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Affect of different ages of second stage juveniles of *Meloidogyne chitwoodi* on the attachment of *Pasteuria penetrans* to the cuticle of nematode.

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ABSTRACT

Pasteuria penetrans is a bacterial parasite of some plant parasite of nematodes which can attach to the cuticle of nematode. Differences in attachment of *P. penetrans* spores to the second stag juvenile (J2) cuticle may provide evidence for nematode cuticle diversity even between different ages of J2s. For investigating this purpose, we studied the effect of different ages of the same population of *Meloidogyne chitwoodi* second stage juveniles (J2) on the attachment of a specific *P. penetrans* spores. Each 200 freshly hatched, 7 and 12 day old J2 were exposed to 40000 spores of *P. penetrans.* The J2s-spores suspension were centrifuged and were examined with a microscope and attached spores were counted. There were significant differences ($P<0.05$) in spore attachment between fresh *M. chitwoodi* J2 and 12-day-old J2. Although there were no significant differences between spore attachment using fresh J2 and 7day-old J2, the numbers of spores attached to the 7-day-old J2 were lower than the numbers attaching to the fresh ones. From this study we can suggest that age of J2s can affect on spores attachment and this may help to have a successful management program.

Key words: Nematode's cuticle, Root knot nematode, *Pasteuria penetrans.*

INTRODUCTION

At present more than ninety species of root-knot nematode (*Meloidogyne*) has been described and about ten species are agricultural pests (Eisenback and Triantaphyllou, 1991). All members are obligate endoparasitic pathogens on plant roots and they are world-wide detected. *M. chitwoodi* has a wide host range among several plant families (Santo *et al*., 1980; O'Bannon *et al*., 1982), including crop plants and common weed species. Most of nematode possess a cuticle, the structure of which may be extremely variable not only between different taxa , but also intra specificity between sexes and developmental stages or different body region of an individual (Decraemer *et al*., 2003). As plant parasitic nematodes migrate through the soil towards a host root they come into contact with an array of different

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microorganism. Cuticle of nematodes is a site of attachment for various bacteria and fungi that are parasitic on nematodes (Bird and Bird, 1991; Spiegel and McClure, 1995). One of them can be *Pasteuria penetrans* which is a bacterial parasite of some plant parasite nematodes like *Meloidogyne* and is a very promising biological control agent against root-knot nematodes. The first stage in the infection process commences when second-stage juveniles (J2) migrating through the soil become encumbered with spores of the bacterium (Davies *et al*., 1991). According to the study of Trudgill *et al.*, (2000); *P. penetrans* can reduce the number of root knot nematodes significantly in some cropping systems. Differences in attachment of *P. penetrans* spores to the cuticle of nematodes and penetration indicate complex interactions between the dynamic cuticle of the nematode and the surface of the bacterial parasite, and observations of patterns of *Pasteuria* attachment provide evidence for nematode cuticle diversity (Davies *et al*., 2001). Another attachment testes have done by Lopez et al.,(2000) and Akhkha *et al*., (2002), they have shown phytohormones as well as other molecules present in root diffusate, trigger a rapid alteration of the surface cuticle of sedentary plant-parasitic nematodes. Therefore in this work, we have evaluated the effects of different ages of *M. chitwoodi* J2 (fresh, 7 and 12 day old) on the attachment of *P. penetrans* to the cuticle of nematode.

MATERIAL AND METHOD

Two week old seedlings of tomato (*Lycopersicon esculentum*) cv. Money Maker (Royal Sluis) were transplanted in 13 cm diam. plastic pots filled with sterilized (100°C, 12 h) soil (sand 87%, loam 9%, clay 4%). Tomato was used because of the ease of culture and it is an annual crop that is an excellent host for *M. Chitwoodi* .With bearmann funnel technique about 2000-3000 hatched juveniles of pure *Meloidogyne chitwoodi* which are obtained from the diagnostic lab of ILVO, Merelbeke, Belgium, were inoculated near the root of tomatoes to culture them. Six or seven weeks after inoculation, the infected roots of the tomato plants were washed and extracted with the Baermann funnel technique. The extraction of nematodes with the Baermann funnel technique (Hooper, 1986) is based on the motility of nematodes and enables them to be separated from soil or organic material. Roots were cut into small fragments which were put on a filter paper (Ederol Rundfilter, 40 g/m2, Munktell Filter AB, Falun, Sweden) that was lined in a sieve (mesh 2 mm). The sieve was put on top of a glass funnel filled with tap water and checked regularly that the water and roots were in continuous contact during the extraction period. The stem of the funnel was connected with a rubber tube that was closed with a clip. Juveniles that hatched from egg masses moved through the filter paper and sieve and accumulated at the bottom of the rubber tube. Nematodes were collected in a conical flask by opening the clip for a few seconds. After one day the nematodes had settled at the bottom of the flask and excess water was removed to concentrate the nematode suspension. The nematode suspension was poured into a counting dish and the number of nematodes was determined. Concentrations of 1000 nematodes/ml were used for the experiments. Juveniles were stored in tap water at room temperature after hatching to obtain 7 and 12-day-old J2. J2s older than 12 days were no longer visibly alive. Spores of *P. penetrans* population RES147 isolated from *M. javanica* (provided by Rothamsted Research, UK) were used. A 0.2 ml aliquot of a 1000 J2/ml suspension was pipetted into a silinized Eppendorf tube (1.5 ml); these are plastic tubes treated to eliminate static electricity and prevent hydrophobic interactions, therefore avoiding spores sticking to the tubes. Then 0.2 ml of a 2×10^5 *P. penetrans* spores/ml suspension were added to the J2s and were spun down at 10,000 rpm for 5 min in an Eppendorf centrifuge machine (Hewlett and Dickson, 1994).The supernatant was removed and re-suspended in 100 µl tap water. Using a pipette, a small amount of the suspension was put on a glass slide, covered with a cover slide and the slide was passed over an alcohol flame, resulting in relaxation of the muscles and prevention of nematodes movement. Spore attachment

was assessed by examining J2s using a microscope (x400, Olympus). Spores adhering to the cuticle of J2s were counted (100 individual J2 per treatment). The experiment was repeated twice.

The experiment was carried out in completely randomize design (CRD), in six replicates. For the statistical analyses, SPSS 16 was used. Data were subjected to analysis of variance (ANOVA) and significant or non-significant differences were determined with LSD-tests ($P < 0.05$).

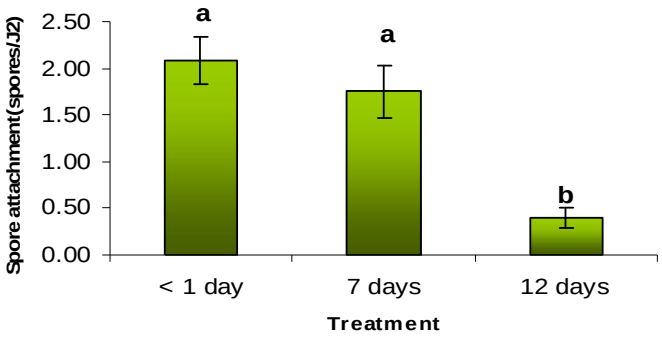
RESULTS

The statistical analysis of results of evaluating the effect of age of J2 on spore attachment is shown in Table 1. Significant differences were found between different age of J2 (fresh, 7, 12 day old) on spore attachment (Table 1 and Fig 1).

Table 1: Effects of variable (age of J2) for the spore attachment on surface cuticle of *Meloidogyne chitwoodi* J2 (Anova P<0.05)

Var.	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.89	2	7.44	**23.64**	**.000**
Within Groups	4.728	15	0.315		
Total	19.6212	17			

Figure 1: Mean number of spore attached to per *Meloidogyne chitwoodi* J2 using different age of juveniles (treatments). Error bars represent standard error. Different letters indicate that means are significantly different from each other at $P < 0.05$.



According to LSD test there were significant differences (P<0.05) in spore attachment to *M. chitwoodi* J2 with significantly fewer spores attaching to 12-day-old J2 compared with the number attaching to 7-day-old or fresh J2. The mean number of spores attaching to fresh J2 was over 2 per J2, whereas the mean number attaching to 12-day-old J2 was less than 0.5 per J2. Although there were no significant differences between spore attachment using fresh J2 and 7-day-old J2, the numbers of spores attached to the 7-day-old J2 were slightly lower than the number attaching to fresh ones. In this experiment the spore

attachment to the cuticle of *M. chitwoodi* J2 of different ages (fresh, 7 days and 12 days) was determined. There were significant differences ($P<0.05$) in spore attachment between fresh *M. chitwoodi* J2 and 12-day-old J2. Although there were no significant differences between spore attachment using fresh J2 and 7-day-old J2, the numbers of spores attached to the 7-day-old J2 were lower than the number attaching to fresh ones.

DISCUSSION

The present work has shown interesting differences between the numbers of spores attaching to cuticle of different age of second stag juveniles (J2s). However, there is a reduction in number of attached spore to the cuticle of J2s when the J2s getting old. Significant difference in *P. penetrans* spore attachment to the nematode cuticle of different ages may be explained by the modification of some component of nematode's cuticle, when the J2s are getting old. Although, we did not analyse the composition of the juvenile cuticle with different ages but from this investigation, we can assume surface coat of cuticle of *Meloidogyne chitwoodi* may alter with different environmental condition like starving and this effect on behaviour of *M. chitwoodi* against different microorganism. This evidence support previous findings of Spiegel *et al.* (1995) who reported that the binding of HRBC (human red blood cell), antibodies to surface cuticle, lectins and neoglycoproteins to *M. javanica* J2 surfaces was found to be dependent on nematode age and temperature. Also our finding, confirms the work of Bert M. Zuckerman and Itzhak Kahane (1983) that showed stage specific differences in cuticle surface carbonhydrates in *Caenorhabditis elegans.* Lopez *et al.*, (2000) and Akhkha *et al.*, (2002) denoted phytohormones as well as other molecules present in root diffusate, trigger a rapid alteration of the surface cuticle of sedentary plant-parasitic nematodes. There is some evidence that starved nematodes, including J2 of *M. javanica* and *Tylenchulus semipenetrans*, can use endogenous amino acids as an energy source (Barrett and Wright, 1998).

Conclusion

We can conclude starving of the nematodes can be result of altering of some component of the nematode's surface coat and this effect on attachment test.As plant parasitic nematodes migrate through the soil towards a host root they may come into contact with *P. penetrans* spores and more knowledge about the condition of spore attachment and diversity of cuticle with different ages of J2s can help to optimise for a successful biological control agent against root knot nematode. Observations of the changes of the surface cuticle of J2s with different ages using suitable probes have be done in further.

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