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Preliminary studies on the effect of the host plant on the susceptibility of *Meloidogyne* nematodes to spore attachment by the obligate parasite *Pasteuria penetrans*

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Summary. Variability in the number of spores of the bacterial parasite *Pasteuria penetrans* which attached to second stage juveniles of a mixed *Meloidogyne javanica* / *M. incognita* population after development on different host plants was recorded. These differences may result from the prevalence of different selected sub-populations with variable susceptibility to the bacterium due to competitive differences in growth and reproductive efficiency between species, races and biotypes. Therefore, the long term effect of the parasite on field populations of *Meloidogyne* is likely to be less consistent under crop rotations than under monoculture.
Key words: bacterial parasite, *Meloidogyne javanica*, *M. incognita*, root knot nematodes, spore adhesion.

Pasteuria penetrans (*Pp*) is an endospore forming bacterium which parasitizes root knot nematodes (Sayre & Starr, 1985) and has potential for biocontrol when added to soil in pots, small plots, container grown crops and polytunnels (Gowen & Tzortzakakis, 1994; Tzortzakakis & Gowen, 1994). Populations of the parasite initially isolated from one species of *Meloidogyne* will attach to others, however, some specificity of spore attachment exists between nematode species and within populations of the same species (Stirling, 1985; Channer & Gowen, 1988; Davies et al., 1988). Problems associated with host specificity could be significantly decreased by using mixtures of isolates of the parasite obtained from different geographical areas and isolated from various *Meloidogyne* species (Channer & Gowen, 1992).

Selection pressure exerted by host plants on field populations of *Meloidogyne* can encourage the predominance of certain species, races or biotypes of the nematodes which may result in the susceptibility to the parasite varying under different crops. Here we report on the influence of different host plants or crop rotations on the susceptibility of mixed *M. javanica* / *M. incognita* populations to spore attachment.

MATERIALS AND METHODS

Meloidogyne populations originating from different areas were inoculated on selected host plants (see below). Subsequently, egg masses were collected and juveniles hatched in extraction dishes. One hundred juveniles (J_2) were collected after a five day hatching period and exposed to *Pp* spore suspensions (3 ml volume of nematode and spore suspensions) in 2.5 cm diameter Petri dishes for 24 h at 28° C. The number of attached spores on J_2 was recorded under an inverted microscope at 400 fold magnification. The spore suspensions were prepared by grinding air-dried roots containing spore infected females and suspending in water, after sieving the root debris through a 38 μm sieve. The spore cultures are from different countries and are kept stored at the Nematology Laboratory, Reading University, UK.

The original sources had been multiplied by the method of Stirling & Wachtel (1980). The tested isolates were: *Pp* 2 (USA), *Pp* 3 (S. Africa), *Pp* M (Malawi) and *Pp* blend (a mixture of the three mentioned isolates plus others from Australia, Papua New Guinea and Ivory Coast) originally isolated from *M. javanica* or *M. incognita* (Channer & Gowen, 1992). Spore attachment tests were conducted for all combinations of nematode populations and host plants described below.

A population of *M. javanica* / *M. incognita* (PNG) originating from Papua New Guinea was allowed to develop on tobacco (*Nicotiana tabacum* cv. NC 95) and pepper (*Capsicum annuum* cv. California Wonder) for one generation. Hatched juveniles were exposed to 16,000 spores of *Pp* blend. A population of *M. incognita* (Tuv) from Tuvalu was allowed to develop on similar host plants for two crop cycles (approximately four generations). Four egg masses were removed from each plant and juveniles from each egg mass were exposed separately to a spore suspension of *Pp* 2 containing 16,000 spores. Spore attachment was recorded on 20 - 40 J_2 per population.

A population of *M. javanica* / *M. incognita* (85% and 15%) originating from a greenhouse in Crete, Greece was tested for its susceptibility to different *Pp* isolates (*Pp* 2, *Pp* 3, *Pp* PNG) after being raised on cucumber (*Cucumis sativus* cv. Bush Champion), marrow (*Cucurbita* spp. cv. Green Bush), cowpea (*Vigna sinensis* cv. Black Eye) and dwarf bean (*Phaseolus vulgaris* cv. Gold Butter) for one generation.

A second population of *M. javanica* / *M. incognita* (80% and 20%) from an adjacent greenhouse was inoculated on cucumbers (cv. Knossos). After the completion of the first generation the plants were uprooted and the galled root systems returned to the soil before planting new seedlings of tomato (*Lycopersicon esculentum* cv. Carouso) and cucumber. The same procedure was repeated and cucumbers and egg plants (*Solanum melongena* cv. Delica) were planted. Juveniles hatched from egg masses after the completion of the three crop cycles were tested for their susceptibility to *Pp* 3, *Pp* M and *Pp* blend. The species composition of the selected populations was determined by perineal pattern morphology on 10 females from each treatment.

With nematode populations originating from Greece 10-15 egg masses were removed from each plant and hatched in extraction dishes. One hundred juveniles were exposed to 20,000 spores of the parasite in 3 separate Petri dishes and the number of attached spores recorded on a total of 30 J_2 (10 from each of the 3 replicate dishes).

RESULTS

Juveniles that hatched from pepper infected with *M. javanica* / *M. incognita* (PNG) were less susceptible than those from tobacco when exposed to 16,000 spores of *Pp* blend. With *M. incognita* (Tuv) three egg masses from pepper resulted in progenies receiving few or no spores whereas the progeny from the three egg masses from tobacco were susceptible. The progenies of one egg mass from pepper were highly susceptible whereas those from tobacco were intermediately susceptible (Table 1).

Results from Greek populations *M. javanica* / *M. incognita* were analysed by the chi square (x^2) tests used to compare the groups of juveniles receiving low (0-5), intermediate (6-10) and high (11-15 and 15) numbers of spores. The four different hosts used selected nematode sub-populations with variable susceptibility for *Pp* 3 and *Pp* 2. The *Pp* blend had probably a broader attachment spectrum since there were no significant differences between treatments (Table 2). After a longer period of selection the effect of host plant on nematode susceptibility was more evident as shown with crop rotations which differentiated significantly the rate of attachment attained by *Pp* 3, *Pp* M and *Pp* blend (Table 3a).

DISCUSSION

Pepper cv. California Wonder is susceptible to all races of *M. incognita*, while tobacco cv. NC 95 to *M. javanica* and races 2 and 4 of *M. incognita* (Hartman & Sasser, 1985). Thus the variable susceptibility of the selected sub-populations of *M. javanica* / *M. incognita* (PNG) and *M. incognita* (Tuv) is probably correlated with prevalence of nematode species or races on the different host plants.

Table 1. Mean number of spores attached and variance on juveniles of *M. javanica* / *M. incognita* (PNG) and *M. incognita* (Tuv) (progenies of four egg masses) being selected on pepper (cv. California Wonder) and tobacco (cv. NC 95) and exposed to spores of *P. penetrans* (average of 20-40 juveniles per population).

Nematode population	*Pp*	Mean spore attachment		Variance	
		Pepper	Tobacco	Pepper	Tobacco
M. javanica / *M. incognita* (PNG)	*Pp* blend	2.20	36.17	2.49	219
		1.13	12.50	0.83	13.41
M. incognita (Tuv)	*Pp* 2	0.35	12.81	0.40	33.49
		1.00	13.38	0.88	20.42
		12.80	6.50	16.02	15.06

Table 2. Number of encumbered juveniles of *M. javanica* / *M. incognita* (Greece) after selection of the original population on different host plants with their respective spore burdens after exposure to three different populations of *P. penetrans* (counts from 10 juveniles from each of 3 replicate dishes).

No of spores/ J2	Number of encumbered juveniles											
	Pp 2				*Pp* 3				*Pp* blend			
	H1	H2	H3	H4	H1	H2	H3	H4	HI	H2	H3	H4
0-5	0	2	1	0	2	2	7	1	1	0	1	1
6-10	3	8	7	4	7	11	14	16	6	6	9	4
11-15	10	10	9	4	8	11	3	9	10	11	10	10
>15	17	10	13	22	13	6	6	4	13	13	10	15
x^2 test	S*				S*				NS*			

H1: Cucumber (cv. Bush Champion); H2: Marrow (cv. Green Bush); H3: Cowpea (cv. Black Eye); H4: Bean (cv. Gold Butter).

S* - significant at $P < 0.05$; NS** - not significant at $P > 0.05$.

Table 3. a) Number of encumbered juveniles of *M. javanica* / *M. incognita* (Greece) after selection of the original population on different crop rotations with their respective spore burdens after exposure to three different populations of *P. penetrans* (counts from 10 juveniles from each of 3 replicate dishes).

No of spores / J2	Number of encumbered juveniles											
	Pp 3				*Pp* M				*Pp* blend			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
0-5	12	3	14	5	14	11	12	14	0	0	9	0
6-10	1	1	1	4	3	14	10	13	0	1	5	11
11-15	5	5	1	6	9	2	6	3	4	2	4	8
>15	12	21	14	15	4	3	2	0	26	27	12	11
x^2 test	S*				S**				S***			

R1: Cucumber-cucumber-cucumber; R2: Cucumber-tomato-cucumber; R3: Cucumber-tomato-egg plant; R4: Cucumber-cucumber-egg plant.. Cucumber - cv. Knossos; Tomato - cv. Carouso; Egg plant - cv. Delica.

S* - significant at $P < 0.01$; S** - significant at $P < 0.05$; S*** - significant at $P < 0.0001$.

b) Identification of nematode populations upon completion of 3 crop cycles based on perineal pattern morphology of 10 randomly selected females.

	M. javanica	*M. incognita*
R1	40%	60%
R2	50%	50%
R3	50%	50%
R4	60%	40%

The reproductive efficiency of *Meloidogyne* species is affected by the presence of other species in the same root system and in a mixed community some species or races can survive and compete more effectively than others (Khan & Haider, 1991). Although the competition between *M. javanica* and *M. incognita* is minimal, and both species coexist (Eisenback, 1985), such interactions may influence reproductive efficiency and population growth (Khan & Haider, 1991). Results with nematode populations from Greece indicated that different host plants or crop rotations may induce nematode interactions in mixed populations of *M. javanica* / *M. incognita* resulting in variable susceptibility to spore attachment due to selection of sub-populations. With crop rotations this could be explained by a variable species selection (Table 3b) but the small size of the sample used for identification and inaccuracy of the methodology based on perineal pattern morphology prevent a firm conclusion. *Meloidogyne* populations from rotations 2 and 3, despite the same species constitution had different responses to *Pp* 3 and *Pp* blend, which may be explained by the selection of races or biotypes (Roberts, 1992).

Despite the lack of accure identification of nematode populations in this study and the influence of other factors our results suggest that *Pp*, produced through an *in vivo* system, when applied to the field provides a reduced chance of consistent control under intensive crop rotations as compared with application to monocultures.

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Tzortzakakis E.A., Channer A.G.De R., Gowen S.R., Goumas D.E. Предварительное изучение воздействия растения-хозяина на чувствительность *Meloidogyne* к прикреплению спор облигатных паразитических бактерий *Pasteuria penetrans*.

Резюме. Число спор паразитических бактерий *Pasteuria penetrans*, прикрепляющихся к поверхности кутикулы личинок 2-го возраста из смешанной популяции *Meloidogyne javanica/M. incognita*, изменяется в зависимости от того, на каком растении-хозяине происходило развитие нематод. Эти различия могут быть объяснены тем, что популяции *Meloidogyne* состоят из отдельных субпопуляций с различной чувствительностью к паразитическим бактериям, причем доля этих субпопуляций обусловливается различиями в скорости роста и размножения видов, рас и биотипов нематод. Долговременное воздействие паразитических бактерий на популяции *Meloidogyne* в полевых условиях будет менее эффективным в севообороте, чем в монокультуре.