FACTORS AFFECTING BIOLOGICAL CONTROL EFFECTIVENESS
OF PASTEURIA PENETRANS IN MELOIDOGYNE JAVANICA AND
THE BACTERIAL DEVELOPMENT IN THE NEMATODE BODY

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ABSTRACT

penetrans in Meloidogyne javanica and the bacterial development in the nematode body. Nematropica
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The invasion and infectivity of Meloidogyne javanica juveniles (J2) encumbered with spore of Pas-
teuria penetrans were influenced by the temperature and the time J2 were in the soil before exposure
to roots. The percentage of infected females decreased as the time juveniles spent in soil increased.
When spore encumbered J2 were maintained at 30°C the decrease in infection was greater than that
at 18°C. The thermal time requirements and the base temperature for P. penetrans development were
estimated. The rate of development followed an exponential curve between 21 and 36°C and the base
temperature for development was estimated by extrapolation to be 18.5°C. The effect of integrating
a nematode resistant tomato cultivar with the biocontrol agent P. penetrans also was investigated. The
ability of the biocontrol agent to reduce numbers of root-knot nematodes was dependent on the den-
sities of the nematode and P. penetrans spores in the soil.

Key words: base temperature, biological control, integrated control, Meloidogyne javanica, resistant cul-
tivar, thermal time.

INTRODUCTION

The obligate endospore forming para-
site Pasteuria penetrans has been attributed
to the partial control of root-knot nema-
todes Meloidogyne spp. in pot experiments
(Channer and Gowen, 1988) and in microplots using either spore-infested soil

RESUMEN

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Pasteuria penetrans de Meloidogyne javanica y el desarrollo de la bacteria en el cuerpo del nemátodo.
Nematropica 34:153-163.

La invasión e infectividad de juveniles (J2) de Meloidogyne javanica estorbados con esporas de Pas-
teuria penetrans fueron afectadas por la temperatura y el tiempo en que los J2 se encontraban en el
suelo antes de ser expuestos a las raíces. El porcentaje de hembras infectadas subía con la cantidad
de tiempo que los juveniles pasaban en el suelo. Cuando J2 estorbados con esporas se mantuvieron
a 30°C la disminución de infección era más que cuando se mantuvieron a 18°C. Se estimaron los re-
quisos de tiempo termal y temperatura básica para el desarrollo de P. penetrans. La tasa de desarrollo
seguía una curva exponencial entre 21 y 36°C y la temperatura básica para el desarrollo fue estimada
por extrapolación a 18.5°C. Se investigó además el efecto de la integración de un cultivar de tomate
resistente y el agente de control biológico P. penetrans. La capacidad del agente de control biológico
para reducir el número de nemátodos agalladores dependió de las densidades del nemátodo y de las
esporas de P. penetrans en el suelo.

Palabras clave: control biológico, control integrado, cultivar resistente, Meloidogyne javanica, temper-
atura básica, tiempo termal.
or powdered roots containing spores (Daudi et al., 1990; Trivino and Gowen, 1996; Trudgill et al., 2000). *Pasteuria penetrans* has also been reported to contribute to supressiveness in groundnut and tobacco monoculture soils (Weibelzahl-Fulton et al., 1996; Chen et al., 1996). *Pasteuria penetrans* also has been used in conjunction with chemicals (Tzortzakakis and Gowen, 1994) in an integrated approach to root-knot nematode management. For development of *P. penetrans* spores must attach to the cuticle of the second stage juvenile (J2). Spores subsequently germinate penetrate body wall, and proliferate within the nematode body. The invasion of roots by spore-encumbered juveniles is negatively correlated to the distance they have to move through soil to locate roots (Stirling, 1984). Movement of spore-encumbered J2 in soil might be impeded by mechanical forces thus affecting their ability to locate roots (Ciancio, 1995). The attachment of 40 spores per juvenile significantly effects their infectivity (Stirling, 1981), and even as few as 15 spores per juvenile can reduce invasion by more than 70% (Davies et al., 1988). There was no differences in the percentage of infection of developing females originating from spore-encumbered juveniles due to the age of juveniles, when these were aged in water at 25°C (Davies et al. 1991). However, maintaining the second-stage juveniles in soil prior to planting had a significant effect on the percentage of infected females (Davies et al., 1988).

Temperature plays a major role in the development of *P. penetrans* (Giannakou et al., 1997; Stirling, 1981). Chen and Dickson (1997) reported 17°C as the minimum temperature for development of *P. penetrans* on a *M. arenaria* population. It might be expected that the thermal requirements of *P. penetrans* will be similar to those of its nematode hosts. Bird and Wallace (1965) reported good growth of *M. javanica* in tomato roots between temperatures of 20 to 30°C with an optimum of 25 to 30°C. Trudgill (1995) fitted the base temperature \( T_b \) for *M. javanica* development at 13°C while the optimum temperature \( T_o \) was 27-28.5°C. Madulu and Trudgill (1994) showed a linear relationship for *M. javanica* development between mean temperatures of 18 to 27°C. They concluded that at 30°C, *M. javanica* development was more rapid than at 27°C but the rate of development was less, indicating that the optimum lies somewhere between 27°C and 30°C (Madulu and Trudgill, 1994). However the upper threshold for *M. javanica* exceeds 35°C (Thomason and Lear, 1961).

The present study was conducted to determine the effect of age on development of *M. javanica* spore encumbered juveniles in two different temperature regimes and to estimate the base temperature and degree-day requirements. The second objective was to evaluate the effect on root-knot nematode population of a resistant tomato cultivar (‘Sinatra’) integrated with the biocontrol agent *P. penetrans*.

**MATERIALS AND METHODS**

**Nematode and Bacterium Cultures**

A population of *Meloidogyne javanica* originally obtained from tomato roots in Malawi was reared on tomato (*Lycopersicon esculentum*) Mill. cv. Tiny Tim and maintained in a temperature-controlled glasshouse at 25-35°C. Eggs were extracted with 1% sodium hypochlorite solution (Hussey and Barker, 1973). Second-stage juveniles (J2) were allowed to hatch in a modified
Biological control of *M. javanica*: Giannakou & Gowen

Baermann funnel. All J2 hatched in the first 3 days were discarded and thereafter, J2 collected after 24 h were used in the experiments. An isolate of *P. penetrans* (Pp3) originally isolated from tomato roots infected with *M. javanica* in South Africa was cultured on *M. javanica* following the procedure described by Stirling & Wachtel (1980). To obtain spore-encumbered J2, fresh juveniles were placed in a spore suspension $3.85 \times 10^5$ spores/ml until 90% of juveniles were encumbered with the adequate numbers of spores when J2 were rinsed with tap water over a 10?m sieve to remove unattached spores. The spore burden was assessed by counting a sample of twenty J2 with the aid of an inverted microscope at ×200 magnification.

*Development of P. penetrans as Affected by the age of juveniles of M. javanica Kept in Soil at Different Temperatures*

The experiment was a factorial design with two treatments of *P. penetrans* (*-Past* and *+Past*), two temperature levels (18 and 30°C) and four different juvenile ages (day of transplanting). Eighty 1-liter plastic pots were filled with loam-based compost (John Innes No 2) and watered with 100 ml tap water. They were then infested with about 1650 J2 in 2.5 ml water to a hole where the root ball of tomato plants was going to be placed. Forty pots were inoculated with spore encumbered J2 (a mean of eight spores per J2) and the other 40 with spore-free-J2. Immediately after inoculation twenty pots of each group were placed in a growth cabinet at 18°C while another 20 of each group were placed in a growth room at 30°C. Tomato plants (one plant per pot) were transplanted after 1, 4, 8 and 12 days after which all plants were placed in the growth room maintained at 30°C. Plants were uprooted and roots washed free of soil 45 days after transplanting. The pots were arranged in a completely randomized design.

*Estimation of Base Temperature and Degree-days for P. penetrans Development*

The thermal time requirements for *P. penetrans* development were determined by measuring the minimum time for completion of the life cycle over a range of constant temperatures. The life cycle was considered the period from the time of root infection by spore-encumbered J2 to the time when mature endospores were first observed in females. Single tomato plants cv. Tiny Tim (4 to 6 leaves) were grown in sterilized loam based soil in clear plastic tubes (3 cm diam. and 10 cm depth) with 10 small drainage holes. The tubes were placed in sterilized sand in two-liter plastic pots without holes. Five hundred juveniles encumbered with a mean of 8 spores each were inoculated into the soil in the tubes. The tubes were maintained at 28°C for 2 days which they were removed. The two-liter plastic pots with tubes were placed in water baths at 21, 24, 27, 30, 33 and 36°C. There were two pots at each temperature each containing 15 tubes. A thermocouple was inserted into one pot in each water bath to monitor and record the soil temperature throughout the experiment. After ten days when the first galls have been formatted, 2 cm pieces of galled roots containing at least 15 to 20 nematodes were removed from roots and examined to monitor *P. penetrans* development. When quartets (Serracin *et al.*, 1997) were first observed root sampling was done on a daily basis. Development of the bacterium was considered to be completed when mature endospores were observed. The rate of development was plotted against temperature and an exponential regression derived.
Effect of a Resistant Cultivar Integrated with the Biocontrol Agent Pasteuria penetrans

**Experiment 1:** Four tomato seedlings (cv. Tiny Tim; four leaves stage), grown in a loam-based compost (John Innes No 2) were transplanted in 20-liter plastic pots and maintained for 40 days in a glasshouse at temperatures 25-40°C. After that period each pot was inoculated with 50,000 *M. javanica* J2. The tops of each plant were removed after 60 days leaving the roots in the pots. The soil containing infected roots was then mixed thoroughly to distribute the egg masses and J2. A total of 24 pots were divided into six groups each representing one treatment. The six treatments were as follows: 1) high density (HD) of *P. penetrans* spores plus susceptible cultivar Shirley, which was highly susceptible to root-knot nematode; 2) low density (LD) of *P. penetrans* spores plus susceptible cultivar; 3) HD of *P. penetrans* spores plus resistant cultivar Sinatra; 4) LD of *P. penetrans* spores plus resistant cultivar; 5) a susceptible cultivar; and 6) resistant cultivar. High and low *P. penetrans* spores densities were 20,000 and 10,000 spores/g of soil, respectively, and this was achieved by applying the appropriate volume of spore suspension in each pot. Tap water without spores was added in control treatments. The soil moisture was kept at field capacity to obtain maximum movement of J2 after hatching and maximize the chances of contact of spores and nematodes. One week later tomato plants (6 to 8 leaf stage) were transplanted. The plants were maintained in the glasshouse for 60 days at temperatures of 18 to 33°C after which the roots were washed free of soil. Galling was assessed according to a 1 to 10 scale (Bridge and Page, 1980). The roots and soil were kept in a glasshouse until they had air dried at 25°C thus all stages of nematodes had been killed either in the roots or soil. After that period the dried roots were ground in a coffee grinder and the resulting powder was distributed in the soil from the same pot. Then the soil in each pot was watered carefully to avoid loss of spores through leaching and inoculated with 50,000 freshly hatched J2 of *M. javanica*. After 2 days in the glasshouse at 23 to 35°C three tomato seedlings (either F1 hybrid cv. Shirley or cv. Sinatra) were transplanted to each pot. After two months the plants were carefully uprooted, the roots within each replicate washed free of soil and were assessed according to the 1 to 10 galling index (Bridge and Page, 1980). All data were subjected to analysis of variance for the first and second crop separately. Means were compared using the LSD test.

**Experiment 2:** Sixty 1.5-liter plastic pots containing commercial loam-based compost (John Innes No. 2) were divided into three groups of 20 pots each and were inoculated with 1,000, 5,000 and 10,000 freshly hatched J2 of *M. javanica*. Immediately, all pots were covered with aluminium foil to avoid water evaporation and placed in a growth room with 16 h day and 8 h night period at temperatures of 31 and 25°C, respectively. After 24 h each pot was inoculated with a spore suspension of *P. penetrans* to achieve a concentration of 40,000 spores/g of soil. Pots were again covered with aluminium foil and after 2 days tomato seedling were transplanted. Ten ‘Shirley’ or ‘Sinatra’ seedlings (five leaf stage) were transplanted to equal numbers of pots in each inoculation group. All pots were maintained in a growth room for 40 days with 16 h day and 8 h night period at temperatures of 31 and 25°C, respectively. The plants were then carefully uprooted, roots were washed free of soil and egg masses were stained by submerging the entire root system in 0.1% phloxine B for 15 min (Southey, 1986).
Then, roots were rinsed with tap water and females and egg masses were counted with the aid of a stereoscopic microscope.

All data were subjected to a factorial analysis of variance of the SAS GLM procedure and treatments compared using the LSD test.

RESULTS

Development of *P. penetrans* as Affected by the Age of the Juveniles of *M. javanica* in Different Temperatures

It was noticeable that the duration between applying nematodes in soil and root infection had influenced the survival rate (Table 1). In pots planted immediately after inoculation more females developed at 30°C without *P. penetrans* than the other treatments (*P* < 0.05). The numbers of females decreased as the inoculum stayed longer in the soil. The decrease was greater for juveniles in pots at 30°C than 18°C. There was no difference in the number of females that developed in plants among the treatments after 4 days (*P* > 0.05). More females were developed in plants on the 8th day at 18°C from J2 non encumbered with *P. penetrans* spores. Similar numbers of females developed in plants on the 12th day for both temperature regimes from J2 non encumbered with *P. penetrans* spores.

Higher percentage of females produced egg masses on all days for both temperature regimes in the non *P. penetrans* treatment compared to the treatment in which J2 were encumbered with *P. penetrans* spores (Table 2). The percentage of females which produced egg masses was not significant on the 1st day for the J2 encumbered with *P. penetrans* at both temperatures while it was constantly higher at 30°C than 18°C on the 4th, 8th and 12th days (*P* < 0.001).

There was no significant difference in the number of *P. penetrans* infected females developed from J2 invading on the 1st day (*P* > 0.05) (Table 3). However, more *P. penetrans* infected females developed from J2 invading on the 4th, 8th and 12th days at 18°C than 30°C (Table 3).

Estimation of Base Temperature and Degree-days for *P. penetrans* Development

Temperature affected the length of *P. penetrans* life cycle. Mature endospores were detected as early as 33 and 34 days at

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Treatment</th>
<th>Age of juveniles (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>18°C</td>
<td>- Pasteuria</td>
<td>199 b</td>
</tr>
<tr>
<td></td>
<td>+ Pasteuria</td>
<td>133 b</td>
</tr>
<tr>
<td>30°C</td>
<td>- Pasteuria</td>
<td>337 a</td>
</tr>
<tr>
<td></td>
<td>+ Pasteuria</td>
<td>205 b</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>122.5</td>
</tr>
</tbody>
</table>

Table 1. Numbers of females per root system of tomato as affected by the age of juveniles, presence of spores and temperature.

In each column, data followed by the same letter are not significantly different.
33 and 30°C, respectively. At 21°C the *P. penetrans* life cycle was completed in 107 days. The rate of development expressed as the reciprocal of the time (in days) taken for the formation of mature endospores at the six temperatures was plotted (Fig. 1). Plotting the duration of development at four constant temperatures, an exponential line can be reasonably fitted between a temperature range of 21 to 30°C. Back extrapolation of this line was done to provide an estimate of the base temperature. The line crosses x axis at 18.7°C.

Effect of a Resistant Cultivar Integrated with the Biocontrol Agent *Pasteuria penetrans* on the Control of Root-knot Nematodes

**Experiment 1:** Root galling was lower in the resistant cultivar than the susceptible one (*P* < 0.001) (Fig. 2). Conversely root galling was not affected by the presence of *P. penetrans* spores in soil in the first crop as shown by the galling index between the high density (HD) and low density (LD) treatments (*P* > 0.05). However, the impact of *P. penetrans* spores in soil was clearly demonstrated in the second

Table 2. Percentage (%) of females with egg masses as affected by the age of juveniles, presence of spores and temperature.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Treatment</th>
<th>Age of juveniles (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>18°C</td>
<td>- Pasteuria</td>
<td>100% a</td>
</tr>
<tr>
<td></td>
<td>+ Pasteuria</td>
<td>42% b</td>
</tr>
<tr>
<td>30°C</td>
<td>- Pasteuria</td>
<td>100% a</td>
</tr>
<tr>
<td></td>
<td>+ Pasteuria</td>
<td>49% b</td>
</tr>
<tr>
<td><em>P</em> &lt;</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>LSD</td>
<td>7.33</td>
<td>7.10</td>
</tr>
</tbody>
</table>

In each column, data followed by the same letter are not significantly different.

Table 3. Percentage of infection of *Meloidogyne javanica* by *Pasteuria penetrans* as affected by the age of juveniles and temperature.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Age of juveniles (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>18°C</td>
<td>56 a</td>
</tr>
<tr>
<td>30°C</td>
<td>41 a</td>
</tr>
<tr>
<td><em>P</em> &lt;</td>
<td>0.05</td>
</tr>
<tr>
<td>LSD</td>
<td>17.13</td>
</tr>
</tbody>
</table>

In each column, data followed by the same letter are not significantly different.
crop since the enrichment of soil with spores reduced the galling level of roots of the susceptible cultivar. Root galling reduction was higher in the HD treatment (4.7) than the LD treatment (7.1) \((P < 0.001)\). There were fewer galls in the resistant cultivar than the susceptible one \((P < 0.001)\). This effect is more pronounced in the second crop due to enrichment of soil with \(P. penetrans\) spores.

**Experiment 2:** An interaction was detected for cultivars, \(P. penetrans\) treatment and inoculation levels \((P < 0.001)\); thus data for the different cultivars are presented separately (Tables 4 and 5). \(P. penetrans\) reduced the number of females in the root systems of the susceptible cultivar \((P < 0.001)\) only in the lowest infestation level \((1,000 \text{ J2})\) while no reduction \((P > 0.05)\) was recorded in the other two infestation levels (Table 4). In contrast, the difference in egg masses was significant between the Pasteuria and non-Pasteuria treatments \((P < 0.001)\) even in the medium and high J2 inoculation levels (Table 4). However as shown by the interaction effect \((P < 0.001)\) this reduction was greater in the low and high inoculation levels. The root invasion was less \((P < 0.05)\) in the resistant tomato cultivar due to presence of \(P. penetrans\) (Table 5). This reduction was higher in the medium and high inoculation levels. Incorporation of \(P. penetrans\) spores in soil reduced egg mass production \((P < 0.001)\). The extent of reduction interacted with inoculation level.

**DISCUSSION**

Aging of J2 in soil resulted in low infectivity. This decrease was greater when J2 were encumbered with \(P. penetrans\) spores. Ciancio (1995) reported that the forces of soil particles might act on \(P. penetrans\) spores attached to J2 as they move in soil and cause spore detachment which would be greater the longer the J2 spend moving in the soil. That older J2 were less infected by \(P. penetrans\) could be due to changes in the nematode cuticle influencing the attachment of spores (Davies et al., 1991). Another reason could be that the more time J2 spend in soil searching for the roots fewer will successfully find and invade roots. However, in field conditions where there is plenty of \(P. penetrans\) inoculum, greater mobility of nematodes at higher temperatures can result in increased rate of spore attachment (Hatz and Dickson, 1992) and presumably infection.

Rate of development has been used in estimating minimal growth temperature for insects (Bastian and Hart, 1991; Jackson and Elliot, 1988) and nematodes (Ferris et al., 1978). Tyler (1933) applied a thermal-time analysis to the development of Meloidogyne spp. and showed that the relationship of the rate of development with temperature was close to linear. Madulu and Trudgill (1994) and Lahtinen et al. (1988) reported the rate of development of \(M. javanica\) and \(M. hapla\) and temperature to be linear. Rate of development of \(P. penetrans\) in \(M. javanica\) females can reasonably fit an exponential regression. This is also indicated by the coefficient of determination.
that is close to 1. Since *P. penetrans* is an obligate parasite it seems logical that its temperature limits for development are between temperature limits of its nematode host without necessarily meaning that their development is in synchrony. The calculated base temperature by back extrapolation is different to that reported by Chen and Dickson (1997). The difference in the base temperature can be explained by the fact that different *P. penetrans* populations were used. Understanding minimal growth temperature and rate of development requirements of *P. penetrans* is of great importance for explaining the presence or absence of *P. penetrans* in different environments. Also understanding minimal growth temperature may facilitate our ability either to amplify the bacterium in the field or improve our ability to cultivate the bacterium *in vivo* (Chen and Dickson, 1997).

The combination of a resistant cultivar and *P. penetrans* incorporation in soil can reduce root-knot nematode population densities. The results of this combination are more pronounced when there is a high

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**Fig. 2.** Effects of resistant and susceptible cultivar integrated with *Pasteuria penetrans* on root galling (1-10 scale) of tomato plants over two cropping seasons. Bars with the same upper or lower case letter are not significantly different according to LSD test (*P*< 0.001).
concentration of *P. penetrans* spores/g of soil. In experiment 1 the initial spore concentration of the first cropping season was 20,000 spores/g of soil. We can conclude that spore multiplication during the first cropping season and incorporation of spores at the beginning of the second cropping season enriched the spore inoculum. Melki *et al.* (1998) showed that a concentration of 40,000 spores/g of soil is needed to provide significant suppression of root-knot nematode populations. We believe that in our experiments spore concentrations in the second were much higher than 40,000 spores/g of soil since the inoculum at the first cropping season was 20,000 spores/g of soil and also considering the fact that one infected by *P. penetrans* female can produce up to $2 \times 10^6$ spores (Giannakou *et al.*, 1999). This spore enrichment,

<table>
<thead>
<tr>
<th>Inoculation level</th>
<th>'Shirley' + Pasteuria</th>
<th>'Shirley' - Pasteuria</th>
<th>'Sinatra' + Pasteuria</th>
<th>'Sinatra' - Pasteuria</th>
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<tr>
<td>1000</td>
<td>155</td>
<td>251</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>5000</td>
<td>436</td>
<td>342</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td>10000</td>
<td>1587</td>
<td>1655</td>
<td>30</td>
<td>49</td>
</tr>
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LSD values for:

<table>
<thead>
<tr>
<th></th>
<th>'Shirley'</th>
<th>'Sinatra'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation level</td>
<td>78.3</td>
<td>8.8</td>
</tr>
<tr>
<td><em>P. penetrans</em></td>
<td>63.8</td>
<td>7.1</td>
</tr>
<tr>
<td>Interaction</td>
<td>110.6</td>
<td>12.2</td>
</tr>
</tbody>
</table>

Values are means of four replicates.

Table 4. Effect of *Pasteuria penetrans* on *Meloidogyne javanica* juveniles invasion on susceptible ‘Shirley’ and resistant ‘Sinatra’ tomato cultivars following inoculation with 1,000, 5,000 or 10,000 second stage juveniles.

<table>
<thead>
<tr>
<th>Inoculation level</th>
<th>'Shirley' + Pasteuria</th>
<th>'Shirley' - Pasteuria</th>
<th>'Sinatra' + Pasteuria</th>
<th>'Sinatra' - Pasteuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>50</td>
<td>213</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>5000</td>
<td>127</td>
<td>299</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>10000</td>
<td>192</td>
<td>517</td>
<td>8</td>
<td>47</td>
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LSD values for:

<table>
<thead>
<tr>
<th></th>
<th>'Shirley'</th>
<th>'Sinatra'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation level</td>
<td>51.8</td>
<td>14.4</td>
</tr>
<tr>
<td><em>P. penetrans</em></td>
<td>42.3</td>
<td>11.7</td>
</tr>
<tr>
<td>Interaction</td>
<td>73.4</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Values are means of four replicates.
cycle after cycle, can create suppressive soils with zero levels of root-knot nematodes (Melki et al., 1998; Weibelzahl-Fulton et al., 1996; Chen et al., 1996). Amplification of *P. penetrans* to suppressive levels can take 3 crop cycles (Oostendorp et al., 1991; Melki et al., 1998) or less (Chen et al., 1996) depending on the initial number of spores/g of soil. Also, nematode infestation levels in soil plays an important role because *P. penetrans* is a density dependent obligate parasite (Giancio, 1995).

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