

Host specificity exhibited by populations of endospores of *Pasteuria penetrans* to the juvenile and male cuticles of *Meloidogyne hapla*

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Pasteuria penetrans (Thorne, 1940) Sayre & Starr, 1985 is an obligate endoparasite of root-knot nematodes, *Meloidogyne* spp., with potential to be developed into a biological control agent. Endospores of the bacterium attach to the cuticle of second-stage juveniles (J2) as they migrate in the soil towards plant roots and germinate when the J2 has established a feeding site and before the first moult. Different populations of endospores of the bacterium exhibit host specificity and one population of a bacterium is able to adhere to one population of the nematode but not to another (Davies *et al.*, 2001). Most studies have focused on the interaction of *P. penetrans* with the obligate parthenogenetic root-knot nematodes *M. arenaria*, *M. incognita* and *M. javanica*. More recently several authors have reported that endospores also attach to *M. hapla* (Carneiro *et al.*, 1999; Wishart *et al.*, 2004), a species that can reproduce by amphimixis. In attachment tests between two populations of *Pasteuria* from Florida, USA, to J2 and males of six populations of *Meloidogyne* spp. from Brazil, only a single male population became encumbered with endospores and these endospores failed to attach to J2 (Carneiro *et al.*, 1999). Therefore, we decided to test the hypothesis that endospores that attach to J2 would fail to attach to males and those that attach to males would fail to attach to J2. The experiment reported here is part of an ongoing study investigating *Pasteuria-M. hapla* interactions.

Three-week-old tomato plants (cv. Tiny Tim) were infected with a strain of *M. hapla* (VW9; Liu & Williamson, 2006) and were kept for 3 months in a glasshouse (20°C, 16 h day 8 h night) for root infection to take place and egg masses and root galls to develop. At harvest, roots were washed and galls and egg masses were then removed from the root system and placed on small hatching trays

in tap water (Whitehead & Hemming, 1965) for J2 and males to emerge. The emerged individuals were checked for endospore contamination before undertaking attachment tests. Two replicate attachment tests were undertaken by mixing 50 µl of water containing either J2 (n = 10) or males (n = 5) using a pipette with a 50 µl suspension of endospores (populations PP3 or RES 147; stock populations 5 × 10⁶ spores per ml in water) in a silicon coated microtube (Hewlett & Dickson, 1993). The mixture of J2 and endospores was centrifuged for 5 min at 9500 g and then observed using a binocular microscope (×400). From each replicated test, ten individual nematodes were identified at random and the endospores adhering to the cuticle were counted. The data were analysed by ANOVA and means and standard errors calculated.

Although large numbers of J2 were produced by *M. hapla*, only 23 males were produced and this limited the extent to which males could be tested in the attachment bioassay. The results of the attachment tests to both *P. penetrans* populations are given in Figure 1. The population PP3 of *P. penetrans* adhered with equal numbers to the J2 and males with a mean of seven endospores adhering to each individual J2 and male. However, males are significantly larger (approximately ×3) than J2 and, therefore, on the basis of spore numbers per unit area of cuticle the attachment is lower on the males than on the J2. Similarly, endospores of *P. penetrans* population RES147 showed a difference in their attachment; J2 were encumbered with less than one endospore per individual compared with over 30 endospores adhering to individual males and this should be taken into consideration when assessing attachment. The ratio between population of endospores (PP3:RES147) adhering to J2 and males is approximately 12:1 and 1:4, respectively. This

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Received: 3 April 2006; revised: 15 April 2006

Accepted for publication: 15 April 2006

Keywords: bacteria, biological control, encumbrance, root-knot nematodes

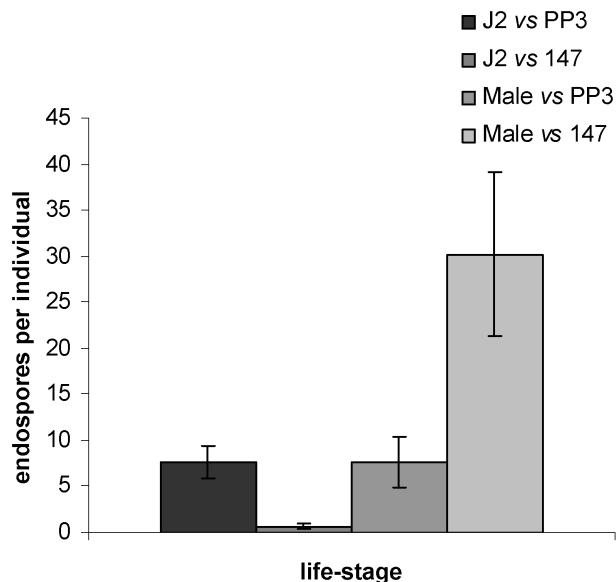


Fig. 1. Mean numbers of endospores of two populations of *Pasteuria penetrans*, PP3 and RES147, adhering to the second-stage juveniles (J2) and males of *Meloidogyne hapla* population VW9.

not only demonstrates a host-stage effect but also a host-specific interaction between the male cuticle and *P. penetrans* populations. Clearly, the nature of the specificity between endospores and J2 and endospores and males is different, and the data indicate that different attachment ligands are present on the cuticle of different lifestages.

Although we did not attempt to investigate whether or not the endospores adhering to the males germinated and infected these individuals and, therefore, were of biological significance, the phenomenon is of interest because it reveals another functional characteristic underpinning investigations of the biochemical nature of cuticle variation. As the genome of *M. hapla* population VW9 is currently being sequenced and the fact that *M. hapla* is meiotically parthenogenetic and, therefore, can reproduce by

amphimixis, this will enable traditional genetics to be undertaken and hence assist in elucidating the functional genetics involved in the molecular nature of cuticle variation and specificity in relationship to *P. penetrans* endospores.

Acknowledgement

Rothamsted Research receives grant-aided support from the Biotechnological and Biological Sciences Research Council of the United Kingdom.

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