RESEARCH COMMUNICATION

Survey of nematophagous fungi in South Africa

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ABSTRACT

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Three hundred and eighty-four samples of leaf litter, soil, faeces from domestic and game animals, compost and aqueous cultures of infective nematode larvae contaminated with unidentified fungi were plated out on water agar, baited with pure infective larvae of *Haemonchus contortus*, incubated and examined for the presence of nematophagous fungi.

Duddingtonia flagrans was isolated from five samples, and 73 samples were positive for other nematophagous fungi.

Keywords: *Arthrobotrys oligospora, Duddingtonia flagrans, Haemonchus contortus*, nematophagous fungi

INTRODUCTION

Pastures are continually contaminated by the free-living stages of various species of parasitic nematodes which infect domestic livestock. The resistance of one such parasite, *Haemonchus contortus*, to anthelmintics in South Africa and other parts of the world, led to the formulation of alternative strategies for its control. Among these was the use of nematophagous fungi such as *Duddingtonia flagrans* for biological control of infective larvae on pastures.

Chlamydospores of *D. flagrans*, fed to livestock, survive the digestive processes and are viable when voided in the faeces. Parts of the fungal mycelium growing from germinating chlamydospores become modified to form three-dimensional adhesive nets which trap and then feed on nematode larvae. On contaminated pastures, *D. flagrans* has been shown to be effective in reducing the number of infective larvae of *H. contortus* and *Trichostrongylus colubriformis* from sheep, *Ostertagia ostertagi* from calves, strongyles from horses, and *Oesophagostomum dentatum* and *Hyostrongylus rubidus* from pigs (Larsen 2000).

The aim of this study was to find a South African strain of *D. flagrans* adapted to local environmental

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conditions that could be used in an integrated worm control programme. In addition (although not the primary aim), the occurrence of other nematophagous fungi in the study area was also recorded.

MATERIALS AND METHODS

The modified sprinkling technique of Larsen, Wolstrup, Henriksen, Dackman, Grønvold & Nansen (1991) was used for the isolation of nematophagous fungi. Soil, faeces, leaf litter and compost samples collected from five provinces in South Africa (Gauteng, Mpumalanga, North West, Limpopo and Free State) were sprinkled in a cross configuration on individual plates of 2 % water agar containing 0.02 % tetracycline hydrochloride to suppress bacterial growth. Approximately 3 mℓ of aqueous cultures of infective nematode larvae contaminated with unidentified fungi were pipetted onto the surface of plates containing the same medium. The plates were incubated at 26 °C, baited at least twice a week for 3 weeks with approximately 3 500 infective *H. contortus* larvae from a pure aqueous culture and examined for signs of nematophagous activity every 2–3 days. The conidia and chlamydospores of some nematophagous isolates were photographed using light microscopy or scanning electron microscopy (SEM). Others were measured and, in some cases, drawn to facilitate identification. Identification was based on the descriptions of type strains (De Hoog 1985; Rubner 1996). The criteria included the type of structure used to trap larvae, the shape, size and numbers of conidia, the configuration of the conidiophore, and the presence or absence of chlamydospores in mature fungal cultures. Conidia growing from trapped larvae on water agar plates were transferred by means of sterile glass inoculation needles to corn meal agar (Difco® Laboratories) plates, to purify the isolate.

Pure cultures of six isolates of nematophagous fungi were sent to the "Centraalbureau voor Schimmelcultures" in the Netherlands for expert identification, to either confirm our findings or identify isolates that were problematic.

Conidia and chlamydospores of *D. flagrans* were prepared for SEM as follows: 1 cm square blocks of corn meal agar cultures were vapour-fixed in osmium tetroxide for 12 h and air-dried for 2 days. The air-dried blocks were mounted on aluminium stubs, sputter coated with gold and viewed in a Philips XL 20 scanning electron microscope.

RESULTS

Five isolates of *D. flagrans* and 73 isolates of other nematophagous fungi were obtained from 384 cultures of soil, faeces, compost, leaf litter and aqueous suspensions of infective larvae contaminated with unidentified fungi. The samples were collected within a 500 km radius of Pretoria, South Africa. The localities included agricultural land, bushveld, urban gardens, nature reserves and a commercial compost processing plant.

The most common nematophagous fungus isolated was *Arthrobotrys oligospora.* The other nematophagous fungi isolated were *Arthrobotrys superba*, *Arthrobotrys dactyloides*, *Arthrobotrys botryospora*, *Arthrobotrys scaphoides* and *Monacrosporium gephyropagum*. Two isolates of *D. flagrans* were isolated from compost and three from leaf litter.

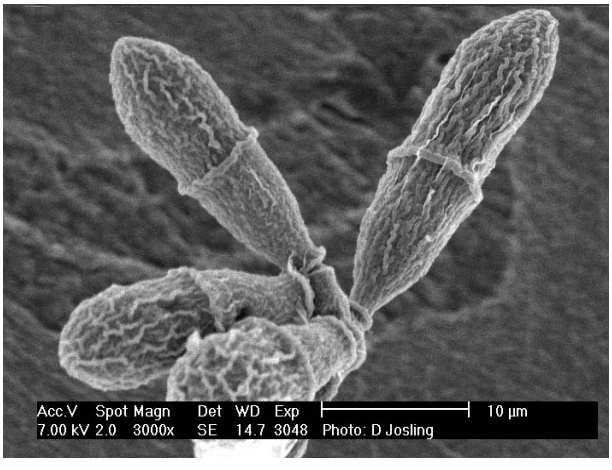

FIG. 1 SEM of conidia from a South African isolate of *Duddingtonia flagrans*

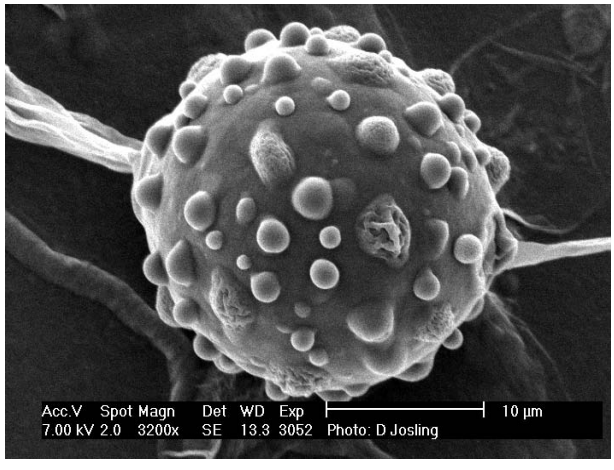

FIG. 2 SEM of a chlamydospore from a South African isolate of *Duddingtonia flagrans*

DISCUSSION

Twenty percent of the samples cultured were positive for nematophagous fungi and *D. flagrans* has for the first time been isolated in Africa**.** This provides the opportunity to compare the local fungus with strains from elsewhere in the world as regards their efficacy in reducing infective parasitic larvae on pastures. The doses of *D. flagrans* chlamydospores required to significantly reduce the number of infective larvae contaminating pasture, are in the order of 10^5–10^6 chlamydospores per kilogram body mass per day (Peña, Miller, Fontenot, Gillespie & Larsen 2002) and the residual effect is limited to 2 days (Waller, Knox & Faedo 2001). Locally isolated strains of *D. flagrans* would need to be cultured in bulk to obtain sufficient quantities of chlamydospores for further studies under African conditions.

Duddingtonia flagrans grows well on cereals and approximately 250 000 chlamydospores have been counted on the surface of a single grain of barley (Grønvold, Wolstrup, Nansen, Henriksen, Larsen & Bresciani 1993).

It has been shown that there is very little genetic variation in isolates of *D. flagrans* from Denmark, the United Kingdom, France, Germany, the United States of America, Australia, Malaysia and India (Faedo 2001). Faedo (2001) suggests that it is possible that *D. flagrans* from countries in Europe was introduced to Asia and Australia in exported livestock. The South African strains of *D. flagrans* had not yet been isolated at the time of Faedo's study but the same hypothesis applies. Chlamydospores from a strain of *D. flagrans* isolated in Denmark have been produced commercially. This survey has demonstrated the presence of local strains of *D. flagrans* in South Africa and together with the limited genetic variation between isolates from different parts of the world, should address any concerns about importing a foreign fungus into this country.

Recent studies in Australia have been focused on finding a practical method of delivery of *D. flagrans* chlamydospores to livestock, for use by large scale commercial farmers (Waller, Faedo & Ellis, 2001; Waller, Knox & Faedo 2001).

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