Seasonal Population Fluctuations of *Meloidogyne* spp. and the *Pasteuria penetrans* Group in Kiwi Orchards

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**ABSTRACT**


Seasonal population fluctuations of the nematodes *Meloidogyne incognita*, *M. arenaria*, and *M. hapla* and the nematode-parasitic bacterium *Pasteuria penetrans* were monitored monthly in two kiwi orchards (A and B) during 1989 and 1990. Nematode numbers in soil fluctuated little, but there was a trend toward reduced numbers at the end of the study, with higher numbers in winter than in summer. The percentage of root-knot nematode juveniles with spores ranged from 0 to 8 (\(x = 2\)) in orchard A and from 0 to 18 (\(x = 7\)) in orchard B. The percentage of parasitized females ranged from 0 to 12 (\(x = 4\)) in orchard A and from 0 to 28 (\(x = 11\)) in orchard B. The total number of females per gram of roots and the percentage with *P. penetrans* were positively correlated in orchard B. Parasitized females and juveniles with spores were also correlated in this orchard during 1990. In vitro tests indicated that spores, whether from orchard A or B, adhered more readily to juveniles from orchard B than from orchard A.

The kiwi crop is relatively new in Spain, where approximately 700 ha are under production. The main growing area is in the northwest part of the country (70% of the hectarage), but new kiwi orchards have been established in the north and northeast in recent years, and thus the crop is currently grown from the Atlantic to the Mediterranean coast. The kiwifruit *Actinidia delicosa* (A. Cheng) Liang & Ferguson is considered a high-value crop because of the high cost of orchard establishment, its lack of fruitfulness for the first three years, and the high demand for the fruit (11).

*Meloidogyne* spp. commonly occur in kiwifruit production areas worldwide (4,5,9,16,25), including those in Spain (10). *Meloidogyne* spp. were found in nine of 16 orchards in Catalonia (northeastern Spain). The high numbers of *Meloidogyne* spp. in kiwi orchards, the profuse root galling, and the sparse appearance of the vines suggest that kiwi plants may be damaged by *Meloidogyne*, although yield loss due to nematodes has not been studied (11,14).

Members of the *Pasteuria penetrans* (Thorne) Sayre and Starr group are obligate parasites and pathogens of plant-parasitic nematodes and are distributed worldwide (15,22). Females of *Meloidogyne* infected by *P. penetrans* have been observed in kiwi orchards during routine sampling in Spain. The spores found in these females were morphologically similar to those described for members of the group that parasitize root-knot nematodes. (Verdejo-Lucas, unpublished).

The study of changes in numbers of nematodes and parasitized nematodes in naturally infested fields may provide important insights into host-parasite dynamics and the potential for biological control from natural infestations. Pests are rarely eradicated by natural enemies, and equilibrium populations are established. Few studies have described the population dynamics of *Pasteuria* and its nematode host, and these studies dealt with different *Pasteuria*-nematode systems, such as *Helicotylenchus lobus* Sher, *Belonolaimus longicaudatus* Rau, and *Heteroderidae Various* Wollenweber (1-3). The percentage of *B. longicaudatus* with spores adhering (74%) remained constant for 16 mo, and it was suggested that the nematode and bacterium may be at equilibrium at that site (3). More studies are needed to find out whether the bacterial parasite will increase over time so as to reach an equilibrium with its nematode host. Spore density can be expected to increase over time, but it is not likely to increase rapidly, because spores produced within the nematode have to be released into the soil after rupture of the nematode cuticle. Thus, the natural increase of *P. penetrans* in root-knot-infested vineyards was slow, and its establishment took considerable time (21). However, in microplots experimentally infested with *M. arenaria* and *P. penetrans*, the population density of *P. penetrans* increased over three annual cropping sequences and was influenced by the cropping sequence itself (13). Mixing the soil in each plot between crops possibly contributed to the increase in population density of *P. penetrans*.

The objective of this study was to describe seasonal fluctuations in population densities of *Meloidogyne* spp. and *P. penetrans* in two kiwi orchards naturally infested by both organisms. These orchards provided an opportunity to investigate the capacity of *P. penetrans* for regulating nematode populations without being introduced, and therefore to assess its potential as a biological control agent under field conditions.

**MATERIALS AND METHODS**

Fluctuations in densities of second-stage juveniles with and without spores in soil and eggs in roots. A total of 960 soil and root samples were collected from around kiwi vines in two adjacent commercial orchards in Tordera, Barcelona. Orchards were selected because *Meloidogyne* and *P. penetrans* were present. The same 20 vines (13 female and seven male plants in orchard A, and 15 female and five male plants in orchard B) were sampled monthly for 2 yr, starting in January 1989. The vines were located in five rows in each orchard, and at least 140 m separated the nearest vines sampled in the two orchards. One soil sample was collected from each vine with an Oakfield tube (2 × 24 cm) on each sampling date. Soil cores from each orchard were combined and mixed (approximately 1,500 cm\(^3\) of soil), and nematodes were extracted from two 250-cm\(^3\) subsamples from each orchard by sieving and sugar flotation (454 g per liter of solution)(8). Nematodes were counted with a Hawksley slide, and numbers of root-knot juveniles with *P. penetrans* spores and numbers of spores per nematode were determined. Roots collected on a screen (147-μm pore diameter) were blotted dry and weighed, and eggs were extracted with NaOCI (0.5% available chlorine) (6) by blending the roots in a blender for 30 sec. Dispersed eggs were collected 10 min later on a screen (25-μm pore diameter) and counted. In addition to *Meloidogyne* spp., the soil from both orchards contained *Criconeella* spp., *Pratylenchus* spp., *Aphelenchus* spp., and *Tylenchorhynchus* spp. Soil from orchard A also contained *Helicotylenchus* spp. and *Trichodorus* spp., and soil from orchard B also contained *Paratylenchus* spp. According to perineal patterns, *M. incognita*, *M. arenaria*, and *M. hapla* were in orchard A, and *M. arenaria* and *M. hapla* were in orchard B. The Mann-Whitney U test

Accepted for publication 29 July 1992.

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(P = 0.001) was used to compare percentages of juveniles with spores in soil at both orchards.

**Parasitism of females.** In 1989, soil remaining after nematode extractions was used for a bioassay. A tomato seedling was planted in each of three replicated pots containing 500 cm³ of soil from each orchard, and plants were maintained in a greenhouse for 8 wk. Numbers of healthy and *P. penetrans*—parasitized females were determined by dissecting 1 g of tomato roots under a microscope. Females with egg masses were considered healthy. Parasitized females were recognized by their characteristic pearly white color. Infection was confirmed by crushing females that lacked egg masses in a drop of water and observing the bacterium under a compound microscope at 400X. In 1990, soil remaining after nematode extractions was sieved to separate kiwi roots. Numbers of healthy and *P. penetrans*—parasitized females were determined by examining 0.5 g of fresh kiwi roots under a microscope as described above. Data were subjected to weighted least-square regression analysis (18) to determine the relationship between the total numbers of females and the percentage of *P. penetrans*—parasitized females. The relationship between numbers of juveniles with spores and parasitized females was also analyzed.

**Spore attachment.** *P. penetrans* and *Meloidogyne* spp. were increased in a greenhouse by planting tomato seedlings in soil from both orchards. Second-stage juveniles and parasitized females recovered from those greenhouse cultures were used as a source of nematodes and spores to determine compatibility between *P. penetrans* and the root-knot nematode populations. The second-stage juveniles consisted of a mix of the *Meloidogyne* species present in each orchard. Spores from individual females of *M. incognita* or *M. arenaria* from orchard A or *M. arenaria* from orchard B (determined by perineal patterns) were released in water, and aliquots of the spore suspensions were added to the surface of 1% water agar plates. Juveniles, 1 day old, from orchard A (a mix of *M. incognita*, *M. arenaria*, and *M. hapla*) or orchard B (a mix of *M. arenaria* and *M. hapla*) were added to the plates (24). All possible combinations were tested, and the number of juveniles with spores was recorded 24 hr later by examining a random sample of 15 nematodes at 400X. The experiment was repeated once. Data were analyzed by the Mann-Whitney U test (P = 0.001).

**Soil characteristics and orchard history and management.** Textural and chemical components of the two orchard soils were characterized (Table 1). Plants, 2 yr old, were transplanted in the orchards in July 1986. Orchard A had 1.7 ha, and orchard B had 1.3 ha. Although kiwi plants from both orchards came from the same nursery, those plants in orchard A came in plastic bags and performed poorly from planting, whereas those planted in orchard B came in pots and established well. The ratio of male to female plants was 1:7 in both orchards; the cultivar Tumuri was used for the male plants, and Hayward for the female plants. The previous crop, *Populus* spp., was uprooted in March 1986, except for a row of trees encircling the orchards as a windbreak. Etoprophos (70 kg/ha), magnesium sulphate (300 kg/ha), potase sulphate (800 kg/ha), calcium superphosphate (2,500 kg/ha), and cattle manure (70–80 t/ha) were incorporated into the soil before orchard establishment. Cattle manure was added annually around the vines during the dormancy.

Supplemental irrigation was provided from mid-April to the end of October, when tensiometer readings were above 15 cm, with each vine receiving a maximum of 70 L/day at the peak of the summer season (August to September). Vines were fertilized twice per week through a sprinkler irrigation system. A zone along the kiwi plant rows was kept weed-free by herbicides. Plants were pruned twice per year in January and July. At the end of March 1988, clover was seeded between the vine rows, and the soil left undisturbed. In November 1988, 95% of the vines suffered badly from frost injury. Plants that did not survive frost damage (four plants in orchard A, and three plants in orchard B) were replaced with 3-yr-old plants in spring 1989. The active vegetative period for kiwifruit in this Mediterranean environment is around 290 days, and plants remain dormant from the end of December to mid-March.

**RESULTS**

Fluctuations in densities of second-stage juveniles with and without spores in soil and eggs in roots. Trends toward reduced numbers of *Meloidogyne* juveniles in soil were observed in both orchards at the end of this 2-yr study (Fig. 1A and C). Seasonal fluctuations had little effect on numbers of *Meloidogyne* juveniles in soil. The overwin-

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**Table 1. Characteristics of soils in two kiwi orchards in Spain from a study determining seasonal population fluctuations of *Meloidogyne* spp. and *Pasteweria penetrans***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Orchard A</th>
<th>Orchard B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (%)</td>
<td>86</td>
<td>74</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Electric conductivity (ds/m)</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>0.77</td>
<td>0.81</td>
</tr>
<tr>
<td>Nitrogen (Kjeldahl, %)</td>
<td>0.043</td>
<td>0.045</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>10.41</td>
<td>10.47</td>
</tr>
<tr>
<td>Phosphorus (Olsen, ppm)</td>
<td>2.02</td>
<td>3.23</td>
</tr>
<tr>
<td>Potassium (ppm)</td>
<td>44.45</td>
<td>34.92</td>
</tr>
<tr>
<td>Magnesium (meq/100 g)</td>
<td>0.72</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Fig. 1. Monthly numbers of second-stage juveniles of *Meloidogyne* spp. in soil in two kiwi orchards in 1989 and 1990. (A) Seasonal fluctuations of second-stage juveniles in orchard A, (B) percentage of second-stage juveniles with spores of *Pasteweria penetrans* in orchard A, (C) seasonal fluctuations of second-stage juveniles in orchard B, and (D) percentage of second-stage juveniles with spores of *P. penetrans* in orchard B. Values are means of two soil extractions.**
tering populations (January to March) were higher, in general, than the summer populations (July to September). The mean *Meloidogyne* juvenile density across all sampling times was 980 ± 520 (mean ± standard deviation) and 1,490 ± 2,340 nematodes per 250 cm² of soil in orchards A and B, respectively (Fig. 1A and C). The larger mean number of nematodes recovered from soil in orchard B was due to the 11,360 and 5,900 individuals recovered in January and March 1989, respectively. If these samples were excluded, the mean was 845 ± 500. In this orchard, a sharp decrease in soil population densities occurred in February 1989, and levels increased in March and decreased again the following months, until the end of the study (Fig. 1C). Seasonal fluctuations had a greater effect on numbers of eggs per gram of root and tended to be lower in winter (December to March) than in other seasons (Fig. 2A and B). Egg production increased at the beginning of the vegetative growing period (April to June), and this trend was clearer in orchard B than in orchard A. The mean numbers of eggs per gram of root across all sampling times was 2,730 ± 2,270 and 1,960 ± 1,800 in orchards A and B, respectively (Fig. 2A and B).

Although nine genera of plant-parasitic nematodes occurred in both orchards, *P. penetrans* was only observed on juveniles and in females of the root-knot nematode. The percentage of juveniles with spores varied greatly in both orchards and ranged from 0 to 8 (X = 2) in orchard A and from 0 to 18 (X = 7) in orchard B. The mean percentage of juveniles with spores in soil across all sampling times was higher (P = 0.001) in orchard B than in orchard A, and the highest proportion (18%) was observed in August 1990 in orchard B. Juveniles with spores were found in all soil samples from orchard B, except those collected in January, July, and October 1989 (Fig. 1D), and more juveniles had spores attached in the second year (8%) of this study than in the first (5%). In orchard A, juveniles with spores were found in eight of the 24 samples collected and were first observed in October 1989 (Fig. 1B). The number of spores attached per juvenile was always low; 70% of the juveniles with spores had fewer than four spores. No correlation was found between juveniles in soil and the percentage with spores at either orchard.

**Parasitism of females.** *P. penetrans* was infecting females of *M. arenaria, M. hapla,* and *M. incognita* from orchard A and females of *M. arenaria* and *M. hapla* from orchard B. The bacterium was first observed parasitizing females in September and January 1989 in orchards A and B, respectively. In both orchards, *P. penetrans* was detected earlier by the bioassay method (parasitized females) than when directly assessed in field soil samples (juveniles with spores) (Table 2; Fig. 1B and D). Bioassay data are only available for 6 mo, because of early death of the tomato plants caused by damping-off microorganisms probably present in the field soil. The number of females per gram of root ranged from 10 to 140 (X = 54) in orchard A and from 3 to 116 (X = 52) in orchard B. Parasitized females ranged from 0 to 12 (X = 4) in orchard A and from 0 to 28 (X = 11) in orchard B, and more (P = 0.001) females were infected by *P. penetrans* in orchard B than in orchard A (18 and 7%, respectively). Parasitized females represented one eighth and one third of the total numbers of females per gram of root in orchards A and B.

**Table 2.** *Meloidogyne* females per gram of root and percentage of females parasitized by *Pasteuria penetrans* as determined by bioassay in two kiwi orchards in 1989

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Orchard A</th>
<th>Orchard B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females per gram of root</td>
<td>Parasitized females (%)</td>
</tr>
<tr>
<td>January</td>
<td>153</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>77</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>161</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>September</td>
<td>53</td>
<td>1</td>
</tr>
</tbody>
</table>

*A tomato seedling was planted in each of three replicated pots with 500 cm³ of field soil from each orchard. The number of healthy and parasitized females per gram of root was determined after 8 wk later. Each value is the mean of three replications.

**Fig. 2.** Reproduction of *Meloidogyne* spp. in roots in two kiwi orchards in 1989 and 1990. (A) Seasonal fluctuations in egg production in orchard A and (B) in orchard B. Values are means of two root extractions.

**Fig. 3.** *Meloidogyne* females per gram of root (solid line) and percentage of females parasitized by *Pasteuria penetrans* (broken line) in roots in two kiwi orchards during 1990. (A) Seasonal fluctuations in orchard A and (B) in orchard B.

**Fig. 4.** Seasonal fluctuations in numbers of second-stage juveniles of *Meloidogyne* spp. with *Pasteuria penetrans* spores per 250 cm² of soil (solid line) and numbers of parasitized females per gram of root (broken line) in orchard B in 1990.
respectively. The percentage of parasitized females was positively correlated ($r = 0.90, P = 0.0001$) with the density of females per gram of root in orchard B (Fig. 3B), but there was no correlation in orchard A (Fig. 3A). Numbers of parasitized females and juveniles with spores were also correlated ($r = 0.78, P = 0.004$) in orchard B (Fig. 4).

Parasitized females with collapsed body walls were localized sometimes deep in the kiwi root tissue. Partially parasitized females (whose body cavities are not filled with spores) and females with a gelatinous matrix and parasitized by the bacterium were also observed.

**Spore attachment.** In laboratory bio-assays, spore attachment was affected by the source of juveniles, but not by the source of spores. Spores from *M. incognita* or *M. arenaria* from orchard A, or *M. arenaria* from orchard B, attached to a much larger ($P = 0.001$) percentage of juveniles from orchard B (a mix of *M. arenaria* and *M. hapla*) (80–100% attachment) than to juveniles from orchard A (a mix of *M. incognita*, *M. arenaria*, and *M. hapla*) (Table 3).

Also, juveniles from orchard B had more spores per nematode when spores were derived from *M. arenaria*, orchard B, than from *M. arenaria* or *M. incognita* from orchard A. Numbers of spores on juveniles from orchard B did not differ when spores came from orchard A (*M. incognita* or *M. arenaria*). The source of spores (*Meloidogyne* species and orchard) did not affect the number of spores on juveniles from orchard A.

**DISCUSSION**

In orchards and other perennial cropping systems, nematode antagonists such as *P. penetrans* may help suppress nematode numbers, particularly when the orchard has existed for many years (20). Although *P. penetrans* was easily detected in these young kiwi orchards, it is unclear whether the bacterium was limiting the nematode-host population density. Apparently *P. penetrans* had little impact on the *Meloidogyne* populations; nevertheless, orchard B was more conducive to *P. penetrans* than was orchard A despite similar levels of root-knot nematode (juveniles, eggs and females), environmental conditions, and management practices. The positive correlations found in orchard B suggest the capacity of *P. penetrans* of regulating nematode populations but only when the density of the bacterial population has reached certain levels. The lack of correlations in orchard A may be due to the very low levels of *P. penetrans* occurring in this orchard (2 and 4% of the soil and root populations, respectively).

Percent parasitism in soil was low in both orchards, but perhaps it will increase with time as orchards reach maturity. Higher frequency of *P. penetrans* was found in old and medium-age vineyards than in younger ones (less than 10 yr old) (21). The density of *P. penetrans* (spores attached per nematode) increased over three cropping sequences in microplots when inoculated with *M. arenaria* juveniles with spores (13). Such an increase in bacterial density influenced peanut yield at the third cropping sequence. Yield was 64% higher in plots with *P. penetrans* than in plots without *P. penetrans*.

Spore dispersal in these kiwi orchards may be slow, since soil is not disturbed. The passive spreading of the spores in soil may occur with rainfall and irrigation water; it has been shown that spores can move 6.4 cm (the maximum distance tested) downward in soil within 3 days with percolating water (12). The fleshy nature of the kiwi roots, on the other hand, may slow down spore dispersal as a result of the deep localization of the parasitized females within the root tissue.

The host-specificity of the *P. penetrans* (15), which may be expressed even at the population level (20), could partially explain the differences observed in parasitism in orchards A and B. *M. arenaria* and *M. hapla* occurred in both orchards, but *M. incognita* was only present in orchard A. The attachment test, in which the juvenile source consisted of a mix of the *Meloidogyne* species occurring in each orchard, indicated that there was a higher compatibility between the bacterium and the populations of the nematode present in orchard B than in orchard A. Spores derived from the same nematode species and spore suspensions used in the attachment test attached to a very low percentage of juveniles (2%) from a pure *M. incognita* population isolated from orchard A (data not shown). The proportion of each *Meloidogyne* species in the orchards was unknown, and determination of the relative abundance of the species proved to be very difficult and unsuccessful.

*P. penetrans* was more abundant in the sandy loam (orchard B) than in the loamy sand (orchard A) soil. Other physical and chemical properties of soil determined were similar in both orchards. Spaul (17) found *P. penetrans* more frequently in sand and loamy sand than in other soil types in sugarcane fields in South Africa. In these sugarcane fields, the percentage of infected females was highest in fields of sand but did not differ in loamy sand and sandy loam soils. Physical or chemical soil properties that affect *P. penetrans* are still unknown but may affect bacterial establishment and dispersal, as has been shown for other nematode–antagonist systems. For instance, transmission of *Hirsutella rhossiliensis* spores to *Heterodera schachtii* was much greater in loamy sand than coarse sand and was attributed to differences in soil pore diameter (7).

Long-term studies on fields naturally infested with *P. penetrans* are necessary to assess levels of natural infestations and the developmental rate of the bacterial population. Both the nematode and bacterium are obligate parasites and have temperature-mediated rates of development (19,23), with the bacterium having a longer generation time at a given temperature. Consequently, there will be a lag time in the bacterial life cycle with respect to that of the nematode. Because life cycles of both organisms are not synchronous, *P. penetrans* may need a long time to reduce the population of its nematode host. Conversely, the progressive buildup of *P. penetrans* could not occur in nature, the bacterium might assure its survival by parasitizing only a low percentage of the nematode population. Examinations of naturally infested fields have shown that nematodes affected by the bacterium did not exceed 25% of the total population (17, 21). Long-term studies will be beneficial in perennial and multicropping systems to identify conditions (rotation schemes, agronomic practices) enhancing the development of natural biological control.

**ACKNOWLEDGMENTS**

I thank J. Rius for allowing this study to be conducted on his property; J. Addi for information on agricultural practices; A. Soler, L. Dunes, and V. Montero for their help in collecting soil samples; J. Pera for preparing this manuscript; and B. A. Jaffe for critical reading of the manuscript and useful suggestions.

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**Table 3. Attachment of spores of *Pasteuria penetrans* from parasitized *Meloidogyne incognita* and *M. arenaria* females to *Meloidogyne* spp. juveniles from two kiwi orchards**

<table>
<thead>
<tr>
<th>Source of parasitized females</th>
<th>Source of juveniles</th>
<th>Juveniles with spores</th>
<th>Juveniles with spores (%)</th>
<th>Spores per juvenile</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. incognita</em></td>
<td>Orchard A</td>
<td>4</td>
<td>27</td>
<td>2 ± 1</td>
</tr>
<tr>
<td></td>
<td>Orchard B</td>
<td>12</td>
<td>80</td>
<td>4 ± 3</td>
</tr>
<tr>
<td><em>M. arenaria</em></td>
<td>Orchard A</td>
<td>4</td>
<td>27</td>
<td>3 ± 2</td>
</tr>
<tr>
<td></td>
<td>Orchard B</td>
<td>15</td>
<td>100</td>
<td>4 ± 2</td>
</tr>
<tr>
<td><em>M. arenaria</em></td>
<td>Orchard A</td>
<td>8</td>
<td>53</td>
<td>4 ± 3</td>
</tr>
<tr>
<td></td>
<td>Orchard B</td>
<td>15</td>
<td>100</td>
<td>7 ± 3</td>
</tr>
</tbody>
</table>

*Based on perineal pattern.
*A* A mix of the *Meloidogyne* species present in each orchard.
*Fifteen nematodes were examined.*

1278 Plant Disease/Vol. 76 No. 12
LITERATURE CITED


