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Host Range of a Population of *Pratylenchus vulnus* in Commercial Fruit, Nut, Citrus, and Grape Rootstocks in Spain[1]

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Abstract: In a host-range study carried out under greenhouse conditions, a total of 37 commercial fruit tree, grape, and citrus rootstocks were tested for their reaction to a population of the lesion nematode, *Pratylenchus vulnus*, in Spain. Twenty-five rootstocks had a Pf/Pi > 1.5. These included almond (Desmayo Rojo, 1143), apple (EM-9, EM-106), avocado (Hass), cherry (Santa Lucia 64, Camil, M × M 14, Masto de Montañana), grape (41-B, Fercal, Ritcher 110), hazelnut (Pauetet), loquat (Nadal), peach (Montclar, GF-305), pear (OHF-333), pistachio (*P. atlantica*, *P. vera*, *P. terebinthus*), plum (San Julian 655-2, Montizo, Pixy, Myrobalan 605), and walnut (Serr). The peach rootstock Nemaguard and the grape 161-49 had Pf/Pi between 1.0 and 1.5 (slightly higher than inoculation level). All the tested citrus (Alemow, rough lemon, Carrizo citrange, sour orange, Troyer citrange, Citrumelo), plus three grape (SO4, *Vitis rupestris*, 1103-P), and the olive rootstock Arbequína had a Pf/Pi < 1.0.
Key words: Citrus, fruit tree crop, grape, host range, lesion nematode, nematode, nut tree crop, *Pratylenchus vulnus*, rootstock.

The most important lesion nematode attacking fruit trees in the Mediterranean region is *Pratylenchus vulnus* Allen and Jensen (10,23). This species parasitizes apple (*Malus silvestris* L.) (2), rose (*Rosa multiflora* L.) (12), quince (*Cyndonia oblonga* Miller), pear (*Pyrus communis* L.), and almond (*Prunus amygdalus* Batsch) in Spain. However, its distribution in commercial orchards and nurseries is unknown. In neighboring France it is widespread and considered a severe pathogen of fruit trees (22,23). In Italy it was reported to damage peach (*Prunus persica* (L.) Batsch), citrus (*Citrus* spp.), and olive (*Olea europea* L.) (8). In the United States, *P. vulnus* damages peach trees throughout the Southeast (1,7, 20). In California, it is considered an important pest of almond, walnut (*Juglans regia* L.), cherry (*Prunus avium* L.), fig (*Ficus carica* L.), grape (*Vitis* spp.), and plum (*Prunus cerasifera* Ehrh.) (3,5,13–15,17,19).

The implementation of nematode management practices in fruit tree crops requires a knowledge of the host suitability of rootstocks to the nematode pest. Previous host-range studies of *P. vulnus* on fruit tree rootstocks, mainly of the genus *Prunus*, were conducted in the United States and France, 29 and 17 years ago, respectively (11,22). Many of those rootstocks are now obsolete or commercially unavailable. Due to the economic importance of *P. vulnus* in the Mediterranean area, an updated host-range study of this pest was conducted in Spain in 1989–1990. The study includes some new and highly popular fruit tree, nut tree, citrus, and grape rootstocks marketed in Spain (6,24), as well as in other European and North African countries.

Materials and Methods

A total of 37 commercial fruit, nut, citrus, and grape rootstocks and varieties were obtained from several sources. The majority of these are among the most widely used rootstocks in Spain. Apple, plum, pear, cherry, and olive were propagated from hardwood cuttings, treated with a 50% alcohol solution containing 2,000 ppm of indolebutyric acid for 10 seconds. Grape cuttings were propagated without any treatment. Cuttings were planted into 200-cm^3 pots containing a 3:1 (v:v) sand and peat mixture and placed in

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a greenhouse for rooting. Almond, peach, hazelnut (*Corylus avellana* L.), loquat (*Eriobotrya japonica* (Thunb.) Lindl.), walnut, and citrus (*Citrus* spp.) were propagated from seeds. *Prunus* seeds were soaked in water for 2 days, stratified in perlite trays, covered with moist paper towels, and maintained in a cold storage room at 4 C for 45 days until radicle emergence. Citrus seeds were treated with a 0.5% NaOCl solution for 1/2 hour, then rinsed and placed in germination trays containing vermiculite. Walnut, loquat, and olive were germinated in sterile sand without any treatment. After 6–8 weeks, germinated seeds and rooted cuttings were transplanted into 2.8-liter PVC pots that contained a pasteurized sandy loam soil (73% sand, 22% silt, 5% clay; pH 7.5; <1% organic matter; cation exchange capacity < 10 meq/100 g soil). Plants were maintained in a greenhouse for 3 to 5 months before nematode inoculation.

A *Pratylenchus vulnus* population isolated from rose in Cabrils, Barcelona, was cultured monoxenically on carrot disks (18). Species identification was made by the Commonwealth Institute of Parasitology, St. Albans, United Kingdom.

Two experiments were carried out. In 1989, 19 fruit tree and grape rootstocks were inoculated with 1,000 nematodes per plant and evaluated after 1 year. In the second trial (1990), 18 fruit tree and citrus rootstocks were inoculated with the same inoculum level and evaluated after 6 months.

Inoculum of *P. vulnus* was recovered from stock cultures by adding water to the cultures and collecting the nematodes on a 0.025-mm sieve (500 mesh). The volume of the nematode suspensions was adjusted to give an initial density (Pi) of 1,000 individuals per plant. Plants with uniform growth were inoculated by adding nematodes to four holes located 4 to 5 cm from the base of the plant. Inoculated pots were placed in a sand bed to minimize temperature and humidity fluctuations. Both experiments were conducted under greenhouse conditions with controlled temperature that fluctuated between 5 and 14 C in winter (to induce dormancy in the first experiment) and 20 to 28 C the rest of the year. Plants were watered as needed and fertilized with full-strength Hoagland's nutrient solution once a week.

At the end of each experiment, soil from each pot was separated from the roots and placed in a large pan with water. Roots were washed in a second pan to remove soil particles, and the resulting suspension was added to the pan containing the soil. The resulting soil suspension was stirred thoroughly and nematodes were extracted from a 250-cm^3 subsample by differential sieving using 0.150-mm, 0.074-mm, and 0.038-mm screens (100, 200, and 400 mesh, respectively) and centrifugal flotation (9). Fresh root weight was determined, and the whole root system was cut into small pieces (1 cm long) and macerated in water in a blender for 30 seconds at consecutive 10-second intervals. This suspension was then concentrated using 0.150-mm, 0.074-mm, and 0.025-mm sieves (100, 200, and 500 mesh, respectively). Root tissue and debris collected on the 0.150-mm sieve were discarded. Nematodes were recovered from the remaining sample by sugar centrifugation. Final population (Pf) was computed as the total number of nematodes extracted from soil and roots for each plant. The *Prunus* rootstocks San Julian 655-2, GF-305, Myrobalan 605 AD, and the pear OHF-333 tested in a previous pathogenicity study (21) were used as reference rootstocks. To determine the relative host suitability among rootstocks, Pf/Pi values were established for each case.

In both experiments, each rootstock was replicated five times in a completely randomized design. Final mean nematode population and standard deviation were calculated, as well as the mean number of nematodes per gram of root.

Results

In the first experiment, 15 rootstocks and (or) selections were found to have Pf

> Pi (Table 1). Final nematode population per plant (soil and roots) in host material ranged from 22,030 nematodes (highest) in the loquat cultivar Nadal to 1,140 in 161-49 grape (lowest). Loquat and the cherry selection Camil reached the highest numbers of nematodes per gram of root (1,950). The grape 161-49 was the only rootstock considered as a poor host (Pf slightly higher than its initial inoculation level). Arbequína olive and the grape rootstocks SO4, *Vitis rupestris* Lot and 1103-P had Pf/Pi < 1.

In the second experiment, 12 rootstocks had a Pf/Pi > 1 (Table 2). Final nematode population in host material ranged from 45,520 in EM-9 apple (highest) to 1,240 in Nemaguard peach (lowest). The highest level of parasitism was recorded in *Pistachia atlantica,* which reached 4,160 nematodes per gram of root. All citrus rootstocks (Alemow, rough lemon, Carrizo citrange, sour orange, Troyer citrange, and Citrumelo) were nonhosts of *P. vulnus.* No nematodes were recovered from the roots, with the exception of Alemow. Small to medium-size lesions were observed in several of the nematode-infected root systems with active growth, especially on *Prunus* and *Malus.* In both experiments, nematode population variability was high in the majority of the tested materials.

Discussion

Of the 37 rootstocks tested in this study, 26 had not been reported as hosts of *P. vulnus.* However, different cultivars of the same plant species that have been tested in other studies were reported to be good hosts (4,11,22).

The *Prunus* cultivars (peach, cherry, plum, and almond) tested were all found to be good hosts for *P. vulnus,* with the exception of the root-knot nematode resistant rootstock Nemaguard, on which the initial inoculum density was barely maintained. The results of this study confirm the need to search for sources of resistance to *P. vulnus* among wild *Prunus* species. *Prunus tomentosa* and *P. fremontii* are the only wild *Prunus* species recorded as resistant to this nematode (22). Other *Prunus* rootstocks that have been found tolerant to the nematode include *P. besseyi, P. cerasifera,* and *P. armeniaca* (20). It can also be concluded that grape has a variable response and that some grape rootstocks, especially hybrid crosses of *V. vinifera* × *V. berlandieri,* are hosts, whereas other hybrids rootstocks are nonhosts. The grape rootstocks SO4, Rupestris de Lot, and the olive Arbequina had Pf/Pi < 1. However, some reproduction of the nematode did occur, because larval stages were recovered from the soil. In general, citrus appears to be a nonhost for the *P. vulnus* isolate tested here. No nematodes were recovered from the roots, with the exception of Alemow (20 specimens), although low numbers were found in the soil.

In host-range studies, discrepancies are likely to occur, particularly when isolates from different geographical regions are used. The peach rootstock Nemaguard is considered to be susceptible to *P. vulnus* in California, causing up to a 23% loss in yield in plum (16) under field conditions. This rootstock was considered a poor host for the isolate from Spain tested in this study, reaching a Pf of 1,240 per plant, slightly over the initial inoculation level (Table 2). The host-susceptibility relation in Nemaguard is not well defined in relation to a range of nematode isolates and field conditions.

Four rootstocks (Myrobalan 605, San Julian 655-2, GF-305, and OHF-333) that were evaluated and used as reference rootstocks in this host-range study reached a lower Pf compared to the high densities reached in a previous pathogenicity study carried out under similar greenhouse conditions (21); however, the amount of nematodes per gram of roots was very similar in both studies. San Julian 655-2 and GF-305 and Santa Lucia 64 have also been evaluated as hosts to *P. vulnus* in France (22), but the number of nematodes per gram of root reported in that study was considerably lower than that reported here. These discrepancies were probably

TABLE 1. Final population density (Pf) of *Pratylenchus vulnus* on 19 commercial fruit tree and grape rootstocks 12 months after inoculation with an initial density (Pi) of 1,000 nematodes per plant.

Plant species	Cultivar or rootstock	Origin	Pf in roots and soil (nematodes/pot)	Pf in roots (nem/g of root)	Pf/Pi
Loquat (*Eriobotrya japonica*)	Nadal	Alicante, Spain	22,030 ± 13,190	1,950	22.0
Pear (*Pyrus communis*)	OHF-333	Oregon, USA	18,110 ± 11,750	1,795	18.1
Plum (*Prunus insititia*)	San Julian 655	INRA, France	16,640 ± 10,780	830	16.6
Cherry (*Prunus mahaleb*)	Santa Lucía 64	INRA, France	14,670 ± 16,820	980	14.6
Cherry (*Prunus candescens*)	Camil	Gembloux, Belgium	13,540 ± 10,470	1,945	13.5
Cherry (*Prunus avium* × *P. mahaleb*)	M × M 14	East Malling, U.K.	10,740 ± 4,220	530	10.7
Walnut (*Juglans regia*)	Serr	USDA, USA	10,733 ± 7,046	300	10.7
Grape (*Vitis vinifera* × *V. berlandieri*)	41-B	Millardet & Grasset, France	10,630 ± 4,850	1,596	10.6
Plum (*Prunus insititia*)	Montizo	S.I.A., Spain	7,100 ± 9,277	570	7.1
Grape (*Vitis vinifera* × *V. berlandieri*	Fercal	INRA, France	5,600 ± 4,330	1,810	5.6
Grape (*Vitis berlandieri* × *V. rupestris*)	Ritcher-110	Ritcher, France	5,160 ± 4,010	1,100	5.1
Plum (*Prunus insititia*)	Pixy	East Malling, U.K.	4,950 ± 3,780	600	4.9
Peach (*Prunus persica*)	GF-305	INRA, France	3,990 ± 4,010	180	3.9
Plum (*Prunus cerasifera*)	Myrobalan 605	S.I.A., Spain	2,430 ± 2,990	130	2.4
Grape (*Vitis riparia* × *V. berlandieri*)	161-49 C	Courders, France	1,140 ± 530	160	1.1
Olive (*Olea europea*)	Arbequína	Tarragona, Spain	930 ± 590	20	0.9
Grape (*Vitis riparia* × *V. berlandieri*)	SO4	Oppenheim, France	800 ± 650	220	0.8
Grape (*Vitis rupestris*)	Rupestris de Lot	Unknown, USA	490 ± 330	150	0.4
Grape (*Vitis berlandieri* × *V. rupestris*)	1103-P	Paulsen, Italy	150 ± 210	20	0.1

Data are means ± standard deviations of five replications.

TABLE 2. Final population density (Pf) of *Pratylenchus vulnus* on 18 commercial fruit tree and citrus rootstocks 6 months after inoculation with an initial density (Pi) of 1,000 nematodes per plant.

Plant species	Cultivar or rootstock	Origin	Pf in roots and soil (nematodes/pot)	Pf in roots (nem/g of root)	Pf/Pi
Apple (*Malus communis*)	EM-9	East Malling, U.K.	45,520 ± 48,048	330	45.2
Apple (*Malus communis*)	EM-106	East Malling, U.K.	21,150 ± 30,890	1,290	21.1
Hazelnut (*Corylus avellana*)	Pauetet	Reus, Spain	14,000 ± 7,490	530	14.0
Cherry (*Prunus cerasus*)	Masto de Montañana	S.I.A., Spain	11,530 ± 10,430	570	11.0
Almond (*Prunus amygdalus*)	Desmayo Rojo	Aragón, Spain	8,440 ± 10,220	240	8.4
Pistachio (*Pistachia terebinthus*)	Terebinto	Tarragona, Spain	8,310 ± 6,260	920	8.3
Pistachio (*Pistachia vera*)	Pistachio	Unknown, Syria	6,390 ± 3,446	190	6.3
Peach (*Prunus persica*)	Montclar	INRA, France	5,890 ± 5,030	630	5.8
Pistachio (*Pistachia atlantica*)	Pistachio	Canary Islands	5,690 ± 3,600	4,160	5.6
Bitter almond (*Prunus amygdalus*)	11-43	Tarragona, Spain	4,610 ± 3,070	480	4.6
Avocado (*Persea americana*)	Hass	California, U.S.A.	3,150 ± 1,140	305	3.1
Peach (*Prunus persica* × *P. davidiana*)	Nemaguard	USDA, USA	1,240 ± 360	140	1.2
Citrus (*Citrus macrophylla*)	Alemow	Unknown, Philippines	810 ± 650	20	0.8
Citrus (*Citrus jambhiri*)	Rough lemon	Unknown, India	780 ± 790	0	0.7
Citrus (*Poncirus trifoliata* × *Citrus sinensis*)	Carrizo Citrange	Texas, USA	480 ± 640	0	0.4
Citrus (*Citrus aurantium*)	Sour orange	Southeast Asia	415 ± 400	0	0.4
Citrus (*Poncirus trifoliata* × *Citrus sinensis*)	Troyer Citrange	California, USA	120 ± 230	0	0.1
Citrus (*Poncirus trifoliata* × *Citrus paradisi*)	Citrumelo	Florida, USA	0	0	0

Data are means ± standard deviations of five replications.

due to the different methods and experimental conditions used to evaluate materials as host. Also, the possibility of a different pathotype of *P. vulnus* should not be discarded.

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