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Pathogenicity of *Conidiobolus* spp. and *Basidiobolus ranarum* to arthropods co-occurring in leaf litter

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ABSTRACT

A novel method is described for the extraction and presentation of mixed arthropods from litter as targets for potentially pathogenic species of *Conidiobolus* and *Basidiobolus* (Zygomycetes: Entomophthorales). All arthropods and test fungi (except *C.* nr. *pumilus* from broad leaf litter) were from the same larch (*Larix*) plantation. For 8 fungus species a bioassay (and repeat), were followed by survivorship comparison tests; Breslow, Logrank (Mantel-Heinszel/Peto) and Wilcoxon (Gehan/Lee-Desau). These were applied to test and control data separately for collembola and mites. Kaplan-Meier plots of Hazard Function were made. Of possible pathogenic effects the most marked was the high mortality of collembola and mites when showered with conidia of *C. coronatus*. *Conidiobolus thromboides* (collembola and mites) and *C. osmodes* (mites only) were associated with enhanced death in one of their two bioassays. Of the other 5 test species, *C. adiaeretus* and *C. iuxtagenitus* gave no indication of pathogenicity; nor did *C. lamprauges*, *C. heterosporus* and *C. pumilus*. Ecological implications are briefly discussed.

Introduction

As part of an ongoing attempt to define the niche differentiation of species of *Conidiobolus* and *Basidiobolus* which occurs in leaf litter of selected target habitats, the most frequently disclosed species (Callaghan 2004; Ian J Hopkins, unpubl.; AAC, unpubl.) were tested for their ability to utilise plant fragments and arthropod cadavers as substrata (Manning *et al.* 2007). No species tested could effectively colonise plant structural polymers or plant debris. In contrast all species were able to colonise arthropod cadavers, usually with the associated formation of resting spores. Results strongly backed the notion that, for at least part of their life, these fungi could be saprotrophs of arthropod substrata. Little firm evidence of any specialisation of target was obtained. The present study experimentally explores the possibility that at least some of the putative saprotrophs may be pathogenic to identifiable sub-groups of arthropods co-occurring in leaf litter. The fungal strains used were all from a small plantation of hybrid larch (*Larix* X *marschlinsii*), at Keele, Staffordshire, UK (Nat. Grid Ref. SJ 821441) in an area repeatedly sampled as a model habitat (Smith & Callaghan 1987; Ian J Hopkins and A.A.Callaghan unpubl.). They include *C. coronatus*, *C. thromboides* and *C. osmodes*, all well known as opportunistic pathogens, sometimes associated with epizootics in above-ground situations (Papierok 1986; Varma *et al.* 1982; Feng *et al.* 1990; Gindin & Ben-Ze'ev 1994). In contrast, other less studied presumptive saprotrophs, include *C. adiaeretus* (Drechsler 1953), *C. iuxtagenitus* (Waters & Callaghan 1989), *C. lamprauges* (Drechsler 1953), *C.* nr. *pumilus* (Drechsler 1955). *Basidiobolus*

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ranarum (Coremans-Pelseneer 1974; Nelson *et al.* 1998) was frequently disclosed along with the *Conidiobolus* spp. Initially the present paper describes a novel method for extracting arthropods, live and relatively unstressed, from the litter. They are channeled and confined in small groups into small compartments for showering with conidia of test fungi and for subsequent monitoring of mortality in test and control arthropods. Target populations of arthropods were of mixed types, predominantly mites and collembola. The timing and extent of mortality over several days allowed at least preliminary comparison of the ability of seven *Conidiobolus* species and *B. ranarum* to kill co-occurring arthropods quickly ('necrotrophy'), more slowly ('biotrophy') or not at all.

Materials and methods

Fungus strains and conidial sources

The eight test isolates (8 spp.) were originally derived from litter of depth about 3 cm. Seven isolates were from one managed habitat described above, and one isolate was from an adjacent habitat (Table 1). Isolates were grown and stored on bottle slopes of malt extract agar (MEA; 1.2 % w/v, malt extract L39, 2 % w/v, agar technical No.3 (Oxoid Ltd, Basingstoke, UK) in deionised water) at 2–5 °C in the dark and subcultured no more than 3 times before use. Sources of primary conidia to be showered onto target arthropods were pre-grown for 2–3 d at 20 °C on MEA in 96-well dishes (Nalge Nunc International, Roskilde, Denmark). They were grown inverted over a matching black-walled 96-compartment tunnel. With fluorescent underlighting, phototropic conidiophores were directed away from the walls. When the sources were eventually placed over 96-well dishes containing the target arthropods (see below) discharge was concentrated onto the centre of each well.

Standardisation of conidial showers

Prior to bioassay, attempts were made to standardise the conidial shower of each fungus species. These involved estimating the densities of deposits around arthropod cadavers placed in the receiving wells and assessment of the areas of different animal types. Several periods of showering were tested. The presence of conidia on the cadavers was monitored by staining them with a mixture of propidium iodide and fluorescein diacetate, and observation by fluorescent microscopy on removed cadavers. Showering for more than 4 h increased the likelihood that all target arthropods received at least one conidium. To assess variation in conidial discharge from different species the density of the spore deposits of *C. lamprauges* and *C.* nr. *pumilus* were used as examples of light and heavy spore showering species, respectively. Monitoring their output at 10 °C led to a final choice of 12–13 h showering for low dischargers and 6 h for species with denser output. Densities of deposits, in relation to sizes of target arthropods were such that, even with the movement of live targets, there was a high probability of each animal having associated conidia. Secondary inoculation of arthropods was possible from scattered globose conidia (primary and repetitional) and from alternative conidia such as capilliconidia of *C.* nr. *pumilus* and *B. ranarum*. Fully formed capilliconidia of the latter fungi were frequently seen on mobile arthropods (notably mites) on the first occasion that arthropods were monitored (on day 1, after 12–13 hours of conidial showering).

Litter sampling and extraction of arthropods

The objectives of the procedures described below were simultaneously to extract and to separate low numbers of fresh, live and relatively unstressed litter arthropods into small compartments to which they were confined in moist

Table 1 – A summary of the outcomes of survivorship comparison statistics from test and control data separated for collembola and mites; sample sizes in parentheses

Test fungi	Code number	Arthropod type			
		Mites[a]		Collembola[a]	
		Exp1	Exp2	Exp1	Exp2
C. adiaeretus	04:407	−(66,122)	−(129,150)	−(35,35)	−(54,56)
C. coronatus	04:427	+(56,76)	+(104,145)	+(55,82)	+(67,146)
C. iuxtagenitus	04:433	−(56,82)	−(68,99)	−(64,105)[b]	−(30,45)
B. ranarum	1/013	−(48,56)	−(51,50)	−(31,34)	−(45,67)
C. thromboides	1/024	−(35,50)	−(34,53)	+(56,71)	−(51,71)
C. osmodes	04:428	+(59,74)	−(68,108)	+(14,40)[c]	+(53,84)
C. lamprauges	1/011	−(72,110)	−(75,88)	−(32,34)	−(9,27)[c]
C. nr. *pumilus*[d]	02:106	−(221,139)	−(135,143)	−(30,24)	+(18,52)[c]

+ = significant ($p < 0.05$); − = not significant.
For all three survivorship comparison tests of Breslow, Logrank (Mantel-Heinszel (Peto)) and Wilcoxon (Gehan (Lee-Desau)).
a Different mixture of target of mites and collembola in each of two experiments; sample sizes of arthropods = (test, control).
b For results of the 3 tests, only the Logrank was significant.
c Ambiguous: low number of test collembola.
d Isolate forming multiple capilliconidia from a mixed broadleaf plantation.