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RESEARCH PAPER

Resistance assessment of grapevine rootstocks used in Chile to the root-knot nematodes *Meloidogyne ethiopica, M. hapla,* and *M. javanica*

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Abstract

E. Aballay, and O. Vilches. 2015. Resistance assessment of grapevine rootstocks used in Chile to the root-knot nematodes *Meloidogyne ethiopica, M. hapla, and M. javanica*. Cien. Inv. Agr. 42(3): 407-413. The rootstocks 1103 P, 101-14, K 5BB, SO4, and 3309 were assessed to determine their resistance to the three common *Meloidogyne* species present in Chilean vineyards, *M. ethiopica*, the most frequent species, *M. hapla* and *M. javanica*. Their response was compared to three ungrafted cultivars, Thompson Seedless, Pinot Noir and Chardonnay. To perform this study, two month-old plants produced from cuttings were inoculated with 5,000 eggs per 3-L pots, filled with steamed substrate and kept in a glasshouse covered with a black mesh to intercept 30% of sun light and avoid heating of the substrates. After a growth period of six months, plants were removed from the pots, and resistance was determined by measuring root weights, the number of galls and eggs per g of root, and second stage juveniles per 250 cm^3 of soil. Results showed that the five rootstocks were resistant to the parasitism of the three *Meloidogyne* species, since their reproduction was limited and differed from two of the cultivars. Chardonnay was the most susceptible cultivar, mainly to the high level of parasitism by *M. ethiopica*, while Pinot Noir was moderately susceptible and Thompson Seedless showed the lowest susceptibility.

Key words: Plant-parasitic nematodes, root pests, *Vitis vinifera*, vineyards.

Introduction

Grapevine (*Vitis vinifera*) is one of the most important crops in Chile, cultivated for table grape as well as wine and liquor production. Several genera and species of plant-parasitic nematodes have been reported to cause economic damage and to be present in the area under cultivation (Aballay *et al*., 2009). *Meloidogyne* species are frequently detected in root systems of grape plants and are especially harmful in wine grape cultivars, such as Chardonnay, Cabernet Sauvignon, Merlot, Sauvignon Blanc and Shiraz. Some table grape cultivars, such as Red Globe and Flame Seedless, are also very susceptible to root-knot nematodes and are frequently associated with the fungi responsible for black-foot disease and grapevine decline (Scheck *et al*., 1998; Montealegre *et al*., 2009). *M. ethiopica* is the most frequently detected root-knot species, and for many years, it was identified as *M. incognita*, *M. arenaria* or *M. javanica,* due to similar

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morphological characteristics and its response to differential host test (Carneiro *et al*., 2004, 2007). More than 99% of the vineyards infested with this genus show the presence of *M. ethiopica* (Carneiro *et al*., 2007). It has been observed that *M. ethiopica* is more aggressive than other *Meloidogyne* species present in Chile, as evidenced by the larger size of galls on the roots, the number of eggs per gram of roots and the premature decline of plants. Multiple classical control methods and strategies are employed for infested soils including the use of fallow, organic amendments, non-fumigant nematicides and others (Alvarez, 2006).

Under replanting conditions, the use of resistant rootstocks is a good alternative, although these control measures are not extensively applied by most growers for several reasons, among them the absence of the insect root pest filoxera *Daktilosphaera vitifolii* in Chilean vineyards (Aballay *et al*., 2009). Additionally, a given rootstock is not resistant and/or tolerant to all nematodes, *e.g., Xiphinema index* or *Mesocriconema xenoplax*. Finally, it is not clear whether rootstocks have a similar level of resistance to all *Meloidogyne* species.

Meloidogyne species may exhibit differences in pathogenicity in the same host (Roberts, 1995). It is noteworthy that the most common species, *M. incognita, M. hapla, M. javanica and M. arenaria* are also present in the country, and previous tests did not consider the relevant presence of *M. ethiopica* (Aballay *et al*., 1997).

The purpose of this study was to assess the resistance of five grapevine rootstocks used in Chile *to M. ethiopica, M. hapla* and *M. javanica*, three species present in vineyards in Chile, and to compare their response with three ungrafted cultivars.

Materials and methods

Five grapevine rootstocks and three commercial cultivars (controls) were evaluated for resistance to three *Meloidogyne* species: 1103 P (*V. berlandieri x V. rupestris*), 101-14 (*V. riparia x V. rupestris*), Kober 5BB (*V. berlandieri* x *V. riparia*), 3309 (*V. riparia* x *V. rupestris*), SO4 (*V. berlandieri* x *V. riparia*), Chardonnay (*V. vinifera*), Pinot Noir (*V. vinifera*) and Thompson Seedless (*V. vinifera*). Plants were obtained from dormant wood cuttings collected in winter from different vineyards and nurseries and rooted in sterile perlite. Once rooted, the plants were transplanted into steamed 1:1:1 (v/v) sand: agriculture soil: organic matter mix in 3.0-L pots and kept in a glasshouse.

After two months of growth, plants were inoculated with the nematode pure species inoculum, obtained from tomato, kiwi plants and grapevines for *M. javanica, M. hapla* and *M. ethiopica,* respectively. Eggs were extracted from the roots according to the method described by Hussey and Barker (1973) and inoculated in a water suspension of 10 mL at a dose of 5,000 eggs per pot with a pipette through 5 holes dug in the soil around the plant.

Once inoculated, plants were grown in a shaded 10 m × 20 m greenhouse covered by a raschel mesh, which intercepted 30% of sunlight and prevented overheating of plants and pots. Plants were watered once or twice per week, depending on the temperature. The maximum and minimum temperature outside the greenhouse in mid-summer were approx. 34 and 15 °C, respectively, and within the greenhouse 28 and 18 °C, respectively.

Each rootstock or grape cultivar was inoculated with the three species of *Meloidogyne*.

The capacity of nematodes to parasitize and reproduce in every rootstock or cultivar was determined at the end of the growing season of the grape plants in early autumn, approximately 6 months after inoculation, by determining the number of second stage juveniles in the soil, along with eggs and galls in the roots. Plants were carefully removed from containers, and the

roots were separated from the canopy, washed and fresh weighed. The number of galls per plant was counted, and eggs were extracted from roots according to the Hussey and Barker method (1973).

The soil from each pot was mixed, and a 250 cm^3 sample was taken to quantify the number of juveniles present, combining the soil sieving and decanting method with Baermann´s funnel (Southey, 1986). Counting was performed using a dissecting microscope Carl Zeiss Stemi 2000 C at 50-90 x magnification.

The experiment was set up in a completely randomized design with factorial structure and 4 replicates, where the two factors are rootstocks-cultivar and nematode species (8 × 3). Data were analyzed separately for each nematode species and rootstock/cultivar with one-way Analysis of Variance (ANOVA). When F values were significant, differences between means were evaluated using Tukey's Multiple Range ($P \leq 0.05$). Experiments were repeated twice, with two sets of pots, and the data presented correspond to the average of both experiments. Minitab® V16 was used for data analysis.

Results

Results from the experiments are presented in Table 1, showing the reproduction parameters with the significance for each combination cultivar – nematode species. The analysis showed that there was an interaction between both factors ($P \leq 0.05$).

Differences were not observed among rootstocks in relation to each of the *Meloidogyne* species, exhibiting the same population levels measured through the three parameters evaluated. This clearly indicates that there were no differences in the reproductive capacities of *M. ethiopica*, *M. hapla* and *M. javanica* on any of the rootstocks ($P \leq 0.05$). Apparently, none of the rootstocks were immune, since the three nematode species were able to reproduce to a limited extent.

Two cultivars were more susceptible to the three *Meloidogyne* species compared with the rootstocks, in spite of differences between them. Thompson Seedless was the most resistant, not showing differences with the five rootstocks for *M. hapla* and *M. javanica* in the number of galls, while it was more resistant than Chardonnay but more susceptible than the rootstocks to *M. ethiopica*.

Table 1. Reproduction of *Meloidogyne* spp. in five rootstocks and three grape cultivars.

	Galls g of roots^{-1}			Eggs g roots^{-1}			Second stage juveniles 250 cm^{-3} soil		
Rootstocks and cultivars	*M. ethiopica*	*M. hapla*	*M. javanica*	*M. ethiopica*	*M. hapla*	*M. javanica*	*M. ethiopica*	*M. hapla*	*M. javanica*
1103 P	0.39 aA	0.33 aA	0.18 abA	12.43 aA	19.83 aA	5.86 aA	7.75 abA	8.50 aA	7.38 aA
SO4	1.04 aA	0.20 aA	0.14 abA	32.36 aA	44.37 aA	41.16 aA	7.50 abA	5.50 aA	4.13aA
101-14	0.15 aA	0.10 aA	0.04 bA	34.66 aA	31.26 aA	23.04aA	5.75 bA	9.25 aA	12.25 aA
K 5BB	0.42 aA	0.35 aA	0.19 abA	48.56 aA	106.41aA	62.66 aA	13.13 abA	7.50 aA	7.75 aA
3309	0.13 aA	0.17 aA	0.43 abA	44.65 aA	31.50 aA	95.26 abA	13.13 abA	7.75 aA	7.38 aA
Pinot Noir	2.63 bA	3.35 bA	0.66 abB	131.92 aA	164.59 abA	102.79 abA	36.50 aA	107.25 bB	14.25 aA
Chardonnay	15.63 cA	3.99 bB	1.54 aC	2,529.52 bA	625.16 bB	198.23 bC	242.88 cA	67.10 bB	29.50 aC
Thompson Seedless	2.48 bA	0.17 aB	0.18 abB	120.01 aA	60.17 aA	113.31 abA	22.50 abA	17.63 aA	22.88 aA

Data are the means of four replicates, in a duplicated experiment.
Upper case letters in rows show the differences between *Meloidogyne* species, and lower case letters in columns compare rootstocks and cultivars.
Means in columns or in rows followed by the same letter do not differ according to Tukey Multiple Range Test ($P \leq 0.05$).

M. ethiopica was the *Meloidogyne* species with the highest rate of reproduction with the three ungrafted cultivars, while *M. hapla* showed similar reproductive capacity as *M. ethiopica* with Pinot Noir.

Chardonnay was the most susceptible cultivar to the parasitism of *M. ethiopica*, since this species reached the highest number of galls, eggs and second stage juveniles of all the nematode-cultivar combinations ($P \leq 0.05$).

Discussion

Most published studies on rootstock resistance evaluation in grapevine host against *Meloidogyne* were performed with the four most common species, *M. incognita, M. hapla, M. javanica* and *M. arenaria*. *M. ethiopica* was identified in Chile in 2003, and in subsequent surveys, it was determined that it is present in most Chilean vineyards, in addition to tomatoes and other crops (Carneiro *et al*., 2007). Morphologically, its perineal patterns are similar to *M. incognita* and *M. arenaria* (Carneiro *et al*., 2004), which explains the misidentification in previous reports (Aballay *et al*., 1997; Alvarez, 2006).

According to our results, *M. ethiopica* is a more aggressive parasite, since the numbers of galls, eggs and second stage juveniles were higher than the other two species. All of the tested rootstocks supported low rates of reproduction for all three *Meloidogyne* species, implying that they are resistant, since they limit the reproduction of the nematodes by 90% compared with the most susceptible cultivar (Taylor and Sasser, 1983; Trudgil, 1991). Working on resistance mechanisms in woody hosts (*Prunus*), Marull *et al*., (1994), reported that root-knot nematodes were able to penetrate the roots in resistant rootstocks but most did not complete their life cycle. Those findings could explain the low numbers of nematodes found at the end of our experiment on *Vitis* host.

Edwards (1989) notes that SO4 and K 5BB are resistant to *M. javanica*; however, Dalmasso and Cuani (1976) report that SO4 is susceptible to *M. hapla*. In our study, all three rootstocks supported significantly lower rates of reproduction than the ungrafted cultivars.

Boubals (1979) classifies 101-14 as resistant to *M. javanica*, the same response as in our study. Meanwhile, Moura *et al*. (2014) note that this rootstock and K 5BB are resistant to *M. incognita* and *M. javanica*.

Muñoz and Gonzalez (2000) described the rootstock 3309 as being susceptible to the parasitism of most species of *Meloidogyne*. In our study, this rootstock showed a low number of galls and juveniles, but the number of eggs was similar to Thompson Seedless and Pinot Noir when infested with *M. javanica*, which could mean that this rootstock may be susceptible when a mixture of species that includes *M. javanica* is present. In previous studies, Mancilla (2004) concluded that under field and glasshouse conditions, SO4, 101-14, Kober 5BB were resistant, while 3309 was more susceptible to *M. ethiopica*.

In other studies (Mckenry and Anwar, 2006) classified 3309 as susceptible to pure populations or mixtures of M. *incognita, M. arenaria* and *M. javanica*, indicating however, that the plants exhibited good growth due to the higher vigor of this rootstock. SO4 was classified as resistant to all three species in the same study.

Cultivars were more susceptible to the three *Meloidogyne* species compared with the rootstocks. The higher sensitivity of Chardonnay to *Meloidogyne* spp. has been reported previously (Aballay *et al.,* 1997), where >65% of roots developed galls when infested with a mixture of species. This higher susceptibility is shown by the number of galls and also by their larger sizes (Alvarez, 2006).

Pinot Noir has been reported as a susceptible cultivar to *Meloidogyne* spp. (Vieira, 1979). However, when compared with Chardonnay reproduction rates, it may be classified as a moderately susceptible cultivar (Taylor and Sasser, 1983).

The response of Thompson Seedless supporting lower rates of reproduction compared with the other two cultivars has been reported previously by Melakeberhan and Ferris (1988) and Walker *et al*., (1994) for *M. incognita* and *M. arenaria* and indicate that this cultivar may be considered moderately resistant to both species (Taylor and Sasser, 1983). According to our results, Thompson Seedless shows a similar reaction when challenged with *M. ethiopica*, which is interesting, considering that this is the most widely distributed species in Chilean vineyards.

The use of rootstocks is a necessary tool to manage the populations of nematodes, irrespective of the fact that they support a limited level of nematode reproduction. Data from Spain (Téliz *et al*., 2007; Gutiérrez-Gutiérrez *et al*., 2011) indicate that *M. arenaria, M. incognita* and *M. javanica*, and the dagger nematode *X. index*, have a reproductive rate higher than 1 in several *Vitis* rootstocks evaluated, including SO4 and 1103 P, indicating that these rootstocks are suitable hosts for nematode reproduction. However, cultivars such as Cabernet Sauvignon support higher levels of reproduction (~8-10 times) compared with the evaluated rootstocks.

In conclusion, these findings suggest that the rootstocks SO4, K 5BB, 1103 P and 101-14 can be grafted with grape cultivars and planted in soils with either a pure populations or a mixture of the species *M. ethiopica, M. hapla and M. javanica*. These rootstocks are not immune, but they are more resistant than ungrafted cultivars. Additionally, the response of Thompson Seedless suggests that it may be planted ungrafted in soils with a low population density of *Meloidogyne* spp.

Resumen

E. Aballay y O. Vilches. 2015. Resistencia de portainjertos de vid utilizados en Chile a los nematodos de la raíz *Meloidogyne ethiopica, M. hapla* y *M. javanica*. Cien. Inv. Agr. 42(3): 407-413. Los portainjertos 1103 P, 101-14, K 5BB, SO4, 3309, de uso frecuente para la plantación de vides en Chile, fueron evaluados para determinar su resistencia a tres especies de nematodos del género *Meloidogyne, M. ethiopica*, la especie de mayor frecuencia en viñedos en Chile, *M. hapla* y *M. javanica*. Junto con ello se evaluó la susceptibilidad de tres cultivares no injertados, Thompson Seedless, Pinot Noir y Chardonnay. Para ello, al inicio de la primavera, plantas nuevas de 2 meses de desarrollo producidas a partir de estacas, cultivadas en macetas de 3 L con sustratos estéril, fueron inoculadas con 5.000 huevos por maceta y mantenidas en un sector habilitado con malla raschel durante 6 meses. Para determinar su resistencia, al término del período de cultivo las plantas se sacaron de las macetas y se determinó su peso radical, número de agallas por masa de raíz, cantidad de huevos por gramo de raíz y número de juveniles de segundo estado en el suelo. Los resultados mostraron que los cinco portainjertos se comportaron como resistentes al ataque de las tres especies de *Meloidogyne*. De las variedades cultivadas, Chardonnay mostró una alta susceptibilidad, especialmente frente a *M. ethiopica*, Pinot Noir se comportó moderadamente susceptible, en tanto que, Thompson Seedless mostró una resistencia moderada.

Palabras claves: Nematodos fitoparásitos, plagas de raíces, *Vitis vinifera,* viñas.

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