Observations on *Bacillus penetrans* infecting *Meloidogyne* in sugarcane fields in South Africa

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SUMMARY

Observations were made on the distribution, level of parasitism and host preference of *Bacillus penetrans* infecting *Meloidogyne* in sugarcane fields in South Africa. Infected female *Meloidogyne* were found in 34% of 73 fields and occurred more frequently in coarse than fine textured soils. Fields of coarse textured soil infested with *B. penetrans* had been cultivated for more years than such fields without the parasite. *Meloidogyne* populations were generally larger in infested fields and data collected from one field show that the level of parasitism was greater at higher densities of the host. *B. penetrans* spores derived from *M. javanica* infected a much larger proportion of the larvae of this species than larvae of *M. incognita* and larvae of an unidentified species of *Meloidogyne*. In contrast, spores from *M. incognita* were equally infective on all three species. An undescribed species of *Meloidogyne* was not infected by spores from *M. javanica*.

RÉSUMÉ

Observations sur Bacillus penetrans *infestant* Meloidogyne *spp. dans les champs de canne à sucre en Afrique du Sud*

Des observations ont été faites sur la répartition, le niveau de parasitisme et la préférence d'hôte de *Bacillus penetrans* infectant *Meloidogyne* spp. dans les champs de canne à sucre en Afrique du Sud. Les femelles de *Meloidogyne* infestées furent observées dans 34% des 73 champs, et ce plus fréquemment dans les sols sableux que dans les sols lourds. La proportion des femelles infestées par le parasite était plus forte dans les champs à sol sableux ; ces derniers champs, infestés par *B. penetrans*, étaient cultivés depuis plus longtemps que les champs non infestés. Les populations de *Meloidogyne* étaient en général plus élevées dans les champs infestés et les résultats obtenus sur un champ particulier indiquent que le niveau de parasitisme plus élevé correspond à une densité plus forte de l'hôte. Les spores de *B. penetrans* provenant de *M. javanica* infestèrent une plus forte proportion de larves de cette espèce que de celles appartenant à *M. incognita* ainsi qu'à une espèce non identifiée de *Meloidogyne*. En revanche, les trois espèces furent également infestés par les spores de *B. penetrans* provenant de *M. incognita*. Une espèce non décrite de *Meloidogyne* ne fut pas infestée par *B. penetrans* obtenu de *M. javanica*.

Bacillus penetrans (Thorne) Mankau is a promising agent for the biological control of certain economically important plant-parasitic nematodes (Mankau, 1975 ; Sayre, 1980*a*). As a result it has aroused much interest (for example Sayre & Wergin, 1977 ; Mankau, 1980 ; Sayre, 1980*b* ; Stirling & Wachtel, 1980 ; Slana & Sayre, 1981, 1982 ; Stirling, 1981 ; Stirling & White, 1982). However more information on the biology and ecology of the parasite is required before its potential can be realized.

B. penetrans infects species of *Meloidogyne* in several countries (Williams, 1967 ; Mankau, 1975, 1980 ; Spaull, 1981*a* ; Stirling, 1981), but, apart from the work of Mankau (1980) and Stirling and White (1982), little is known of its distribution within a particular region or of its importance in the field.

Stirling and Wachtel (1980) described a method by which 90% or more of second stage larvae of *Meloidogyne javanica* could be infected with spores of *B. penetrans*. While using this method I obtained only 50% infection of a mixture of *M. incognita* and *M. javanica*. Since 97% of the infected larvae each had more than twenty spores attached to the cuticle, a low spore concentration was not responsible for the relatively low infection. The spores originated from infected females of both *M. incognita* and *M. javanica*.

This paper reports observations on the distribution and level of parasitism of *B. penetrans* infecting *Meloidogyne* on sugarcane in South Africa. It also describes experiments performed to explain the relatively low level of infection of the mixed populations of *Meloidogyne* species.

Materials and methods

DISTRIBUTION OF *B. penetrans*

The galled roots of tomatoes, that had previously been used to detect the presence of *Meloidogyne* in sugarcane fields in South Africa (Spaull, 1981*b*),

were used in the present study to determine the distribution of *B. penetrans*. Infected plants had been grown for 8-12 weeks in 2 l of soil taken from a composite sample of 40 cores from each of 81 fields. The roots were washed and fixed in boiling lactophenol stained with 0.01% cotton blue. Female *Meloidogyne* without egg sacs were dissected from the galls and squashed on microscope slides with a cover glass. They were then examined for the presence of *B. penetrans*, either as spores or as other stages in the life cycle (Sayre, 1980*b*). Approximately 2 500 females were examined. The probability of correctly recording the presence or absence of parasitized female *Meloidogyne* is reduced when only a few individuals are examined. An arbitrary limit of ten females was chosen as the minimum. Eight samples from which less than ten females were recovered were therefore not included in the analysis ; none of these contained infected females. The number of *Meloidogyne* larvae in the soil and roots of sugarcane in each of the fields was derived from data summarized by Spaull (1981*b*).

The approximate number of years that the soil had been cultivated was ascertained for 60 of the 73 fields included in the analysis.

Level of infection of *Meloidogyne* spp. by *B. penetrans*

A 1 000 m² area in a field of sugarcane that had previously been used for a nematicide trial was selected for study. The trial site comprised twenty plots in four blocks arranged in a rectangle, four plots wide by five plots long. Each plot was composed of five rows of sugarcane, 1 m apart and 10 m long. The soil contained 2% clay and, apart from short periods of fallow, had been under sugarcane for more than 30 years. Root samples taken during the course of the trial had shown that *B. penetrans* infecting *Meloidogyne* was present and that there was a marked gradient in the number of *Meloidogyne* larvae across the site. The gradient was not related to any of the treatments. When the sugarcane was harvested, twenty months after the trial began, five root samples were collected per plot. *Meloidogyne* larvae were extracted from 10 g subsamples by incubating the root pieces in a 3% solution of hydrogen peroxide (130 vol.) for six days in a polythene bag. The larvae were separated from the root pieces with a 140 mm diameter Baermann tray. Females were extracted from 30 g subsamples of roots by blending plus sugar flotation (Coolen & D'Herde, 1972) after incubating the roots in hot EDTA (pH 10.5) for 24 hours (Stynes, 1976). The number of larvae and females with and without *B. penetrans* were recorded.

Host preference of isolates of *B. penetrans*

Four experiments were performed to compare the infectivity of *B. penetrans* from different hosts on different species of *Meloidogyne*.

Experiment 1 : B. penetrans was isolated from infected *M. incognita* and *M. javanica* females on sugarcane roots and subsequently maintained on a mixture of these two species on tomatoes. A suspension of the spores was prepared by first air drying the roots, grinding them in a coffee grinder, sieving out the larger fragments and then mixing the resulting spore-laden powder with water (Stirling & Wachtel, 1980). The infectivity of the spores was tested on four populations of *M. incognita* (1-4), three of *M. javanica* (1-3) and a population of an unidentified species here referred to as *Meloidogyne* sp. G ; each population was derived from the progeny of a single female.

One hundred egg sacs of each of the eight populations were placed on 80 mm diameter Baermann trays for 48 hours. The hatched larvae, collected in 20 ml of water, were added to 80 ml aliquots of the spore suspension and agitated for 24 hours by bubbled air. A 35 ml sample was then removed and the larvae killed with formalin. Approximately 120 individuals were mounted in lactophenol on microscope slides and the number with and without spores attached were counted. This experiment was not replicated.

To multiply the populations of *B. penetrans* that infected larvae of four of the *Meloidogyne* populations, freshly hatched individuals were added to the remainder of the spore suspension and agitated for 24 hours. The infected larvae were then inoculated around the roots of tomatoes grown in sterile sandy soil. The tomatoes were maintained in a glasshouse until 1 300 degree days above 10° had accumulated and the spores of the parasite were mature (Stirling, 1981). The roots were then washed and air dried for use in the following three experiments.

Experiment 2 : Spores of *B. penetrans* were obtained from parasitized *M. javanica* population 3 and a suspension prepared as previously described. Infectivity of the spores was tested against the eight *Meloidogyne* populations and a population of an undescribed species (*Meloidogyne* sp. SC). Three hundred egg sacs from each of the nine populations were placed on 80 mm diameter Baermann trays for 48 hours. Thirty ml aliquots of the hatched larvae of each population were added to 40 ml aliquots of the spore suspension in three conical flasks. The flasks were agitated on a rotary action mixer for six hours after which the larvae were killed and counts made as described for Experiment 1.

Experiment 3 : Spores were obtained from infected females of *M. incognita* population 3 and tested against larvae of this population and of *M. javanica* population 3. The procedure was the same as described for Experiment 2.

Experiment 4: A mixture of spores from infected *M. incognita* populations 1 and 2 was tested against the larvae of these two populations, of *M. javanica* population 3 and of *Meloidogyne* sp. G. The procedure was the same as described in Experiment 2.

Results

Distribution of *B. penetrans*

B. penetrans infecting *Meloidogyne* was recorded in 34% of the sugarcane fields and occurred much more frequently in fields of sand and loamy sand than of other soil types (Tab. 1). The proportion of females infected with the parasite was greatest in fields of sand. Significantly more *Meloidogyne* larvae were extracted from sugarcane roots from

Table 1

Incidence of *Meloidogyne* infected with *Bacillus penetrans* in different soils

Soil textural class	*Number of field soil samples with tomatoes galled by* Meloidogyne	*Proportion of samples with* Meloidogyne *infected with* B. penetrans (%)	*Proportion of females without egg sacs* that were infected *per infested field* (%)
Sand	19 (1) *	63.2	16.8
Loamy sand	15 (3)	53.3	3.8
Sandy loam	18	16.7	7.5
Sandy clay loam	10 (3)	10.0	2.2
Clay loam and clays	11 (1)	9.1	2.4

* Figures in parenthesis represent number of field samples from which less than ten females were recovered.

Table 2

Mean number of *Meloidogyne* larvae in sugarcane fields and age of fields with and without *Bacillus penetrans* (Bp)

	Soil texture of fields					
	Sand		*Loamy sand*		*All textures (including sands) and loamy sands)*	
Number of fields	*No Bp (7)*	*Bp (12)*	*No Bp (7)*	*Bp (8)*	*No Bp (48)*	*Bp (25)*
Larvae/100 ml soil	14	23	11	57	15	32
Larvae/10 g roots	1 987	511	13	286	327	337
Average age of fields in years	12 (n = 7)*	29 (n = 8)	13 (n = 6)	27 (n = 7)	21 (n = 40)	25 (n = 20)

* n = number of fields for which the approximate age was known.

fields of loamy sand with *B. penetrans* than from those without the parasite ($p < 0.05$) (Tab. 2). Also, greater numbers of larvae were extracted from the soil from parasite-infested fields of a sandy texture (sands plus loamy sands) than from uninfested fields ($p < 0.05$). The large mean number of larvae recovered from sugarcane roots in fields of sand without *B. penetrans* was due to the 12 416 individuals obtained from one field. If this field is excluded, the mean of 1 987 larvae/10 g roots given in Table 2 falls to 249. Similarly the mean number of larvae from roots in all fields without *B. penetrans* would be reduced from 327 to 70. Thus the data show that populations were generally larger in fields with *B. penetrans* than in those without (Tab. 2).

The average age of fields of sandy soil with *B. penetrans* was more than twice that of unifested fields ($P < 0.05$) (Tab. 2). However when fields of all textures are considered there is little difference between the average ages. The number of *Meloidogyne* larvae recovered from the soil and roots of sugarcane was not correlated with the age of the field.

Level of infection of *Meloidogyne* spp. by *B. penetrans*

Based on perineal patterns, approximately 89% of the *Meloidogyne* in the trial site were *M. incognita* and 11% *M. javanica*. The average proportion of females infected with *B. penetrans* was 20.6% ($\pm$ 13.6 st. dev.) and the average proportion of larvae infected during the incubation period in the polythene bags was 23.1% ($\pm$ 17.0). Females of the two species were equally infected. The relationship between number of females per plot and proportion infected can be expressed by a natural log regression line, $y = -3.669 + 8.015 \log x$ ($P < 0.05$) ($r = 0.4933$) (Fig. 1). Grouping the plot data into their respective blocks showed not only the distinct gradient in numbers of nematodes but also a similar gradient in proportion of individuals infected from block 1 to block 4 (Tab. 3). No gradients were apparent when the plots were grouped across the blocks.

Host preference of isolates of *B. penetrans*

Table 4 summarises the results of the four experiments. Spores of *B. penetrans* which originated from a mixed culture of *M. javanica* and *M. incognita* (experiment 1) infected a much greater proportion of the larvae of *M. javanica* than those of *M. incognita*. Infection of *Meloidogyne* sp. G was intermediate, although many of the spores did not appear to be as firmly attached to the cuticle as they were on larvae of the other populations. When larvae of each of the eight *Meloidogyne* populations were mixed with spores from parasitized *M. javanica* (experiment 2), a similar pattern of infection occurred except that *Meloidogyne* sp. G was less heavily infected. Spores did not attach to larvae of *Meloidogyne* sp. SC.

Spores derived from *M. incognita* infected larvae of this species and those of *M. javanica* to the same extent (experiment 3). However, the level of infection of *M. javanica* larvae was much lower than that in the first two experiments. In the fourth experiment larvae of *M. javanica*, *M. incognita* and *Meloidogyne* sp. G were equally infected by spores from *M. incognita*. The level of infection of *M. javanica* larvae was lower than that in experiments 1 and 2 while the reverse was true for the two *M. incognita* populations. *Meloidogyne* sp. G was more susceptible to spores derived from *M. incognita* than to those from *M. javanica*.

Spores of *B. penetrans* from *M. javanica* females that infected *M. javanica* larvae were of the same diameter as those from *M. incognita* females that attached to *M. incognita* larvae (3.98 µm $\pm$ 0.17 and 3.96 µm $\pm$ 0.13 respectively, n = 50).

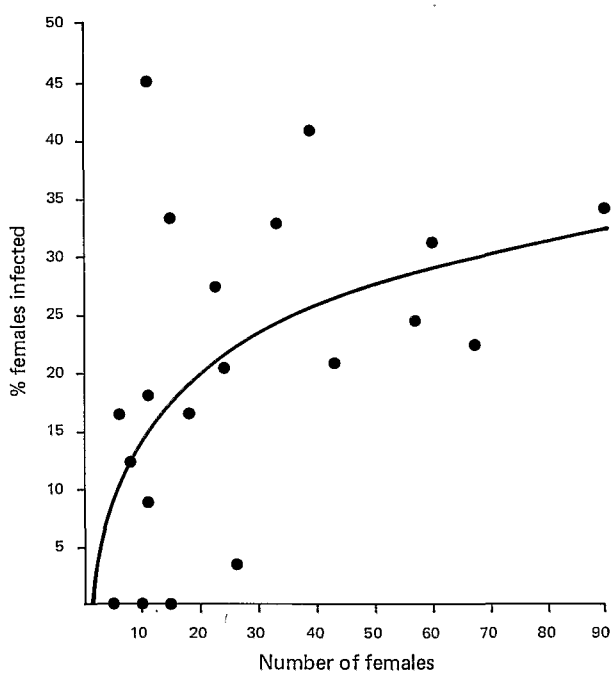

Fig. 1. Relationship between number of female *Meloidogyne* per plot and proportion infected with *Bacillus penetrans* ($y = -3.669 + 8.015 \log_n x$; $r = 0.4933$).

Discussion

Species of *Meloidogyne*, which are considered to be among the most important nematode pests of sugarcane in South Africa, are particularly common in the coarse textured soils (Spaull, 1981*b*). To find *B. penetrans* infecting *Meloidogyne* in over half of the fields of sand and loamy sand was therefore of considerable interest. But the log curve relating number of female *Meloidogyne* to percentage infected (Fig. 1) shows that there was a proportional increase in infection as the host density increased. This suggests that, at the time of sampling, the parasite was not limiting the *Meloidogyne* populations but merely removing surplus individuals. This suggestion may also apply to other infested fields since the result of the survey showed that there were generally more *Meloidogyne* larvae in fields of sandy soil with

Table 3

Level of infection of *Meloidogyne* by *Bacillus penetrans*

	Blocks (each of five plots)			
	1	*2*	*3*	*4*
Mean number of larvae/10 g roots	2 541	1 440	1 419	357
Mean number of females/30 g roots	57	29	19	9
% larvae infected	40	30	11	12
% females infected	31	25	16	16

Table 4

Percentage of *Meloidogyne* larvae infected with spores of *Bacillus penetrans* originating from different *Meloidogyne* populations

	Origin of spores			
	Experiment 1	*Experiment 2*	*Experiment 3*	*Experiment 4*
Meloidogyne *populations*	M. javanica *and* M. incognita *(field)*	M. javanica *population 3*	M. incognita *population 3*	M. incognita *population 1 & 2*
M. incognita pop. 1	15.7	8.7	—	43.2
M. incognita pop. 2	29.1	10.6	—	45.2
M. incognita pop. 3	25.0	13.7	21.4	—
M. incognita pop. 4	5.2	13.5	—	—
M. javanica pop. 1	100.0	92.1	—	—
M. javanica pop. 2	99.4	92.2	—	—
M. javanica pop. 3	99.5	96.2	21.3	45.4
Meloidogyne sp. G	52.0	7.2	—	50.0
Meloidogyne sp. SC	—*	0.0	—	—
L.S.D. ($P < 0.05$)		6.45	14.57	37.95
L.S.D. ($P < 0.01$)		8.87	33.62	57.68
Coefficient of variation		10.00	19.50	41.30

* Not tested.

B. penetrans than in those without. Possibly the fields were sampled in a relatively early phase in the build-up of the parasite population and the host population will decline in time.

In contrast with the observations reported here, *Meloidogyne* populations in South Australia tend to be lower in vineyards infested with *B. penetrans* than in vineyards where the parasite is rare or absent (Stirling & White, 1982). However, as in the Australian vineyards, the incidence of *B. penetrans* in sandy soils was greater in fields that had been cropped for many years than in younger fields. Stirling and White (1982) suggested that this relationship was a result of the slow rate of spread of the parasite and the time taken for it to reach concentrations that could be detected. This may also be true for the sugarcane fields in South Africa.

The average age of the 28 fields of sandy soils and of the 32 fields of loam and clay soils was 21 years (range 3-60) and 23 years (6-80) respectively. Thus, differences between the ages of fields of different soil textures do not explain the greater frequency of occurrence of *B. penetrans* in sandy soils. Rather, this may be due to a combination of the greater incidence and density of the host in such soils, a higher level of infection in fields where host density is greater and consequently an increased chance of detection in these fields.

The average level of infection of larvae extracted from root samples by the hydrogen peroxide incubation method was similar to that of the females (23 and 21 % respectively). This suggests that the concentration of spores in the peroxide solution, and hence the level of infection of larvae, was related to the proportion of infected females in the same root sample. However, the variation in the ratio of proportion of infected females to proportion of infected larvae per plot was very large (coefficient of variation = 62%). It is therefore unlikely that larvae obtained in this way could be used to assess the level of parasitism in females.

In the first host preference experiment, a much smaller proportion of the larvae of *M. incognita* were infected by *B. penetrans* than were those of *M. javanica* (Tab. 4). This explains the original observation that the average level of infection of a mixed culture of the two species was lower than expected when the technique of Stirling and Wachtel (1980) was used. Slana and Sayre (1981) found that spores of *B. penetrans* from *M. incognita acrita* adhered more readily to larvae of this species than to larvae of other species of *Meloidogyne*. The spores from *M. javanica* used in the second experiment showed a similar preference for the larvae of the host species. However, no such preference was found with spores from *M. incognita* which infected larvae of this species and of *M. javanica* equally (experiments 3 and 4 ; Tab. 4). The results suggest that there were two biotypes of *B. penetrans* in the original culture, one that readily infects *M. javanica* and, possibly, to a lesser extent, *M. incognita*, and another that is equally infective on both *M. javanica* and *M. incognita*.

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