INHIBITION OF VERTICILLIUM SUCHLASPORIUM AND OTHER NEMATOPHAGOUS FUNGI BY BACTERIA COLONISING HETERODERA AVENAE FEMALES

by

L.V. LOPEZ-LLORCA and B. BOAG*

Summary. Pseudomonas fluorescens and a bacterium with similar characteristics (isolate A) were obtained from females of Heterodera avenae which caused growth-inhibition 'halos' in colonies of Verticillium suchlasporium or Acremonium sp. from naturally infected young females of H. avenae. These bacteria partially inhibited the growth of the egg-parasites Paecilomyces carneus and Acremonium sp. on agar. Isolate A also inhibited the growth of V. suchlasporium while Cladosporium cucumerinum, a fungal plant pathogen, was unaffected indicating a certain degree of specificity in the mycostasis. The percentage of nematodes infected by nematophagous fungi belonging to the genus Verticillium was less in young females than for newly formed cysts. The young females may have been initially protected by mycostasis which gradually decreased allowing the Verticillium spp. to infect the newly-formed cysts. The importance of mycostasis in the understanding of the process by which H. avenae is infected by nematophagous fungi under field conditions has still to be investigated.

Nematophagous fungi colonise the roots of the plants (Kerry et al., 1984) where they attack female and cyst nematodes killing their eggs (Kerry, 1981). These egg-parasites especially Verticillium spp. are good candidates to be developed for the biocontrol of cyst-nematodes (Kerry, 1986).

However, in order to use these fungi effectively as biocontrol agents it is necessary to study their physiology, especially in relation to the factors that may affect their survival in the rhizosphere and soil, where their hosts (female nematodes and cysts) live. Fungi in soils are subjected to antagonism by different organisms, but especially other fungi and bacteria. This phenomenon has been referred to as soil mycostasis (Jackson, 1957; Ko and Lockwood, 1970). Mankau (1962) found for the nematode-trapping fungus Arthrobotrys arthrobotryoides, reductions in the germination of conidia up to 90% in citrus soil. He also found that conidia of nematode-trapping fungi exposed to mycostatic factors produced traps directly from the spore without the development of mycelium. Kerry (1984) considered endoparasitic fungi producing adhesive spores or resting spores germinating only in the presence of the host to be less sensitive to mycostasis. Kerry (1984) also discussed the convenience of nutrient sources for overcoming mycostasis in applications of nematophagous fungi to control nematode populations in soil. Rhizosphere microorganisms, and specially pseudomonad bacteria have been found to inhibit the growth of several microorganisms including fungi (Schroth et al., 1983; Schippers et al., 1987a).

In this paper we report the growth inhibition of V. suchlasporium, an egg-parasite of cyst nematodes (Gams, 1988) and other nematophagous fungi by a rhizosphere bacterium, isolated from females of the cereal cyst nematode Heterodera avenae Woll.

Materials and methods

Heterodera avenae females and newly-formed cysts were obtained in pots by growing susceptible oats (cv. Peniarth) in naturally infested soil (Spittalfield, Blairgowrie, Scotland) known to contain endoparasitic fungi (Lopez-Llorca and Duncan, 1986).

The soil used for the pot-experiments was collected from eight points of the same field. Samples were bulked and stored at 5°C in the dark for at least 5 weeks before use to break the diapause and ensure hatching of H. avenae (Fisher, 1981). The soil was used directly from the cold room to fill (30 x 30 x 25 cm) square pots which were sown with oats. When the seed had germinated, seedlings were thinned so as to have approximately 20 plants per pot. Pots were then left in a greenhouse at 15°C.
Approximately ten oat plants were removed with their roots and adhering soil from three pots at 6, 7, 10, 13, 15, 16, 17, 18 and 25 weeks after sowing. The tops of the plants were severed and the roots left for 15 min in tap water to loosen soil and debris. Afterwards, the roots systems were washed gently in tap water and checked under the microscope for the presence of white females and/or newly-formed cysts.

Nematodes were picked-off the root systems, and separated into white females and newly-formed brown cysts. White females were separated, according to their size and shape, into two age categories viz. young females (YW) (small and elongated) and older females (OW) (‘lemon-shaped’ and bigger than YW). Females and cysts from the roots were washed twice in sterile distilled water (SDW) and plated on to 90 mm plastic petri dishes containing a growth-restricting medium (‘Medium 1’, Lopez-Llorca and Duncan, 1986) slightly modified (no rose bengal was included and penicillin was used instead of aureomycin). An average of 10 females or cysts per dish were plated. Plates were incubated at 20°C in the dark for 6-7 days. Cysts and females were then inspected for the presence or absence of fungi which were identified.

The plates with females in which fungi were developing were also scored for the presence of ‘halos’ of growth inhibition.

Bacterial colonies which developed from young females of H. avenae when plated on modified Medium 1, and those females showing mycostasis, were subcultured on nutrient agar (Oxoid) and single colonies isolated on the same medium. Bacterial colonies which differed in appearance, were sent to the Commonwealth Mycological Institute (CMI) for identification. The different bacterial colonies were also plated on corn meal agar (CMA), potato dextrose agar (PDA) and water agar (WA) (Oxoid) with egg-parasites from infected eggs of H. avenae and other fungi at 20°C in the dark, to test their effects on the growth of pure cultures of these fungi.

**Results**

The results for the fungal colonisation of H. avenae white females and newly-formed cysts (‘brown cysts’) are presented in Fig. 1. The percentage of white females colonised by fungi increased between weeks six and seven until week ten when the infection rate remained relatively constant while the percentage of brown cysts infected by fungi was consistently above 90%.

The infection of the white females with Verticillium species alone gradually rose between weeks 6 and 25 while the Verticillium infection in the brown cysts increased from around 20% at week 15 to nearly 50% in week 17 before decreasing to about 20% at week 25.

When plated on growth-restricting medium a large proportion of young females did not develop fungus (Fig. 1a).
and some of these apparently had no microorganism associated with them. In others, small, shiny globules developed which suggested that they were bacterial colonies, although the media contained antibiotics. Where nematodes with and without fungi were present on the same plate growth inhibition ‘halos’ were associated with nematodes that had bacteria-like colonies, irrespective of whether fungal colonies had developed. In one instance a female developing Verticillium, itself contained a shiny colony with antifungal activity against the fungus.

Mycostasis ‘phenomena’ were most frequently observed in young females, six halo area being recorded from six agar plates while only one halo was observed from five plates containing cysts (Table I).

Colonies from three females of H. avenae showing antifungal action against females developing V. suchlasporium or Acremonium sp. were subcultured and single colonies isolated. Three colony types were observed. Isolate B was sent to the Commonwealth Mycological Institute (CMI) and was identified as Pseudomonas fluorescens (Trevisan) Migula, biovar 1 or A (Dr J.F. Bradbury, CMI, personal communication). Colonies of isolate A had similar characteristics to isolate B (P. fluorescens and probably were also a pseudomonad bacterium, but isolate A colonies were not identified and are referred to as ‘isolate A’.

The bacterium ‘isolate A’ and P. fluorescens inhibited the growth of the egg-parasites Paecilomyces carneus and Acremonium sp. but not that of the phytopathogen Cladosporium cucumerinum. Isolate A also inhibited the growth of V. suchlasporium (Table II).

Figures 2a and 2b shows the growts inhibition of Acremonium cerealis (from H. avenae females) by the bacterium P. fluorescens on CMA and WA. Figures 2c and 2d show the same features on the same media for V. suchlasporium and the bacterium ‘isolate A’. The plates were incubated at 20°C in the dark and photographed 20 days after inoculation.

### Table I - Occurrence of mycostasis in fungal colonies associated with young and old Heterodera avenae females.

<table>
<thead>
<tr>
<th>Plate number</th>
<th>Total number of females</th>
<th>Young females</th>
<th>Old females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. infected with fungi</td>
<td>No. infected with fungi and mycostasis</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table II - Antifungal activity of the two bacterial isolates Pseudomonas fluorescens (PF) and isolate A (A) from H. avenae females on the growth of Paecilomyces carneus, Verticillium suchlasporium, Acremonium sp. and Cladosporium cucumerinum on different agar mediums.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Agar medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water agar</td>
</tr>
<tr>
<td>Paecilomyces carneus</td>
<td>PF + *</td>
</tr>
<tr>
<td>Verticillium suchlasporium</td>
<td>A +</td>
</tr>
<tr>
<td>Acremonium sp.</td>
<td></td>
</tr>
<tr>
<td>Cladosporium cucumerinum</td>
<td>PF -</td>
</tr>
</tbody>
</table>

* +, growth inhibition observed; –, no growth inhibition observed; + / –, little growth inhibition observed; NT, not tested.

**Discussion**

Mycostasis by bacteria has also been observed in other invertebrate pathogens such as entomopathogenic fungi. Dillon and Charnley (1986) reported the inhibition of the entomophagous fungus Mortierellum anisopila by insect gut bacterial flora.

The results of the present investigation indicate that P. fluorescens, and another bacterium, could colonise females of H. avenae and inhibit the growth of fungal egg-parasites. This is consistent with the observations of several other workers who also found that rhizosphere bacteria can act as natural antagonists of fungi (Schroth et al., 1983; Lambert et al., 1987; Schippers et al., 1987a, 1987b). Ganesan and Gnanamanickam (1987) inoculated plants with P. fluorescens and provided protection against the phytopathogenic fungus Sclerotium rolfsii.

The mechanism by which fluorescent pseudomonads inhibit fungi in the soil seems to be by competition for iron. The bacteria release siderophores; these are secondary metabolites with a strong affinity for iron (Fe + 3) (Schippers et al., 1987a, 1987b).
Fig. 2A-2D - Antifungal effect of rhizosphere bacteria on egg-parasites of H. avenae, 20 days after inoculation at 20°C in the dark: A, B, growth inhibition of Acremonium cerealis (a) by Pseudomonas fluorescens (b); (A in 1% water agar, B in corn meal agar). C, D, growth inhibition of V. suchlasporium (v) by bacterium 'isolate b' (C in 1% water agar, D, in corn meal agar).
The growth of *C. cucumerinum*, a fungal plant pathogen and not involved in cyst-nematode parasitism, was unaffected by the bacteria which inhibited growth of *V. suchlasporium* and other egg-parasites. This might indicate a certain degree of specificity in the mycostasis induced by the bacteria tested. The growth of the egg-parasitic fungi was never completely inhibited by bacteria. *V. chlamydosporium* has been shown to have antibacterial activity against several species (Filipello-Marchisio, 1976) which may explain why the antagonistic bacterium (*P. fluorescens*) was found together with egg-parasitic fungi in some *H. avenae* females.

Peterson and Katzenelson (1964, 1965) also observed differences in the degree of colonisation of wheat and soybean rhizosphere by the nematode trapping-fungus *A. oligospora*. The poorer colonisation of wheat rhizosphere by the fungus was associated with the presence of bacterial flora antagonistic to the fungus, absent in soybean rhizosphere.

Differences recorded in the root-colonisation of several species of cereals by the egg-parasites in Scotland (Lopez-Llorca, 1988) may also be due to rhizosphere mycostatic bacteria.

The percentage of nematodes colonised by nematophagous species of *Verticillium* increases from females to newly-formed cysts, supporting the view that these fungi have a major role in egg-parasitism. The increase in the occurrence of *Verticillium* with time may also indicate that the fungus overcomes mycostasis and perhaps competes with the ‘other fungi’, probably by producing inhibitory substances (Leinhos and Buchenauer, 1986), and thus becoming the dominant egg-parasite.

The mycostatic action of rhizosphere bacteria was mainly restricted in this study to young females (6 weeks after sowing). Mycostasis may act to protect young females from fungal parasitism, but its effects appeared to decrease progressively. However, the results shown here indicate that mycostasis is a process operating in soils which may be relevant to parasitism of cyst nematodes by egg-parasites such as *V. suchlasporium* and others.

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**Literature cited**


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