Nematol. medit. (1991), *19*: 225-227

Department of Agriculture, University of Reading — Reading, RG6 2AT, U.K.

OBSERVATIONS ON THE DETACHMENT OF SPORES OF *PASTEURIA PENETRANS* FROM PRE-PARASITIC SECOND-STAGE JUVENILES OF *MELOIDOGYNE* SPP.

by
H.A. RATNASOMA[1], S.R. GOWEN and N.G.M. HAGUE

Summary. Some spores of *Pasteuria penetrans* adhering to second-stage juveniles of *Meloidogyne* spp. detached when nematodes were placed in moist loamy soil and when kept in water. No detachment was observed from dead nematodes. Detachment could occur before or after the invasion of roots by nematodes. Detachment was enhanced by pectinase.

Pasteuria penetrans (Thorne) Sayre *et* Starr has potential as a biological control agent especially against root- knot nematodes (Stirling, 1984). However, as the spores are non- mobile the efficacy of the bacterium is partly related to the concentration and distribution of spores in soil and the chance contact of nematodes with spores as the juvenile stages move towards host roots. The nature of the spore attachment process is so far not understood and needs further investigation. It has been suggested that it can be either a process of mechanical- suction-cup (Mankau and Prasad, 1977) or an interlocking process between the fibrillar outer coat of the spore and the nematode cuticle (Stirling *et al.*, 1986). There is no ultrastructural evidence to show that it is due to an adhesive substance (Mankau and Prasad, 1977), Stirling *et al.* (1986) revealed that even dead nematodes could be encumbered with spores of *Pasteuria* although the number attached was small. On live nematodes higher rates of attachment have been associated with increased spore concentrations (Stirling and Wachtel, 1980; Davies *et al.*, 1988), temperature (Stirling, 1981) and by the sonication of spores (Stirling *et al.*, 1986; Davies *et al.*, 1988).

During casual observations it was noticed that spores become detached from cuticles of juveniles and so studies were pursued to test whether attachment of spores was permanent or transient. The detachment of spores from cuticles of nematodes under normal circumstances has not previously been recorded. If some of the spores are loosely attached to the cuticles of nematodes, spores may become detached when the nematodes migrate through the soil. Therefore, four studies were conducted to investigate whether detachment could happen in water, in soil or at the time of root invasion. As pectinase was used in the extraction of nematodes from roots its effect on detachment of *P. penetrans* spores was also studied.

Materials and methods

Juvenile root-knot nematodes, *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub.) Chitw. and various *P. penetrans* isolates were collected from cultures which had been maintained in the greenhouse at the University of Reading. The isolates of *P. penetrans* were from cultures obtained from G. Stirling (PP1), R.M. Sayre (PP2), V. Spaull (PP3), A. Daudi (PP Malawi) and J. Bridge (PP-PNG).

Seeds of *Lycopersicon esculentum* Mill. cv. Tiny Tim were washed overnight in tap water, then shaken for about 5-7 minutes in 0.001% mercuric chloride solution, rinsed in sterile distilled water and placed on Petri plates containing 1.5% water agar which were then sealed. When roots were 1-2 cm long, they were cut at the hypocotyl and transferred singly to Petri dishes containing sterile Gamborg's B_5 medium (Imperial Laboratories — 'Europe' Ltd., Hampshire). All the plates were sealed and the whole procedure was done under asceptic conditions. Petri dishes were incubated at 25°C for about 1-2 weeks until enough side roots were produced.

[1] Present address: Department of Export Agriculture, Matale, Sri Lanka.

In the first experiment four live *M. incognita* juveniles with 30 spores attached were placed individually in glass cavity block each containing 1 ml of sterile distilled water. Another set of four live nematodes with a similar number of attachments was added together into one glass cavity block containing 1 ml of sterile distilled water. Simultaneously four heat-killed nematodes that had been placed in a spore suspension and had seven spores attached on their cuticles were picked and placed in four cavity block each containing 1 ml of sterile distilled water. After 5 days the number of spores retained on each live and heat-killed nematode was recorded. The pH of the sterile distilled water was 8.9.

Detachment of *P. penetrans* spores from nematodes incubated for 5 days in soil at 28°C was studied on about 100-200 second- stage juveniles of *M. javanica*. Nematodes were placed in a cavity block containing about 1 ml of a spore suspension (6,500 spores/ml) of a blend of *P. penetrans* isolates of PP1, PP2, PP3 and PP(Malawi). Twenty-five juveniles with five spores attached were hand-picked after 12 hours and poured with a little water onto an extraction dish filter (7.5 cm diameter) containing a 3 cm layer of sterilized loamy soil. One ply of paper towel tissue layer was placed between filter and soil before nematodes were added into the soil. Soil moisture was then brought to near field capacity taking care not to allow any water to leach from the soil. Then the extraction filter was placed in a closed plastic box and incubated at 28°C for 5 days. On the 6th day, the extraction filter was placed on a saucer and water was added to the saucer until the water level touched the soil layer. One to two drops of hydrogen peroxide were added to the saucer in an attempt to recover as many nematodes as possible (Gowen and Edmunds, 1973) and the extraction filter was left at room temperature (12-25°C) for 24 hours. Nematodes which moved through the filter into the saucer were collected on a sieve (24 μm) with a little water and poured into a Petri dish. Altogether, 16 nematodes were recovered from soil and each was examined (x400) for counting the attached spores.

To determine the effect of pectinase on detachment of spores of *P. penetrans* from the cuticles of *M. javanica* a spore suspension (3.2×10^5 spores/ml) of *P. penetrans* (PP-PNG) prepared by crushing infected females of a Sri Lankan population of *M. javanica* was serially diluted six times. Approximately 100 second-stage juveniles of *M. javanica* were added into each of six Petri dishes containing 10 ml of the spore dilutions. After 12 hours, nematodes encumbered with one spore, 25 spores and approximately 100 spores were picked and placed separately in glass cavity blocks containing 1 ml of diluted (1:9) pectinase solution (pectinase 3 x L, NOVO Industry, Denmark). Six nematodes were used in each treatment. Spores retained on each nematode were recorded after 24 hours.

Detachment of *P. penetrans* spores in the root invasion process was investigated on Tomato cv. Tiny Tim; excised root cultures were inoculated separately with about 35 surface sterilized second-stage juveniles of *M. javanica* that has been encumbered with one or five spores after having been placed in an axenically produced suspension of PP-PNG. Spore suspensions were prepared by surface sterilizing infected females for 5 minutes in 0.001% mercuric chloride, rinsing three times in sterile water and crushing them in fresh sterile distilled water. After 8 days roots from three Petri plates of each treatment were removed, cut into 2-2.5 cm pieces and soaked in tap water for 3 days. Then roots under each treatment were macerated at a low speed for 2.5 second and the suspension thus obtained was passed through 250 μm, 125 μm and 24 μm sieves respectively. Nematodes retained on the 24 μm sieve were collected in Petri plates. The total number of nematodes recovered from each treatment was recorded and the number of spore attachments on each nematode was examined at x400 magnification.

Results

Spores became detached from juveniles when held in sterile distilled water. After five days, the four nematodes kept in isolation had 26, 22, 19 and 26 spores attached and those kept in a group had 18, 23, 20 and 30 spores attached. The proportion of spore retention was 77.5% and 75.8% respectively. No spore detachment was observed from the heat-killed nematodes.

P. penetrans endospores became detached from cuticles when nematodes were placed in loamy soil at 28°C or during the 24 hour period extraction. Of the sixteen nematodes recovered from soil after five days, two had the original burden of spores, eleven had less than five spores and three nematodes had no spores attached.

Pectinase affected the spore attachment of *P. penetrans* on *M. javanica* juveniles. On the six nematodes encumbered with approximately 100 spores an average of eighteen spores remained attached after 24 hours and the others with 25 spores or a single spore lost their attachments during this period.

Six of the seven nematodes with a single attachment had no spore attached after invading roots growing in sterile culture. When the nematodes had a burden of five spores, four of the six nematodes recovered had no spores attached and the reamining two had one and two spores respectively.

Discussion

It seems that spores of *P. penetrans* can detach from juveniles and that this is not necessarily caused by contact with other nematodes. As none became detached from dead nematodes, it is possible that spores may be detached during movement. Detachment of spores by pectinase suggests that a substrate which can be degraded by pectinase

is involved in the attachment process. Possibly this could be a form of carbohydrate although it is not thought to be a sugar (Stirling *et al.*, 1986).

It was also found that spores became detached from nematodes when they were added to soil confirming Stirling's observation (1984) that successful infection appears to require more than five spores attached per nematode cuticle, and that low infection may be the result of spore detachment rather than non-viability of the attached spores. However, for the root invasion study it is possible that spores may have been removed from the juveniles during the maceration and seiving and not during invasion. One implication of this work could be a need to use higher spore doses of *P. penetrans* in soil treatments. Spore detachment could be one of the reasons for inconsistent control in the field apart from the variability of nematode susceptibility to infection by a particular strain of *P. penetrans* (Channer and Gowen, 1988).

Part of this work was funded by the Overseas Development Administration through the Natural Resources Institute.

Literature cited

CHANNER A.C. and GOWEN S.R., 1988 — Preliminary studies on the potential of *Pasteuria penetrans* to control *Meloidogyne* species. Brighton Crop Protection Conference — Pests and Diseases, 1988, Vol. 3: 1209-1214.

DAVIES K.G., KERRY B.R. and FLYNN C.A., 1988 — Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. *Ann. appl. Biol.*, *112*: 491-501.

GOWEN S.R. and EDMUNDS J.E., 1973 — An evaluation of some simple extraction techniques and the use of hydrogen peroxide for estimating nematode populations in banana roots. *Plant Disease Reporter*, *57*: 678-681.

MANKAU R. and PRASAD N., 1977 — Infectivity of *Bacillus penetrans* in plant-parasitic nematodes. *J. Nematol.*, *9*: 40-45.

STIRLING G.R., 1981 — Effect of temperature on infection of *Meloidogyne javanica* by *Bacillus penetrans*. *Nematologica*, *27*: 458-462.

STIRLING G.R., 1984 — Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. *Phytopathology*, *74*: 55-60.

STIRLING G.R., BIRD A.F. and CAKURS A.B., 1986 — Attachment of *Pasteuria penetrans* spores to the cuticles of root-knot nematodes. *Revue Nématol.*, *9*: 251-260.

STIRLING G.R. and WATCHEL M.F., 1980 — Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica*, *26*: 308-312.

Accepted for publication on 7 May 1991.