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Short communication. Identification of the Poncirus biotype of *Tylenchulus semipenetrans* in Valencia, Spain

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Abstract

High population densities of *Tylenchulus semipenetrans* Cobb were detected on mature *Poncirus trifoliate* (L.) Raf. rootstocks in a commercial citrus orchard in Rafelguaraf, Valencia, Spain. Until now, this rootstock was considered resistant to populations of *T. semipenetrans*. The biotype of the nematode was characterised according to its host range. Under greenhouse conditions, it proved to be very capable of reproduction on the Bennecke and Rubidoux cultivars of *P. trifoliate* and on rough lemon (*Citrus jambhiri* Lush) at seven months post-infestation. It was also able to infest grapevine 141-R (*Vitis berlandieri* x *V. rupestris*), but not cv. Arbequina olive (*Olea europaea* L.). These results suggest this Valencia population of *T. semipenetrans* behaves similarly to the *Poncirus* biotype.

Additional key words: citrus nematode, citrus rootstocks, differential host test.

Resumen

Nota corta. Identificación del biotipo Poncirus de *Tylenchulus semipenetrans* en Valencia, España

En un huerto comercial de cítricos en la localidad de Rafelguaraf, Valencia, se detectó una alta densidad de poblaciones de *Tylenchulus semipenetrans* sobre árboles adultos de *Poncirus trifoliate* (L.) Raf. Este patrón estaba considerado hasta ahora en España como resistente a poblaciones de *T. semipenetrans*. Se caracterizó el biotipo de la población Valencia de *T. semipenetrans* de acuerdo a su rango de hospedantes diferenciales. Siete meses después de la infestación bajo condiciones de invernadero, la población Valencia demostró una alta capacidad para reproducirse sobre los cultivares Bennecke y Rubidoux de *P. trifoliate* y sobre limón rugoso (*Citrus jambhiri* Lush). Además fue capaz de infestar la vid 141-R (*Vitis berlandieri* x *V. rupestris* Scheele), pero no el olivo (*Olea europaea* L.) cv. Arbequina. Estos resultados observados sobre los hospedantes diferenciales demuestran que la población Valencia se comporta de modo similar al biotipo *Poncirus*.

Palabras clave adicionales: ensayo diferencial de hospedantes, nematodo de los cítricos, patrones de cítricos.

The citrus nematode (*Tylenchulus semipenetrans* Cobb) — the cause of slow decline in citrus plants— is found worldwide and attacks nearly all commercial citrus rootstocks. Baines *et al*. (1969) reported the host preference of field populations of this pest and demonstrated the existence of different biotypes on citrus plants. Since then, all *T. semipenetrans* biotypes have been differentiated by host specificity (Baines *et al*., 1974; O'Bannon *et al*., 1977). An extended classification of *T. semipenetrans* biotypes was proposed by Inserra *et al*. (1980), including: i) a citrus biotype that reproduced poorly on *Poncirus trifoliate* (L.) Raf. but which infested *Citrus* spp., olive, grape, and persimmon; ii) a Mediterranean biotype with the same characteristics as the citrus biotype except for its ability to infest olive; iii) a *Poncirus* biotype capable of infesting *Citrus* spp., *P. trifoliate* (and its hybrids) and grapevines, but not olive.

To date, the citrus biotype has been found in the United States and Italy (Baines *et al*., 1974; Inserra *et al*., 1980), the Mediterranean biotype has been detected in the Mediterranean, South Africa and perhaps India (Inserra *et al*., 1980; Gottlieb *et al*., 1986; Verdejo-Lucas, 1992), and the *Poncirus* biotype has been found in California, Japan, Italy and Israel (Baines *et al*., 1974; Gottlieb *et al*., 1986).

In Spain, *T. semipenetrans* is the most common and abundant phytoparasitic nematode of citrus orchards;

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several surveys estimate more than 90% to be infested (Bello *et al*., 1986). A number of studies have reported an increase in the dissemination of the Mediterranean biotype in Spain, as well as the possible presence of other biotypes (Verdejo-Lucas, 1992; 1997). Certainly, the Mediterranean biotype has adapted to reproduce well on Troyer and Carrizo citranges (Verdejo-Lucas *et al*., 1997). Both of these rootstocks have been used in Spain to replace bitter orange (*Citrus aurantium* L.) rootstocks since the early seventies (Forner and Pina, 1992).

P. trifoliate has been used as a source of resistance to some populations of *T. semipenetrans, Phytophthora parasitica* Dast., and citrus tristeza virus (CTV). This rootstock is highly resistant to many Spanish populations of *T. semipenetrans* (Verdejo-Lucas *et al*., 1997), but it is not often used commercially in Spain since it performs poorly on the calcareous or alkaline soils which dominate the country's citrus-growing areas. It is, however, used in Alcira and Carcagente (Valencia), Málaga, Huelva and Badajoz, where soils are more favourable (Forner and Pina, 1992).

High population densities of *T. semipenetrans* were detected in a commercial citrus orchard in Rafelguaraf (Valencia) containing 40-year-old Washington navel cv. Thompson orange trees [*Citrus sinensis* (L.) Osbeck] on *P. trifoliate* rootstocks. This paper reports the result of a differential host study under glass to characterise this population.

The soil was a sandy clay loam (20% clay, 20% silt, and 60% sand, pH 6.07). In preliminary sampling, high population densities of *T. semipenetrans* [1,800-2,564 adult females per gram of root; 560-630 second-stage juveniles (J_2) and males per 100 cm^3 of soil] were detected in roots and soil samples collected from this orchard. Five citrus trees were selected based on the results of these preliminary samples. The nematode inoculum (eggs and J_2) was obtained from infested citrus roots that were washed gently in water, cut into 0.5 cm-long segments and thoroughly mixed. Roots samples (Ø < 2 mm) were macerated for 30 s at 8000 rpm in an electric miniblender (Greco and D'Addabbo, 1990). The resulting suspension was passed through 354, 100 and 30 µm sieves. Eggs and J_2 were separated by repeated passage through a 30 µm sieve.

The following host plants were used to characterise the biotype of the nematodes: *P. trifoliate* cvs. Bennecke and Rubidoux, rough lemon (*C. jambhiri* Lush), olive (*Olea europaea* L.) cv. Arbequina, and grapevine 141-R (*Vitis berlandieri* x *V. rupestris* Scheele). Seeds of *P. trifoliate* and rough lemon were germinated in seedbeds. Olive seeds were germinated at 14°C in the dark for 45 days for stratification (Antunes, 1999). One month-old seedlings of *P. trifoliate* cultivars and rough lemon, 45 day-old grapevine seedlings, and newly rooted softwood vine cuttings were planted in plastic pots (1.5 L capacity) containing steam-sterilised sphagnum moss (pH 6.5) mixed 2/3:1/3 (v v^{-1}) with a quartz sand potting mixture. All plants were fertilised with 2.5 g L^{-1} of a commercial formulation of micronutrients (16:8:12:2 $N:P_2O_5:K_2O:MgO$; slow release fertiliser, eight months longevity). All plants were maintained in a greenhouse at 23 ± 5°C for eight months before adding the nematodes. Each pot was infested with approximately 10,000 eggs and J_2. These were delivered into the growth substrate in four places at 3 cm from the base of the plant using a syringe. The experiment had a randomised complete block design with five replicates of each host plant. All plants were maintained in the greenhouse for seven months at 23 ± 5°C before evaluation.

At harvest time, the roots were washed and processed to extract all life cycle stages of *T. semipenetrans*. The numbers of females and eggs + J_2 were expressed per gram (fresh weight) of fibrous root. Nematode counts were log (X + 1) transformed for statistical analysis (ANOVA and Duncan's multiple range test). All calculations were performed using Statgraphics Plus 3.1 software.

Seven months after infestation, the *P. trifoliate* Bennecke and Rubidoux plants were found to be highly susceptible to the nematode. Significant differences ($P \leq 0.05$) were seen between these cultivars and the other host plants in terms of the mean numbers of females, eggs and J_2 per gram of root (Table 1). Large population were detected on the susceptible *C. jambhiri* rootstock, but densities were lower than those for the Bennecke and Rubidoux cultivars.

Table 1. Density of the Valencian population of *Tylenchulus semipenetrans* per gram of fresh root

Host	Females[1]	Eggs + J_2
Citrus jambhiri	153 b[2]	3,063 a
Poncirus trifoliate		
— cv. Bennecke	552 a	4,723 a
— cv. Rubidoux	469 a	4,792 a
Grape 141-R	16 c	22 b
Olive cv. Arbequina	0 d	0 c

[1] Population at seven months post-infestation. [2] Values are means of five replicates. Figures in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Although the nematode reproduced on grapevine 141-R, the mean numbers of females, eggs and J_2 per gram of root were lower ($P \leq 0.05$) than those recorded for the other rootstocks. No life cycle stages of the pest were detected on olive cv. Arbequina (Table 1). In summary, the Valencian *T. semipenetrans* population showed a strong ability to reproduce on Bennecke and Rubidoux cultivars of *P. trifoliate* and on *C. jambhiri*. It was also able to infest the grapevine tested, but it could not develop on olive.

The Valencian nematode behaves similarly to the *Poncirus* biotype defined in previous studies (O'Bannon *et al*., 1977; Inserra *et al*., 1980); the latter readily reproduces on citrus varieties as well as on *P. trifoliate* and its hybrids. The differential susceptibility of the Bennecke and Rubidoux cultivars of *P. trifoliate* to the Valencia population was comparable to that reported for different biotypes of *T. semipenetrans* (Baines *et al*., 1974). Other studies report that the *Poncirus* biotype produces minor infestations of grape rootstocks or *Vitis* species (Baines *et al*., 1974; Inserra *et al*., 1980; Gottlieb *et al*., 1986).

To date, only the Mediterranean biotype of *T. semipenetrans* has been identified in the citrus-growing regions of Spain (Verdejo-Lucas, 1992; Verdejo-Lucas *et al*., 1997), where the bitter orange (*Citrus aurantium* L.) has been used as a rootstock for decades. The field and greenhouse data provided in a previous study (Verdejo-Lucas *et al*., 1997) suggest that the Mediterranean biotype of the nematode has adapted to reproduce well on Troyer and Carrizo citranges. The continuous exposure of the nematode to partially resistant rootstocks may enhance its ability to infest and reproduce on these citrange rootstocks. Since biotype designation is mainly determined by host plant range rather than by climatic, edaphic or other environmental factors (Baines *et al*., 1974; Gottlieb *et al.,* 1986), this may assist in the appearance of other biotypes.

The Valencia population was isolated from a 40 year old orchard containing *C. sinensis* trees growing on *P. trifoliate* rootstocks, and in which the acidic soils (pH 6.07) provided favourable conditions for both *P. trifoliate* growth and the reproduction and development of the nematode. This *Poncirus* biotype may have risen under the conditions at Rafelguaraf because of the presence of a suitable host and in response to selection pressure. The other known *Poncirus* biotype populations have all developed in areas where *P. trifoliate* rootstocks or resistant hybrids are commonly used (Baines *et al*., 1969). A different biotype of *T. semipenetrans*, reported from Florida (Duncan *et al*., 1994), may have developed in response to the selection pressures at work in that part of the world.

Further studies are needed to determine the existence of the *Poncirus* biotype in other locations in Rafelguaraf, as well as in other citrus-growing areas with similar conditions where *P. trifoliate* or its hybrids are used as rootstocks (Forner and Pina, 1992). An objective of a breeding program underway at the Agricultural Research Institute of Valencia (IVIA) is to breed rootstocks tolerant to CTV that are adapted to Spanish conditions (particularly calcareous soils and increased salinity). Many of the hybrids obtained in the program have *P. trifoliate* as the male parent because of its tolerance to this virus (Forner and Alcaide, 1994). Twenty six citrus hybrid rootstocks were reported resistant to the Mediterranean biotype in greenhouse tests (Verdejo-Lucas *et al*., 2000). Citrus breeding for nematode resistance is performed with native nematode populations in mind. Future screenings of hybrid citrus rootstocks for resistance to *T. semipenetrans* should therefore take into account both the *Poncirus* and Mediterranean biotypes.

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