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THE PARASITIC ACTIVITY OF *MELOIDOGYNE INCOGNITA* AS AFFECTED BY THE DYNAMICS OF ACQUISITION OF *PASTEURIA PENETRANS* SPORES

by
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Summary. Eggs of *Meloidogyne incognita* were exposed to two concentrations of blends of *Pasteuria penetrans* isolates, 3.10^4 (Pp-low) and 10^5 spores/g soil (Pp-high), for 2, 6, 9 and 17 days. Spore burdens of juveniles increased with the time of exposure and in proportion to spore concentrations. Data of the number of galls produced by period of exposure suggested that most root invasion occured between 7 and 15 days with the lower spore density and 15-30 days with the higher following transplanting. Root damage was suppressed by 61% with the former and 80% with the latter spore concentration ($P<0.01$). The number of egg masses produced was also significantly suppressed, 83% and 94% respectively, with the lower and higher spore concentrations ($P<0.01$).

Pasteuria penetrans (Thorne) Sayre *et* Starr is an obligate endospore-forming organism parasite of plant-parasitic nematodes. The host-specificity (Stirling, 1985; Davies *et al.*, 1988) and ability of *P. penetrans* to withstand nematicides and extreme environmental conditions (Dutky and Sayre, 1978; Stirling *et al.*, 1986; Nishizawa, 1989) make it one of the few organisms which have shown the best biocontrol features. Though reported on 205 nematodes species belonging to 96 genera, it is on *Meloidogyne spp.* that the biocontrol agent presents the best potential (Stirling, 1984; Dube and Smart, 1987).

Endospores of *P. penetrans* become attached on second-stage juveniles of root-knot nematodes as the latter migrate between soil particles. Spores germinate when encumbered juveniles invade roots of the host-plant (Sayre and Wergin, 1977). Stirling (1984) indicated that at least 40 spores per juvenile were necessary to reduce nematode motility and root invasion. Davies *et al.* (1988) and Sayre and Gherma (1990) reported, respectively, that 15 and 26 spores/juvenile would be required to alter both nematode movement and root penetration.

The present experiment was conducted to determine how spore concentration and time of exposure of nematodes could affect the spore burdens of juveniles and root infection by the parasite.

Materials and methods

An isolate of *Meloidogyne incognita* (Kofoid *et* White) Chitw. from vegetable plots in the suburb of Abidjan, Côte d'Ivoire was used. The nematode was propagated on tomato (*Lycopersicon esculentum* Mill. cv. Tiny Tim) in sterilised soil in a glasshouse for 45 days.

Eggs were extracted from tomato roots in 1% sodium hypochlorite (NaOCl) as described by

Hussey and Barker (1973). The resulting egg suspension was quantified under a compound microscope by counting three 1-ml aliquots. The suspension was diluted to obtain an inoculum level of 500 eggs in 0.5 ml of tap water.

To achieve consistent levels of attachment (Tzortzakakis and Gowen, 1994), a blend of *P. penetrans* isolates was applied. The blend was made of isolates designated Pp1 from Australia, Pp2 (USA), Pp3 (South Africa), Pp4 (Papua New Guinea), Pp5 (Côte d'Ivoire) and PpB7 (Australia). The isolates have been mass-produced for several years at Reading University, as described by Stirling and Wachtel (1980), on both *M. incognita* and *M. javanica* inoculated on tomato plants.

Tomato roots were air-dried and ground in an electric mill. The concentration of spores in the root powder was determined by grinding in a ceramic mortar containing 1 ml of distilled water, 0.5 g of root powder with a pestle. The slurry was diluted in 50 ml of distilled water and decanted through a 38 µm sieve. The resulting endospore suspension was quantified with a counting chamber under a stereo microscope at x450.

Treatments were 500 eggs of *M. incognita* alone; 500 eggs of *M. incognita* $+3.10^4$ spores/g soil and 500 eggs of *M. incognita* $+10^5$ spores/g soil. Each treatment was replicated four times. For convenience, the rate of 3.10^4 spores/g soil will be referred to as Pp-low, and 10^5 spores/g soil as Pp-high. Treatment replicates were arranged in a completely randomised design on a glasshouse bench at 28 °C.

Inoculation consisted of mixing in a polyethylene bag spore-laden root powder and nematode eggs with 334 g of sandy loam soil (5.1% clay, 5% silt, 87.9% sand, 2% organic matter, pH 5.2). The mixture was poured into 7.5 cm-diam. pots, watered and kept moist for 48 hours.

Roots of four weeks old tomato seedlings were washed free of nursery soil and transplanted in treated potting soil. Four, seven and fifteen days later, four seedlings in each treatment were unpotted, their roots washed free of previous soil, and transferred in 11 cm-diam. pots filled with sterilised sandy loam soil free of both *Pasteuria* and nematode. The rest of the seedlings were allowed to grow continuously in nematode and *Pasteuria* treated soil.

After the transfer of seedlings from nematodes and bacteria treated to non-treated potting soil, the former soil was subjected to elutriation (Seinhorst, 1962). Twenty randomly chosen juveniles per replicate were examined under an inverted stereo microscope (x400) to determine their spore burdens. Juveniles were classified as unencumbered, lightly encumbered (1 to 9 spores/juvenile) and heavily encumbered (more than 10 spores/juvenile).

Thirty days after transplanting tomato seedlings from the nursery to nematode and *Pasteuria* treated potting soil, the experiment was terminated to prevent reinfestation from the second generation. Plants were unpotted, their roots rinsed with tap water and egg masses stained with a solution (15 mg/l) of phloxine B (Hartman and Sasser, 1985). Galls and egg masses on the roots of each plant were counted. Females were extracted from galls free of egg masses, crushed between slide and cover slip and observed for the presence of endospores under a stereo microscope.

Results and discussion

Exposure to 10^5 spores/g soil, resulted in most juveniles (94%) encumbered with at least 10 spores. In pots treated with 3.10^4 spores/g soil instead, only two-third of juveniles had that spore burden (Fig. 1).

Both spore concentrations suppressed significantly root galling ($P<0.01$) (Fig. 2) and egg mass production ($P<0.05$) (Fig. 3). Suppression was greater at 10^5 spores than 3.10^4 spores/g. soil. No endospores were found in females of *Meloidogyne* free of egg masses after 30 days at 28 °C. Hatz and Dickson (1992) observed a mature sporangium at 30 °C only after 40 days.

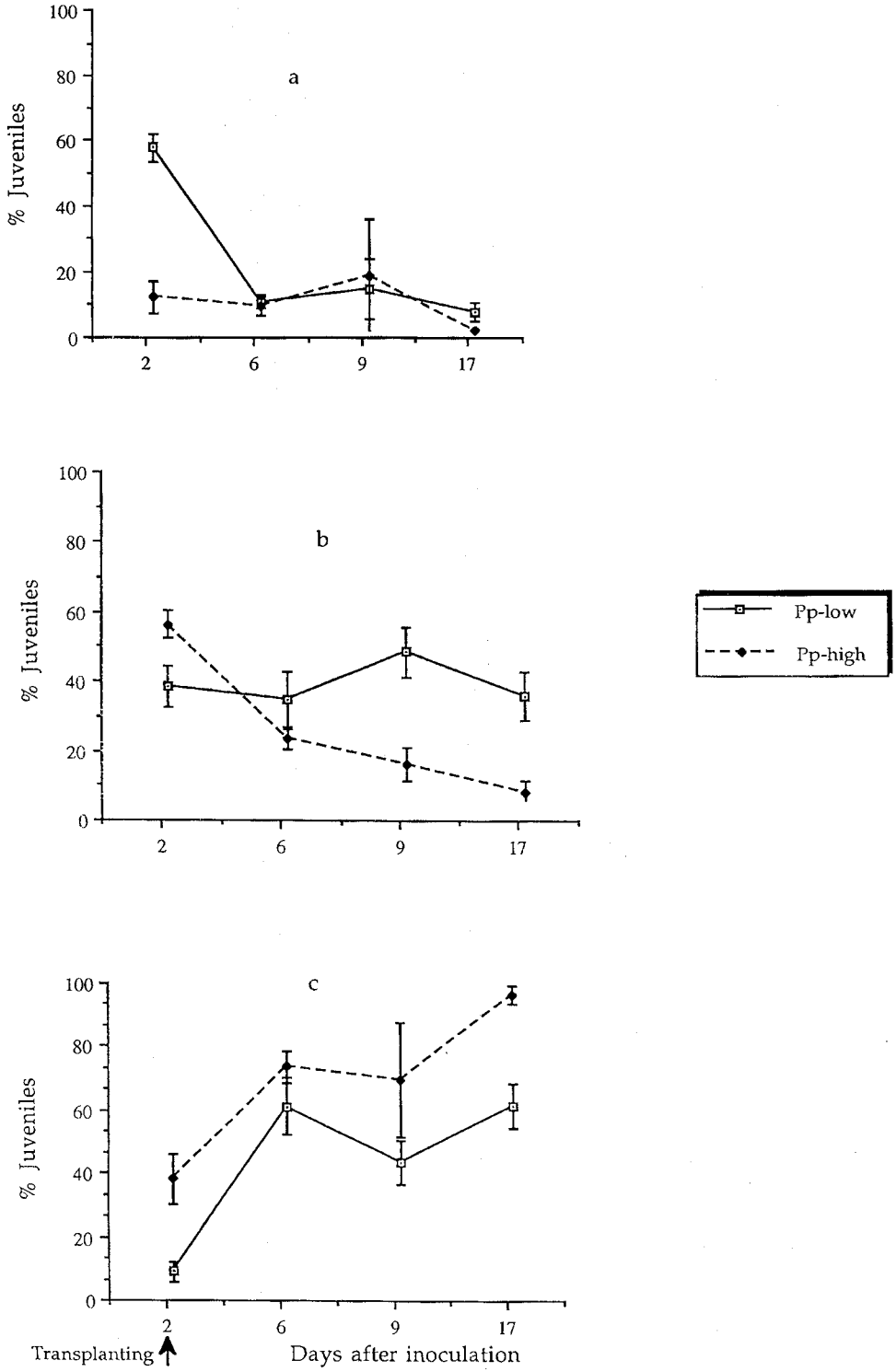

Fig. 1 - Spore burdens of juveniles of *Meloidogyne incognita* as affected by spore concentration and time of exposure to *Pasteuria penetrans* (Pp-low = 3×10^4 spores/g soil; Pp-high = 10^5 spores/g soil): a. unencumbered juveniles; b. juveniles with 1-9 spores; c. juveniles with 10+ spores.

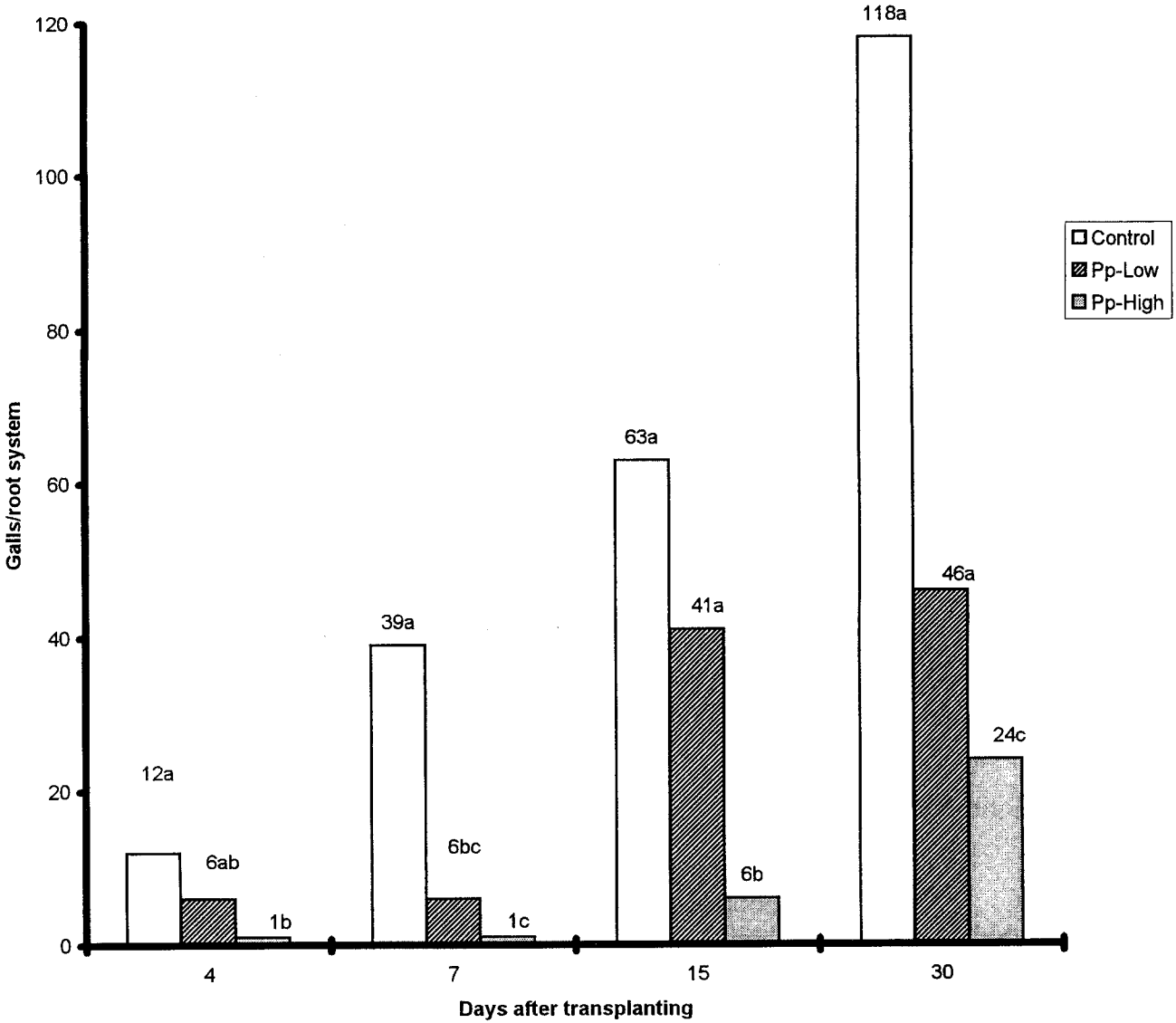

Fig. 2 - Infectivity of *M. incognita* as affected by spore concentration of *P. penetrans* and time of exposure to the bacterium (Pp-low=$3x10^4$ spores/g soil; Pp-high=10^5 spores/g soil). Values followed by the same letter are not significantly different ($P<0.05$).

Spore acquisition by second-stage juveniles occurred rather rapidly in soil. Only two days after inoculation, some juveniles were found encumbered with more than 20 spores. Moreover, extending the time of exposure of nematodes to spores had a significant effect on spore attachment which increased accordingly, and in proportion to spore concentration (Stirling *et al.*, 1990).

Spore burdens of nematodes affected root damage. Assessing root invasion from the number of galls per plant recorded, it appears that juveniles readily invaded roots in control pots whereas in *Pasteuria*-treated pots, infection was both retarded and suppressed proportionally to spore concentration. For an annual crop with a short cycle, these actions of the microbial agent may be of importance as yield components

could be determined before the build-up of subsequent generations, should reproduction not be inhibited by encumbering spores.

Retardation and suppression of root infection could be accounted for by the burden of spores which may physically impede nematode movement. Consequently, encumbered juveniles would take longer to locate their host-plants. As shown by the present data, in soil treated with the lower spore concentration, root galls occurred mainly between 7 and 15 days following inoculation; with the higher concentration instead, root infection took place during the last two weeks of exposure to the host-plant. Furthermore, in their slow movement towards the host-plant, spore encumbered juveniles may deplete most of their energy reserves so that root invasion, which requires more energy than motility, could be suppressed (Van Gundy *et al.*, 1967; Reversat, 1981), as in the present experiment.

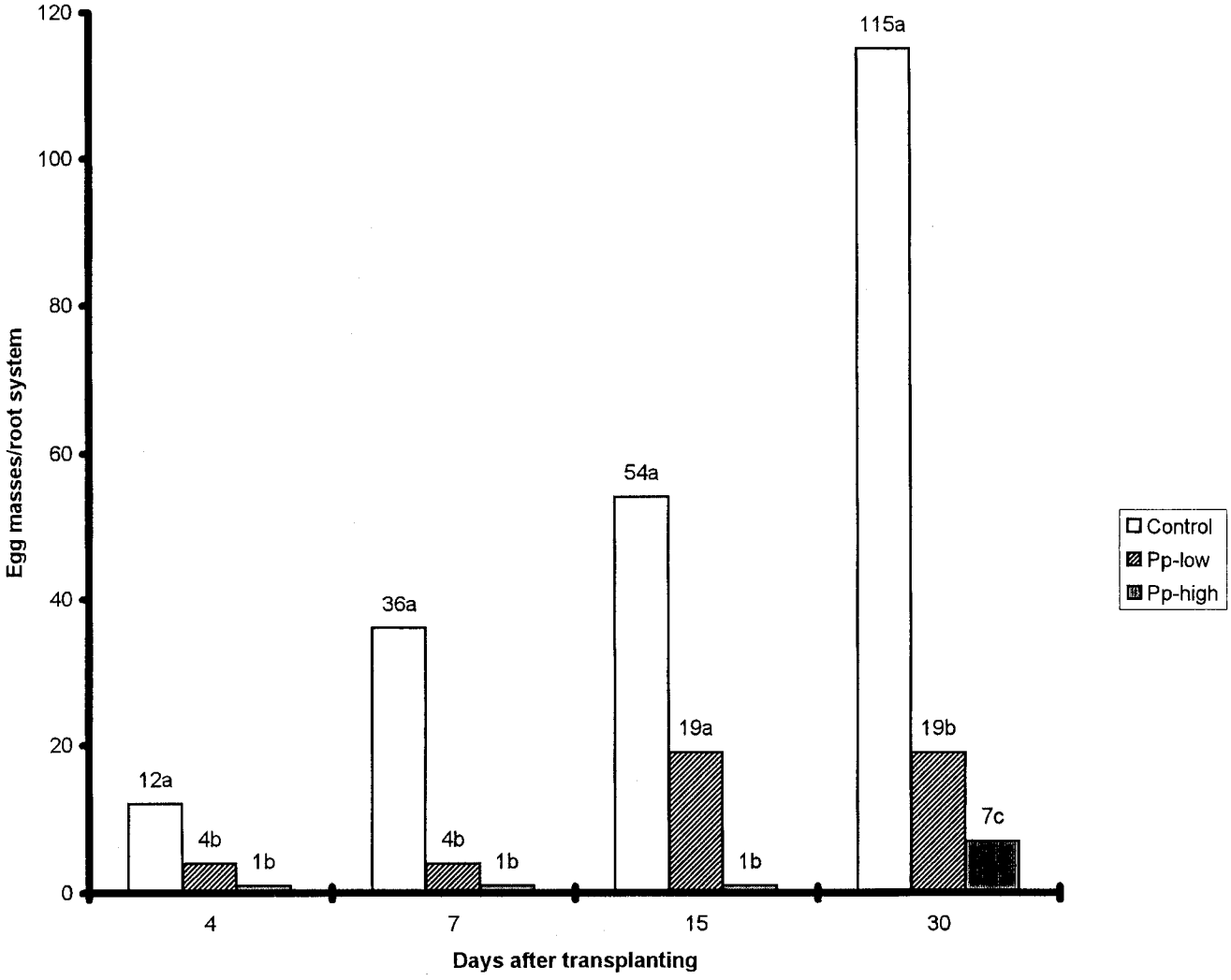

Fig. 3 - Production of egg masses by *M. incognita* as affected by spore concentration of *P. penetrans* and time of exposure to the bacterium (Pp-low=$3x10^4$ spores/g soil; Pp-high=10^5 spores/g soil). Values followed by the same letter are not significantly different ($P<0.05$).

The occurence of a greater proportion of lightly encumbered juveniles (1 to 9 spores) with Pp-low (34%) than Pp-high (6%) could account for the differential incidence of the bacterium recorded between the two levels of spore treatments. Data reported here suggest that spore burdens of 1 to 9 spores/juvenile may have a limited effect not only on nematode infectivity but also on nematode reproduction. According to previous work (Sayre and Wergin, 1977; Stirling, 1984) only 20 to 30% of attached spores germinate. The concentration of 10^5 spores/g soil in which up to 94% of juveniles were encumbered with at least ten spores acted as an efficient buffer against the parasitic activities of root-knot nematodes. These results are consistent with those of Stirling *et al.* (1990) who indicated that 10^5 spores/g soil could be the critical level of *P. penetrans* needed to control nematodes in the field.

This study shows that in assessing the pathogenicity of *Pasteuria* isolates it is important to determine the proportion of juveniles encumbered with at least ten spores, this being the spore burden mostly likely to impede nematode motility and preclude both infection and reproduction.

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