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Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne-Pasteuria penetrans* interaction

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Summary — When a population of *Meloidogyne* spp. from Tuvalu was exposed to an isolate of *Pasteuria penetrans* from South Africa (PP3), the progeny of those nematodes which escaped infection were less susceptible to spore attachment than the original nematode population. *M. graminicola* and *M. naasi* were initially poor hosts of PP3. Exposure of *M. graminicola* to large numbers of spores resulted in multiplication of the isolate, and the spore population thus produced gave significantly greater attachment than the original PP3 population to *M. naasi*, but not to *M. graminicola*. Attachment by the new PP3 population to *M. incognita* (*ex* Bangladesh) was significantly reduced. Out of four populations of *M. incognita* and *M. javanica*, one, *M. incognita ex* Barbados, was also initially a poor host of PP3. Exposure of *M. incognita* (*ex* Barbados) to large numbers of spores resulted in the multiplication of a *P. penetrans* isolate which gave significantly greater attachment to *M. incognita ex* Barbados than the original PP3 population. However this isolate gave significantly reduced attachment to the other three *Meloidogyne* populations. When isolates of *P. penetrans* from several sources were blended, the consistency of spore attachment between and within a range of *Meloidogyne* populations was increased. It is concluded that populations of *P. penetrans* and *Meloidogyne* can be genetically heterogeneous with respect to specificity and susceptibility respectively. Thus biological control is unlikely to be consistent or durable unless populations of *P. penetrans* with a broad spectrum of host specificity can be deployed.

Résumé — ***Sélection pour un accroissement de la résistance de l'hôte et de la spécificité pathogénique dans l'interaction* Meloidogyne-Pasteuria penetrans** — Lorsqu'une population de *Meloidogyne* spp. provenant de Tuvalu est exposée à un isolat de *Pasteuria penetrans* provenant d'Afrique du Sud (PP3), la descendance des nématodes ayant échappé à l'infestation est moins sensible à l'adhérence des spores que ne l'était la population originelle. *M. graminicola* et *M. naasi* sont initialement des hôtes médiocres pour la souche PP3. L'exposition de *M. graminicola* à de grandes quantités de spores conduit à la multiplication de l'isolat; la population de spores ainsi produite montre une adhérence plus importante que celle de la population PP3 originelle, tout au moins envers *M. naasi*, mais non envers *M. gaminicola*. L'adhérence à *M. incognita* (orig. Bangladesh) de la population nouvelle de PP3 ainsi créée est significativement diminuée. Parmi quatre populations de *M. incognita* et *M. javanica*, l'une — *M. incognita* orig. Barbade — était également un hôte faible pour PP3. L'exposition de *M. incognita* (orig. Barbade) à un grand nombre de spores conduit à la multiplication d'un isolat de *P. penetrans* qui montre une adhérence à *M. incognita* (orig. Barbade) significativement plus élevée que celle de la population PP3 originelle. Cependant cet isolat fait montre d'une adhérence significativement plus faible envers les trois autres populations de *Meloidogyne*. Lorsque des isolats de *P. penetrans* de différentes origines sont mélangés, la cohérence de l'adhérence des spores entre et à l'intérieur des éléments d'une gamme de populations de *Meloidogyne* est accrue. Il est conclu que les populations de *P. penetrans* et de *Meloidogyne* peuvent être génétiquement hétérogènes au regard, respectivement, de la spécificité et de la sensibilité. Ainsi, le contrôle biologique peut difficilement être constant et durable à moins que ne puissent être utilisées des populations de *P. penetrans* ayant un vaste spectre de spécifité envers l'hôte.

Key-words : *Meloidogyne, Pasteuria,* resistance, pathogenicity, biological control.

Pasteuria penetrans (Thorne) Sayre & Starr, an obligate bacterial parasite of nematodes, has potential as a biological control agent (Mankau, 1973; Stirling, 1984; Brown, *et al.*, 1985; Bird & Brisbane, 1988). However, isolates of the organism seem to be highly specific, not only to particular nematode genera (Starr & Sayre, 1988) but to different populations of the same nematode species (Spaull, 1984; Stirling, 1985; Davies *et al.*, 1988). An implication of this specificity is that biological control may fail where a *P. penetrans* isolate deployed in the field is not compatible with the *Meloidogyne* population present. The experiments described below address the problem of specificity and its implications for the consistency and durability of biological control.

It is evident, given the large differences in susceptibility to infection reported for different populations of *M. javanica* and *M. incognita* (Stirling, 1985), that field populations of *Meloidogyne* spp. may show bimodal or multimodal distributions in spore load per nematode, corresponding to the presence of two or more nematode types differing in susceptibility to spore attachment. Natural selection could thus favour the build-up of

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types more resistant to *P. penetrans* at the expense of those more susceptible. This hypothesis is tested in the first experiment.

If *Meloidogyne* populations from a given field can exhibit variation to *P. penetrans* infection which is genetically determined, then it is reasonable to assume that populations of *P. penetrans* spores in the field may also be variable in their specificity. If the ability of a spore sub-population to attach to a particular *Meloidogyne* isolate can preclude it from attaching to another *Meloidogyne* isolate, then it could be postulated that culturing a population derived from a field sample of *P. penetrans* on different *Meloidogyne* types may result in a shift in the host specificity of that population. This hypothesis is tested in the second and third experiments.

The successful deployment of *P. penetrans* as a biological control agent could be greatly complicated by variability and selection. If blending *P. penetrans* isolates from different sources improved the consistency of attachment within and between different *Meloidogyne* populations, this might provide a means of minimizing the problem of nematode resistance to spore attachment in the field.

Materials and methods

Juvenile nematodes were obtained by hatching a suspension of eggs on a sieve covered with filter paper, placed over a dish of tap water, and incubated at 28 °C. The egg suspension was prepared from *Meloidogyne*-infected tomato roots using the method described by Barker (1985).

P. penetrans inoculum, in the form of dried plant roots containing the cadavers of spore-laden female nematodes, was produced using methods developed by Stirling and Wachtel (1980). A suspension of spores for experimental purposes was prepared by adding a few drops of water to 100 mg of dried root material in a mortar, grinding with a pestle to form a slurry-like paste, then adding 100 ml of tap water and sieving through a 37 µm mesh to remove residual root tissues. The concentration of spores per ml was assessed using a haemocytometer and diluted as necessary.

Juvenile nematodes were exposed to *P. penetrans* spore suspensions for a specified length of time at specified temperatures. Experiments involving long assessments were terminated with the addition of a few drops of 5 % formalin to each experimental receptacle. The use of an inversion microscope at a magnification of 200 × made it possible to count spores on nematodes without having to remove them from the experimental Petri dish.

The frequency with which spores attach to juveniles within a nematode population rarely conforms to a normal distribution. Further, frequency distributions can vary markedly between one nematode/*P. penetrans* combination and another, invalidating statistical comparison using parametric tests. In cases where significant differences between treatments are not immediately evident from visual comparison of the histograms, chi-squared (χ^2) tests were carried out on differences in the proportion of low (< 5) and high (≥ 5) numbers of spores per nematode.

The origins and identities of the *Meloidogyne* and *P. penetrans* populations used in the experiments are shown in Tables 1 and 2.

Table 1. The origins and identities of *Meloidogyne* populations used in the experiments.

Population name	Identification	Country of origin	Source
Tuvalu	*M. incognita* *M. arenaria*	Tuvalu	F. Pullen
Bangla	*M. incognita*	Bangladesh	J. Bridge
Barbados	*M. incognita*	Barbados	S. Gowen
PNG	*M. incognita* *M. javanica*	Papua New Guinea	J. Bridge
SL	*M. javanica*	Sri Lanka	J. Bridge
RES	*M. incognita*	USA	B. Kerry
Graminicola	*M. graminicola*	Bangladesh	J. Bridge
Naasi	*M. naasi*	UK	R. Cook

Table 2. The origins of *P. penetrans* isolates used in the experiments.

Population name	Nematode	Country of origin	Source
PP1	*Meloidogyne* sp.	Australia	G. Stirling
PP2	*M. incognita*	USA	R. Sayre
PP3	*M. javanica* *M. incognita*	South Africa	V. Spaull
PP4	*M. javanica* *M. incognita*	Papua New Guinea	J. Bridge

Experiment 1 — selection for increased resistance to spore attachment by *P. penetrans* in a population of *Meloidogyne* from Tuvalu

Ca 53 000 juveniles of *M. incognita* (Tuvalu), obtained from several culture plants, were exposed to a high, but unrecorded, concentration of PP3 suspension in a total volume of 267 ml contained in a shallow dish. After 16 hours in a incubator at 28 °C, 50 nematodes were removed and the number of spores on each counted. The remaining encumbered nematodes were applied to four tomato plants in the greenhouse. Concurrently, juveniles hatched from the same culture plants, but unexposed to *P. penetrans*, were applied to two other tomato plants.

Five weeks later 10-15 egg-masses were removed from each of the two *P. penetrans*-free culture plants and from two of the plants where the nematode population was infected with *P. penetrans*. These egg masses were hatched in four separate watchglasses. Appoximately 100 juveniles were removed from each watchglass and pipetted into four other watchglasses containing 0.2 ml of the same PP3 suspension used 5 weeks previously to infect the original population. The total volume in each watchglass was 2.5 ml. After 22 h at 28 °C, fifteen nematodes from each watchglass were examined for the number of spores adhering to them.

EXPERIMENT 2 — THE EFFECT ON SUBSEQUENT HOST SPECIFICITY OF CULTURING A *P. PENETRANS* ISOLATE ON *M. INCOGNITA* OR *M. GRAMINICOLA*

Groups of 400 juveniles of either *M. incognita* (Bangla), *M. graminicola* or *M. naasi* were introduced into 100 ml flasks. Each flask contained either PP1 cultured on *M. incognita* (Bangla), PP3 cultured on *M. incognita* (Bangla) or PP3 cultured on *M. graminicola*, at concentrations of 30 000 spores/ml in a total volume of 25 ml. Three replicate flasks were used for each treatment combination. After 24 h at 23 °C, with periodic bubbling of air into the flasks to facilitate mixing, 30 nematodes from each treatment were examined for the number of spores attaching to them.

EXPERIMENT 3 — THE EFFECT ON SUBSEQUENT HOST SPECIFICITY OF CULTURING A *P. PENETRANS* ISOLATE ON DIFFERENT *M. INCOGNITA* POPULATIONS

Three nematode populations, *M. incognita* (Tuvalu), *M. incognita* (Barbados) and *M. incognita/M. javanica* (PNG) were introduced to soil heavily laden with PP3 spores. At harvest, female nematodes were extracted from plant roots in each treatment and examined for infection. Spores from infected individuals were retained and diluted to give three suspensions of equivalent concentration. A fourth suspension was also prepared from PP3 spores which had not been cultured on any nematodes in Reading since arriving from South Africa.

Sixty juvenile nematodes of *M. incognita* (Barbados), *M. incognita* (Tuvalu), *Meloidogyne/M. javanica* (PNG) and *M. javanica* (SL), in 0.5 ml, were pipetted into 2.5 cm Petri dishes containing 1.5 ml of the spore suspensions at a final spore concentration of 15 000 spores/ml. Two replicate dishes were used for each treatment combination. After 20.5 h at 28 °C, 20 nematodes from each treatment combination were examined for the number of spores attached to them.

EXPERIMENT 4 — THE EFFECT OF BLENDING DIFFERENT *P. PENETRANS* ISOLATES ON THE VARIABILITY OF SPORE ATTACHMENT WITHIN AND BETWEEN DIFFERENT *MELOIDOGYNE* POPULATIONS

Sixty juvenile nematodes of *M. incognita* (Barbados), *M. incognita* (Tuvalu), *M. incognita/M. javanica* (PNG), *M. incognita* (RES) and *M. javanica* (SL), in 0.5 ml, were introduced into 3.5 cm Petri dishes containing 1.5 ml of the following spore suspensions at concentrations of 15 000 spores/ml : *(i)* PP3 (original); *(ii)* PP2; *(iii)* PP1, PP2, PP3, PP4 - blended (blend 2); *(iv)* four equal parts of : [PP3 original], [PP2], [PP3 *ex M. incognita* (Barbados)] and [PP1, PP2, PP3 + PP4] — blended (blend 1).

Two replicate dishes were used for each spore-nematode combination. After 24 hours at 28 °C, 20 nematodes from each treatment combination were examined for the number of spores attached to them.

Results

EXPERIMENT 1

The distribution of spore attachment on the population of nematodes selected from the progeny of those individuals which resisted infection is distinctly bimodal (Fig. 1 B) : 80 % of the nematodes received no spores at all, while 20 % received seventy or more spores each. In contrast, the distribution of spore attachment on the progeny of the Tuvalu population which had not previously been exposed to spores was more even (Fig. 1 C). Comparisons with Fig. 1 A should be made with caution since the attachment test on the original nematode population from Tuvalu was carried out under different experimental conditions.

EXPERIMENT 2

PP1 and PP3, both *ex M. incognita* (Bangla), gave similar forms of distribution on all three nematode populations (Fig. 2), and (χ^2) tests on the proportion of low and high numbers of spores per juvenile obtained on the three nematode species suggest that these two *P. penetrans* populations do not differ significantly from one another. In contrast PP3 *ex M. graminicola* gave noticeably different distributions. Statistical comparison of PP3 *ex M. graminicola* and PP3 *ex M. incognita* reveals that these differences are significant on *M. incognita* (χ^2 : $P < 0.001$) and *M. naasi* (χ^2 : $P < 0.001$), but insignificant on *M. graminicola.*

EXPERIMENT 3

Culturing PP3 on *M. incognita* (Barbados) resulted in a change in its spectrum of host specificity (Fig. 3). In comparison to the original PP3 population, PP3 *ex* Barbados gave significantly greater attachment to *M. incognita* (Barbados) (χ^2 : $P < 0.001$) and significantly lower attachment to the other three *Meloidogyne* populations (χ^2 : $P < 0.001$ in each case). In contrast culturing PP3 on *M. incognita* (Tuvalu) resulted in no significant changes in host specificity. Culturing PP3 on *M. incognita/M. javanica* resulted in significantly increased spore attachment to *M. incognita/M. javanica* (PNG) and also to *M. incognita* (Barbados), while attachment

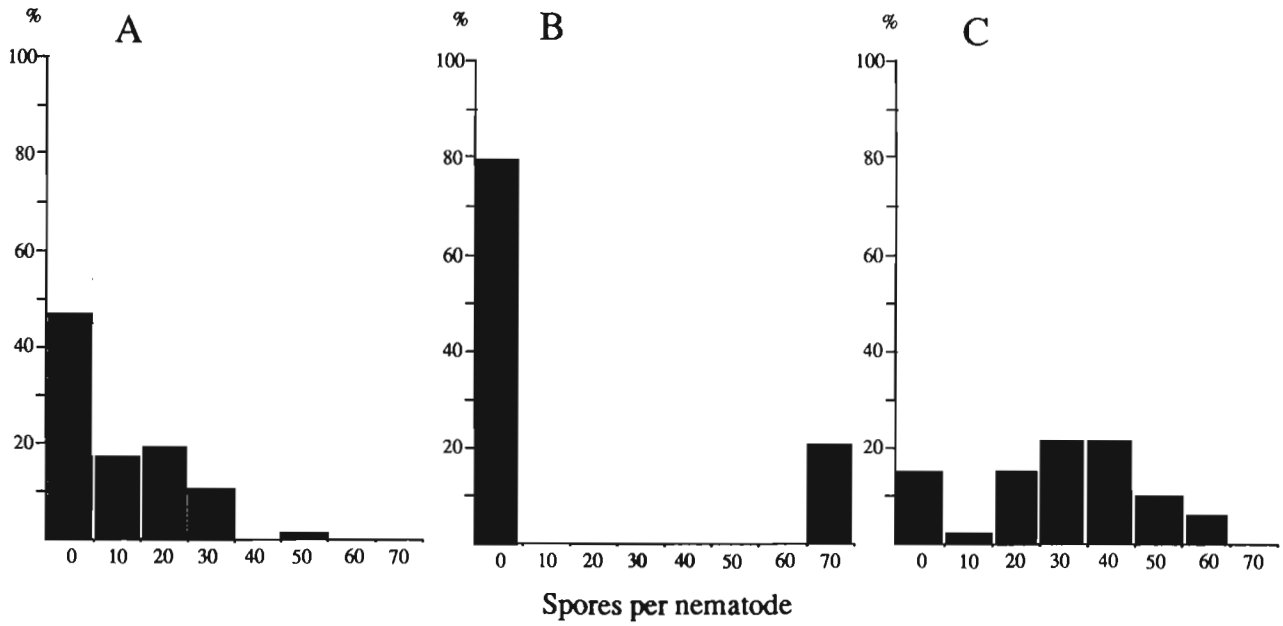

Fig. 1. A demonstration of selection for increased resistance to spore attachment by *Pasteuria penetrans* (PP3) in *Meloidogyne incognita* (Tuvalu). Frequency distributions for spore attachements on 60 nematodes from each treatment combination. A : A field sample population; B : Progeny of field sample population that resisted infection; C : Progeny of field sample population not previously exposed to infection.

levels to *M. incognita* (Tuvalu) and *M. javanica* (Sri Lanka) remained unchanged.

EXPERIMENT 4

The host ranges of PP3 and PP2 *ex* PNG are strikingly different : χ^2 tests reveal significant differences between them on all the nematode populations tested (χ^2 : $P < 0.001$), except *M. incognita* (RES) (Fig. 4). It is notable that PP2 *ex* PNG shows a similar spectrum of activity to PP3 *ex* Barbados in the previous experiment.

The mean values of attachment obtained by the blends over the diferent nematode populations are more similar to each other than the values obtained over these populations by either of the unblended *P. penetrans* isolates. χ^2 tests reveal no significant differences between the distributions obtained by Blend 1 over the five different *Meloidogyne* populations tested. However in the case of Blend 2, *M. incognita* (RES) differs significantly from *M. javanica* (Sri Lanka) and from *M. incognita/M. javanica* (PNG) (χ^2 : $P < 0.05$). Overall, less nematodes acquired either very low or very high spore burdens when exposed to the two blends : there is a marked tendency for more bell-shaped, symmetrical distributions of spore attachment to result when juvenile nematodes are exposed to blends of *P. penetrans* rather than single isolates.

Discussion

Variation in susceptibility to spore attachment between different *Meloidogyne* populations has already been documented (Stirling, 1985; Davies *et al.*, 1988). However, nothing has been detailed on variation in susceptibility to spore attachment within *Meloidogyne* populations.

We have found that the variance in spore attachment per nematode tends to be greater on field sample than on single egg mass populations of *Meloidogyne* (Channer & Gowen, unpubl.). Since single egg mass populations are likely to be more genetically uniform, such differences in variance could be due to genetic variation in susceptibility to attachment between different nematode types in field populations. Netscher (1978) and Fargette (1987) found that 25 % and 18 %, respectively, of *Meloidogyne* field populations were mixtures of *Meloidogyne* biotypes. It is thus conceivable that natural selection could favour the build-up of a nematode sub-population more resistant to *P. penetrans* at the expense of one more susceptible. This hypothesis is supported by the results of Experiment 1. Firstly, the distribution of spores on the original nematode population is over-dispersed (*sensu* Bliss, 1953) : there is greater variation in the number of spores per nematode than would be expected

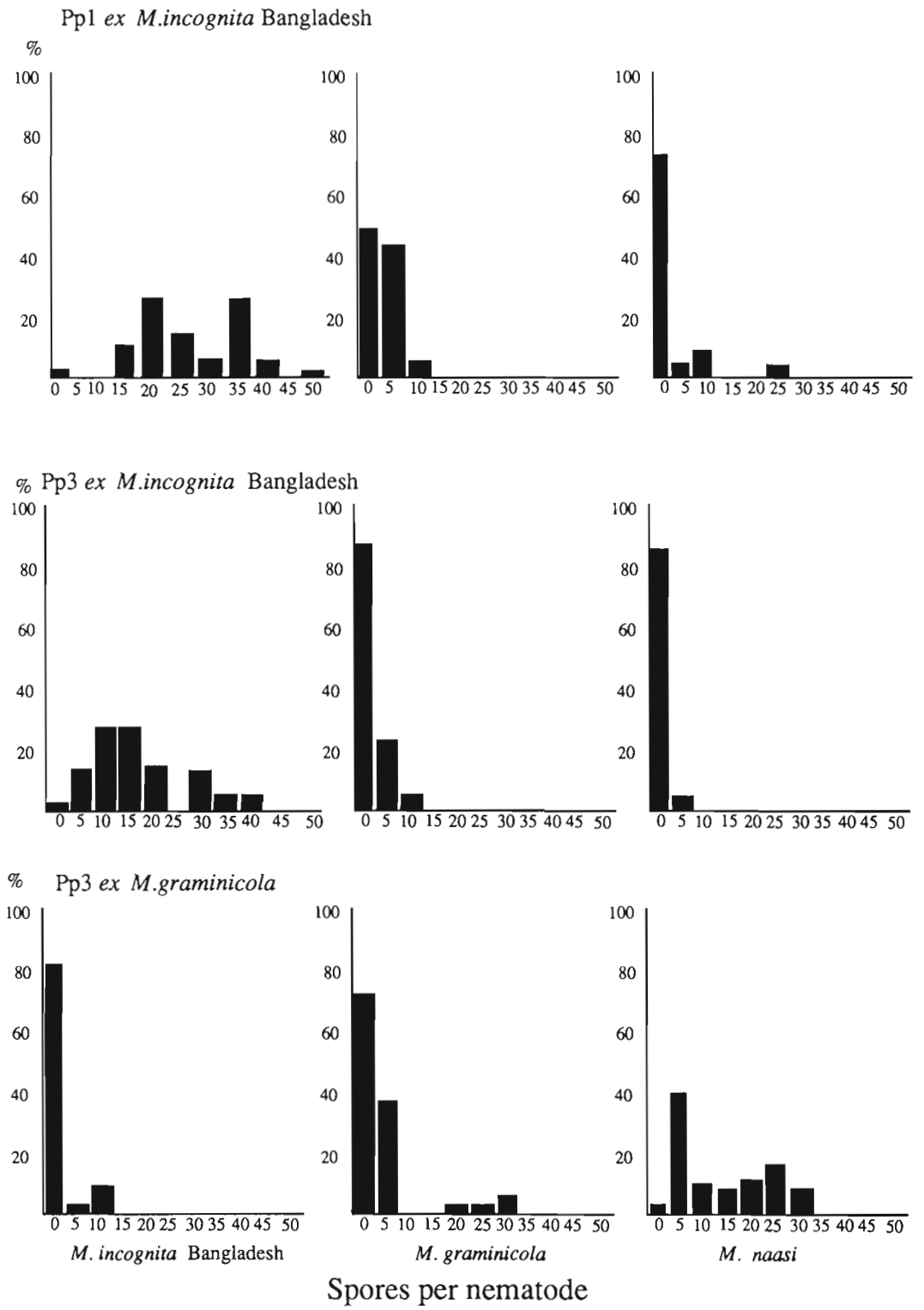

Fig. 2. Frequency distributions of the numbers of spores per nematode arising from combinations of PP1, PP3 *ex Meloidogyne incognita* and PP3 *ex M. graminicola* on *M. incognita, M. graminicola* and *M. naasi.* (Spore attachments on 30 nematodes for each treatment combination.)

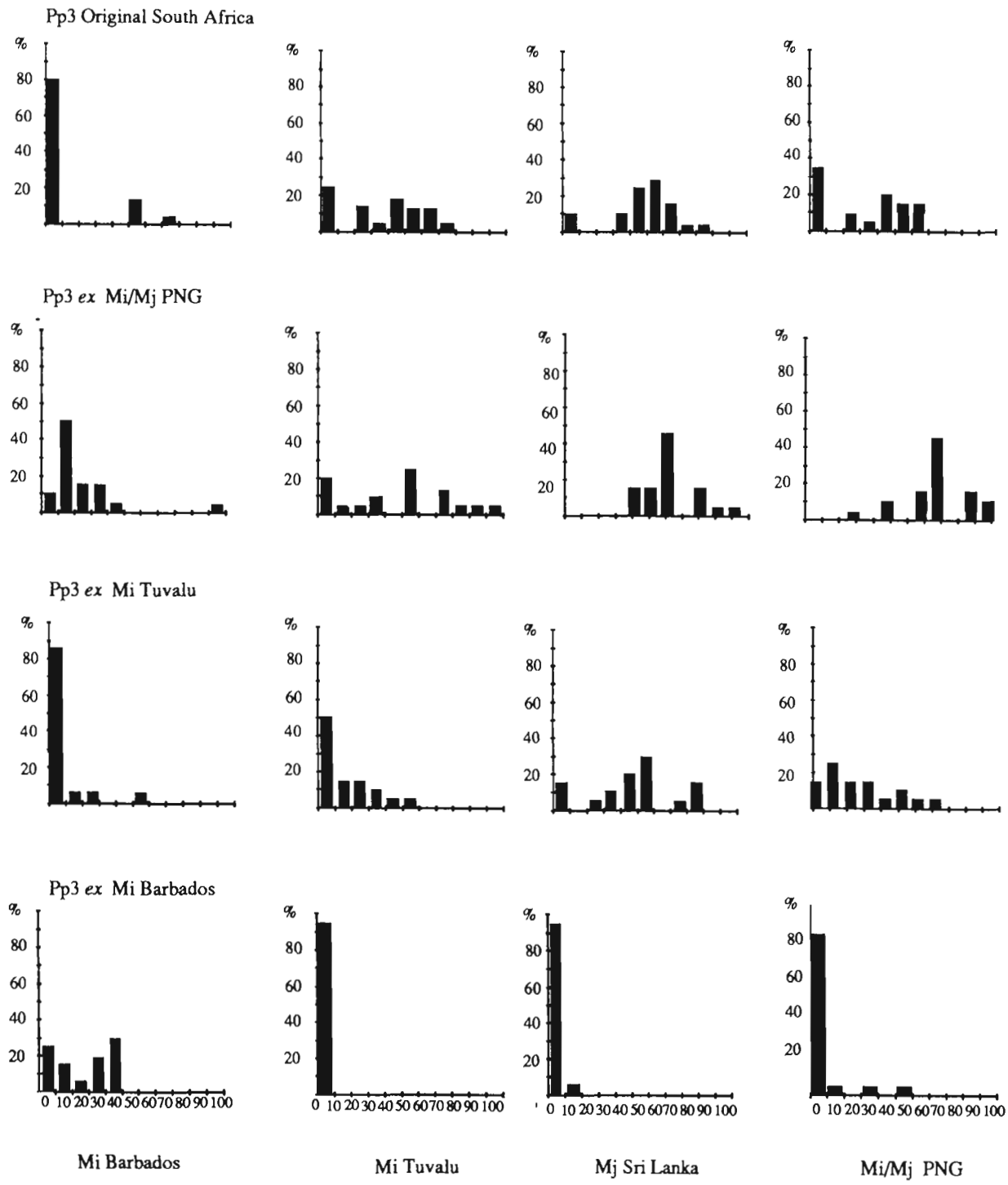

Fig. 3. Frequency distributions of the number of spores per nematode arising from combinations of different *Meloidogyne* populations exposed to PP3 populations previously cultured on different nematode hosts. (Spore attachments on 20 nematodes for each treatment combination.)

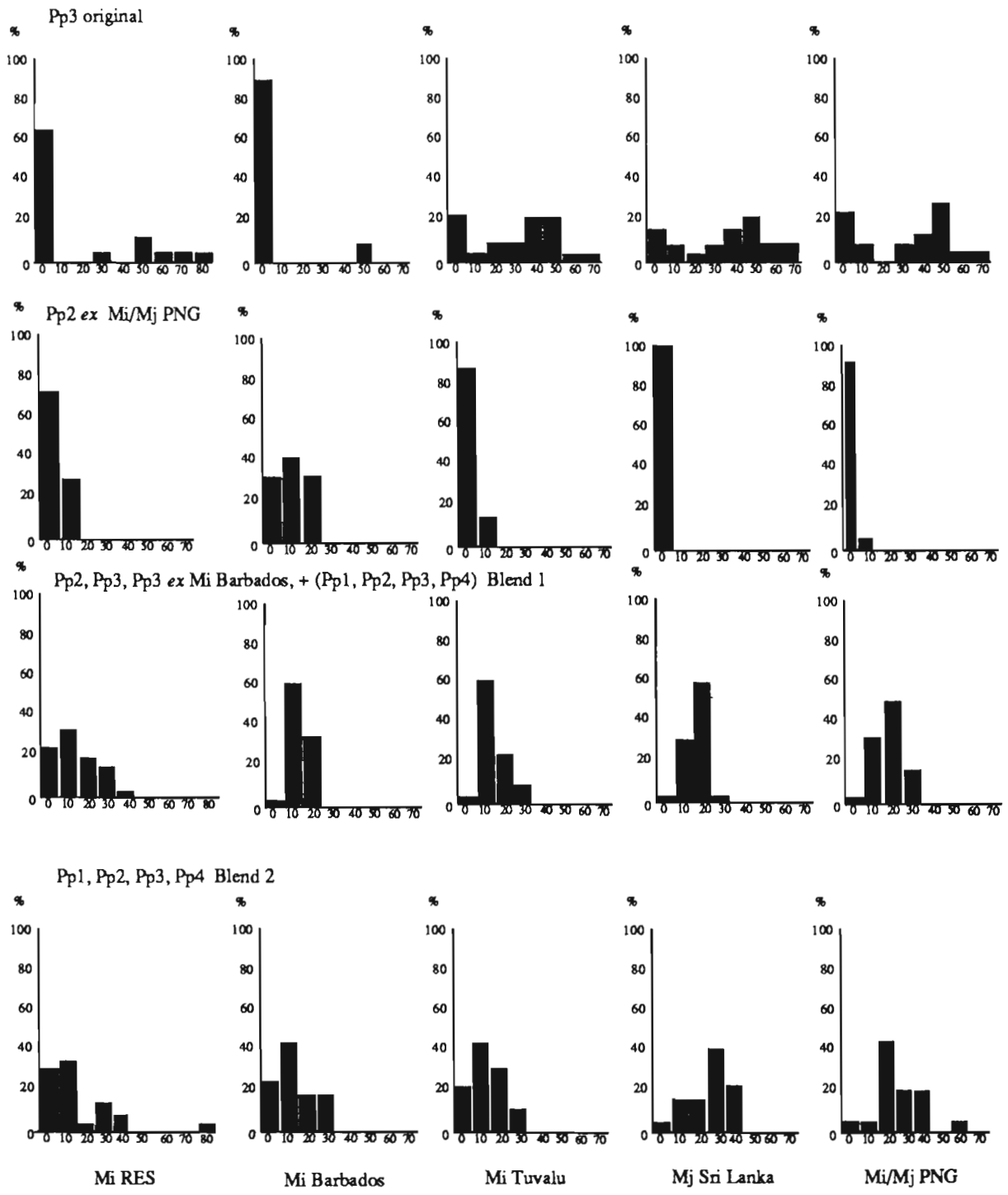

Fig. 4. Frequency distributions of the number of spores per nematode arising from combinations of five *Meloidogyne* populations exposed to two single isolates and two blends of *Pasteuria penetrans*. (Spore attachments on 20 nematodes from each treatment combination.)

to occur by chance. When the progeny of those nematodes in this population which had escaped infection were re-exposed to spores, a marked decrease in mean spore attachment was obtained, with a large number of nematodes acquiring no spores at all. Evidently the susceptible sub-population had declined relative to the resistant one. While a sample of the original nematode population from Tuvalu was identified by examination of perineal patterns as *M. incognita*, the population with the higher proportion of resistant individuals was identified as a mixture of *M. incognita* and *M. arenaria*. The implication is that *M. arenaria* is resistant to spore attachment by PP3 and present at low levels in the original population. Infection of this population resulted in an increase in *M. arenaria* relative to *M. incognita*, such that *M. arenaria* individuals were more likely to be sampled in the examination of perineal patterns.

Davies *et al.* (1988) showed that spores of *P. penetrans* from six populations of *Meloidogyne* only adhered to species of *Meloidogyne*, and adhered in greatest numbers to the species from which they had been originally isolated. However Stirling (1985), in a study of four populations of *P. penetrans*, and fifteen single egg mass populations of *Meloidogyne* spp., concluded that spore attachment was not related to the species from which spores were obtained, nor the species of the recipient nematode. The evidence presented here is that *Meloidogyne* host populations can sometimes influence the subsequent host specificity of *P. penetrans* isolates. This is shown clearly in Experiment 3, where the ability of PP3 *ex M. incognita* Barbados to attach to the host nematode on which it was last cultured increases dramatically, while its ability to attach to the *Meloidogyne* populations on which PP3 was originally more aggressive diminishes. This suggests a degree of mutual exclusivity; that when attachment increases on one nematode population, it may necessarily diminish on another. Culturing PP3 on *M. incognita* (Tuvalu) had no significant effect on its subsequent spectrum of specificity. The difference in behaviour between these two *M. incognita* populations confirms the assertion by Stirling (1985) that the recognition process in spore attachment is determined by characteristics which are variable within *Meloidogyne* species. When PP3 was cultured on the mixed population of *M. incognita/M. javanica* from Papua New Guinea, spore attachment was subsequently improved in all the nematode populations tested. This improvement was significant on the PNG population and also the *M. incognita* population from Barbados. It may be that the PNG population is constituted by two sub-populations, one similar to *M. incognita* (Barbados), and the other similar to *M. incognita* (Tuvalu) and *M. javanica* (Sri Lanka) in their susceptibility to PP3.

One explanation for apparent switches in host specificity is that both the genus *Meloidogyne* and species within it are genetically heterogeneous with respect to susceptibility to spore attachment, while *P. penetrans* is genetically heterogeneous with respect to host specificity. This spore selection hypothesis may also explain why culturing PP3 on *M. graminicola* increased spore attachment to *M. naasi* more than to *M. graminicola*. There may be a minority nematode sub-population in *M. graminicola* (*M. g.* A) which is more similar in susceptibility to the majority *M. naasi* sub-population (*M. n.* A) than it is to the majority *M. graminicola* sub-population (*M. g.* B). If *M. g.* A was much more susceptible to PP3 than *M. g.* B, then the majority of spore types carried forward to predominate in the next generation would be PP3 *ex M. g.* A. And if the proportion of A to B types was much greater in *M. naasi* than in *M. graminicola*, then *M. naasi* would manifest greater susceptibility than *M. graminicola* when exposed to PP3 *ex M. g.* A.

An alternative explanation for switches in host-specificity is that *P. penetrans* undergoes some form of host-induced adaptation. This implies that *P. penetrans* can recognise a host type it has successfully infected, and produce spore progeny with an enhanced ability to re-infect that particular host type.

Clearly, elucidation of the specificity of spore attachment may shed light on genetic variability in *Meloidogyne* species and *P. penetrans*, which could be correlated with electrophoretic, monoclonal antibody or DNA-probe tests. The development of single spore lines is required to ascertain the host specificity of homogeneous *P. penetrans* populations and the extent to which individual *P. penetrans* strains differ in their spectra of specificity. In fact such studies in the *P. penetrans-Meloidogyne* interaction may provide a model system for the study of variability in the relationship between an obligately biotrophic micro-organism and its host.

The final experiment demonstrates that using a blend of *P. penetrans* isolates reduces the inconsistency of spore attachment within and between *Meloidogyne* populations. Since only five spores per juvenile are required to ensure infection (Stirling, 1984) this suggests that blends, rather than single isolates, should be deployed if durable biological control is to be achieved in the field.

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References

BARKER, K. R. (1985). Nematode extraction and bioassays. *In :* Barker, K. R., Carter, C. L. & Sasser, J. N. (Eds). *An Advanced Treatise on Meloidogyne, Vol. II Methodology.* North Carolina State University, USA : 17-35.

BIRD, A. F. & BRISBANE, P. F. (1988). The influence of *Pasteuria penetrans* in field soils on the reproduction of root-knot nematodes. *Revue Nématol.*, 11 : 75-81.

BLISS, C. I. (1953). Fitting the negative binomial distribution to biological data. *Biometrics*, 9 : 176-199.

BROWN, S. M., KEPNER, J. L. & SMART, G. G. (1985). Increased crop yields following application of *Bacillus penetrans* to field plots infected with *Meloidogyne incognita*. *Soil Biol. Biochem.*, 17 : 483-486.

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

FARGETTE, M. (1987). Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes observed in West African populations and their characterisation. *Revue Nématol.*, 10 : 45-56.

MANKAU, R. (1973). Utilization of parasites and predators in nematode pest management ecology. *Proc. Tall Timbers Conf. Ecol. Animal Habitat Manag.*, 4 : 129-143.

NETSCHER, C. (1978). Morphological and physiological variability of species of *Meloidogyne* in West Africa and implications for their control. *Medeed. Landbhogesch. Wageningen*, 78 : 1-46.

SPAULL, V. W. (1984). Observations on *Bacillus penetrans* infection, *Meloidogyne* in sugarcane fields in South Africa. *Revue Nématol.*, 7 : 277-282.

STARR, M. P. & SAYRE, R. M. (1988). *Pasteuria thornei* sp. nov. and *Pasteuria penetrans sensu stricto emend.*, mycelial and endospore-forming bacteria parasitic, respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne*. *Annals Inst. Pasteur/Microb.*, 139 : 11-31.

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

STIRLING, G. R. (1985). Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne*. *Nematologica*, 31 : 203-209.

STIRLING, G. R. & WACHTEL, M. F. (1980). Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica*, 26 : 308-312.