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Decreased ability of *Pasteuria penetrans* spores to attack to successive generations of *Meloidogyne javanica* [1]

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Summary – The decreasing ability to attachment of *Pasteuria penetrans* spores on a field population of *Meloidogyne javanica* from Malawi and single egg mass lines of the same species from Crete was recorded in the laboratory. When the bacterial parasite does not provide sufficient control of the nematode, a selection of *Pasteuria* spores with high attachment potential against the target nematode might be an alternative for delaying the decrease of spore attachment ability.

Résumé – ***Décroissance de l'aptitude des spores de* Pasteuria penetrans *à l'attaque de générations successives de* Meloidogyne javanica** – La décroissance de l'aptitude à l'attaque des spores du parasite bactérien *Pasteuria penetrans* est mise en évidence au laboratoire vis-à-vis d'une population sauvage de *Meloidogyne javanica* originaire du Malawi et de lignées provenant d'une seule masse d'œufs de cette même espèce originaire de Crète. Dans le cas où le parasite bactérien ne permet pas un contrôle suffisant du nématode, une sélection de spores de *Pasteuria* à haut potentiel d'adhésion vis-à-vis du nématode cible pourrait constituer une alternative à la décroissance de l'aptitude à l'adhésion des spores.

Key-words : attachment, biological control, *Pasteuria penetrans, Meloidogyne javanica,* spore adhesion.

Pasteuria penetrans (Pp) is an endospore forming bacterium pathogenic to root-knot nematodes (Sayre & Starr, 1985) with potential for biocontrol when employed in container grown crops, small plots and polytunnels (Stirling, 1984; Brown *et al.*, 1985; Dube & Smart, 1987; Gowen & Ahmed, 1990; Gowen & Tzortzakakis, 1994; Tzortzakakis & Gowen, 1994 *b*). Previous work demonstrated that a mixed population of *Meloidogyne javanica* and *M. incognita* was subjected to selection pressure for decreased susceptibility to spore attachment and infectivity after exposure to a population of the parasite for four generations. That could be successfully prevented when a mixture of parasite isolates (*Pp* blend) was used instead of a single isolate (Tzortzakakis & Gowen, 1994 *a*). Several authors addressed the problem of specificity of spore attachment on several *Meloidogyne* populations and concluded that for durable control, *Pp* isolates with a broad spectrum of host specificity should be used (Stirling, 1985; Davies *et al.*, 1988 *a, b;* Channer & Gowen, 1992). This investigation aims to study the effectiveness of a mixture of parasite isolates (*Pp* blend) in preventing selection for decreased susceptibility to spore attachment in *Meloidogyne* species with narrow genetic diversity, such as a population or single egg mass lines.

The studies were carried out in pot experiments at the University of Reading, UK and the Plant Protection Institute of Heraklion, Greece.

Materials and methods

Pp ISOLATES AND NEMATODE POPULATIONS ORIGINS

Spore suspensions were prepared according to the method of Stirling and Wachtel (1980). Powdered tomato roots containing *Pp* infected females were wet ground in a mortar and pestle and the resulting slurry passed through a 38 μm sieve to separate the spores from coarse plant material. The suspensions prepared at the begining of the experiments were stored in a domestic refrigerator. The origins of spore isolates were : *Pp* 1 (Australia), *Pp* 2 (USA), *Pp* 3 (S. Africa), *Pp* PNG (Papua New Guinea), *Pp* M (Malawi) and *Pp* IC (Ivory Coast). The tested *Meloidogyne* populations were identified morphologically (perineal pattern of mature female, tail length and hyaline portion of J2) and by the North Carolina differential host test (Hartman & Sasser, 1985; Jepson, 1987).

The nematode population used in the first experiment was a population of *M. javanica* originally isolated from

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a field site in Malawi and maintained on tomato cv. Tiny Tim for many years in a glasshouse at Reading University.

The populations used in the second experiment were single egg mass lines of *M. javanica* originating from two plastic mini-greenhouse frames in Crete and maintained for one generation on tomato cv. Rutgers in a growth chamber at the Plant Protection Institute of Heraklion, Greece. These populations were chosen from several populations of the same species after testing for susceptibility to spore attachment.

EXPERIMENT 1

Juveniles (J2s) were collected from egg masses incubated on extraction filters (Southey 1986) for 4 days and were immersed in a spore suspension (3×10^4 spores/ml) at 28 °C. Spore attachment was recorded on 50 individuals under an inverted microscope at $\times 400$. The minimum level of spore attachment before plant inoculation was that at which at least 80 % of the nematodes were encumbered with more than five spores. Nematodes from the same source left in tap water under the same conditions served as controls (original population which was not exposed to spores). Juveniles contaminated with spores of *Pp* blend (the six isolates blended) and *Pp*-free controls were inoculated on tomato plants cv. Tiny Tim. Cross contamination was avoided by placing saucers beneath individual plant pots and watering by hand with care. Each treatment was replicated four times. After a 5-week-growing period in a glasshouse with air temperature 25-30 °C, the egg masses were collected from the roots and incubated in extraction dishes. The same procedure of exposure to spores was repeated for four generations using highly concentrated spore suspensions (up to 10^5 spores/ml) to hasten the selection and achieve the desired attachment level. Plant inoculation was done with 1000-4000 nematodes per pot depending on the size of the seedlings.

At the end of each crop cycle 100 J2s collected from the egg masses of each plant (selected and original populations) were incubated in 3 ml spore suspensions of *Pp* blend and *Pp*2 containing a total of 20 000 spores in 2.5 cm Petri dishes at 28 °C. Spore attachment was recorded on 20 J2s from each of the four replicate dishes after 24 h. The J2s finally collected after the completion of the fourth generation were also exposed to *Pp*3, *Pp*PNG, *Pp*M and *Pp*IC under the same conditions.

EXPERIMENT 2

The procedure was the same as that described in the first experiment. The plants grew for six weeks in a growth room with air temperature 26-30 °C. Attachment tests were conducted as previously described, using the *Pp* blend (used for selection) and some of the individual isolates (*Pp*3, *Pp*PNG, *Pp*M, and *Pp*IC) in 5 cm diameter Petri dishes containing 30 000 spores of the parasite in a total volume of 5 ml. The experiment was terminated after recording spore attachment on the first generation J2s.

Data were transformed to square roots before analysis when the value for the standard error of the mean was higher than a mean value (Mead & Curnow, 1990). Comparisons of main effects were made using the F value calculated by two factor analysis of variance between nematode populations and *Pasteuria* isolates.

Results

EXPERIMENT 1

Significantly fewer spores of *Pp* blend and *Pp*2 attached to the J2s of the selected nematode population after the completion of the third ($P < 0.01$) and fourth generations ($P < 0.001$) (Table 1). The selected population indicated also decreased susceptibility when exposed to four of the *Pp* isolates ($P < 0.001$) composing the blend (Table 2).

EXPERIMENT 2

Several single egg mass populations of *M. javanica* originating from plastic tunnels in Crete were preliminary tested indicating mostly either high (> 15 spores/J2) or low (0-5 spores/J2) susceptibility to spore attach-

Table 1. *Number of attached spores of* Pasteuria penetrans *(*Pp *blend and* Pp *2) on the original population of* Meloidogyne javanica *(Malawi) and the selected population previously exposed to* Pp *blend for four generations.*

	Generations							
	1		2		3		4	
	Pp blend	*Pp*2	*Pp* blend	*Pp*2	*Pp* blend	*Pp*2	*Pp* blend	*Pp*2
Original	10.88	10.88	10.30	7.95	10.77	10.82	13.52	7.58
Selected	9.47	8.67	9.13	8.56	8.77	7.02	1.51	2.35
	NS		NS		**		*** a	

Number of spores attached to 20 J2s per dish (average of four replicate dishes); a : analysis on square root transformed data; **, *** significant differences at $P < 0.01$ and 0.001, respectively, between original and selected nematode population; NS : non significant difference.

Table 2. *Number of attached spores of different* Pasteuria penetrans (Pp) *isolates on the original population of* Meloidogyne javanica *(Malawi) and the selected population previously exposed to* Pp *blend for four generations.*

	*Pp*3	*Pp*PNG	*Pp*M	*Pp*IC
Original	1.12	9.03	9.37	3.65
Selected	0.13	2.30	1.41	0.32
	***	***	***	***

Number of spores attached to 20 J2s per dish (average of four replicate dishes); analysis on square root transformed data; *** indicates significant differences between original and selected population for each isolate of *P. penetrans* at $P < 0.001$.

ment. The fecundity of the highly susceptible populations was brought down to zero after developing on tomato roots for one generation whereas the populations exhibiting low susceptibility produced abundant egg masses. The susceptibility of two populations which were finally chosen for selection process was intermediate between the two extremes (approximately 50 % of the J2s had more than five spores). These populations produced fewer egg masses when parasitized by spores of the blend, and infected females were recovered from galls without egg masses.

The nematode populations which were subjected to selection pressure by the *Pp* blend for one generation were found to differ significantly from the original populations for their susceptibility to spore attachment ($P < 0.001$) after exposure to spores of *Pp* blend, *Pp*3, *Pp*PNG, *PpM* and *Pp*IC (Table 3). Population 2 was not exposed to *Pp*PNG due to the limited number of J2s hatched from the egg masses of the selected population.

Table 3. *Number of attached spores of different* Pasteuria penetrans (Pp) *isolates on two single egg mass populations of* Meloidogyne javanica *(Crete) and the selected populations previously exposed to* Pp *blend for one generation.*

	Pp blend	*Pp*3	*Pp*PNG	*Pp*M	*Pp*IC
POPULATION 1					
Original	17.09	17.81	2.05	5.97	5.68
Selected	10.83	11.64	1.00	5.15	1.76
	***	***	***	***	***
POPULATION 2					
Original	9.22	10.21	ND	3.42	4.87
Selected	3.98	5.46	ND	1.40	2.17
	***	***		***	***

Number of spores attached to 20 J2s per dish (average of four replicate dishes); analysis on square root transformed data; ND not determined; *** indicates significant differences between original and selected populations for each isolate of *P. penetrans* at $P < 0.001$.

Discussion

Channer and Gowen (1992) stated that single egg mass populations of *Meloidogyne* are likely to be more genetically uniform for their susceptibility to spore attachment compared to field populations and this difference could be due to variability in the different nematode types occuring in the field. The present study demonstrates that parasite selection could favour the development of a nematode subpopulation with decreased susceptibility at the expense of one more susceptible, in populations composed by a single species (*M. javanica* from Malawi) or originating from a single egg mass (*M. javanica* from Crete). The process on single egg mass populations could be swift with obvious differences appearing after the brief period of one generation. The implication is that even a mixture of parasite isolates (*Pp* blend) may not provide long term nematode control in a species of *Meloidogyne* or a single egg mass line. It might be argued that under field conditions, when *Pp* is applied either in spot or band treatments, individual nematodes have greater chances of " escape " than those in pot tests. In the field, the multiplication of susceptible nematodes which did not come into contact with spores will delay the prevalence of the nematode subpopulation selected by the parasite. The parasite may also be selected for increased attachment ability to a particular nematode. Nevertheless, the risk of selecting nematode populations with decreased susceptibility to mixtures of *Pasteuria* isolates would justify the effort of seeking spore isolates with better attachment capabilities within the *Pp* group. Selection and proliferation of specific spores for a particular nematode population may be feasible by repeated culturing of the parasite on the target nematode. The selected bacterium can probably recognize a host type it has successfully infected and produce spore progeny with an enhanced ability to reinfect that particular host (Channer & Gowen, 1992; Tzortzakakis & Gowen, 1994 *a*).

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