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Development of *Pasteuria penetrans* in *Meloidogyne javanica* females as affected by constantly high vs fluctuating temperature in an in-vivo system

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Abstract: Growth room and glasshouse experiment was conducted to investigate the effect of constant and fluctuating temperatures on the development of *Pasteuria penetrans* a hyperparasite of root-knot nematodes. Tomato plants (*Lycopersicon esculentum* Mill) were inoculated with *Meloidogyne javanica* second-stage juveniles attached with endospores of *P. penetrans* and were grown in growth room at 26–29 °C and in glasshouse at 20–32 °C. The tomato plants were sampled from the growth room after 600 degree-days based on 17 °C/d, accumulating each day above a base temperature of 10 °C and from the glasshouse after 36 calendar days. Temperature affected the development of *P. penetrans* directly. The rate of development at constant temperature in growth room was faster than that in the glasshouse at fluctuating temperatures.

Key words: *Pasteuria penetrans*, *Meloidogyne javanica*, Development and tomato plants
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INTRODUCTION

Pasteuria penetrans is an obligate, endospore-forming bacterial parasite of *Meloidogyne* spp. and has shown potential as a biological control agent against root-knot nematodes (Chen *et al*., 1996; Dickson *et al*., 1994) in pot experiments (Channer and Gowen, 1988) and micro-plot experiments (Daudi *et al*., 1990; Trivino and Gowen, 1996). There are practical and technical problems associated with mass production of *P. penetrans* inoculum. In-vivo systems, although proven (Stirling and Wachtel, 1980), are unlikely to be adopted for large-scale treatments in most farming systems. If greater use is to be made of in-vivo production techniques, refinements are needed which can only be developed with improvements based on a better understanding of the biological factors involved.

The durations and temperatures when mature endospores are first seen in female root-knot nematodes have been recorded (Stirling, 1981). Furthermore, Davies *et al*.(1988) and Giannakou *et al*.(1997) showed that temperature fluctuations in the glasshouse might retard the development of the parasite. The objective of this work was to determine the effect of different temperature schemes on the development of *P. penetrans* and number of endospores on a per nematode basis at constant temperatures known to be suitable for *P. penetrans* and in a fluctuating temperature regime such as might occur in a glasshouse production system.

MATERIALS AND METHODS

Pasteuria penetrans, originally collected from South Africa but cultured for over 10 years on root-knot nematode hosts at the University of Read-

ing, was established on a population of *M. javanica* from Pakistan. A spore suspension was prepared by squashing with fine forceps infected females in 0.5 ml water in a small Eppendorf tube and homogenising for 30 s. To the suspension was added 11000 freshly hatched *M. javanica* J2s which resulted in a spore concentration of 1.22×10^4 spores/ml. After agitation for 2–3 min this was divided between four Petri dishes, which were placed in an incubator at 28 °C to enable a large surface air interface to prolong nematode activity. Spore attachment was monitored hourly until 90% of juveniles were encumbered with 6–10 spores. Then suspensions were combined in one beaker and poured through a 20 μm sieve to separate nematodes from remaining spores in suspension. Eight hundred spore-encumbered J2s were inoculated on 6-week old tomato plants (*Lycopersicon esculentum* Mill) cv. Tiny Tim, a dwarf, determinate variety, growing in a loam-based proprietary compost (John Innes No. 2) in 500 cm^3 plastic pots into which they had been transplanted 1 week earlier. For 48 h after inoculation, all plants were kept in the growth room at 26–29 °C to ensure good invasion of nematodes. Then half of the plants were transferred to a glasshouse at 20–32 °C. Each treatment was replicated five times using a randomized block design. All plants were usually watered daily sufficiently to prevent wilting. The daily temperature was recorded by setting electronic temperature loggers (Tiny Talk-II ISO EN 9002) and a probe of each was inserted in soil near to the pots. After three weeks, all pots in the glasshouse were put in horticultural planter bags [92 cm×28 cm×27 cm] containing loam-based compost. The plants from the growth room and glasshouse were sampled after 600 degree-days (equivalent to 36 d) based on 17 °C/d, accumulating each day above a base temperature of 10 °C.

After harvesting, all roots were placed in a freezer for 5 d. Upon thawing, the roots readily disintegrated when probed with needles and female root-knot nematodes were collected from the separated root tissue. Fifty endospore-filled females were then individual squashed with fine forceps in a small Eppendorf tube containing 0.5 ml of water and homogenised for 30 s. The suspensions in the Eppendorfs were then rinsed into small glass bottles with 0.5 ml of water. Three aliquots per sample were taken to count the numbers of developing stages (micro colonies, fragmented micro colonies, doublets) and mature endospores of *P. penetrans* at ×400 magnification using a haemocytometer.

RESULTS

The temperature had great effect on the development of *Pasteuria penetrans*, a hyperparasite of root-knot nematodes. The rate of development was found faster and began earlier in the growth room at constantly higher temperature than in the glasshouse at fluctuating temperatures. There was a highly significant ($P<0.001$) difference in the number of micro colonies and was almost three times higher in the glasshouse treatment than in the growth room treatment (Fig.1). At fluctuating temperatures the parasite development was not only slower but was more prolonged than that at the constant temperature. There was a highly significant ($P<0.001$) difference in the number of mature endospores per female (Fig.2). The highest number of endospores was produced in the growth room. There was a greater difference between glasshouse and growth room in the development of *P. penetrans*. At 36 calendar days (600 degree-days) in the glasshouse treatment, the development of *P. penetrans* was slower than that in the growth room treatment, which was clearly indicated by the fewer doublets and mature endospores. However, more micro colonies and fragmented micro colonies were found in the glasshouse treatment than in the growth room treatment (Fig.1).

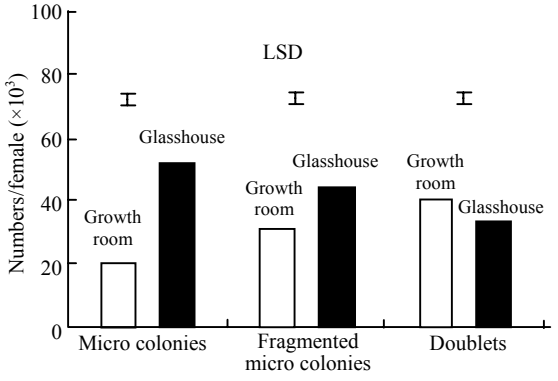

Fig.1 Effect of constant and fluctuating temperatures on the development of *Pasteuria penetrans* at the growth room and the glasshouse. Vertical bars represent the least significant difference (LSD) of means (data are means of five replicates)

DISCUSSION

This research was done to determine the conditions necessary to maximize the production of endospores of *Pasteuria penetrans* on a per nematode basis. It was hypothesised that more spores would be produced in nematodes at constantly higher temperature than at fluctuating temperature. Hatz and Dickson (1992) reported that with increasing temperature the development of *P. penetrans* and the formation of mature endospores began earlier. After 600 degree-days in the growth room, it has been observed that continuous higher temperature promoted the faster development of nematodes which indirectly promoted the development of hyper parasite *P. penetrans*, as clearly indicated by the higher number of endospores produced at constantly higher temperature in the growth room (Fig.2).

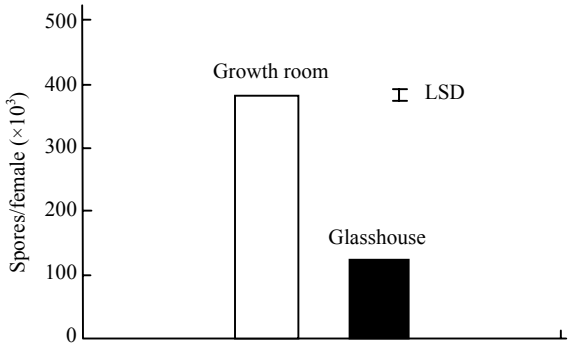

Fig.2 Effect of constant and fluctuating temperatures on the production of endospores of *Pasteuria penetrans* per female in the growth room and the glasshouse. Vertical bar represents the least significant difference (LSD) of means (data are means of five replicates)

Plants in the glasshouse at fluctuating lower temperature were healthier than those in the growth room and produced bigger root systems. It could explain that healthier and bigger root systems of the glasshouse plants provided more space and less food competition than those in the growth room where the slower the nematode development indirectly affected the development of *P. penetrans*.

Giannakou *et al.*(1999) found that a bigger root system provides better conditions for the development of female nematodes which in turn provides a better medium for the development of the parasite. Studies by Hatz and Dickson (1992) also showed that there is coincident development by host (nematode) and hyperparasite (*Pasteuria*). These results show that in-vivo systems of production are feasible. In field-grow production there will be less diurnal temperature fluctuation and more natural conditions than in a glasshouse or the growth room.

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