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Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control

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ABSTRACT

Wireworms, the subterranean larval stage of click beetles (Coleoptera: Elateridae), are an important pests of potatoes throughout the world. Laboratory assays were done to identify virulent strains of entomopathogenic nematodes (EPN) and fungi against wireworm, *Agriotes lineatus* (L.) (Coleoptera: Elateridae). A fungus, *Metarhizium anisopliae* (Metsch), Sorokin strains V1002 and LRC181A, caused 90 and 100% mortality of *A. lineatus*, 3 weeks post-inoculation. Other *M. anisopliae* strains caused mortality ranging between 10 and 70%, whereas strains of *Beauveria bassiana* (Balsamo) Vuillemin and *Paecilomyces fumosoroseus* (Wize) were non-pathogenic to *A. lineatus*. The EPN, *Heterorhabditis bacteriophora* Poinar strain UWS1, caused significantly greater mortality (67%) to *A. lineatus* than other EPN species (0–50%), 3 weeks post-inoculation. The organophosphate insecticide Mocap 10G gave 100% control. The present results suggest that *M. anisopliae* strain V1002 has considerable potential for the control of the wireworm tested.

1. Introduction

Wireworms, the subterranean larval stages of click beetles (Coleoptera: Elateridae), are major pests of arable crops including potatoes in many parts of the world (Jansson and Seal, 1994). In the United Kingdom, the most important species are *Agriotes obscurus* (L.), *Agriotes lineatus* (L.) and *Agriotes sputator* (L.) (Gratwick, 1989; Parker and Howard, 2001). Wireworm damage to potato tubers can drastically reduce the yield and quality of the potato crop. Where tuber samples have >10–15% tubers damaged by wireworms, it is uneconomic to grade out the damaged tubers, rendering the crop unmarketable (W.R. Bill Parker, personal communication). Current control of wireworm on potato is dependent on a combination of cultural methods and the use of soil-applied insecticides, most of which are relatively ineffective (Parker et al., 1990; Kuhar et al., 2003; Kuhar and Alvarez, 2008) or pose a risk to human health and the environment. There is considerable interest in the development of benign alternatives with much attention focusing on entomopathogenic fungi and nematodes. The latter have been successfully used against a wide range of soil pests with the exception of wireworm (Ansari et al., 2003; Grewal et al., 2005; Georgis et al., 2006; Morris, 1985; Edit and Thurston, 1995). Entomopathogenic fungi such as *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin, have shown promise for the control of soil pests (Copping and Menn, 2000; Butt et al., 2001; Ansari et al., 2004a). To date, Canadian strains of *M. anisopliae* have been identified which are pathogenic to wireworm (Kabaluk et al., 2005, 2007), but the search for more aggressive strains of entomopathogenic nematodes (EPN) and fungi continues. The aim of this study was to evaluate the virulence of native and exotic strains of EPN and entomopathogenic fungi against wireworm.

2. Materials and methods

2.1. Wireworm

Fourth and fifth instars of *A. lineatus* were collected from a heavily infested potato field (150–350 larvae/m^2), Rhydlewis, Llandysul, Carmarthenshire, UK. They were identified based on morphological characters to species or genus level using the keys of Klausnitzer (1994); whenever possible, larval instar was

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determined by measuring head capsule width. This site had not been treated with insecticides during the previous year. Wireworms were stored individually in moist sandy loam soil in 24-well plates (Falcon No. 3047, Hamburg, Germany) with a carrot slice (1.5 cm diameter) for larval nutrition at 15 °C for one week to recognize unhealthy larvae before use in experiments.

2.2. Nematode and fungal strain and culture

Nematode and fungal species/strains used in this study are summarized in Table 1. All EPN used in this study were cultured in last instar larvae of the greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) and the emergent infective juveniles (IJs) harvested and stored in distilled water at 10 °C for 7–30 days (Kaya & Stock, 1997). Similarly, all the entomopathogenic fungi used in this study were passaged through *G. mellonella* larvae, to ensure the cultures were not attenuated, and re-isolated on oatmeal dodine agar medium. Single spore colonies were transferred to Sabouraud Dextrose Agar and incubated at 25 ± 1 °C for 15 days. Conidia were harvested and the final concentration adjusted to 1×10^8 conidia/ml with 0.03% Tween 80 (Fisher Scientific Ltd, Leicestershire, UK). Conidia had over 95% viability as determined by the plate count technique described by Goettel and Inglis (1997).

2.3. Screening of fungal strains

Larvae were dipped in 10 ml of the fungal suspension containing 1×10^8 conidia/ml for 20 s. Control larvae were dipped in 0.03% Aqueous Tween only. Ten larvae were placed in a 30 ml plastic cup (surface area: 15.9 cm^2) filled with 25 g sandy loam soil (collected from the same field where wireworms were collected). A slice of carrot was placed in each container as food source and was replaced weekly. Containers were maintained under controlled conditions (22 ± 1 °C; 60–70% RH). Larval mortality was assessed every week for 3 weeks post-inoculation. Dead larvae from fungal treatments were incubated at 25 °C and 100% RH for 7–10 days. The cause of death was confirmed by examination of the fungal outgrowth of the cadaver. Each treatment was replicated three times and the whole bioassay was conducted twice.

Table 1
The origin of entomopathogenic nematodes and fungus species/strains screened against the *Agriotes lineatus*.

Species/strains	Host or source of origin	Geographic origin
Entomopathogenic nematodes		
Heterorhabditis bacteriophora UWS1	Soil, *Galleria* bating; grassland	UK
H. bacteriophora (nema-green®)	e-nema GmbH-biological plant protection, Germany	Germany
H. megidis (Larvanem-M)	Koppert Biological Systems, The Netherlands	The Netherlands
H. downesi	Soil, *Galleria* bating; grassland	Ireland
Steinernema feltiae (Entonem)	Koppert Biological Systems, The Netherlands	The Netherlands
S. carpocapsae (Millenium)	Becker Underwood, Littlehampton, West Sussex, UK	UK
Entomopathogenic fungus		
Metarhizium anisopliae		
V275 (=[a]BIPESCO 5)	*Cydia pomonella* (Lepidoptera: Torticidae)	Austria
[b]ARSEF4556	*Boophilus* sp. (Acari: Ixodidae)	USA
ARSEF3297	*Boophilus* sp. (Acari: Ixoididae)	Mexico
V1001	*Agriotes* sp. (Coleoptera: Elateridae)	UK
V1002	*Agriotes* sp. (Coleoptera: Elateridae)	UK
997	S. Keller	Switzerland
602	S. Keller	Switzerland
LRC181A	Todd Kabaluk	Canada
LRC112A	Todd Kabaluk	Canada
LRC219	Todd Kabaluk	Canada
Beauveria bassiana (Bgard)	Botaniguard	USA
Paecilomyces fumosoroseus PFEEP9901	PreFeral (FuterEco)	Spain

[a] BIPESCO, Biocontrol of Important Soil Dwelling Pests by Improving the Efficacy of Insect Pathogenic Fungi.
[b] ARSEF, US Department of Agriculture. Agricultural Research Service, Collection of Entomopathogenic fungus culture, Ithaca, NY, USA.

2.4. Screening of EPN

Experiments were conducted in 30-ml cups filled with 25 g of sandy loam soil and a carrot slice as a food source for the larvae. Ten larvae which had been held at 15 °C for 1 week were added into each cup. Nematodes (1000 IJs/cup = 63 IJs/cm^2) were inoculated onto the soil surface of each cup in 1 ml of water and the final moisture content was standardized at 12%. Control cups received 1 ml of water only. Cups were capped and kept in controlled temperature room at 22 ± 1 °C in the dark. Mortality was assessed at weekly intervals for 3 weeks post-inoculation. Dead larvae were dissected under a stereomicroscope to confirm that the mortality was due to nematode infection. Dead larvae were also kept on White trap (Kaya and Stock, 1997) to observe the nematodes emergence from infected cadavers. Each treatment was replicated three times with 10 larvae per replicate and the whole bioassay was conducted twice.

The chemical insecticide Mocap 10G (10% w/w ethoprophos; Bayer CropScience, UK) is recommended at the rate (60 kg/ha) for wireworm control in potato (Bayer CropScience, 2002); therefore, Mocap 10G was also included in the above assays.

2.5. Data analysis

Percentage data were normalized using arcsine transformation and subjected to analysis of variance (ANOVA) using software for statistical analysis (SPSS, 2003). Differences among treatments were compared using Tukey's mean separation test ($P < 0.05$). Larval mortality data were not corrected for control mortality as no larvae died in control treatments.

3. Results

3.1. Fungal virulence

There were significant differences among fungal strains in their virulence against *A. lineatus* ($F = 18.0$; df = 13, 42; $P <= 0.001$) 3 weeks post-inoculation. The *M. anisopliae* strains V1002 and LRC181A were highly pathogenic causing 90–100% mortality, followed by strains V1001 and LRC112A which caused 70% mortality (Fig. 1). The other isolates of *M. anisopliae* caused between 10 and 63% mortality, but neither *B. bassiana* nor *Paecilomyces fumosoroseus* were pathogenic to *A. lineatus* (Fig. 1).

3.2. Nematode virulence

There were significant difference among EPN strains in their virulence against *A. lineatus* ($F = 139.27$; df = 7, 47; $P <= 0.001$) 3 weeks post-inoculation. The most aggressive EPN were the UK strain of *Heterorhabditis bacteriophora* (UWS1) which caused 67% mortality (Fig. 2). The other EPN strains tested did not statistically differ in the control they gave which ranged between 0% and 50%, but they were significantly different from the untreated control (Fig. 2). No mortality was observed with *Steinernema feltiae*. Mocap