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Dispersal of *Beauveria bassiana* by the activity of nettle insects

Nicolai V. Meyling [a,*], Judith K. Pell [b], Jørgen Eilenberg [a]

[a] *Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*
[b] *Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK*

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Abstract

Recent studies have shown that the entomopathogenic fungus *Beauveria bassiana* occurs naturally on the phylloplanes of several plants, including nettles. Insects could, by their activity, be contributing to this inoculum by dispersing it from other sites. The potential of nettle aphids *Microlophium carnosum* and their predator *Anthocoris nemorum* to disperse conidia of *B. bassiana* from soil to nettles and from sporulating cadavers in the nettle canopy was investigated in laboratory experiments. In petri dish assays, aphids showed potential to distribute *B. bassiana* from soil to nettle leaves. Predators dispersed inoculum from both soil and cadavers to nettle leaves in petri dishes. In microcosms, aphids did not disperse *B. bassiana* from the soil or from cadavers confined in the canopy, but *A. nemorum* were able to transfer inoculum from soil into the nettle canopy and to distribute conidia from cryptic cadavers. In some instances, infections were initiated in aphids and predators as a consequence of dispersal.

Keywords: Entomopathogenic fungi; Hypocreales; *Urtica dioica*; *Microlophium carnosum*; Aphididae; *Anthocoris nemorum*; Anthocoridae; Soil; Phylloplane

1. Introduction

The cosmopolitan entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) infects insects from most orders and the fungus is ubiquitous in soil (Keller and Zimmerman, 1989). Recently, propagules of *B. bassiana* were documented to occur frequently on phylloplanes of hedgerow vegetation and this new aspect of *B. bassiana* distribution raises the question of dispersal pathways to the foliage (Meyling and Eilenberg, 2006). Besides dispersal by wind currents (Shimazu et al., 2002) and rain splash from soil surfaces (Bruck and Lewis, 2002b) insects could potentially contribute to the distribution of fungus inoculum. Aphid predators are known to disperse conidia of the aphid entomopathogen *Pandora neoaphidis* (Remaudière and Hennebert) Humber (Zygomycota: Entomophthorales) thereby enhancing infection rates in aphid populations (Pell et al., 1997; Roy et al., 1998, 2001). Regarding hypocrealean entomopathogenic fungi, *Paecilomyces fumosoroseus* (Wize) Brown and Smith was dispersed by the ladybird *Hippodamia convergens* Guerin (Coleoptera: Coccinellidae) to Russian wheat aphids *Diuraphis noxia* Kurdjumov (Homoptera: Aphididae) in laboratory experiments (Pell and Vandenberg, 2002). Likewise, *B. bassiana* infections were initiated in European corn borers *Ostrinia nubialis* Hübner (Lepidoptera: Crambidae) through dispersal by the fungivorous beetle *Carpophilus freemani* Dobson (Coleoptera: Nitidulidae) (Bruck and Lewis, 2002a). Within the soil environment, which is a well-known reservoir of *B. bassiana* inoculum (Keller and Zimmerman, 1989), the fungus can be dispersed and vectored by collembolans (Dromph, 2001, 2003). Dispersal of *B. bassiana* by insect activity has been developed and exploited for pest management using the auto-dissemination strategy (Meadow et al., 2000; Dowd and Vega, 2003; Vickers et al., 2004).

We hypothesised that fungal inoculum on phylloplanes could originate from the soil as well as from fungus infected cadavers confined in cryptic places within the nettle canopy

* Corresponding author. Fax: +45 3528 2670.
E-mail address: nvm@kvl.dk (N.V. Meyling).

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such as leaves rolled by lepidopteran larvae. Laboratory studies were conducted on the potential of insects to disperse *B. bassiana* inoculum from soil to phylloplanes and within the canopy from cryptic sources of inoculum. We selected the stinging nettle system, *Urtica dioica* L. (Urticaceae), since earlier studies showed that nettle plants harboured the greatest number of *B. bassiana* propagules compared with other hedgerow plants (Meyling and Eilenberg, 2006). Nettle aphids *Microlophium carnosum* (Bukton) (Homoptera: Aphididae) and the predator *Anthocoris nemorum* (L.) (Heteroptera: Anthocoridae) are common insects on nettles in Northern Europe (Davis, 1973) and *A. nemorum* is one of the most important predators of *M. carnosum* (Perrin, 1976). The dispersal potential of these species was investigated in petri dish and microcosm experiments where the sources of *B. bassiana* were either soil inoculated with conidia or sporulating cadavers placed in cryptic positions in the nettle canopy.

2. Materials and methods

2.1. Insects and plants

For experiments 2.4, 2.5 and 2.7, adult *A. nemorum* were collected by sweep netting nettles in April and May at Rothamsted Research, Hertfordshire, UK, and insects were maintained and reared as described by Meyling and Pell (2006) in a controlled environment room at Rothamsted Research. In experiments using field collected adults they were used within 3 weeks of collection. Laboratory reared adults were used in experiments 2–4 weeks after adult eclosion. Nettle plants were grown from seed in a glasshouse. The plants for microcosm experiments were 3 weeks old while detached leaves for petri dish experiments were removed from 5 to 7 weeks old plants. Nettle aphids, *M. carnosum*, were reared on 4–6-week-old nettle plants in cages in the insectary at Rothamsted Research (18 °C, L16: D8).

For experiment 2.6, adult *A. nemorum* were collected on nettles in September at Bakkegården, Northeast Zealand, Denmark, and kept in ventilated plastic jars at 20 °C, L16: D8, in a climate cabinet for up to 3 weeks prior to use. For this experiment nettle plants were grown outside in Denmark in large pots under ambient conditions and pea aphids, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), were reared on broad bean, *Vicia faba* L. (Fabaceae), in cages placed in a glasshouse at 20–23 °C, L16: D8.

2.2. Suspensions of B. bassiana conidia

The *B. bassiana* isolate KVL 03-90 was originally isolated from an adult *A. nemorum* collected on stinging nettle at Bakkegården, Denmark, in June 2002. Stock cultures on Sabouraud dextrose agar (SDA) (Oxoid Ltd, Basingstoke, Hampshire, UK) were stored at 5 °C and subcultures onto SDA made for use in experiments as required. Only single transfers from stock cultures were made. Subcultures were incubated in darkness at 25 °C. Conidia suspensions were made from sporulating plates, 3–5 weeks after subculturing, by adding 0.03% Tween 80 (Acros Organics, Morris Plains, New Jersey, USA) to the petri dish and scraping the surface. The suspensions were filtered through four layers of muslin and the conidia concentration determined by counting a diluted sample in a Neubauer bright-line haemocytometer at 400× magnification. For all experiments, suspensions containing 1×10^8 conidia per ml were used. The germination rate of conidia from each suspension was assessed by counting the proportion of germinated conidia after incubation on SDA at 25 °C for 24 h. Germination was always over 95%.

2.3. B. bassiana-infected aphid cadavers

Cadavers of pea aphids, *A. pisum*, were prepared by dipping single leaflets of broad bean into 30 ml 1×10^8 conidia per ml suspension. The petiole of each leaflet was embedded in 3% water agar in the bottom of a 30 ml medicine cup. Two or three second instar pea aphid nymphs were transferred to every inoculated leaflet. The aphids were kept for 5 days at 20 °C in a climate cabinet; by this time the aphids had died from fungal infection. Cadavers were transferred to petri dishes and incubated at 25 °C in darkness and under humid conditions for 5–6 days until they sporulated profusely and could be used in experiments.

Cadavers of nettle aphids, *M. carnosum*, were prepared by dipping single detached nettle leaves as described above. The nettle leaves were air-dried on tissue paper and then embedded abaxial side facing up in 2% water agar in 9 cm petri dishes. Ten to fifteen large nymphs of *M. carnosum* were placed on each leaf and then incubated at 18 °C, L15.5 : D8.5, for 8–9 days until aphids died due to infection. Dead, infected aphids were transferred to petri dishes and incubated at 25 °C in darkness and under humid conditions for 5–6 days until they sporulated profusely and could be used in experiments.

2.4. Petri dish experiment to quantify dispersal of B. bassiana from soil to leaves

Soil from a commercial potting medium mix (75% medium grade peat, 12% sterilised loam, 3% medium grade vermiculite and 10% 6 mm screened, lime free grit, Petersfield Products, Leicester, U.K.) was sterilised in an autoclave at 115 °C and 10 psi. for 20 min. This soil was used in all experiments involving dispersal of *B. bassiana* from soil to leaves. The water content as wet weight (w.w.) of the soil was determined by weighing 10 samples and then reweighing them after drying in an oven at 100 °C overnight. A suspension of 1×10^8 conidia per ml was prepared and added to 100 g of soil which was mixed by hand in a clean plastic bag to reach a level of 1×10^7 conidia per g of dry soil. Distilled water was subsequently added to reach a water content of 50% w.w. Control soil was prepared by adding an amount of sterile 0.03% Tween 80 similar to the volume of