *Beauveria bassiana* from phylloplanes of all the hedgerow plants investigated contributes to our knowledge of the spatial distribution of this fungus in semi-natural habitats associated with agro-ecosystems. Apart from isolation of *B. bassiana* from elm bark and corn this is, to our knowledge, the first successful isolation of naturally occurring *B. bassiana* propagules from the surfaces of non-crop herbaceous plants (Doberski & Tribe 1980; Bruck & Lewis 2002b; Arnold & Lewis 2005). CFUs of *B. bassiana* were isolated in May, when the leaves of trees and herbaceous vegetation were only newly developed, and occurrence continued until September. This suggests that *B. bassiana* inoculum is present throughout the growing season in Denmark and is thus present whenever potential hosts are active. In general,

Table 1 – The observed percentages of leaf surfaces (adaxial and abaxial) from which *Beauveria bassiana* could be obtained at the two Danish localities of Taastrup in 2003 and Østrup in 2004. Within rows, pairwise comparisons of percentages were made by standard χ^2 tests. When no test value is given (-) 50 % of the expected values were below five

	% Occurrence		χ^2	*P*
	Adaxial	Abaxial		
Taastrup, May 2003				
Hawthorn	5.0	2.0	-	-
Nettle, upper leaves	9.0	10.0	0.0582	0.8094
Nettle, lower leaves	17.0	20.0	0.2985	0.5849
Grasses	5.0	5.0	0	1
Taastrup, July 2003				
Hawthorn	5.6	6.7	0.0969	0.7556
Nettle, upper leaves	8.6	10.0	0.0848	0.7709
Nettle, lower leaves	18.8	28.1	2.3510	0.1252
Grasses	1.1	3.2	-	-
Taastrup, Sept. 2003				
Hawthorn	9.0	22.0	6.4516	0.0111
Nettle, upper leaves	9.0	30.0	14.047	0.0002
Nettle, lower leaves	40.0	45.0	0.5115	0.4745
Grasses	6.0	13.0	2.8497	0.0914
Østrup, Sept. 2004				
Hawthorn	23.0	16.0	1.5608	0.2116
Nettle, upper leaves	38.0	23.0	5.3072	0.0212
Nettle, lower leaves	69.7	49.5	8.3898	0.0038
Grasses	14.0	14.0	0	1

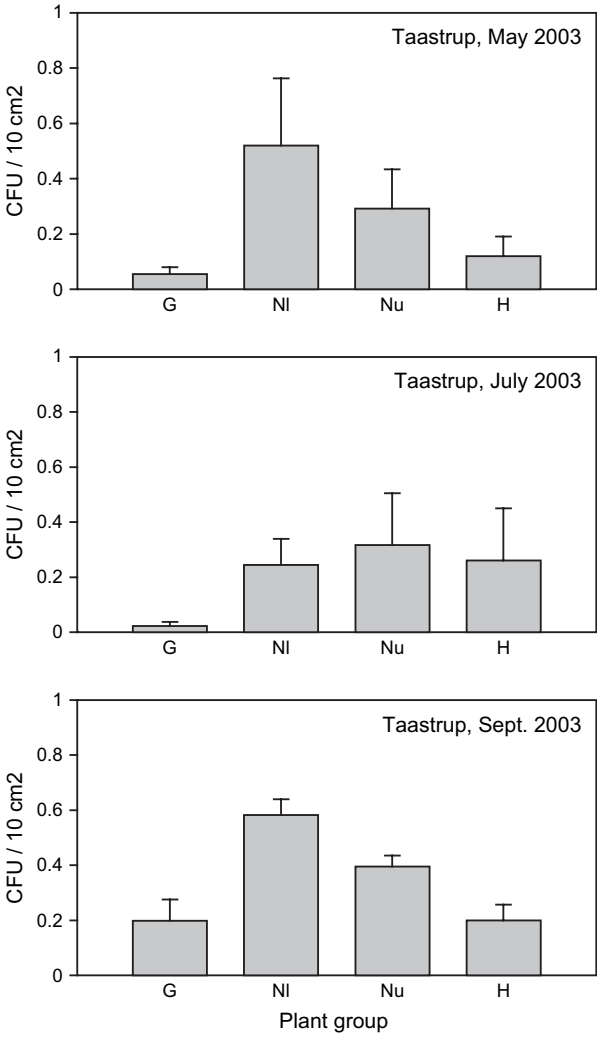

Fig 3 – Mean observed densities (+SE) of CFUs of *Beauveria bassiana* 10 cm^{-2} leaf area at Taastrup, Denmark, in May, July and Sept. 2003. Plant groups as in Fig 1. The numbers of CFUs per leaf were analysed by a mixed linear regression model. The test statistics are provided in the text.

pathogen abundance depends on host population dynamics through the season and it is known that an increase in the quantity of *B. bassiana* inoculum is dependent on an increase in host population densities over time in modelling studies (Anderson & May 1981; Knudsen & Schotzko 1999; Long *et al.* 2000). The observed increase in *B. bassiana* inoculum levels on phylloplanes in Sept. 2003 could thus reflect increase in density of the fungus as response to increased host populations, though this would require further study over several years.

The source of inoculum of *B. bassiana* observed in the present study could have been the soil reservoir or sporulating cadavers within the plant canopy. Inoculum may take several paths from the source to the plants. From the soil environment conidia can disperse on wind currents (Hajek 1997) or onto aerial plant parts through rain splash (Bruck & Lewis 2002b). However, the observation of *B. bassiana* on hawthorn leaves 1.5 m above ground suggests that this was not as a result of rain splash. Viable inoculum of *B. bassiana* has been isolated from air samples (Airaudi & Marchisio 1996; Shimazu *et al.* 2002; Ulevicius *et al.* 2004) and the observed presence of *B. bassiana* on leaf surfaces could have resulted from deposition from the air. Moreover, insects are capable of dispersing conidia of *B. bassiana* by their activity, both within the soil (Dromph 2001) and on plants (Bruck & Lewis 2002a). Nettles harbour a vast community of associated insects (Davis 1973, 1975) and nettle insects are able to disperse *B. bassiana* conidia from the soil onto nettle plants under laboratory conditions (N.V.M. unpubl.).

Once on the plant surface, exposed fungal inoculum is affected by abiotic factors. Conidia of *B. bassiana* that were applied to plant surfaces as microbial control agents were degraded by solar radiation and removed by rain, although they persisted longer on the inner canopy compared to the outer canopy (Daoust & Pereira 1986; Inglis *et al.* 1993, 1995, 2000, 2001; James *et al.* 1995). Protection by the canopy could explain the greater frequency of occurrence and amount of inoculum on lower nettle leaves compared to upper nettle leaves in the present study.

An evaluation of the significance of *B. bassiana* on phylloplanes to the population dynamics of the fungus is challenging. Does the occurrence contribute to the life-cycle of *B. bassiana* by providing a population of conidia for new infections, or does it rather represent an ecological dead-end for viable conidia? A proportion of free-living infective stages of

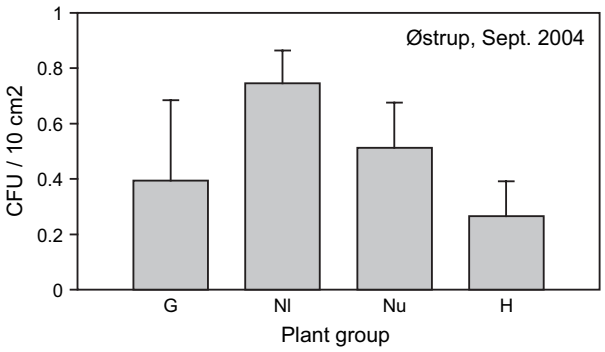

Fig 4 – Mean observed densities (+S.E.) of CFUs of *Beauveria bassiana* 10 cm^{-2} leaf area at Østrup, Denmark, in Sept. 2003. Plant groups as Fig 1. The numbers of CFUs per leaf were analysed by a mixed linear regression model. The test statistics are provided in the text.

invertebrate pathogens are expected to die without infecting hosts and pathogens that rely on hosts to increase their densities often decay if they disperse too far away from those hosts (Anderson & May 1981; White *et al.* 2000). To become infected, insects could acquire infective propagules of *B. bassiana* from leaf surfaces. In laboratory experiments, Colorado potato beetles *Leptinotarsa decemlineata* (*Coleoptera: Chrysomelidae*) acquired *B. bassiana* conidia from sprayed leaves resulting in infection (Fernandez *et al.* 2001). However, the inoculum level was much greater than in the present study. We hypothesise that multiple events of conidium acquisition by stressed or weak hosts could be sufficient for successful infection. Indeed, insects associated with grasses and nettles are commonly infected with *B. bassiana* in the field (N.V.M., pers. obs.).

Another possible fate of the *B. bassiana* conidia could be horizontal transmission of the infective stage from an endophytic association with plants. Endophytic fungi are thought to be ubiquitous in many plant families and an endophytic association between *B. bassiana* and corn is well characterised (Bing & Lewis 1991, 1992; Saikkonen *et al.* 1998; Wagner & Lewis 2000; Arnold & Lewis 2005). This association between plants and entomopathogens is considered to provide protection for the plant from insect herbivores (Elliot *et al.* 2000). Conidia of *B. bassiana* germinated on phylloplanes of corn and infected the leaves in laboratory experiments and it is possible that the fungus has a broader host range for this endophytic activity (Wagner & Lewis 2000; Arnold & Lewis 2005). This aspect requires further study.

Characterisation of selected single conidium isolates by UP-PCR showed that several genotypes of *B. bassiana* were present simultaneously at the two field sites. Furthermore, no association between banding pattern and plant species were found suggesting that the observed *B. bassiana* genotypes are ubiquitous on plant surfaces. Wang *et al.* (2004) identified several genotypes of *B. bassiana* isolated from insect cadavers collected in a single forest in China, and there was a tendency to a shift in genetic diversity through the season (Wang *et al.* 2004). In the present study, one genotype seemed to be most common at both sites, however further studies are required to draw conclusions about local genetic structure.

In conclusion, this study revealed significant new information adding to the future understanding of the ecology of *B. bassiana*. Large numbers of genetically variable isolates of *B. bassiana* were obtained using the leaf imprinting method described. The method thus represents a quick and easily applicable tool for obtaining large numbers of indigenous *B. bassiana* isolates from any terrestrial ecosystem, for example for screening of strains for their potential as biological control agents of specific pests.

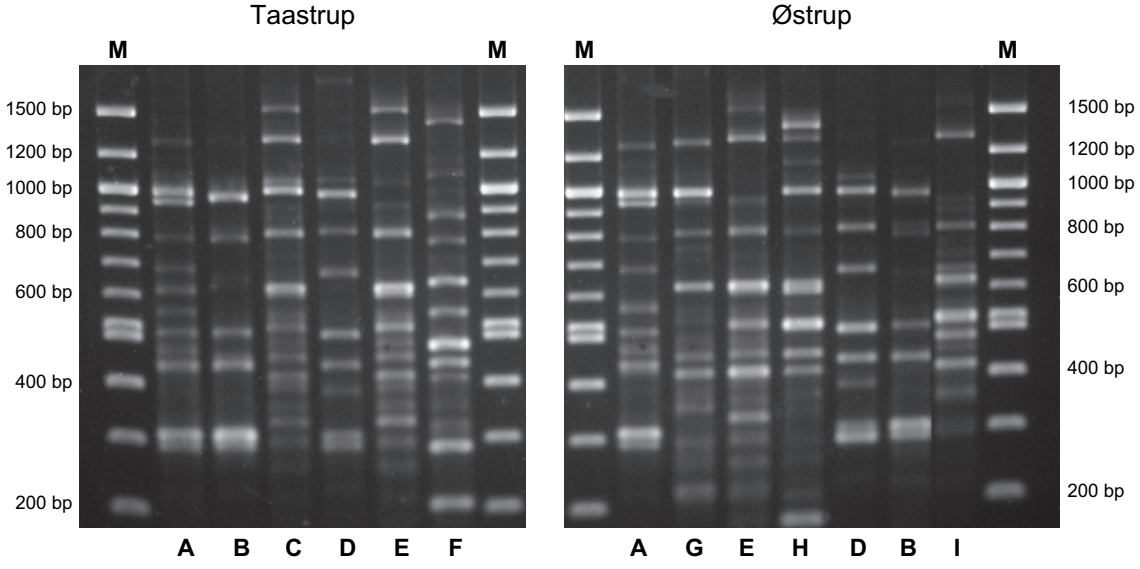

Fig 5 – UP-PCR banding patterns of selected single conidium isolates of *Beauveria bassiana* from Taastrup and Østrup using UP primer L15/AS19. Letters below lanes denote characteristic banding patterns; patterns with the same letter are considered to be the same. 'M' above a lane represents size marker.

Table 2 – Distribution of different banding patterns of selected single conidium isolates of *Beauveria bassiana* ($n = 25$) collected at the two localities Taastrup and Østrup in Sept. 2003 and 2004, respectively, divided amongst the four sampled plant groups. Banding patterns were based on distinct bands obtained by Universally Primed PCR (primer L15/AS19) presented in Fig 5

Taastrup, Sept. 2003

	Number of isolates						
	Banding pattern by UP-PCR						
Plant group	*n*	A	B	C	D	E	F
Hawthorn	8	4	4				
Nettle, upper leaves	8	6	1	1			
Nettle, lower leaves	3	1	1		1		
Grasses	6	3	1			1	1

Østrup, Sept. 2004

	Number of isolates							
	Banding pattern by UP-PCR							
	n	A	B	D	E	G	H	I
Hawthorn	6	3					2	1
Nettle, upper leaves	6	1		1	2	1	1	
Nettle, lower leaves	7	5	1				1	
Grasses	6	4			2			

Acknowledgements

We thank Christina Wolsted, Karen M. Kjeldsen and Martin R. Davidsen for valuable technical assistance in the field and laboratory. N.V.M. was supported by a PhD grant from The Royal Veterinary and Agricultural University.

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