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Forum article

The importance of tropical root-knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent

David L. TRUDGILL [1,*], George BALA [2], Vivian C. BLOK [1], Andrew DAUDI [3], Keith G. DAVIES [4], Simon R. GOWEN [5], Mireille FARGETTE [6], Joseph D. MADULU [7], Thierry MATEILLE [8], Werner MWAGENI [7], Casper NETSCHER [9,†], Mark S. PHILLIPS [1], Abdoussalam SAWADOGO [10], Carmen G. TRIVINO [11] and Effie VOYOUKALLOU [12]

[1] *SCRI, Invergowrie, Dundee, Scotland DD2 5DA UK*
[2] *CARDI, Central Experiment Station, Centeno, Trinidad, West Indies*
[3] *Ministry of Agriculture, BRS, PO Box 5748, Limbe, Malawi*
[4] *IACR Rothamsted, Harpenden, Herts., AL5 2JQ, UK*
[5] *University of Reading, Earley Gate, Berks.,RG6 2AT, UK*
[6] *Centre de Biologie et de Gestion des Populations, IRD (previously ORSTOM), Laboratoire de Nématologie, BP 5045, 34032 Montpellier, Cedex 1 France*
[7] *Research and Training Institute, Tumbi, Po Box 306, Tabora, Tanzania*
[8] *ORSTOM, BP 1386, Dakar, Senegal*
[9] *ORSTOM, Gadjan Mada University, Sekipk 3, Yogyakarta 5528, Indonesia*
[10] *LVP, BP 403, Bobo-Dioulasso, Burkina Faso*
[11] *INIAP, Boliche Experimental Station, Box 7069, Guayaquil, Ecuador*
[12] *NARF, Institute of Subtropical Plants and Olive Trees, Agrokipio, Chania, Crete*

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Summary – The conclusions of a collaborative study of the occurrence and importance of root-knot nematodes (RKN, *Meloidogyne* spp.) and of their control agent, *Pasteuria penetrans*, in parts of Europe, Africa, South America and the Caribbean are presented. Root-knot nematodes were estimated to reduce the yields of a wide range of horticultural crops by > 25% in Ecuador, Malawi and Tanzania, and by *ca* 10% in Trinidad and Tobago. The greatest proportion of infected crops were observed in Ecuador (205 of 207) and the least in Trinidad and Tobago (70 of 174). The mean gall index was greatest in Ecuador (5.5). Levels of galling were least in Senegal (1.6), even though 89% of crops were infested and virulent *M. mayaguensis* was widespread. In all countries, *M. incognita* and *M. javanica* were the most abundant species, but *M. hispanica* occurred widely in Burkina Faso, even in newly cultivated areas in the Sahile. Several new esterase phenotypes were detected, especially in Ecuador and Malawi. Juveniles (J2) collected from the soil during the surveys were examined for attached spores of *P. penetrans*. It was widespread (20 to 60% of RKN populations), except in Malawi and Tanzania (< 10% were infected), and was found for the first time in Crete (Greece). Generally, < 50% of the J2 carried spores. The occurrence of *P. penetrans* was sometimes correlated with soil type *e.g.*, in Senegal it was least frequent in sandy soils. Laboratory assays of the binding of spores of isolates of *P. penetrans* to populations of RKN indicated large variations in specificity and substantial interactions; differences between populations within a species of RKN were sometimes almost as great as those between species. In microplot trials in which an "exotic" isolate of *P. penetrans* was introduced (*ca* 10^3 spores per g soil), its incidence was not increased by increasing the frequency or intensity of the growing of RKN-susceptible crops. However, in two such trials at sites in Tanzania and Ecuador naturally infected with *P. penetrans*, there were large increases in the proportions of spore-encumbered J2 (up to 100% encumbered) and in the yields of spores (up to 3.3×10^6 spores per mg dry root) in those plots amended with an "exotic" isolate. In these plots, numbers of J2 in the soil were decreased and damage by RKN was suppressed; gall indices were decreased (from > 8 to < 3) and yields were increased (by up to 30%). No such changes were observed in the unamended control plots. Increased suppression of RKN was also

* Corresponding author, e-mail: dtrudg@scri.sari.ac.uk
† deceased

observed in a field trial, even in plots where RKN-susceptible and non-host crops were alternated. Increased suppression following amendment with the "exotic" isolate of *P. penetrans* was not observed at sites not previously infected with *P. penetrans*. Regression analysis of the results from the microplot and field trials indicated that tomato yields were decreased by > 5% for every increase of one in the gall index. Yields were increased by alternating tomato with leguminous crops in some trials, but not in others. It is proposed that, in natural infections, mutual selection produces a dynamic balance between the *P. penetrans* and the RKN whereby levels of infection are rarely suppressive. However, the introduction of an "exotic" isolate of *P. penetrans,* with a different attachment profile, can disturb this balance, resulting in a greatly increased proportion of infected J2 and females, increased yields of spores and more suppression of RKN populations.

Résumé – ***Importance des nématodes à galles tropicaux*** **(Meloidogyne *spp.*)** ***et facteurs affectant l'utilité de*** **Pasteuria penetrans, *agent de contrôle biologique*** – Ce travail présente les conclusions d'une étude, menée en collaboration par plusieurs équipes de recherche, sur la présence et l'importance des nématodes phytoparasites du genre *Meloidogyne* et de leur parasite bactérien, *Pasteuria penetrans*, dans certains pays d'Europe, d'Afrique, d'Amérique du Sud et des Caraïbes. Les réductions de rendement de cultures maraîchères très diverses dues à ces nématodes atteignent 25% en Equateur, Malawi et Tanzanie, et 10% à Trinidad et Tobago. La plus forte proportion de parcelles infestées a été rencontrée en Equateur (205 sur 207) et la plus faible à Trinidad et Tobago (70 sur 174). C'est en Equateur que l'indice de galles moyen (égal à 5,5) était le plus élevé. Même si cet indice était faible en moyenne au Sénégal (1,6), 89% des cultures étaient infestées dans ce pays, en grande partie par l'espèce virulente *M. mayaguensis*. *M. incognita* et *M. javanica* sont les plus répandues dans tous les pays prospectés. Cependant, *M. hispanica* est très répandu au Burkina Faso, même dans des zones récemment cultivées en maraîchage en région sahélienne. Plusieurs phénotypes estérasiques nouveaux ont été détectés, spécialement en Equateur et au Malawi. Les juvéniles de second stade (J2) extraits des échantillons de sol collectés lors des prospections ont été examinés pour détecter la présence de spores de *P. penetrans* sur leur cuticule. Trouvé pour la première fois en Crète (Grèce), *P. penetrans* est très répandu dans les autres pays prospectés, infestant 20 à 60% des populations de *Meloidogyne* spp., sauf au Malawi et en Tanzanie où moins de 10% des populations sont atteintes. Le plus souvent, moins de 50% de J2 portent des spores bactériennes. Le taux de parasitisme des J2 par *P. penetrans* est influencé par les types de sols, comme par exemple au Sénégal où il est très faible dans les sols sableux grossiers. Des expériences en laboratoire portant sur l'attachement de spores de divers isolats de *P. penetrans* à des populations de *Meloidogyne* spp. ont révélé une grande variation de la spécificité et des interactions; les différences observées pour diverses populations d'une même espèce de *Meloidogyne* sont parfois presque aussi marquées que celles observées entre espèces. L'incidence parasitaire d'un isolat "exotique" de *P. penetrans* introduit dans des microparcelles (ca. 10^3 sporespar g. de sol) n'a pas été accrue par l'augmentation de la fréquence ou de la densité de plantation des cultures sensibles à *Meloidogyne* spp. employées. Cependant, dans deux microparcelles natullement infestées en *P. penetrans*, situées en Equateur et en Tanzanie, l'introduction d'un isolat "exotique" s'est traduite par un accroissement de la proportion de J2 infestés (jusqu'à 100%) et de la production de spores ((jusqu'à 3, 3×10^6 spores par mg [poids sec] de racines), d'une diminution de la population de J2 dans le sol, et d'une disparition des dégâts racinaires; les indices de galles moyens ont diminué (de plus de 8 à moins de 3) et les rendements des cultures ont augmenté (jusqu'à 30%). De tels changements n'ont pas été observés dans des sols non amendés en isolats "exotiques" de *P. penetrans*. Un meilleur contrôle des populations de *Meloidogyne* spp. a également été observé au champ, même lorsque la séquence culturale alternait des cultures sensibles et des cultures non-hôtes du nématode. Dans des parcelles non infectées en *P. penetrans*, la baisse des populations de *Meloidogyne* spp. n'a pas été observée après introduction d'un isolat "exotique" de la bactérie. Des analyses de régression portant sur les données obtenues en microparcelles ou au champ montrent que les rendements en tomate diminuent de plus de 5% chaque fois que l'indice de galle augmente d'une unité. Ces rendements ont parfois été améliorés lorsque des cultures de légumineuses alternaient les cultures de tomate. Ainsi, sur la base des analyses nématologiques et agronomiques faites en fin de cycles culturaux, il est suggéré que, dans les cas d'infestations naturelles en *P. penetrans*, des sélections mutuelles entraîneraient un équilibre dynamique entre les populations de la bactérie et celles du nématode, représentatif d'une densité-dépendance retardée. En revanche, l'introduction inondative d'isolats "exotiques" de *P. penetrans*, aux propriétés parasitaires différentes de celles des populations natives, pourraient rompre temporairement cet équilibre en faveur d'un accroissement de la proportion de nématodes (J2 et femelles) infestés et de la production de spores bactériennes, ainsi qu'un meilleur contrôle des populations de *Meloidogyne* spp. La capacité des populations de *P. penetrans* à survivre dans les sols et à contrôler durablement les populations de *Meloidogyne* spp. dépendraient de la spécificité entre les organismes, des propriétés des sols et des systèmes de culture.

Keywords – damage, gall index, heterogeneity, specificity, suppression survey.

The research described here derives from a coordinated, 4-year, European Union-funded study involving 11 centres in Europe, Africa, South America and the Caribbean. Its purpose was to assess the occurrence and importance of species of root-knot nematodes (RKN; *Meloidogyne* spp.), and of their parasite *P. penetrans*, and to explore factors that influence the effectiveness of *P. penetrans* as a biocontrol agent of RKN. Selected experiments and re-

sults are presented. The full report is in the Library of the Scottish Crop Research Institute.

Meloidogyne incognita, *M. javanica* and *M. arenaria* are a closely related group of mitotically parthenogenetic species of root-knot nematodes (RKN) causing widespread damage, especially in developing countries (Sasser, 1979). Their wide host ranges (Jepson, 1987) make them difficult to control by rotation, and resistant cultivars are of variable value because of the occurrence of virulent races and species mixtures (Fargette & Braaksma, 1990; Roberts, 1992). They also have rapid rates of multiplication on good hosts, further increasing the difficulty of preventing crop damage as small populations increase and become damaging during one growing season (Ehwaeti *et al.*, 1998). Nematicides are used to control various species of RKN in developed agriculture but are mostly inappropriate for subsistence farmers in developing countries. Consequently, there is a great need to increase the control options for managing RKN and biological control has been an active area of research.

Chen and Dickson (1998) reviewed the biocontrol potential of a specific parasite of the *Meloidogyne* spp., the actinomycete-like *Pasteuria penetrans*. Its spores lie free in the soil and bind on contact to the migratory RKN juveniles (J2). Unless heavily encumbered with spores, most infected J2 are still able to invade host roots, establish feeding sites and develop into enlarged, adult females. *P. penetrans* proliferates within the developing nematode and, on its death, each infected female releases about 2 million spores of *P. penetrans*. These spores can withstand drying and are persistent (Giannakou *et al.*, 1997). Increased suppression of RKN and/or increased crop yields associated with *P. penetrans* have been reported by Mankau (1980), Brown *et al.* (1985), Bird and Brisbane (1988), Madulu *et al.* (1994) and Chen *et al.* (1996).

Pasteuria penetrans has been reported from most of the world, but its occurrence and abundance seem to be variable. This variability is thought to be due to several factors, including differences in the specificity of isolates of *P. penetrans* to populations and species of *Meloidogyne* (Stirling, 1985; Channer & Gowen, 1992; Davies & Danks, 1993). Soil and climatic conditions are thought to influence the occurrence of *P. penetrans* (Stirling & Wachtel, 1980; Stirling, 1981; Brown & Smart, 1985; Oostendorp *et al.*, 1990; Hatz & Dickson, 1992). Also, as RKN probably owe their current worldwide distributions to recent spread, some regions are likely to have been colonised by introductions of RKN free from *P. penetrans*.

The objectives of the research reported here were to *i)* conduct surveys to determine the occurrence and importance of species of RKN and of *P. penetrans* in relation to soil and environmental factors; *ii)* examine variations in the binding of *P. penetrans* to the species and populations of RKN; *iii)* conduct field and microplot trials to assess the damage caused by RKN and the utility of *P. penetrans* in the integrated management of RKN. This last objective tested the hypothesis that suppression could be increased by increasing the intensity of cropping with RKN susceptible crops, as has been observed for cereal cyst nematode, *Heterodera avenae* (Kerry & Crump, 1998).

The research is of particular value because it involved many different investigations, countries and centres, using similar experimental protocols. Consequently, RKN damage/gall indices relationships obtained in a field trial and national surveys to estimate the extent of root galling could be used to demonstrate widespread damage due to RKN. Differences in the effectiveness and specificity of isolates of *P. penetrans* and variations in the susceptibility of populations of RKN were also demonstrated. Most importantly, whilst suppression was not achieved at all sites, evidence for strong suppression of RKN by *P. penetrans* was obtained at two sites. It was shown that the continuous growing of susceptible hosts was not required and that *P. penetrans* could be integrated with the growing of poor and non-hosts for the RKN.

Materials and methods

Surveys and soil factors

Structured surveys were made over 3 years (1993-1996) in Burkina Faso, Crete, Ecuador, Malawi, Senegal, and Trinidad and Tobago. It was also planned to survey parts of Indonesia, but this was prevented by the illness and subsequent death of one of the participants (C. Netscher). The aims were to characterise the occurrence and importance of species of *Meloidogyne* and of *P. penetrans* in field-grown vegetable crops. In some of these surveys, soil physico-chemical characteristics were analysed and correlated with the occurrence of *P. penetrans* to compare its distribution amongst the countries and to understand the influence of environmental factors on its efficiency as a biocontrol agent.

Field selection for surveys

In the first year, each laboratory sampled local fields, in the second year they sampled a similar number of

fields in their region, and in the third they sampled the rest of the country. Fields were selected at random within known areas of vegetable production. A wide range of crops was surveyed (Table 1). Sampling varied according to the size of each country and the accessibility of the vegetable producing areas, but the distribution of the fields sampled was heterogeneous and generally included the main horticultural areas (Table 1).

Sampling methods

Crops approaching maturity were selected and at least ten sub-samples (soil cores, 10-20 cm deep in the rhizosphere) were taken across each field along an M-shaped path. During soil sampling, ten plants were up-rooted, the degree of galling recorded on a 1 to 10 scale (Bridge & Page, 1980) and soil and root samples taken.

Nematode extraction from the soil

In Burkina Faso and Senegal, *Meloidogyne* second stage juveniles (J2) were extracted by elutriation (Seinhorst, 1962). In Ecuador, Malawi and Tanzania, J2 were extracted using the Baermann tray technique (Southey, 1986). In Crete and Trinidad, the J2 were extracted by decanting and sieving (Barker, 1985). In all laboratories, the J2 were counted under an inverted microscope and the numbers determined per ml of soil. The first 20 J2 encountered were examined for spores of *P. penetrans*.

A susceptible tomato plant was planted in a mixed subsample of soil from each field to obtain females and egg-masses for subsequent species identification.

Nematode extraction from the roots

In Burkina Faso and Senegal, nematodes were extracted from the roots using a mist chamber (Seinhorst, 1950). Elsewhere, roots (5 g) were chopped into short segments and placed on a Baermann funnel for at least 7 days. The numbers of J2 extracted per g root and the level of spore encumberment were determined. If *P. penetrans* was detected the remaining root samples were dried, powdered and a sample sent to Dr K.G. Davies (IACR Rothamsted).

Identification of Meloidogyne *species*

Species were identified by perineal pattern and esterase phenotype (Esbenshade & Triantaphyllou, 1985). At least ten single females per population were examined/tested.

Soil analysis

Soil samples were sent to Senegal where the texture of soils with *P. penetrans* was analysed (Table 2). The proportions of clay (0-2 μm), fine silt (2-20 μm), coarse silt (20-50 μm), fine sand (50-200 μm), coarse sand (200-2000 μm) and organic matter were determined, and the pH, concentrations of phosphorus, exchanged calcium, magnesium, sodium and potassium, the exchange capacity, the wilting point 4 (pF = 4.2 — the permanent wilting point, expressed as % moisture) and the conductivity (increases with increasing salinity) were measured.

PASTEURIA PENETRANS/MELOIDOGYNE INTERACTIONS

Specificity of spore binding

The ability of spores of *P. penetrans* to adhere to J2 of species of RKN was assessed using a standard method. Suspensions of spores were prepared by grinding 0.1 g of dried tomato roots, containing *P. penetrans*, in tap water (50 ml) with a pestle and mortar to release the spores. Samples (0.25 ml) of the spore suspension were then placed in a 24-well tissue culture dish and freshly hatched (within 2 days) J2 (*ca* 100 in 0.25 ml) were added. After incubation overnight the nematodes were removed, 20 to 40 J2 mounted between two coverslips and the numbers of spores attached determined. Three separate studies were made.

i) The compatibility between a single population of *P. penetrans* (PP1, from Australia) and some of the field populations of RKN isolated during the surveys was assessed in each laboratory. Population PP1 was chosen for its previously observed ease of culture and relatively high level of attachment. Tomato roots containing PP1 infecting *M. incognita* race 2 were prepared at IACR-Rothamsted using the method of Stirling and Wachtel (1980). Dry root powder was dispatched to each of the participating laboratories for them to undertake standardised attachment tests.

ii) Two single egg-mass lines, one of *M. incognita* and one of *M. arenaria*, known to have different responses to both *P. penetrans* attachment and to antibody labelling (Davies & Danks, 1993), were tested for their compatibility to 24 populations of *P. penetrans* (15 from Burkina Faso, four from Ecuador, and one each from Trinidad, Senegal, Malawi and Tanzania). PP1 was used as a control. The level of spore attachment to 20 J2 of each *P. penetrans* population was assessed.

iii) Six populations of *P. penetrans* from around the world (including PP1) were tested against 39 single egg-mass lines of RKN derived from North and South America, the Caribbean, Europe, Africa, Asia and Australia, including some collected during the present surveys. Some lines were tested more than once, and some lines were derived from the same population. The binding of spores to

Table 1. *Numbers of sites and samples taken from crops in regions of seven countries for* Meloidogyne *spp. and* Pasteuria penetrans.

Countries Regions	Sites	Samples	Crops (No samples)			
Burkina Faso						
South Sudanese region	12	78	tomato	(63)	eggplant	(67)
Central Sudanese region	10	30				
Sahelian region	10	22				
Total		130				
Crete						
Chania	1	3	tomato	(19)	melon	(2)
Falasarma	1	3	pepper	(1)	cucumber	(1)
Koudoura	1	8				
Plakias	1	2				
Tibaki	1	7				
Total		22				
Ecuador						
Coast region	62	104	tomato	(166)	green pepper	(10)
Highland region	36	70	melon	(10)	watermelon	(5)
Oriental Amazonia	12	20	cabbage	(4)	lettuce	(4)
Galapagos	1	13	cucumber	(3)	pea	(3)
Total		207	other	(2)		
Malawi						
Blantyre	35	37	tomato	(99)		
Karonga	4	8				
Kasungu	2	8				
Lilongwe	11	12				
Machinga	2	2				
Mzuzu	14	14				
Salima	16	16				
Shire Valley	2	2				
Total		99				
Senegal						
Cap Vert	5	100	tomato	(61)	cabbage	(13)
Niayes	5	24	okra	(19)	African eggplant	(17)
Senegal valley	8	20	sweet potato	(13)	potato	(15)
Sereer region	8	56	water melon	(11)	other	(28)
Total		200				
Tanzania						
Tumbi	33	33	tomato, cucumber, sesbania, eggplant,			
Tabora	33	33	okra, cabbage, onion, sweet potato,			
Tanzania	23	23	tobacco, amaranthus (89)			
Total		89				
Trinidad & Tobago						
St George	15	42	celery	(23)	pumpkin	(3)
St Andrew	2	2	okra	(14)	sweet pepper	(3)
Caroni	4	13	lettuce	(13)	other	(11)
Nariva	1	3	cucumber	(10)		
Mayaro	1	5	tomato	(9)		
Victoria	2	1	bodi	(4)		
St Patrick	1	2	melongue	(6)		
Tobago	4	5	shadow beni	(4)		
Total		73				

20 J2 was assessed for each combination. The RKN lines comprised ten of *M. incognita* including one population each of race 1, 3 and 4, 11 of *M. javanica,* seven of *M. arenaria,* three each of *M. hispanica* and *M. mayaguensis,* one of *M. hapla* and four of unidentified esterase phenotypes.

MICROPLOT AND FIELD TRIALS

Microplot trials were designed to test the hypothesis that suppressiveness due to *P. penetrans* could be increased by intensifying the growing of crops susceptible to RKN. The field trials sought to determine whether *P. penetrans* could be integrated with resistant/non-host, leguminous crops. Susceptible tomato was grown periodically in all plots to facilitate comparisons between treatments. Crop growth and yields, and gall indices were determined at harvest. Soil samples were taken, J2 extracted and groups of at least 20 examined to determine the proportion encumbered with spores of *P. penetrans.* The harvested roots were dried and numbers of spores of *P. penetrans* determined. The remainder of the dried roots were re-incorporated to ensure as many *P. penetrans* endospores as possible were returned to the soil.

Microplot trials

Microplot experiments were completed in Ecuador, Trinidad and Tanzania. A trial was initiated in Senegal in concrete microplots using a sandy soil naturally infested with *M. incognita* (not infected with *P. penetrans*). Some of the plots were inoculated at planting with root powder containing *P. penetrans* as described below for Ecuador. However, although some J2 infection was observed initially, this rapidly decreased and the trial was abandoned. The trials that were completed are described below. Treatments differed slightly because of the local needs, preferences and availability or suitability of crops.

Ecuador. The trial was on clay loam soil naturally infested with *M. incognita* and a low level of *P. penetrans.* Plots, 1 m^2 and separated by paths, grew an initial, non-experimental planting of 12 *M. incognita*-infected tomato plants/plot for 8 weeks. This produced a heavy infestation index (8-10; Bridge and Page, 1980) of *M. incognita.* The plots were then dug to 15 cm deep and tomato root powder containing spores of *P. penetrans* incorporated into half of the plots. The *P. penetrans* came from a population of *M. incognita,* from Fumisa in Los Rios Province, being multiplied on tomato in the glasshouse at Boliche Research Station (Trivino, 1996). The rates of application were 4.4×10^7 (normal cropping density) and 8.8×10^7 (high cropping density) spores per m^2, respectively. The plots were planted in four of the cropping cycles (cycles 1, 2, 4, and 5) with susceptible bean (*Phaseolus vulgaris* cv. INIAP 272), at densities of 12 and 20 (4×3 and 4×5) plants per plot for normal and high densities, respectively. For cycles 3 and 6, susceptible tomato was planted at six and 12 plants per plot for normal and high densities, respectively. There were six replicates. The trial lasted 2 years in which there were six crops, each of *ca* 3 months duration.

Trinidad. Plots were 1 m^2 on a clay (56%) soil not naturally infested with RKN. Soil from an adjacent field, naturally infested with *M. incognita* infected with *P. penetrans,* was added to all plots as 4 dm^3 soil per plot. The plots were then planted with lettuce to increase the RKN populations. Tomato crops were four and nine plants per plot for normal and high densities, respectively. The plots cropped normally grew six crops (four of susceptible tomato, two of celery) over 3 years. Plots with a high frequency of planting grew extra tomato crops in June 1994 and May 1995, giving a total of eight crops (six of tomato, two of celery). Each tomato crop was grown for only 2 or 3 months — consequently full fruit yields were not available. Instead, top weights and fruit numbers were recorded. There were six replicates.

Tanzania. Six blocks of 2×2 m were established on a sandy loam soil in a field previously cultivated with vegetables and naturally infested with *M. javanica* and a low level of *P. penetrans.* All blocks were initially planted with susceptible tomato (cv. Moneymaker) to increase RKN populations. Each 4 m^2 block was then subdivided into four 1 m^2 plots. Twelve plots were inoculated with spores of *P. penetrans,* at an estimated rate of 4.4×10^7 spores per plot incorporated into the top 15 cm of soil. The *P. penetrans* was a mixture combining PP1 provided by Dr K. Davies (IACR Rothamsted) and a local population from Tabora. The plots were planted with tomato at densities of four and eight plants/m^2 for normal and increased densities, respectively. The trial lasted 3 years and plots either grew five successive crops of susceptible tomato (cv. Moneymaker) or resistant tomato (cv. Rossol), each with six replicates.

Field trials

Rotational field trials with five or more cropping cycles were established in Ecuador, Trinidad, Senegal, Tanzania and Crete. Where possible, each trial was on a site naturally infested with RKN infected with *P. penetrans.* The trials compared rotations involving good and poor or non-hosts, including legumes. The crops and cultivars varied

with country but, to enable cross-comparisons, susceptible tomato was grown in all plots for the third and fifth cropping cycles.

Ecuador. The trial was adjacent to the microplot trial and the site was also infested with *M. incognita* infected with *P. penetrans*. Six blocks were established, each with four plots (2.2 × 2.2 m). Local infestation was enhanced by planting with tomato plants (28 per plot) previously infected with the local population of *M. incognita*. All plots received spores of *P. penetrans* (4.4×10^7 per m^2) as for the microplots. There were four rotational treatments, each with six replicates and five cropping cycles. In three of the rotations susceptible tomato cv. Walter (crops 1, 3, 5) was alternated (crops 2, 4) with *i)* susceptible *Phaseolus* beans cv. INIAP 472; *ii)* non-host peanut cv. INIAP Boliche; or *iii)* poor host maize cv. INIAP 526. In rotation *iv*, the first tomato crop was replaced by a fallow; thereafter susceptible bean was alternated with susceptible tomato as for rotation *i*.

Trinidad. The trial was on land known to suffer from damage by *M. incognita* but free of *P. penetrans*. Initially, the experimental area was cropped with cucumber to increase the infestation of *M. incognita*. Spores of *P. penetrans* (the same population as for the microplot trial) were applied (approximately 1.1×10^7 per m^2) to all plots (2 × 2 m) before planting tomato in crop cycles 1 and 3. There were four rotations, each with five replicates and five cropping cycles. These were susceptible tomato (crop cycles 1, 3, 5; 16 plants per plot) alternated in cycles 2 and 4 with *i)* poor host maize; *ii)* susceptible beans (cv. Contender) and *iii)* a resistant legume (bodi; *Vigna unguiculata*). Rotation *iv* comprised continuous susceptible tomato for all five cropping cycles.

Senegal. Plots, 4 × 5 m containing 80 tomato plants in the first cycle, were established on a sandy clay soil. The site was infested with *M. javanica* heavily infected with *P. penetrans*. Results are presented for three rotations over seven cropping cycles, each with six replicates in a completely randomised block design. In all plots, susceptible tomato was cropped in cycles 1, 3, 4, 6, and 7. In cropping cycles 2 and 5 either *i)* susceptible *Vigna* beans, *ii)* non-host peanut, or *iii)* poor host millet were grown.

Tanzania. Six plots 7 × 3 m initially grew susceptible tomato cv. Moneymaker to establish a uniformly high population of *M. javanica* with a low level infection of *P. penetrans*. For the second crop cycle, each plot was divided into three 7 × 1 m plots and grew susceptible carrot, cucumber or tomato. For the third and subsequent crops, the plots were further sub-divided to provide 12 treatments, each with six replicates. In the third and fourth cropping cycles, carrot, cucumber or tomato were each followed by two crops of peanut (a non-host), resistant tomato, susceptible tomato or susceptible bean. The entire trial was then planted with susceptible tomato for the fifth and sixth cropping cycles.

Crete. The experiment was in a polythene tunnel infested with *M. javanica* and a small proportion of *M. incognita*, in a sandy loam soil free of *P. penetrans*. Spores of *P. penetrans* (a mixture of three populations from Australia and S. Africa; see Channer and Gowen, 1992) were applied (1.8×10^8 spores per m^2) as root powder to individual planting sites. There were four rotational treatments, each with five replicates and five cropping cycles. Plots were 2.1 × 1.4 m and in cropping cycles 1, 3 and 5 grew two rows of six susceptible tomato plants alternated in cycles 2 and 4 with susceptible bean, resistant bean or pepper (a host for *M. incognita*, but not for *M. javanica*). Two rotations grew resistant bean, but one was fallowed for the first cropping cycle.

Results

SURVEYS AND SOIL FACTORS

Distribution of Meloidogyne *spp. and* P. penetrans

The occurrences of RKN and of *P. penetrans* are summarised in Table 2. RKN were found in almost all fields sampled in Ecuador, Malawi and Tanzania and mean gall

Table 2. *Numbers of survey samples infected with root-knot nematodes (RKN), mean gall indices, and of second stage juveniles (J2) with spores of* Pasteuria penetrans.

Country	Samples	With RKN	Mean gall index	With *P. penetrans*	% of J2 encumbered
Crete	22**	22**	3.8	11*	14
Burkina Faso	130	69	2.4	44*	52
Ecuador	207	205	5.5	67*	45
Malawi	99	90	5.3	9	16
Senegal	200	178	1.6	53*	8
Tanzania	89	89	5.7	5	11
Trinidad & Tobago	174	70	4.9	15*	8

* Physico-chemical properties analysed.
** Selected for presence of RKN; most vegetable production areas treated with methyl bromide.

Table 3. *Occurrence of* Meloidogyne *spp. in surveys of seven countries (% of sites), including species mixtures so results may total > 100%.*

Country	*M. incognita*	*M. javanica*	*M. arenaria*	*M. mayaguensis*	*M. hispanica*	Unidentified
Crete	74	26	0	0	0	0
Burkina Faso	63	23	0	1	13	4†
Ecuador	89	26	2	0	0	3††
Malawi	61	29	0	1	2	15†††
Senegal	29	71	7	31	0	0
Tanzania	0	100††††	0	0	0	0
Trinidad & Tobago	93	13	3	11	0	0

† Possibly *M. chitwoodi*.
†† Includes *M. hapla*.
††† Five unidentified esterase phenotypes.
†††† Only Tabora region. Species in other areas not identified.

indices ranged from 5.3 to 5.7, indicating widespread, substantial damage (see Fig. 3 for relationship between gall index and yield). In Trinidad and Tobago, RKN were less frequent, but the mean gall index (4.9) indicates substantial damage to infested crops. In Senegal and Burkina Faso, although RKN were still widespread, the mean gall indices were lower. The values for Crete represent targeted sampling of polythene tunnels not treated with methyl bromide.

The most frequently occurring species was *M. incognita*. *M. javanica* was the second most widespread species and was most frequent in Senegal and Tanzania (Tabora region) (Table 3). *M. mayaguensis* was more abundant than *M. incognita* in Senegal, and was also found in Burkina Faso, Malawi, and Trinidad. Several new esterase phenotypes were identified and, whilst some may be variants of described species, others may represent undescribed species.

P. penetrans was comparatively rare in the two East African countries, but 64% of RKN populations were infected in Burkina Faso, *ca* 30% in Ecuador and Senegal and 22% in Trinidad and Tobago (Table 2). The distribution of *P. penetrans* in Ecuador varied, with only 9% of RKN populations from the Highland region being infected, compared with 52% from the coastal region. In Senegal, *P. penetrans* was more frequent in the Sereer region than in the Niayes region. In Crete, *P. penetrans* was found in *ca* 25% of polythene tunnels. Although surveys were not completed in Indonesia, *P. penetrans* appeared to be rare.

The proportion of infected juveniles and levels of spore encumberment were generally low in all countries (Table 2), but in a few fields in Ecuador > 80% of juveniles were infected with means of up to nine spores per juvenile. More generally in Ecuador < 60% of juveniles were encumbered with means of 0.5-3.5 spores per juvenile. In Burkina Faso, *P. penetrans* was found associated with RKN in newly irrigated areas, which were recently desert. Overall, few juveniles in any country carried more than ten spores.

Soil factors

Although the soils in each country were heterogeneous, a comparison of the mean soil data for each country (Table 4) shows that, generally, Ecuador has the heaviest soils, with high proportions of clay, whereas Senegal has sandy soils and those in Burkina Faso and Trinidad are intermediate. Soils in Crete have high proportions of coarse sand and clay with fewer intermediate particles. In Malawi, almost 50% of the fields surveyed are sandy loams, 40% sandy clays or sandy clay loams, 5% clays, and the remainder are sands (Daudi, unpubl.). Results were not available for Tanzania.

The N-P-K status of soils was influenced by the mineral and organic amendments applied, but it is apparent that the soils from Ecuador were fertile compared with those from Senegal which were very poor (0.5% organic carbon). The exchange capacity is consistently higher in Ecuador than in Burkina Faso, Trinidad, Crete and especially Senegal. All the pH values are similar and below 7, except in Crete.

Principle component analysis indicates that, in fields in Senegal with *P. penetrans*-infected RKN populations, as the proportion of fine particles (clays and fine silts) in-

Table 4. *Mean physico-chemical characteristics of soils with* Pasteuria penetrans *and* Meloidogyne *infestation in five countries.*

Characteristics	Ecuador	Burkina Faso	Trinidad	Crete	Senegal
Texture (%)					
Clays	30.2	18.4	18.4	18.9	4.6
Fine silts	24.9	14.1	18.5	13.0	1.5
Coarse silts	11.8	16.0	18.9	5.4	3.3
Fine sands	18.8	27.3	29.9	16.1	49.7
Coarse sands	11.3	23.2	12.9	46.2	41.5
Minerals (meq%)					
Ca	19.9	7.03	6.96	19.57	3.1
Mg	6.05	2.73	1.28	2.35	0.68
Na	0.77	0.09	0.15	0.98	0.34
K	1.45	0.5	0.65	1.11	0.14
Cl	0.24	0.09	0.15	0.72	na
SO_4	0.65	0.2	0.41	2.25	na
HCO_3	0.39	0.19	0.27	0.7	na
Al	0.53	0.38	0.49	0.34	na
P(‰)	139.7	117.4	360.5	571.5	186.1
Organic matter (%)					
C	2.1	1.1	1.3	1.8	0.5
N	0.21	0.11	0.15	0.18	0.07
pH	6.7	6.4	6.6	7.3	6.3
Exchange capacity (meq%)	25.3	8.3	9.2	13.1	3.4
Conductivity (mS)	0.39	0.33	0.3	0.8	0.22
WP4 (%)	16.6	7.7	7.4	6.4	2.2
Meloidogyne J2/dm^3	23000	15000	3500	4100	2500
% J2 with *P. penetrans*	42	24	15	14	5

na = not analysed.

creased, so did the proportion of spore-encumbered J2. However, in Ecuador, the proportion of J2 with *P. penetrans* was independent of the soil factors analysed or of the *Meloidogyne* population density, whereas in Burkina Faso the abundance decreased as the clay and organic matter content increased (results not shown).

Pasteuria/*Meloidogyne* interactions

Specificity of spore binding

i) The binding of *P. penetrans* isolate PP1 ranged from none to > 40% of J2 encumbered with populations of RKN from Burkina Faso, Senegal and Trinidad and Tobago, (Table 5). PP1 bound uniformly well to all populations from Malawi (> 40% encumbered) and, in varying degrees, to all populations of RKN from Ecuador and Greece. However, it did not adhere to 64% of populations from Senegal, and to only a minority of populations from Burkina Faso and Trinidad and Tobago (Table 5).

Where the *Meloidogyne* populations tested were identified as a single species, differences in levels of PP1 spore attachment were observed within species (results summarised below). In Ecuador, populations of *M. incognita* had levels of spore attachment which ranged from > 90% to < 10% of the J2 with spores. In Burkina Faso, some pure populations of both *M. incognita* and *M. javanica* had > 80% of J2 encumbered with spores, others had < 15% with spores, and two populations identified as mixtures of both species had no J2 invested with spores. In Malawi and Trinidad and Tobago, populations of both species of *Meloidogyne* were observed with 100% of the J2 invested with spores, whereas in Senegal most populations of both species had few (< 10%) or no J2 encumbered with spores.

Table 5. *Percentage of root-knot nematode populations encumbered by spores of* Pasteuria penetrans *PP1 in laboratory assays in six different countries.*

% J2 encumbered	Burkina Faso	Ecuador	Crete	Malawi	Senegal	Trinidad & Tobago
> 40%	21	44	0	100	5	7
< 40%	61	56	100	0	31	86
0	18	0	0	0	64	7
Number of populations	64	64	15	9	42	13

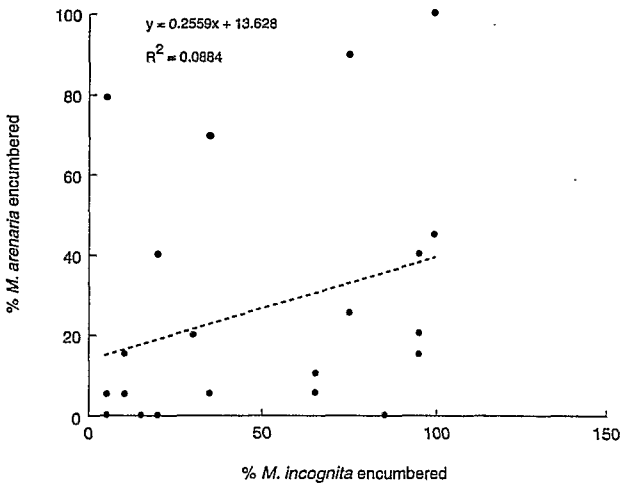

Fig. 1. *Percentage of juveniles of* Meloidogyne arenaria *encumbered with spores of 23 isolates of* Pasteuria penetrans *regressed against the percentages of juveniles of* M. incognita *that were encumbered.*

ii) The assay comparing the binding of 23 isolates of *P. penetrans* to one population of *M. arenaria* and one of *M. incognita* indicated big differences in specificity. All isolates bound to the *M. incognita*, but only two isolates encumbered all of the J2 examined whereas three isolates encumbered only one of 20 J2. Five isolates did not encumber any J2 of *M. arenaria*, and only two isolates bound more to *M. arenaria* than to *M. incognita*. Regressing the percentages of J2 of *M. arenaria* that were encumbered by each isolate of *P. penetrans* against those for *M. incognita* showed that there was no correlation (Fig. 1); a linear regression accounted for only 9% of the variation.

iii) The test involving 39 lines of RKN and six populations of *P. penetrans* also indicated significant ($P \leqslant 0.001$) differences between populations of *P. penetrans*: population Aus92 attached to only 36% of the RKN lines compared with 72% with Sen92 (Table 6). There were also similar significant differences between lines of RKN; none of the *P. penetrans* populations attached to six lines of RKN (races 3 and 4 of *M. incognita*, a line of *M. incognita* from North Carolina, and one each of *M. arenaria*, *M. javanica* and *M. mayaguensis*). Three further lines, two of them *M. arenaria*, were encumbered only by population PPI. In contrast, all populations of *P. penetrans*, except Aus92, heavily encumbered (> 60% of J2) the same 11 lines of RKN, including *M. incognita*, *M. hispanica*, *M. javanica* and two lines with unidentified esterase phenotypes. There were significant interactions ($P \leqslant 0.001$) between the remaining 19 lines of RKN (including one of *M. hapla*) and the populations of *P. penetrans* both in proportions of J2 encumbered and mean rates of spore attachment. Two lines derived from the same population of *M. hispanica* from Burkina Faso were similarly encumbered by the six populations of *P. penetrans*. Similarly, there were only small differences between two lines from a population of *M. javanica* from Burkina Faso, but two lines from a population of *M. incognita* from Guyana differed considerably in that five of the populations of *P. penetrans* bound well to one line (> 90% J2 encumbered) but only three populations bound (two only poorly, < 40%) to the second.

Microplot and field trials

Microplot trials

Numbers of J2 in soil, incidence of *P. penetrans* infection, intensity of root galling, tomato yields and numbers spores in roots at harvest are presented, where available, for the three microplot and five field trials.

Ecuador

Numbers of J2 in the soil. At the end of the first cropping cycle, J2 population densities were similar for all treatments, ranging from means of nine to 15 per cm^2 of soil. After the fifth and sixth cropping cycles, in those

Table 6. *Encumberment of 39 lines (35 only for PP1) of* Meloidogyne *spp. (RKN) exposed to six isolates of* Pasteuria penetrans*; the numbers of lines of each species with none, <50% or >50% J2 encumbered are given.*

	Populations of *P. penetrans* No lines of RKN					
	Aus 92	PP1	Brazil	Thies	NC	Sen 92
Not encumbered						
M. incognita	7	4	4	5	3	3
M. javanica	8	2	2	2	2	2
M. arenaria	5	1	5	3	3	3
M. mayaguensis	3	2	2	1	2	2
M. hispanica	1	0	0	0	0	0
Other	1	0	1	2	1	1
Total	25	9	14	13	11	11
<50% J2 encumbered						
M. incognita	3	2	2	1	3	1
M. javanica	3	3	2	3	1	1
M. arenaria	2	5	1	2	3	3
M. mayaguensis	0	1	1	2	1	1
M. hispanica	2	0	0	0	0	0
Other	3	3	2	1	2	1
Total	13	14	8	9	10	7
>50% J2 encumbered						
M. incognita	0	2	4	4	4	6
M. javanica	0	5	7	6	8	8
M. arenaria	0	0	1	2	1	1
M. mayaguensis	0	0	0	0	0	0
M. hispanica	0	3	3	3	3	3
Other	1	2	2	2	2	3
Total	1	12	17	17	18	21

plots to which *P. penetrans* had not been added the densities of J2 were similar to those at the end of the first cycle. In contrast, in the plots amended with the exotic isolate of *P. penetrans*, the numbers of J2 had decreased to less than four per cm^2, significantly less ($P \leqslant 0.001$) than at the end of the first cropping cycle. Plant density had no effect.

P. penetrans *incidence*. At the start of the trial, *ca* 20% of juveniles in all plots carried spores of *P. penetrans* (Fig. 2). In those plots not amended with the exotic isolate of *P. penetrans*, this level of encumberment remained almost constant throughout the six cropping cycles. However, in the plots amended with the exotic isolate, the levels of encumberment were *ca* 60% at the end of the first cropping cycle and progressively increased (Fig. 2) so that in the final three cropping cycles > 90% of the juveniles were spore encumbered, more ($P \leqslant 0.001$) than in the unamended plots. Only in the amended plots were there J2 with more than ten spores per juvenile. Increasing plant densities and amounts of *P. penetrans* added had no additional effect.

Gall index. In those plots not amended with *P. penetrans*, mean gall indices in the fourth to sixth cycles remained high (> 8). In those plots to which it was added, gall indices were decreased ($P \leqslant 0.001$) to 5.3 or less. Gall indices tended to be slightly higher in the plots planted to a high crop density than in the low density plots (Fig. 2).

Yields. Amendment with *P. penetrans* generally led to an increased yield of beans and tomatoes over the six crops (Table 7). In the sixth and final crop cycle, tomato yields were 30% greater in plots amended with the exotic isolate of *P. penetrans* than in non-amended plots. Mean final tomato yield was greatest in the *P. penetrans*-amended, high plant density plots which yielded

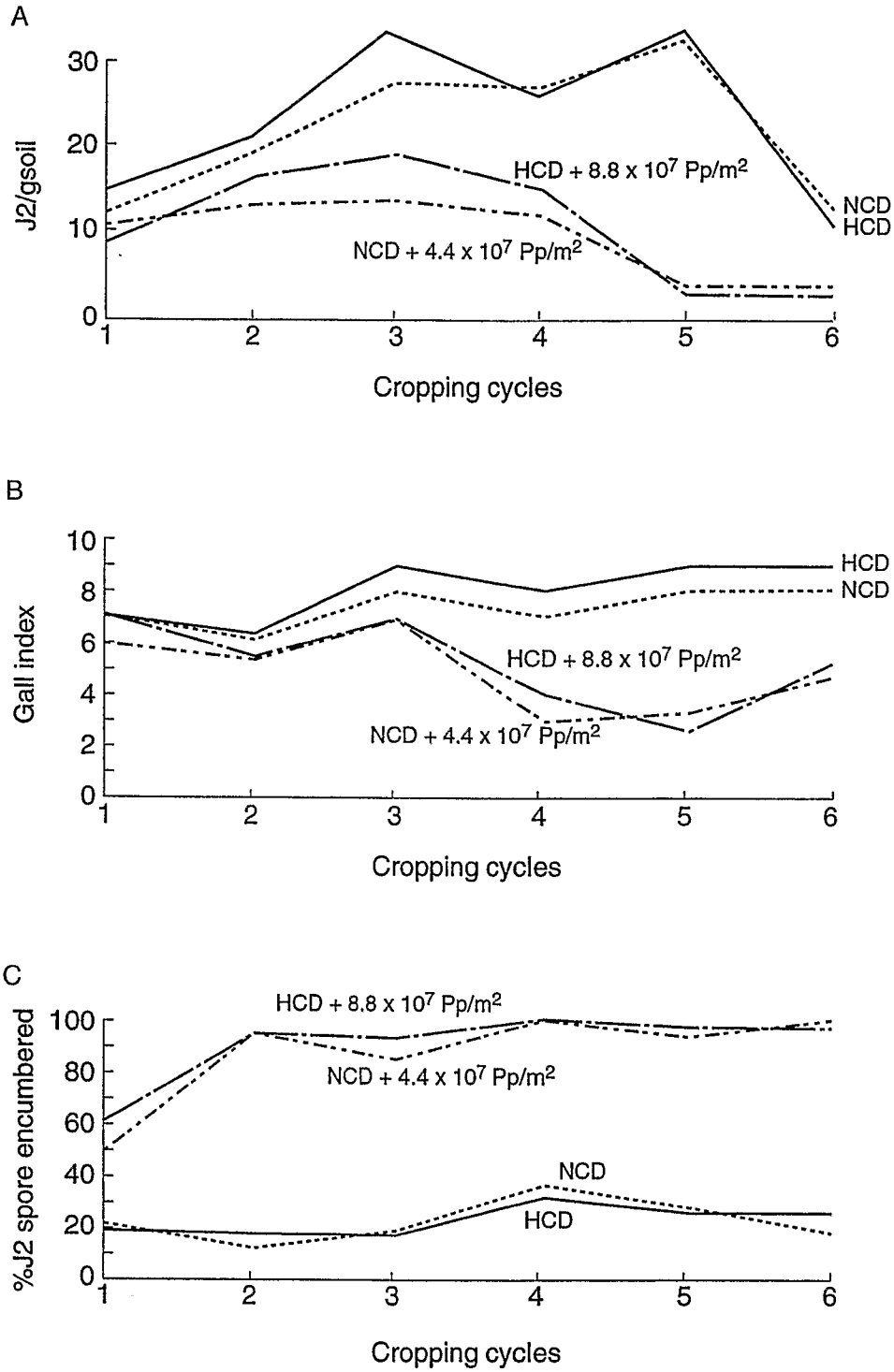

Fig. 2. *Changes over six cropping cycles in the microplot trial in Ecuador in A: numbers of J2 per g soil; B: gall indices; and C: percentages of juveniles in the soil encumbered with spores of* Pasteuria penetrans (Pp). *Results are presented for normal (NCD) and increased frequency of cropping (HCD) without and with the addition of the exotic isolate of* Pasteuria penetrans *(+4.4 or 8.8 × 10^7 spores per m^2, respectively).*

Table 7. *The effect of* Pasteuria penetrans *added at two spore densities and two densities of cropping on the yields of bean and tomato during six cropping cycles in the microplot trial in Ecuador.*

Treatments			Cropping cycles & Rotational crops Yields (kg/plot)					
Cropping densities	*Pasteuria* (spores/g soil)		1. Bean	2. Bean	3. Tomato	4. Bean	5. Bean	6. Tomato
Normal	–		0.29	0.35	0.30	0.35	0.54	4.80
High	–		0.38	0.49	0.15	0.42	0.51	4.15
Normal	1000		0.28	0.46	0.27	0.48	0.58	5.81
High	2000		0.44	0.57	0.88	0.59	0.53	5.88
Cropping density		SED	0.05	0.03	0.43	0.04	0.04	0.57
P. penetrans		SED	0.05	0.03	0.43	0.04	0.04	0.57
Interactions		SED	0.06	0.05	0.60	0.05	0.50	0.81

Table 8. *Mean numbers ($\times 10^4$) of* Pasteuria penetrans (Pp) *spores per mg dried root powder in the microplot trial in Ecuador.*

Treatments			Cropping cycles & Rotational crops *Pp*/mg dry root powder $\times 10^4$					
Cropping densities	*Penetrans* (spores/g soil)		1. Bean	2. Bean	3. Tomato	4. Bean	5. Bean	6. Tomato
Normal	–		1.0	4.0	2.0	8.0	9.3	5.8
			(1.00)	(2.00)	(1.41)	(2.28)	(3.04)	(2.04)
High	–		0.8	2.3	2.3	7.3	8.5	4.1
			(0.92)	(1.51)	(1.52)	(2.70)	(2.91)	(2.02)
Normal	1000		36	126	137	286	30.8	29.3
			(6.00)	(11.22)	(11.68)	(16.91)	(5.54)	(5.41)
High	2000		50	164	176	331	46	38.8
			(7.07)	(12.80)	(13.24)	(18.19)	(6.80)	(6.22)
Cropping density		SED	0.15	0.63	0.46	0.50	0.17	0.25
P. penetrans		SED	0.15	0.63	0.46	0.50	0.17	0.25
Interactions		SED	0.22	0.89	0.65	0.71	0.17	0.36

Numbers in parentheses are square root transformed *Pasteuria* spore production, SED apply to these means.

42% more than the equivalent treatment without *P. penetrans*.

A linear regression of the relation at the end of the final cropping cycle between gall indices and tomato yields produced a significant ($P = 0.018$, $r^2 = 0.96$) negative correlation, with yield decreased by 5% for every unit increase in the gall index.

Spores in roots. The numbers of spores per mg dry root, estimated for each crop before incorporating the roots into the plots (Table 8), indicated an immediate and large effect of the amendment with root powder containing *P. penetrans*. Numbers of spores in the third cycle were 76-fold greater for amended than unamended plots. The harvested dry tomato roots from amended plots in the fourth cropping cycle weighed a mean of 4.45 g compared with 4.25 g for the unamended plots. The maximum spore yield (for the amended plants growing at high density) was 7.4×10^9 spores per plant, equivalent to 1.1×10^{12} spores per m^2 or *ca* 4.4×10^6 spores per g soil.

Trinidad

This trial only examined the effect of the different cropping density/frequency because the site was not naturally infested and all plots were amended with *M. incognita* infected with *P. penetrans* from an adjacent field.

P. penetrans *incidence.* The proportion of J2 encumbered with spores increased from a mean of 5.2% in 1993 to 11.9% in 1994 and to 13.4% in 1995. However, in 1996

Table 9. *Gall index, fresh top weight and fruit number for dry season tomato crops in the microplot trial in Trinidad.*

Cropping		1993	1994	1995	1996
Density	Frequency				
		Gall indices			
Normal	Normal	1.0	6.0	5.8	0.3
Increased	Increased	0.2	6.0	5.5	0.2
Increased	Normal	0.5	7.1	6.0	0.5
Increased	Increased	0.8	7.0	4.9	0.2
LSD (for years $P \leqslant 0.05$)		0.8			
		Top weight (g/plant)			
Normal	Normal	83	234	205	570
Normal	Increased	93	258	208	555
Increased	Normal	110	258	228	614
Increased	Increased	88	244	188	573
LSD (for seasons only $P \leqslant 0.05$)		60			
		Fruit number per plant			
Normal	Normal	17.5	2.3	18.0	36.0
Normal	Increased	17.7	29.5	18.7	30.2
Increased	Normal	22.5	26.8	18.4	2.4
Increased	Increased	18.3	27.1	17.1	30.0
LSD (for seasons only $P \leqslant 0.05$)		6.0			

it declined to 4.9%. Differences in cropping density and frequency did not have a consistent effect.

Gall index. There were no significant effects of cropping treatments on root gall indices, but there were large differences between years. In the first cropping year (1993), mean gall indices were < 1, in 1994 and 1995 they had greatly increased and ranged from 4.85 to 7.1, but in 1996 they were again < 1 (Table 9).

Yields. Top fresh weight, and tomato numbers at the end of the dry season tomato crops in 1993 to 1996, were unaffected by cropping density/frequency or by interactions between the treatments and seasons. However, there were differences between seasons (Table 9).

Spores in roots. Root populations of *P. penetrans* were measured in 1994 and 1995 only, but were not significantly affected by either cropping density/frequency. However, they differed between years, with overall means of 5.2×10^4 and 13.0×10^4 spores per mg of dry root in 1994 and 1995.

Tanzania

Plant density had no significant effects, except on yields where plots with a normal plant density generally had greater yields than those planted at high density. Consequently, the results presented are means of both planting densities.

Table 10. *Numbers of* Meloidogyne javanica *J2 per g soil and percentage encumbered with spores of* Pasteuria penetrans *in the microplots amended or not with an exotic isolate of* P. penetrans *and planted with cv. Moneymaker in Tanzania.*

Cycle	J2/g soil		% spore encumbered	
	Unamended	Amended	Unamended	Amended
1	13	10	–	–
2	17	5	–	–
3	18	2	3	49
4	16	2	7	73
5	20	2	28	91
LSD ($P \leqslant 0.05$)	4		7	

–, Not assessed.

Numbers of J2 in soil. In the susceptible tomato (cv. Moneymaker) plots, the numbers of J2 in the soil were greater ($P \leqslant 0.01$) in the unamended plots than in those amended with the exotic isolate of *P. penetrans* (PP1 mixture) for all cropping cycles except the first and the last (Table 10).

P. penetrans *incidence.* Levels of spore attachment were assessed only for the last three cropping cycles. Few J2, and none encumbered with spores, were observed in the plots growing resistant tomato (cv. Rossol). In plots growing cv. Moneymaker amended with *P. penetrans,* the proportion of J2 with spores increased from 49% in the third to 91% in the fifth cycle. In the unamended plots, lower but increasing levels of spore encumberment were observed, probably indicating contamination from adjacent amended plots (Table 10).

Gall index. Resistant cv. Rossol was lightly galled (< 1) only in the first two cropping cycles, and was not included in the statistical analysis. Susceptible cv. Moneymaker amended with the exotic isolate of *P. penetrans* was significantly ($P \leqslant 0.05$) less galled than the unamended, and the gall index progressively decreased in the amended plots, but not in the unamended (Table 11).

Yields. In the *P. penetrans* amended plots yields of cv. Moneymaker progressively increased in all crop cycles except the last, and were greater ($P \leqslant 0.001$) than those of the unamended plots (Table 11). By the fifth cycle they were double those of the unamended. Yields of resistant cv. Rossol (Table 11) were increased ($P \leqslant 0.05$) by *P. penetrans* amendment in the first three cropping cycles, the reasons for which are unclear. Regressing yields for cv. Moneymaker in the fifth cropping cycle against gall

Table 11. *Mean gall indices and yields for plots amended or not with an exotic isolate of* Pasteuria penetrans *growing cvs. Moneymaker and Rossol in the microplot trial in Tanzania.*

Crop cycle	Gall index		Yields (kg/plot)			
	Moneymaker		Moneymaker		Rossol†	
	Unamended	Amended	Unamended	Amended	Unamended	Amended
1	8.8	7.3	10.4	14.3	7.0	10.9
2	8.4	6.0	11.9	16.2	9.1	12.2
3	9.2	4.2	10.8	18.7	9.6	11.0
4	8.8	3.5	11.2	18.9	10.0	11.3
5	9.2	2.9	10.2	20.1	11.2	12.3
LSD ($P \leqslant 0.05$)	1.4			1.4		

† Few galls observed.

Table 12. *Percentage J2 encumbered by spores of* Pasteuria penetrans *and numbers of J2 per g soil under tomato cv. Moneymaker (cycles 1, 3, 5) in the field trial in Ecuador.*

Rotation	Cropping cycles					% spore encumbered			J2 per g soil		
	1	2	3	4	5	1	3	5	1	3	5
1	T	B	T	B	T	80*	82	90	107	50	3
2	T	P	T	P	T	80*	92	90	125	25	3
3	T	M	T	M	T	82*	98*	100*	87	94*	5
4	F	P	T	P	T	47	89	100*	94	64	7

T = Tomato, B = Bean, P = Peanut, M = Maize, F = Weed fallow.
* Significantly greater ($P \leqslant 0.05$) than the lowest value in each cycle (based on transformed results).

indices gave a significant ($P = 0.027$, $r^2 = 0.95$) negative linear regression in which yield was decreased by 6.1% for every increase of one in the gall index.

Rotational field trials

The rotation trials had no controls without *P. penetrans*. Comparisons are therefore between particular sequences of crops.

Ecuador

All plots were initially heavily infested with *M. incognita* lightly infected with *P. penetrans*. All plots were further amended by the addition of dried tomato root containing the same isolate of *P. penetrans* as for the microplot trial.

Numbers of juveniles in soil (tomato crops) and percentage spore encumbered. The numbers of J2 in the soil progressively decreased from a mean of 103 per g in the first cycle to 3 per g in the fifth cycle (Table 12). *P. penetrans* became well established; at the end of the first cycle 47-82% of J2 were encumbered with spores and this increased to at least 90% by the end of the experiment (Table 12). The proportions of spore-encumbered J2 were greatest ($P \leqslant 0.05$) for the rotation involving maize (rotation 3).

Gall index. Mean gall indices decreased from 9.7 in the first cropping cycle to 5.2 in the fifth. Gall indices in the third and fifth cycles were greater ($P \leqslant 0.05$) for the rotation involving maize than for those with peanut.

Yields. Tomato yields progressively increased with each cycle but, whilst yields in the third and fifth cycles tended to be higher in rotations that included peanut or *Phaseolus* bean than in that with maize (Table 13), the differences were not significant. However, in the third and fifth cycles, when the rotational crops would have had an effect, the mean tomato yields from the plots with peanut as the rotational crop averaged 19.6 kg compared with 16.1 kg in the rotation with maize. These differences were supported by differences ($P \leqslant 0.05$) in mean shoot weight measurements (data not shown) which, in cropping cycles 3 and 5, averaged 1.26 kg for the rotations involving peanut, 1.21 kg for the susceptible bean rotation and 0.88 kg for the maize rotation. Because of the variability of the tomato

Table 13. *Gall indices and yields for tomato cv. Moneymaker (cycles 1, 3, 5) in the field trial in Ecuador.*

Rotation	Cropping cycles					Gall indices			Yields (kg/plot)		
	1	2	3	4	5	1	3	5	1	3	5
1	T	B	T	B	T	10	8*	5	13.6	13.6	22.0
2	T	P	T	P	T	9	4	4	13.6	14.8	27.5
3	T	M	T	M	T	10	9*	7*	14.9	11.0	21.2
4	F	P	T	P	T	–	5	5	–	10.8	25.3

* Significantly greater ($P \leqslant 0.05$) that the lowest value in each cropping cycle.

Table 14. *Tomato yields (cycles 1, 3, 5) in the field trial in Trinidad at a site initially free of* Pasteuria penetrans.

Rotation	Treatments and cropping cycles					Yields (kg/plant)		
	1	2	3	4	5	1	3	5
1	Tomato	Maize	Tomato	Maize	Tomato	0.98	1.02	0.35
2	Tomato	Bean[1)]	Tomato	Bean[1)]	Tomato	0.92	0.90	0.31
3	Tomato	Bodi[2)]	Tomato	Bodi[2)]	Tomato	0.97	0.95	0.32
4	Tomato	Tomato	Tomato	Tomato	Tomato	0.87	0.78	0.27
L.S.D. ($P \leqslant 0.05$)							0.08	

[1)] Susceptible.
[2)] Resistant.

yields in the third and fifth cropping cycles, and the contribution of the leguminous crops to increased yield, the regressions against gall indices were not significant.

Trinidad

The site was naturally infested with *M. incognita* only. All plots were amended with the isolate of *P. penetrans* used in the microplot trial. The data presented are all for the tomato crops in the dry seasons (cropping cycles 1, 3, 5).

Juvenile numbers in soil and gall index. Numbers of juveniles remained small (data not shown, but generally < 20/kg soil). As in the microplot trial, there were differences ($P \leqslant 0.001$) between years in the mean gall indices. In the tomato crops grown in cropping cycles 1, 3, 5 these were 3.0, 2.8 and 0.3 respectively. There were no significant differences between rotations.

Yields. Yields per tomato plant were greatest in cycle 1 and least in cycle 5 (Table 14). Plants rotated with bodi (resistant legume) yielded less ($P \leqslant 0.05$) than those rotated with maize or continually cropped with tomato.

Senegal

The trial site was naturally infested with *M. javanica* infected with *P. penetrans*, and plots were not amended by the addition of an exotic isolate.

Table 15. *Numbers of J2 per ml soil and percentage encumbered with* Pasteuria penetrans *in the field trial in Senegal.*

Cropping cycle	Tomato/bean		Tomato/peanut		Tomato/millet	
	Number per ml	% infected	Number per ml	% infected	Number per ml	% infected
1	0.2	75	0.4	96	0.3	91
3	2.0	70	2.1	40	1.6	59
4	0.8	78	0.3	71	0.5	55
6	7.3	40	7.3	71	4.4	71
7	3.4	55	4.3	69	3.7	53

Juvenile numbers in soil and percentage spore encumbered. The numbers of nematodes in the soil after each tomato crop initially increased from a low level but, whilst there were differences ($P \leqslant 0.05$) between cycles, numbers were unaffected by rotational treatments (Table 15). Initially, a high percentage of J2 (> 75%) were encumbered with spores of *P. penetrans* but there was a tendency for the proportion to decrease over time.

Gall index and yields. Gall indices were not affected by rotational treatments, but they differed ($P \leqslant 0.01$) between cropping cycles, increasing from means of 2.7 and 3.9 in the third and fourth cycles to 7.0 and 5.2 in the sixth

Table 16. *Tomato yields (kg per plot) in the Senegal field trial.*

Cycle[1)]	Rotation		
	Tomato/bean	Tomato/peanut	Tomato/maize
1	11.9	13.6	16.3
3	90.2	85.9	93.6
4	31.3	25.8	31.4

[1)] No fruit was produced in cycles 6 and 7.

and seventh cycles. Fruit numbers and weights were measured in seasons 1, 3 and 4 but no fruit were produced in cycles 6 and 7. There were significant differences between seasons in fruit weight but not fruit number. There were no effects of cropping treatments (Table 16).

Tanzania

This trial site was naturally infested with *M. javanica* infected with a low level of *P. penetrans* and was not further amended. Results are presented for the fifth and sixth cropping cycles when all plots grew susceptible tomato cv. Moneymaker. The results are means grouped according to the crops grown in the third and fourth cycles (non-host peanut, resistant tomato cv. Rossol, susceptible tomato cv. Moneymaker, or susceptible bean).

Juvenile numbers in soil and percentage spore encumbered. There were significant differences as a result of the previous cropping ($P \leqslant 0.001$) in cycles 3 and 4 but there was no detectable effect of the cropping in cycle 2. Tomato following peanut and resistant tomato produced the fewest J2 whilst susceptible tomato produced the most (Table 17). The percentages of J2 encumbered with spores of *P. penetrans* were generally low (< 11%), with many plots having no infection.

Gall index and yield. There was an effect of previous cropping in cycles 3 and 4 on both gall indices and yields ($P \leqslant 0.001$). Crops following peanut had least galling and those continuously cropped with tomato cv. Moneymaker, most. Galling following resistant tomato was not significantly different from that on peanut. There were significant interactions between previous crops and cycles ($P \leqslant 0.001$) with increase in galling between cycles following peanut and susceptible tomato but not following resistant tomato or susceptible bean (Table 18).

Yields were smaller in the sixth cycle than in the fifth ($P \leqslant 0.001$) and there was a significant effect of the crops grown in cycles 3 and 4 ($P \leqslant 0.001$). Crops following peanut produced the greatest yield, and the least were where the susceptible cv. Moneymaker had been grown throughout. The yields from tomato following

Table 17. *Numbers of juveniles per g soil and percent infected with* Pasteuria penetrans *after the final two tomato crops in the field trial in Tanzania.*

Cropping cycle		Rotation			
		Peanut	Resistant tomato	Susceptible tomato	Susceptible bean
5	Number per g	0.11	0.08	3.71	0.55
	% infected	0.0	0.0	6.4	0.0
6	Number per g	0.09	0.38	2.30	1.44
	% infected	5.9	3.0	1.4	10.8

Multiplicative LSD ($P \leqslant 0.05$) for numbers/g = ×9.1.
Numbers were converted to logs for analysis. Detransformed values are presented here and means are significantly different ($P \leqslant 0.05$) if one is > 9.1 times greater than the other.

Table 18. *Gall indices and tomato yields (kg/plot) in the field trial in Tanzania.*

Cropping cycle		Rotation			
		Peanut	Resistant tomato	Susceptible tomato	Susceptible bean
5	Gall index	1.4	2.4	5.8	5.2
	Yield	8.8	8.9	6.5	7.4
6	Gall index	4.1	3.4	8.6	5.4
	Yield	8.8	6.1	4.9	7.7

LSD gall index ($P \leqslant 0.05$) cycle × crop ⩽ 1.078.
LSD yield ($P \leqslant 0.05$) cycle × crop ⩽ 0.42.

a susceptible bean crop were greater than those that followed either resistant or susceptible tomato (Table 18).

When individual plot yields for the fifth cycle were regressed against their respective gall indices there was a significant ($P \leqslant 0.001$, $r^2 = 0.86$) negative correlation (Fig. 3); tomato yields in the fifth year were decreased by 5.2% for every increase of one in the gall index. Consequently, a gall index of 5 indicates a 26% reduction in tomato yield. The results for the sixth cycle, indicated a similar relation ($P = 0.04$), a gall index of 5 indicating a 23% reduction in tomato yield.

Crete

The site was naturally infected with *M. javanica* and a small proportion of *M. incognita* but was free of *P. penetrans*. All plots were amended with an exotic mixture of *P. penetrans*. Results are for the susceptible tomato crops (cycles 1, 3 and 5) which alternated in cycles 2 and 4 with; *i)* susceptible bean, *ii)* resistant bean, *iii)* pepper (non-host

for *M. javanica* but a host for *M. incognita*), and *iv)* an initial fallow followed by resistant bean alternated with tomato.

Juveniles in soil. Numbers of J2 were small at the time of sampling and consequently levels of spore encumberment were not determined.

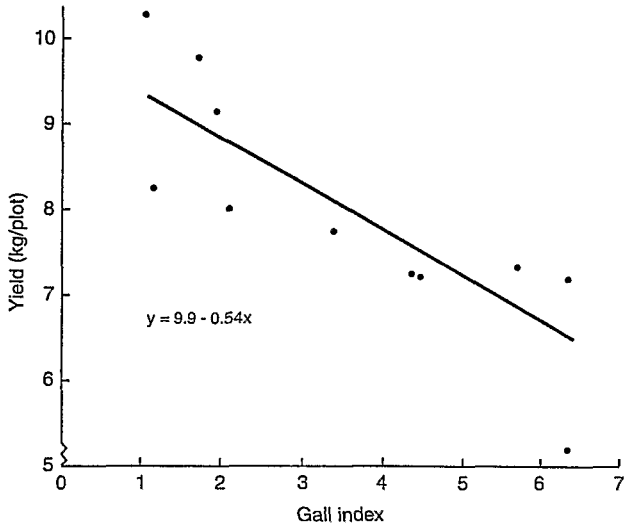

Fig. 3. *Yields (kg per plot) of tomato cv. Moneymaker in the fifth cropping cycle regressed against gall indices for individual plots in the rotational field trial in Tanzania.*

Gall index, height of plants and spores in roots. There were differences in gall indices between rotations ($P \leqslant 0.001$) with rotation 4 having the lowest gall index throughout the experiment (Table 19). There were significant differences in tomato plant height between cropping cycles but not between rotations (Table 19). The yields of *P. penetrans* spores per mg dry tomato root differed significantly between rotations, but these differences were not consistent between cropping cycles (Table 19).

Discussion

The widespread distribution and importance of root-knot nematodes (RKN) to tropical agriculture was confirmed by the International *Meloidogyne* project (Sasser *et al.*, 1983). The coordinated study reported here has further advanced knowledge of RKN in several important ways. Whereas previous reports of damage were often based on subjective estimates (*e.g.*, Sasser, 1979), we have compatible survey and experimental data from which estimates can be derived. Tomato yields in the microplot trial in Ecuador and the microplot and field trials in Tanzania were decreased by an estimated 5 to 6% for every increase of one in the gall index. If it is assumed that, for the same gall index, the yield reduction for all the vegetable crops surveyed is similar to that for tomato, then overall yield losses due to damage by RKN exceed 24%

Table 19. *Effect of rotation on mean root galling, plant height and numbers of* Pasteuria penetrans *($\times 10^3$) per mg dry root of tomato cv. Moneymaker in Crete.*

Cropping cycle		Rotation			
		1 Susceptible bean	2 Resistant bean	3 Pepper	4 Fallow & resistant bean
1	Gall index	5.1	4.4	5.5	–
	Height (cm)	56.5	54.5	55.3	–
	P. penetrans	8.0	6.8	9.5	–
3	Gall index	6.1	4.9	4.3	3.5
	Height (cm)	68.2	66.6	64.4	65.4
	P. penetrans	7.5	13.3	5.8	6.3
5	Gall index	6.0	5.1	4.9	4.5
	Height (cm)	65.7	66.4	63.7	62.2
	P. penetrans	14.6	11.0	7.8	13.4
LSD ($P \leqslant 0.05$ within rotations)		Galls 0.8 Height (cm) 5.3 *P. penetrans* 3.1			

Table 20. *Yield losses due to damage by* Meloidogyne *spp. estimated from data in Table 2 and Fig. 3.*

Country	% crops infected	Mean gall index	% loss
Crete	–	–	–
Burkina Faso	53	2.4	6.4
Ecuador	99	5.5	27.2
Malawi	91	5.3	24.1
Senegal	89	1.6	7.1
Tanzania	100	5.7	28.5
Trinidad & Tobago	40	4.9	9.8

in Tanzania, Malawi and Ecuador and are > 10%, 6 % and 7% in Trinidad and Tobago, Burkina Faso and Senegal, respectively (Table 20).

It was demonstrated that, at two sites (Ecuador and Tanzania) where *P. penetrans* occurred naturally, enhanced control of RKN was obtained in plots where the natural infection was augmented by the introduction of an exotic isolate of *P. penetrans*. It was also shown that *P. penetrans* can be integrated with the growing of non host or resistant crops and, perhaps most importantly, our results provide several indications regarding the factors that influence the effectiveness of *P. penetrans*.

Surveys

The survey results confirm the widespread and serious damage caused by RKN (especially *M. incognita*) to a wide range of crops. However, much more needs to be done, including similar studies in regions (*e.g.*, Asia) not covered by the present surveys. The detection of eight new esterase phenotypes, whose species are uncertain, also emphasises how much more remains to be done. And the unexpected widespread occurrence of *M. hispanica* in Burkina Faso further points to the relative ignorance of the distribution and importance of different RKN species and the need for more studies. The detection of *M. mayaguensis*, first described from Puerto Rico in 1988 (Rammah & Hirschmann, 1988), in Trinidad and Malawi and the observation that it is widespread in Senegal, are further causes for concern because of its apparent pathogenicity and virulence (Fargette & Braaksma, 1990).

A lack of convincing estimates of the damage caused to crops grown by local farmers has been one of the factors restricting funding of research to control RKN in developing countries (S. Eden-Green, pers. comm.). By using the same method of assessing gall indices in the surveys and in the microplot and field trials, it was possible to estimate the likely extent of yield losses in the countries surveyed. However, because a variety of gall index schemes have been used by others, it is difficult to extend our results to their survey results. We used the scheme of Bridge and Page (1980) because it provides an appropriate range relevant to estimating the likely damage. Also, the different categories of damage are clearly indicated and include lateral as well as main roots. To ensure that the results of future investigations are comparable, we suggest that other researchers adopt the gall index scheme of Bridge and Page (1980) as the standard method.

The widespread occurrence of species mixtures potentially complicates control based on cultural methods. In both Ecuador and Trinidad, *ca* 20% of populations were detectable mixtures of species. In Senegal, 20% were mixtures of two species and 6% of three species. Because of the small numbers of nematodes identified to species in each sample, the true values are probably considerably greater. The existence of races varying in host range and virulence (Sasser *et al.*, 1983) further complicates cultural control. It is not surprising therefore that, despite their expense, farmers in Ecuador (Trivino, 1996) and Trinidad (Bala, pers. comm.) indicated that they frequently used nematicides, further increasing the significance of the high gall indices observed.

The surveys indicate that, except in Burkina Faso, the majority of RKN populations were not detectably infected with *P. penetrans*. Interpreting these observations was aided by laboratory experiments that showed spores of *P. penetrans* are readily eluted from sandy soils subjected to water percolation following heavy rainfall (Mateille *et al.*, 1996). These results could account for the absence of *P. penetrans* from the cultivated layer in many fields in Senegal. However, the absence of *P. penetrans*, for example from large parts of Tanzania and Malawi, and possibly much of Indonesia (and other parts of Asia?) may be because it has never been introduced, rather than because of adverse soil and environmental factors.

Nematode factors may also account for the variation in occurrence of *P. penetrans*. The mean soil J2 populations of *Meloidogyne* spp. in the samples where *P. penetrans* occurred, were much greater in Ecuador and Burkina Faso than in Crete, Trinidad and especially Senegal, and there was a clear linear correlation between the proportion of the juveniles infected with *P. penetrans* and the total population of J2 (Mateille *et al.*, 2000).

SPECIFICITY

There is evidence for surface heterogeneity in both parasite and host. Davies and Redden (1997) used a panel of monoclonal antibodies raised to a population of *P. penetrans* to demonstrate that there were substantial differences in the surface antigens of spores both within and between isolates of *P. penetrans*. Davies and Danks (1993) used the same panel of antibodies to label spores attached to J2 of the four races of *M. incognita* and two of *M. arenaria* and confirmed surface differences in the spores attached to the populations representing the different races. This suggests that there are complementary differences in the surfaces of these RKN populations. Attempts to block spore attachment with antibodies, lectins and several other treatments were, at best, only partially successful, suggesting that several components are involved in attachment (Davies *et al.*, 1996; Speigel *et al.*, 1996).

Stirling (1985) reported differences between four isolates of *P. penetrans* in the ability of their spores to attach to J2 from 15 single egg-mass populations of RKN. Trivino (1996) compared the binding to 207 *Meloidogyne* populations of the isolate of *P. penetrans* used in the trials in Ecuador with that of isolate PP1 and also reported differences. The isolate from Ecuador bound well (ten or more spores/J2) to 67% of the populations and PP1 to 62%, but they had different specificities. Our experiments (Table 6) confirmed these differences in *P. penetrans* specificity and further showed that, as observed by Trivino (1996) and Carneiro *et al.* (1999), there are also large differences between populations of RKN in their susceptibility to encumberment. Our results further demonstrate that differences within RKN species can be as great as those between species, and that some populations of RKN were much less readily encumbered than others, irrespective of the isolate of *P. penetrans*.

Verdejo-Lucas (1992) observed variable but consistently low levels of natural infection of RKN by *P. penetrans* in two kiwi orchards. Our survey results indicated that, whilst *P. penetrans* was widespread in some countries, the proportions of J2 encumbered were generally low. Laboratory experiments indicate that the invasive ability of J2 is decreased by *P. penetrans*, especially if they are heavily encumbered (Brown & Smart, 1985; Davies *et al.*, 1988) but, in our surveys and field trials, relatively few J2 were observed to be heavily encumbered (> 10 spores/J2). Many factors could be involved in limiting the proportion of spore-encumbered J2, including low levels of spores in the soil, restricted J2 migration (Stirling *et al.*, 1990), and soil temperature (Hatz & Dickson, 1992). However, our results show that individuals within populations of RKN differ in their susceptibility to encumberment by different isolates of *P. penetrans*. Such differences provide opportunities for selection, and Tzortzakakis and Gowen (1994) demonstrated selection for decreased attachment in a mixed population of *M. incognita* and *M. javanica* repeatedly challenged by a particular isolate of *P. penetrans*. Channer and Gowen (1992) and Tzortzakakis and Gowen (1996) further showed that such selection could occur within a single population of a species, and even a single female line. Conversely, selection within an isolate of *P. penetrans* for increased spore binding was also demonstrated. Consequently, the relation between *P. penetrans* and RKN hosts appears to be dynamic and these observations, and the survey results, lead to the hypothesis that, in natural infections, an equilibrium evolves in which the level of infection is not greatly suppressive. However, although this hypothesis is supported by some of the microplot and field trial results, it requires further testing.

Consequently, when seeking to introduce *P. penetrans* to suppress RKN, it is essential to test and select an isolate that binds well to the local population of RKN. Over time, it may be necessary to introduce new isolates of *P. penetrans* because of selection of the RKN for decreased spore binding. Introducing a mixture of isolates may be an attractive option in the short-term, but could enhance rates of selection for a population of RKN that is not recognised by a wide range of isolates.

MICROPLOT AND FIELD TRIALS

The microplot trials tested the hypothesis that suppression would increase in proportion to the intensity of cropping of RKN susceptible crops. This hypothesis was not supported. However, suppression (characterised by *i)* the proportion of spore-encumbered J2 increasing to high levels, *ii)* gall indices decreasing and *iii)* yields increasing) was observed in three trials; the microplot trial in Tanzania and both field and microplot trials in Ecuador. But suppression was only observed in those trials and plots where the natural, background infection of *P. penetrans* was augmented by the introduction of an exotic isolate (this includes isolates from different parts of a country). The observation that suppression was not observed in the adjacent, unamended plots leads to the conclusion that the introduction of an exotic *P. penetrans* was essential for the induction of suppression.

The amount of *P. penetrans* added as an amendment (*ca* 10^3 spores/g soil in both Tanzania and Ecuador) was

chosen as being too small to be suppressive immediately. Chen *et al.* (1996) reported that an inoculum of 10^5 spores/g soil was required to decrease galling in the first year on peanut, and that it took 3 years for an initial inoculum of 10^3 spores/g soil to increase to a level where it reduced galling. In other studies, suppression of RKN was observed with 2.5×10^4 spores/g (Tzortzakakis & Gowen, 1994), 1.1×10^4 spores/g (Gowen *et al.*, 1998), 10^4 to 4×10^4 spores (Melki *et al.*, 1998). Gowen and Tzortzakakis (1994) concluded that 10^4 spores per g soil are required to ensure that high proportions of J2 are encumbered. However, even though much smaller amounts were used in our trials, some suppression of *M. javanica* was observed in the first cropping cycle in the Tanzanian microplot trial, and to a lesser extent in that in Ecuador. Suppression in both microplot trials progressively increased up to the fifth/sixth cropping cycles, presumably because of the huge numbers of endospores produced by successive crops (up to 1.8×10^6 per mg dry tomato root, which is equivalent to adding $> 3 \times 10^5$ spores per g soil).

Suppression was not observed in the field trial in Tanzania, the microplot trial in Trinidad and the field trial in Senegal. Two of these three ineffective trials were at sites where RKN was naturally infected with *P. penetrans* but, unlike the trials where suppression was observed, the soil was not amended with an exotic isolate. These results do not conflict with the suggestion that the introduction of an exotic isolate made the crucial difference in the trials where suppression was observed. However, suppression did not always result from the introduction of an exotic isolate as suppression was not observed in the field trials in Trinidad or Crete, both of which were amended but which were at sites where *P. penetrans* did not occur naturally. This suggests that the environment has to be conducive to *P. penetrans* for suppression to develop.

The field trials sought to explore the integration of *P. penetrans* with poor and resistant hosts and legumes. Suppression was achieved only in the trial in Ecuador, and the results indicate that *P. penetrans* can be effectively integrated with non-hosts, with an additional yield benefit if it is a legume. Alternating poor host maize or non-host peanut with tomato did not affect the increase in the proportion spore encumbered J2 compared with susceptible bean. The peanut rotation produced the greatest tomato yields and smallest gall indices. Growth after susceptible bean was greater and gall indices less than after poor host maize. In Trinidad and Senegal, rotating tomato with legumes did not increase tomato yields, indicating that different integrated management strategies are required for different environments.

Utility as a biocontrol agent

The above observations are consistent with the hypothesis that suppression is most likely to follow from the introduction of an exotic isolate, or mixture of isolates, of *P. penetrans* at sites where the soil and environment are favourable, one indication of which is that *P. penetrans* is already present. However, the absence of *P. penetrans* may not indicate that the environment is unfavourable, only that it has never been introduced. The exotic isolate must be one that binds well to the local RKN. This hypothesis assumes that in natural infections, through a dynamic process of mutual selection, the RKN and *P. penetrans* achieve a balance, typically one that is not strongly suppressive. All the above suggestions require further, careful testing.

Overall, *P. penetrans* appears to be an ideal biocontrol agent for integrating with other control measures. Only a small amount of root powder, containing an exotic isolate, was required to initiate suppression. This root powder can be produced readily by local farmers. It is also relatively easy to screen exotic isolates for their binding to the target nematode population, and there is substantial variation available from which to choose. Once introduced, provided the environment is conducive, very large numbers of spores can be produced and they are very persistent (Giannakou *et al.*, 1997), and can withstand solarization and nematicides (Walker & Wachtel, 1988).

Even so, our results also demonstrate that *P. penetrans* will not be effective in all situations. This is because of the effects of and interactions between soil and environmental factors. Additionally, where *P. penetrans* is initially effective, the RKN may select for reduced recognition, and some populations of RKN, particularly of *M. mayaguensis*, appear to be comparatively resistant to infection by most isolates of *P. penetrans*. However, it is unclear how such selection and resistance, which must involve the surface properties of the J2, can evolve comparatively rapidly in species which reproduce by mitotic parthenogenesis and which otherwise show relatively little heterogeneity (Blok *et al.*, 1995).

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References

BARKER, K.R. (1985). Nematode extraction and bioassays. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (Eds). *An advanced treatise on* Meloidogyne. *Vol. II: Methodology*. Raleigh, USA, North Carolina University Graphics, pp. 19-35.

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

BLOK, V.C., PHILLIPS, M.S., MCNICOL, J.W. & FARGETTE, M. (1995). Genetic variation in tropical *Meloidogyne* spp. as shown by RAPD-PCR. *Fundamental and Applied Nematology* 20, 127-133.

BRIDGE, J. & PAGE, S.L.J. (1980). Estimation of root-knot infestation levels on roots using a rating chart. *Tropical Pest Management* 26, 296-298.

BROWN, S.M., KEPNER, J.L. & SMART, G.C. (1985). Increased crop yield following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita*. *Soil Biology and Biochemistry* 17, 483-486.

BROWN, S.M. & SMART, G.C. (1985). Root penetration by *Meloidogyne incognita* juveniles infected with *Bacillus penetrans*. *Journal of Nematology* 17, 123-126.

CARNEIRO, R.M.D.G., RANDIG, O., FREITAS, L.G. & DICKSON, D.W. (1999). Attachment of endospores of *Pasteuria penetrans* to males and juveniles of *Meloidogyne* spp. *Nematology* 1, 267-271.

CHANNER, A.G. DE R. & GOWEN, S.R. (1992). Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne-Pasteuria penetrans* interaction. *Fundamental and Applied Nematology* 15, 331-339.

CHEN, Z.X., DICKSON, D.W., MCSORLEY, R., MITCHELL, D.J. & HEWLETT, T.F. (1996). Suppression of *Meloidogyne arenaria* race1 by soil application of endospores of *Pasteuria penetrans*. *Journal of Nematology* 28, 159-168.

CHEN, Z.X. & DICKSON, D.W. (1998). Review of *Pasteuria penetrans*: biology, ecology and biological control potential. *Journal of Nematology* 30, 313-340.

DAVIES, K.G., KERRY, B.R. & FLYNN, C.A. (1988). Observations on the pathology of *Pasteuria penetrans*, a parasite of the root-knot nematodes. *Annals of Applied Biology* 112, 491-501.

DAVIES, K.G. & DANKS, C. (1993). Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M. arenaria* related to the adhesion of the bacterium *Pasteuria penetrans*. *Parasitology* 105, 475-480.

DAVIES, K.G., AFOLABI, P. & O'SHEA, P.S. (1996). Adhesion of *Pasteuria penetrans* to the cuticle of root-knot nematodes (*Meloidogyne* spp.) inhibited by fibronectin, a study of electostatic and hydrophobic interactions. *Parasitology* 112, 553-559.

DAVIES, K.G. & REDDEN, M. (1997). Diversity and partial characterisation of putative virulence determinants in *Pasteuria penetrans*, the hyperparasite root-knot nematodes. *Journal of Applied Microbiology* 83, 227-235.

EHWAETI, M.E., PHILLIPS, M.S. & TRUDGILL, D.L. (1998). Dynamics of damage to tomato by *Meloidogyne incognita*. *Fundamental and Applied Nematology* 21, 627-635.

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. (1985). Use of esterase phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17, 6-20.

FARGETTE, M. & BRAAKSMA, R. (1990). Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 3. A study of some "B" race lines and their taxonomic position. *Revue de Nématologie* 13, 375-386.

GIANNAKOU, I.O., PEMBROKE, B., GOWEN, S.R. & DAVIES, K.G. (1997). Effects of long-term storage and above normal temperatures on spore adhesion of *Pasteuria penetrans* and infection of the root-knot nematode *Meloidogyne javanica*. *Nematologica* 43, 185-192.

GOWEN, S.R., TZORTZAKAKIS, E.A. & CHANNER, A.G. DE R. (1998). Control of the root-knot nematode *Meloidogyne javanica* by the parasite *Pasteuria penetrans* as influenced by initial nematode population densities. *Nematologica* 44, 369-379.

HATZ, B. & DICKSON, D.W. (1992). Effect of temperature on attachment, development and interactions of *Pasteuria penetrans* on *Meloidogyne arenaria*. *Journal of Nematology* 24, 512-521.

JEPSON, S.B. (1987). *Identification of root-knot nematodes* (Meloidogyne *species*). Wallingford, UK, CAB International, pp. 265.

KERRY, B.R. & CRUMP, D.H. (1998). The dynamics of the decline of the cereal cyst nematode, *Heterodera avenae*, in four soils under intensive cereal production. *Fundamental and Applied Nematology* 21, 617-625.

MADULU, J.D., TRUDGILL, D.L. & PHILLIPS, M.S. (1994). Rotational management of *Meloidogyne javanica* and effects on *Pasteuria penetrans* and tomato and tobacco yields. *Nematologica* 40, 438-455.

MANKAU, R. (1980). Biological control of *Meloidogyne* populations by *Bacillus penetrans* in West Africa. *Journal of Nematology* 12, 230.

MATEILLE, T., DUPONNOIS, R., DABIRE, K., N'DIAYE, S. & DIOP, M.T. (1996). Influence of the soil on the transport of the spores of *Pasteuria penetrans* by water and on their avail-

ability to parasitise nematodes genus *Meloidogyne*. *European Journal of Soil Biology* 32, 81-88.

MATEILLE, T., TRUDGILL, D.L., TRIVINO, C., BALA, G., SAWADOGO, A. & VOUYOUKALOU, E. (2000). Multisite study of the interaction of soil factors on the root-knot nematode (*Meloidogyne* spp.) parasitism by *Pasteuria penetrans*. *Biological Control*, in press.

MELKI, K., GIANNAKOU, I.O., PEMBROKE, B. & GOWEN, S.R. (1998). The cumulative build-up of *Pasteuria penetrans* spores in root-knot nematode infested soil and the effect of soil applied fungicides on its infectivity. *Fundamental and Applied Nematology* 21, 679-683.

OOSTENDORP, M., DICKSON, D.W. & MITCHELL, D.J. (1990). Host range and ecology of isolates of *Pasteuria* spp. from the south-eastern United States. *Journal of Nematology* 22, 525-531.

RAMMAH, A. & HIRSCHMANN, H. (1988). *Meloidogyne mayaguensis* n. sp. (Meloidogynedae), a root-knot nematode from Puerto Rico. *Journal of Nematology* 29, 56-69.

ROBERTS, P.A. (1992). Current status of the availability, development and use of host plant resistance to nematodes. *Journal of Nematology* 24, 213-227.

SASSER, J.N. (1979). Economic importance of *Meloidogyne* in tropical countries. In: Lamberti, F. & Taylor, C.E. (Eds). *Root-knot nematodes* (Meloidogyne *species*) *systematics, biology and control*. London, UK, Academic Press, pp. 359-374.

SASSER, J.N., EISENBACK, J.D. & CARTER, C.C. (1983). The international *Meloidogyne* project — its goals and achievements. *Annual Review of Phytopathology* 21, 271-288.

SEINHORST, J.W. (1950). De betekenis van de toestand van de grond voor het optreden van aanstasting door het stengelaaltje *Ditylenchus dipsaci* Kühn Filipjev. *Tijdschrift Over Planteziekten* 56, 289-349.

SEINHORST, J.W. (1962). Modifications to the elutriation method for extracting nematodes. *Nematologica* 8, 117-128.

SOUTHEY, J.F. (1986). *Laboratory methods for working with plant and soil nematodes*. London, UK, HMSO, pp. 202.

SPIEGEL, Y., MOR, M. & SHARON, E. (1996). Attachment of *Pasteuria penetrans* endospores to the surface of *Meloidogyne javanica* second-stage juveniles. *Journal of Nematology* 28, 328-334.

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

STIRLING, G.R. (1981). Effect of temperature on infection of *Meloidogyne javanica* by *Bacillus penetrans*. *Nematologica* 27, 158-162.

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

STIRLING, G.R., SHARMA, R.D. & PERRY, J. (1990). Attachment of *Pasteuria penetrans* spores to the root-knot nematode *Meloidogyne javanica* in soil and its effects on infectivity. *Nematologica* 36, 246-252.

TRIVINO, C.G. (1996). *The occurrence of* Pasteuria penetrans *infecting root-knot nematodes* (Meloidogyne *spp.*) *in vegetable fields in Ecuador and its potential role in nematode management*. Reading, UK, Ph.D. Thesis, University of Reading, pp. 138.

TZORTZAKAKIS, E.A. & GOWEN, S.R. (1994). Resistance of a population of *Meloidogyne* spp. to parasitism by the obligate parasite *Pasteuria penetrans*. *Nematologica* 40, 258-266.

TZORTZAKAKIS, E.A. & GOWEN, S.R. (1996). Decreased ability of *Pasteuria penetrans* spores to attach to successive generations of *Meloidogyne javanica*. *Fundamental and Applied Nematology* 19, 201-204.

VEDEJO-LUCAS, S. (1992). Seasonal population fluctuations of *Meloidogyne* spp. and the *Pasteuria penetrans* group in kiwi orchards. *Plant Disease* 76, 1275-1279.

WALKER, G.E. & WACHTEL, M.F. (1988). The influence of soil solarization and non-fumigant nematicides on infection of *Meloidogyne javanica* by *Pasteuria penetrans*. *Nematologica* 34, 477-483.