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OCCURRENCE OF *PASTEURIA* SPP. IN NORTHEASTERN SPAIN

by
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Summary. The occurrence of *Pasteuria* spp. in Spanish soils is reported. A total of 160 soil samples were collected from vegetable crops, kiwi and citrus orchards, and deciduous fruit trees. Bacteria were found associated with six nematode genera but they were only observed within females of *Meloidogyne* spp., second-stage juveniles and males of *Tylenchulus semipenetrans*, and juveniles of *Pratylenchus* spp.

Pasteuria spp. are obligate parasites of nematodes with potential as biological control agents (Stirling, 1991). These organisms are widespread in different soil habitats and have been observed on nematodes belonging to various taxa including plant-parasitic, predatory and free-living or microbivorous species. According to a recent report, the host range of the *Pasteuria*-group includes 102 nematode genera and 236 identified species (Ciancio *et al.*, 1994). In Spain, members of the *Pasteuria penetrans* group have been reported infecting *Meloidogyne* in kiwi orchards (Verdejo-Lucas, 1992). Also, they have been observed on *Tylenchorhynchus brassicae*, *Aphelenchoides composticola*, and in the pseudocoelom of *Helicotylenchus* spp. and *Xiphinema pachtaicum* in the Canary Islands (Sturhan, 1985, 1988).

In the past eight years, the Department of Patología Vegetal of Institut de Recerca i Tecnologia Agroalimetàries has made surveys for the detection and identification of plant-parasitic nematodes on vegetable crops, in kiwi and citrus orchards, and on deciduous fruit trees in order to determine potential nematological problems. During examination of soil samples from these surveys, the presence of *Pasteuria* spp. was noticed and recorded. This paper reports the occurrence of *Pasteuria* spp. in annual and perennial crops.

Materials and methods

During 1988-1996, soil samples were collected from vegetables crops (tomato *Licopersicon esculentum* L, cucumber *Cucumis sativus* L., lettuce *Lactuca sativa* L., watermelon *Citrullus lanatus* Tunb., garlic *Allium sativum* L. and French bean *Phaseolus vulgaris* L.) (93 fields), kiwi *Actinidia deliciosa* (A. Cheng) Liang and Ferguson (17 fields), citrus *Citrus* spp. (20 fields), and deciduous fruit trees which included peach *Prunus persica* (L.) Batsch, cherry *Prunus avium* L., apple *Malus domestica* Borkh, and pear *Pyrus communis* L. (30 fields). The fields with vegetable crops were located in the province of Barcelona, the kiwi orchards in Barcelona and Tarragona, the citrus orchards in the

provinces of Tarragona and Valencia and the deciduous fruit trees in the provinces of Lerida, Gerona, and Zaragoza (Fig. 1).

Composite soil and root samples were collected to a depth of 30 cm with a soil auger. The samples were sieved to separate roots from soil and 250 cm^3 soil subsamples were processed to extract nematodes from the soil by differential sieving and centrifugation-sugar flotation (Jenkins, 1964). Nematodes in roots were extracted by blender maceration (McSorley *et al.*, 1984). The nematodes were counted and identified at the genus level by light microscopy at 100X. The presence of *Pasteuria* spores adhering to nematode cuticles was confirmed at 400X with a light microscope. Sporangia or vegetative stages within the nematode were determined by crushing the parasitized nematodes in a drop of water and the bacterial developmental stages observed with a 1000X oil immersion objective.

Results and discussion

Spores of *Pasteuria* spp. were observed adhering to and/or within the body cavities of six genera of plant-parasitic nematodes that were associated with different plant hosts from different geographical localities (Table I). Samples from 160 fields were examined and *Pasteuria* spp. were observed in 19 of these fields (12%). In all cases, the bacteria were only associated to one of the several nematode genera that occurred simultaneously in each field.

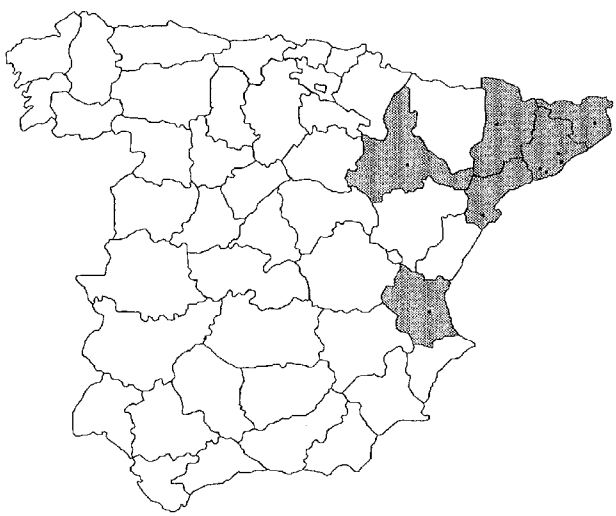

Fig. 1 - Map showing the provinces surveyed and the geographic distribution of *Pasteuria* in northeastern Spain during 1988-1996.

In vegetable crops, 11 genera of plant-parasites were detected, *Tylenchorhynchus* and *Meloidogyne* being the genera most frequently found. *Pasteuria* spp. were observed on the cuticle of *Aphelenchoides*, *Helicotylenchus*, *Pratylenchus*, *Meloidogyne* spp. and *Tylenchorhynchus* in six out of the 93 samples examined (Table I). However, mature sporangia within the nematode were observed only in females of the root-knot nematode that infected tomato plants. *Meloidogyne incognita* (Kofoid *et* White) Chitw. (two fields), *M. arenaria* (Neal) Chitw. (one field) and *M. javanica* (Treub) Chitw. (one field) were parasitized by *Pasteuria* spp. The percentage of second-stage juveniles (J2) with spores ranged from 16 to 50%. The spore diameter of these isolates was 3.5-4 µm which is similar to those described for members of the *Pasteuria penetrans* group that infect root-knot nematodes (Mankau, 1975, Sayre and Starr, 1985). Although the bacteria were present in these naturally infested tomato fields, they were not abundant and they could not be detected at monthly intervals. Seasonal differences in the occurrence of *Pasteuria* spp. on vegetable crops were not observed. However, the highest frequency of J2 with spores was recorded when high populations densities of the nematode were present in the fields (Sorribas, 1996). The low frequency of ocurrence of *Pasteuria* spp. in vegetable crops could by explained by the broad biocide effect of the soil fumigants that are usually applied to control weeds, soil pests and diseases in these crops. Thus, 53% of the fields had been fumigated either with methyl bromide or metham sodium just before the initiation of the survey.

TABLE I - *Association of* Pasteuria *spp. with plant-parasitic nematodes in annual and perennial crops in northeast Spain.*

Crop	Number of fields sampled	Percentage of fields with *Pasteuria* spp.	Nematode host
Vegetables	93	7	*Aphelenchoides* spp. *Helicotylenchus* spp. *Meloidogyne* spp.* *Pratylenchus* spp. *Tylenchorhynchus* spp.
Kiwi	17	35	*Aphelenchoides* spp. *Meloidogyne* spp.* *Pratylenchus* spp. *Tylenchorhynchus* spp.
Deciduous fruits	30	6	*Helicotylenchus* spp. *Pratylenchus* spp.*
Citrus	20	20	*Tylenchulus semipenetrans**

* *Pasteuria* sporangia present in the pseudocoelom.

In kiwi orchards, up to 12 different nematode genera were detected. *Tylenchorhynchus* spp. and *Meloidogyne* spp. were the genera most commonly found followed by *Pratylenchus* spp. *Pasteuria* spp. were found in 35% of the fields sampled. Bacterial spores were observed on the cuticle of *Aphelenchoides* sp., *Meloidogyne* spp., *Pratylenchus* sp. and *Tylenchorhynchus* sp., but they only infected species of *Meloidogyne* such as *M. incognita*, *M. arenaria* and *M. hapla* Chitw. Parasitism of *M. hapla* by *P. penetrans* was restricted to kiwi orchards and the nematode species was not found in any other crop. In two of the naturally infested orchards, the percentage of J2 with spores ranged from 2 to 18% and the percentage of infected females/g root from 7 to 18% (Verdejo-Lucas, 1992). *Pratylenchus* spp., *Paratylenchus* spp., and *Helicotylenchus* spp. were the nematodes most abundantly associated with deciduous fruit trees. The percentage detection of *Pasteuria* was low (6%). Spores were found within a *Pratylenchus* individual in a peach orchard, and on *Helicotylenchus* in apple and peach orchards. In citrus orchards, *Tylenchulus semipenetrans* Cobb was the only plant-parasitic nematode found. Juveniles and males of *T. semipenetrans* were parasitized by *Pasteuria* spp. in four of the citrus orchards. This *Pasteuria* completed its life cycle within the vermiform stages of the nematode (J2 and males), and was never detected on the cuticle or within the adult female stage. Fewer than 15% of the total number of nematodes recovered from soil samples collected in spring, summer, autumn or winter had spores attached. Most nematodes had 1-2 spores attached to their cuticle. The morphology of the spores was similar to that described for *Pasteuria* isolates parasitizing the citrus nematode in Iraq (Fattah *et al.*, 1989), Florida (Kaplan, 1994), and Italy (Ciancio *et al.*, 1994).

This report adds to the host range and geographical distribution of *Pasteuria* spp. The host-parasite association of *Pratylenchus* spp. and *T. semipenetrans* with *Pasteuria* spp. had not been reported for Spain before. An association with *Aphelenchoides* sp., *Helicotylenchus* sp. and *Tylenchorhynchus* sp. had been report-

ed from the Canary Islands (Sturhan, 1985, 1988) but not from continental Spain. The compilation of data presented here indicate that the frequency of occurrence of *Pasteuria* spp. is relatively low in northeast Spain.

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