Vitis spp. Rootstocks Are Poor Hosts for *Meloidogyne hapla*, a Nematode Commonly Found in Washington Winegrape Vineyards

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Abstract: The majority of winegrape (*Vitis vinifera*) vineyards in Washington are planted with own-rooted grapevines, as opposed to grapevines grafted onto rootstock varieties. The plant-parasitic nematode *Meloidogyne hapla* (common name: northern root-knot nematode) is commonly found in Washington winegrape vineyards, and own-rooted grapevines are susceptible to this nematode. Before rootstocks are used to manage *M. hapla* or other horticultural characteristics in Washington, their host status for *M. hapla* should be defined. In greenhouse experiments, 10 commercially available rootstock varieties were evaluated for their *M. hapla* host status. Additionally, the reproductive potential of different *M. hapla* populations collected from Oregon and Washington, and of another root-knot nematode, *M. chitwoodi*, on rootstock varieties and own-rooted *V. vinifera* Chardonnay was evaluated. The rootstocks Salt Creek, Freedom, Harmony, St. George, Riparia Gloire, 101-14 Mgt, 3309C, 110R, 420A, and Matador were poor hosts for *M. hapla*. Populations of *M. hapla* varied in reproductive potential and virulence on own-rooted Chardonnay. An *M. hapla* population collected from a *V. vinifera* vineyard in Paterson, WA had 33 to 78% greater reproduction than the other *M. hapla* populations. An *M. hapla* population collected from a *V. vinifera* vineyard in Alderdale, WA was consistently more virulent than the other *M. hapla* populations. Own-rooted Chardonnay and the rootstock Matador were poor hosts for *M. chitwoodi.* This is the first report of the host status of several grapevine rootstocks for *M. hapla*.

Key words: root-knot nematode, semiarid, virulence, *Vitis vinifera*

Over 30 different winegrape varieties are cultivated on ~21,043 ha of vineyards in Washington (NASS 2017). Most of these vineyards are planted with own-rooted varieties of *Vitis vinifera*, as opposed to grapevines grafted onto rootstock varieties. The periodic occurrence of sub-zero cold winter temperatures, particularly rapid drops in temperature during vine cold hardiness acclimation and deacclimation, can result in cold injury to vines (Ferguson et al. 2014). Recent examples of such weather events occurred during the "Halloween Freeze" (31 Oct) of 2002 and the "Thanksgiving Freeze" (24 Nov) of 2010, when temperatures dropped to -11.5 and -17.3°C, respectively (AgWeatherNet; weather.wsu.edu). When vines are own-rooted, vineyards can be retrained during the season immediately following cold damage, resulting in only a one-year loss in crop (Moyer et al. 2011). However, when cold damage occurs to vines that are grafted onto a rootstock variety, the growing season immediately following a cold event is either spent field-grafting a scion onto the rootstock variety or removing the remaining rootstocks entirely and replanting. This process can result in a crop loss for up to two to three years following a damaging cold event.

The modern Washington winegrape industry underwent its first rapid vineyard expansion in the 1980s, followed by an additional period of rapid growth from 1993 to 1999 (NASS 2017). Thus, many vineyards are either past or approaching the end of their productive lifespans and are scheduled for replanting within the next several years. Plant-parasitic nematodes are commonly found in Washington vineyards and could be a concern for replanting. Surveys conducted in eastern Washington found *Meloidogyne hapla*, the northern root-knot nematode, to be the most abundant nematode present, found in 60% of the surveyed vineyards (Zasada et al. 2012). The proposed threshold is 100 *M. hapla*/250 g soil (Santo, unpublished data, 2000), a density exceeded in 26% of surveyed winegrape vineyards in Washington. While *M. hapla* is the predominant species found in the region, *M. chitwoodi*, another other common Pacific Northwest *Meloidogyne* species, is also widespread in other crop production systems (Zasada et al. in press). Own-rooted *V. vinifera* varieties have been shown to be good hosts for *M. hapla* (Howland et al. 2015). Unfortunately, given the preference for own-rooted vines in

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Washington, replant situations where susceptible vines are placed into sites with high nematode pressure is a concern for vineyard establishment and productive lifespan.

Meloidogyne spp., or root-knot nematodes, are a significant production and economic constraint to grapevines worldwide (Jenser et al. 1991, Arredondo 1992, Nicol et al. 1999). As sedentary endoparasites, these nematodes remain stationary inside the roots of a host plant for the majority of their lifespan. Adult females lay their eggs outside the roots in a gelatinous matrix and a single egg mass can contain up to 400 to 500 eggs. The infective stage is the second-stage juvenile, which hatches from eggs and migrates through the soil in search of a root tip to penetrate. Once within the root tip, the juvenile migrates up the root, where it ultimately establishes a feeding site and completes its lifecycle. In the United States, *Meloidogyne* spp. have been reported to reduce grapevine yields by up to 20% (Anwar and McKenry 2000). Seven species of *Meloidogyne* are found on grapevines, but only four species, *M. incognita*, *M. hapla*, *M. javanica*, and *M. arenaria*, are considered damaging (Esnard and Zuckerman 1998, Esmenjaud and Bouquet 2009).

Most winegrape-producing regions use rootstocks to manage plant-parasitic nematodes when they are identified as a production constraint. Breeding for resistance to *Meloidogyne* spp. has been the primary goal of some rootstock programs over the years. The cultivars Harmony and Freedom were the first *Meloidogyne*-resistant rootstocks to come from a breeding program (Weinberger and Harmon 1966). 101-14 Mgt and Ramsey (= Salt Creek) are also considered resistant to *Meloidogyne* spp. (Nicol et al. 1999, Ferris et al. 2012). Other rootstocks more recently developed with resistance to *Meloidogyne* spp. include UCD GRN1, 2, 3, 4, and 5 (Ferris et al. 2012), USDA 10-17A, USDA-23B, USDA 6-19B, RS-3, and RS-9 (Anwar et al. 2002, Gu and Ramming 2005a, 2005b), and Matador, Minotaur, and Kingfisher (Cousins 2011). In a summary of the literature on nematode-resistant rootstocks, *M. hapla* was not included (Ferris et al. 2012). Very little is known about the response and host status of rootstocks to *M. hapla*, and no breeding programs focus on developing rootstocks with resistance to *M. hapla*.

The host status of rootstocks for the industry-prevalent *M. hapla* must be known for the Washington wine industry to deploy rootstocks effectively for management of nematodes and other desired horticultural characteristics. The research presented here is a first step in this direction. The objectives were: 1) to determine the host status of *Vitis* rootstocks for *M. hapla*, 2) to determine whether *M. hapla* populations from Washington and Oregon differ in virulence on *Vitis* rootstocks and own-rooted *V. vinifera* Chardonnay, and 3) to compare the ability of *M. hapla* to parasitize own-rooted Chardonnay and the rootstock Matador with that of *M. chitwoodi*.

Materials and Methods

Experiment 1: Determining host status of rootstocks for *M. hapla*. Nine rootstocks, including Salt Creek, Freedom, Harmony, St. George, Riparia Gloire, 101-14 Mgt, 3309C, 110R, and 420A (Sunridge Nurseries, Inc., Bakersfield, CA) (Table 1), were evaluated for host status to a single population of *M. hapla*. Own-rooted *V. vinifera* Riesling was included as a susceptible control (Howland et al. 2015). In March 2014, dormant, non-rooted cuttings of each rootstock and the own-rooted Riesling were grouped relative to stem diameter to ensure vine uniformity. Using pruning shears, vines were cut into three node segments, with the basal internode cut diagonally. The basal internode was dipped in rooting hormone (1% indole-3-butyric acid, 0.5% 1-napthalaneacetic acid; Dip'N Grow) to stimulate root growth. Cuttings were inserted in a perlite and vermiculite mixture (Santo and Hackney 1980) and placed on a bench with a heating pad for two months, where they were misted with water every 30 min.

In April 2014, the grape cuttings were removed from the mist bench and placed in a greenhouse under a shade cloth to be hardened-off. A week later, established grape cuttings of each rootstock or own-rooted Riesling with uniform root systems were transplanted into 3.7 L pots containing a steam-pasteurized 1:1 sand:Willamette loam soil. Buds were removed until only a single bud/shoot remained, and any developing inflorescences were removed to promote root growth. The grapevines were fertilized initially with a 9-45-15 NPK starter fertilizer (Jack's Professional) at a rate of 4 g/L, delivering 336 mg/L N. Four weeks later, the grapevines were fertilized with a 20-20-20 NPK fertilizer (Jack's Professional) at a rate of 16 g/L, delivering 150 mg/L N; vines were fertigated biweekly thoughout the experiment. The grapevines were grown in a greenhouse under a 16 hr photoperiod for the duration of the experiment; temperatures were set to 25°C during the day and 20°C at night.

In late May 2014, vines were inoculated with nematodes. The *M. hapla* population was originally collected from a *V. vinifera* vineyard in Veneta, OR (designated Veneta). To establish the population in culture, soil was collected from the vineyard, placed in a 2 L clay pot, and a 3- to 4-wk-old tomato (*Solanum lycopersicon* Mill. Rutgers) was planted in each pot. After approximately four to five months, plants were removed from the pots, roots were washed free of adhering soil, and single egg masses were picked and placed on new tomato plants. These plants were maintained for an additional three to four months with these single-female lines used as inoculum. Inoculum was obtained by destructively harvesting tomato

Table 1 Parentage of *Vitis* rootstocks evaluated against *Meloidogyne hapla* and *M. chitwoodi*.

Rootstock	Species
Salt Creek	*Vitis* × *champinii*
Harmony	1613 C (*V. solonis* × Othello) × *V. champinii*
Freedom	1613 C (*V. solonis* × Othello) × *V. champinii*
St. George	*V. rupestris*
Riparia Gloire	*V. riparia*
101-14 Mgt	*V. riparia* × *V. rupestris*
3309C	*V. riparia* × *V. rupestris*
110R	*V. berlandieri* × *V. rupestris*
420A	*V. berlandieri* × *V. riparia*
Matador	101-14 Mgt × (*V. mustangensis* × *V. rupestris*)

plants and collecting eggs from washed roots by agitating the root system in a 0.05% NaOCl solution for 3 min (Hussey and Barker 1973). The egg suspension was then poured over nested 250- and 25-µm-sieves, with eggs being retained on the 25-µm-sieve. A 1 mL subsample of the egg suspension was placed on a counting slide to determine the total inoculum concentration. The suspension was then diluted until the concentration equaled 9000 eggs/3.7 L pot, or a density of three *Meloidogyne* eggs/g of soil. The inoculum was applied to each grapevine by pipetting 5 mL suspension into four holes, 6 cm deep around the base of the vine. The holes were covered and plants were watered regularly starting the next day. The rootstocks and own-rooted Riesling were arranged in a randomized block design on a greenhouse bench with treatments replicated six times; the experiment was conducted twice with trials separated in time (inoculation was offset by a week) and space (trials were conducted in different greenhouses).

Plants were destructively harvested in October 2014. For each vine, the shoot was removed, placed in a paper bag, dried at 70°C for five days, and weighed. Roots were shaken free of soil and a 50 g subsample of soil from each pot was collected to extract second-stage juveniles (J2) using the Baermann funnel method (Ingham 1994). Roots were then gently rinsed free of soil. *M. hapla* eggs were extracted from the entire root system as described above. The number of eggs in 1 mL of the 50 mL egg suspension was determined using an inverted microscope. The remaining roots were oven-dried like the shoots and weighed.

Experiment 2: Determining *M. hapla* population virulence differences. Four rootstocks, Harmony, St. George, 3309C, and Riparia Gloire (Sunridge Nurseries, Inc.), were evaluated for host status to four populations of *M. hapla*. Own-rooted *V. vinifera* Chardonnay was included as a susceptible control (Howland et al. 2015). The Veneta population was used, as well as three other *M. hapla* populations: two collected from *V. vinifera* vineyards in Paterson, WA and Alderdale, WA, respectively (designated Paterson and Alderdale), and the third collected from a *Vitis labruscana* Concord vineyard in Prosser, WA (designated Prosser). The establishment of nematode cultures was as described in Experiment 1. In March 2015, dormant, unrooted cuttings of each rootstock and own-rooted Chardonnay were grouped relative to stem diameter to ensure vine uniformity, and rooted as described above. The same experimental methods described in Experiment 1 were used to root, establish, and maintain vines in pots, and for nematode inoculation of vines. The genotype and *M. hapla* population treatment combinations were arranged in a randomized block design on a greenhouse bench with treatments replicated five times; the experiment was conducted twice, and trials were separated in time (inoculation was offset by a week) and space (different greenhouse benches). Plants were destructively harvested in October 2015 as described above.

Experiment 3: Comparing host status of *M. hapla* versus *M. chitwoodi*. The rootstock Matador (Inland Desert Nursery, Benton City, WA) was evaluated for host status for a single population each of *M. hapla* and *M. chitwoodi*. Own-rooted *V. vinifera* Chardonnay was included as a susceptible control. The *M. hapla* Paterson population was used, as well as an *M. chitwoodi* Race 1 population originally collected from a potato field in Prosser, WA. The establishment of nematode cultures was as described in Experiment 1. In March 2017, dormant, unrooted cuttings of each rootstock and own-rooted Chardonnay were grouped relative to stem diameter to ensure vine uniformity and rooted as described above. The same experimental methods as in Experiment 1 were used to establish and maintain vines in pots and for nematode inoculation of vines. The genotype and *M. hapla*/*M. chitwoodi* treatment combinations were arranged in a randomized block design on a greenhouse bench with treatments replicated six times. The experiment was conducted twice, and trials were separated in time (inoculation was offset by a week) and space (different greenhouse benches). Plants were destructively harvested in October 2017 as described above.

Data analysis. *Meloidogyne* data are presented as eggs/g root. In addition, reproduction factor values, where RF = final nematode population (eggs + J2)/initial nematode population (9000 eggs/pot), were calculated. An RF value > 1 indicates that the plant is a good host, while an RF value < 1 indicates a poor host (Oostenbrink 1966). Data were analyzed using a mixed linear model analysis of variance (ANOVA) in JMP (SAS Institute Inc.). In all analysis, trial was considered a random factor while all other treatments were fixed factors. When the trial × treatment interaction was significant ($p < 0.001$), the trials were analyzed separately. To meet ANOVA assumptions, nematode data were $\log_{10}$ (x+1)-transformed prior to analysis. Statistically significant differences among treatments were computed by Tukey's honest significant difference test with significance at $p < 0.05$.

Results

Experiment 1: Determining host status of rootstocks for *M. hapla*. Differences were observed among the rootstocks in above- and below-ground biomass (Table 2). Shoot weight of Freedom was significantly smaller than that of Salt Creek, 420A, and own-rooted Riesling, which did not differ from each other. Riparia Gloire had the largest root system, which was similar to that of Freedom, 101-14 Mgt, and 420A. 3309C had the smallest root system, which was similar in size to that of Salt Creek, Harmony, St. George, and 110R. The susceptible control, own-rooted Riesling, had a significantly greater density of *M. hapla* eggs/g of root and RF value than the rootstocks (Table 2). Among the rootstocks, there were no differences in the measured *M. hapla* parameters: all rootstocks were poor hosts (RF < 1; less-then-replacement reproductive rate) for *M. hapla*.

Experiment 2: Determining *M. hapla* population virulence differences. In both trials, the rootstocks evaluated against the four *M. hapla* populations, 3309C, Riparia Gloire, St. George, and Harmony, were all poor hosts for the populations, with RF values ranging from 0 to 0.38 and *M. hapla* eggs/g root ranging from 0 to 565. To determine if the *M. hapla* populations varied in virulence on a susceptible host, the data from the own-rooted Chardonnay was

analyzed independently of the other rootstock varieties. The results from the trial repetitions were significantly different ($p = 0.001$), therefore, they were analyzed separately (Figure 1); however, similar trends were observed. In the first trial of the experiment, root parasitism by *M. hapla* Alderdale resulted in a significantly smaller root system at the end of the experiment than the other *M. hapla* populations (Figure 1A). In this trial, the *M. hapla* Paterson population had a greater final population density on own-rooted Chardonnay than the other populations, with 41% more eggs/g root recovered then the next highest population density in *M. hapla* Alderdale. The RF value of *M. hapla* Paterson was at least two times greater than that of the other *M. hapla* populations (Figure 1B). While *M. hapla* Alderdale produced more eggs/g root than *M. hapla* Prosser and Veneta, the RF values were similar.

Table 2 Reproduction of *Meloidogyne hapla* on *Vitis* rootstocks and on own-rooted *Vitis vinifera* Riesling in Experiment 1.

Rootstock	Shoot dry wt (g)	Root dry wt (g)	*M. hapla* eggs/g of root	RF[a]
Salt Creek	23.0 ab[b]	9.1 d	21 b	0.0 b
Freedom	15.5 e	15.3 ab	18 b	0.0 b
Harmony	18.9 cde	9.3 d	12 b	0.0 b
St. George	15.6 e	10.2 cd	8 b	0.0 b
Riparia Gloire	19.7 bcd	17.5 a	470 b	0.6 b
101-14 Mgt	21.5 abc	16.7 ab	547 b	0.6 b
3309C	16.0 de	8.2 d	13 b	0.0 b
110R	21.2 abc	10.3 cd	17 b	0.0 b
420A	24.1 a	13.8 abc	14 b	0.0 b
Riesling 90	20.5 abc	13.5 bc	22,302 a	20.7 a
p values	<0.001	<0.001	<0.001	<0.001

[a]Reproduction factor (RF) values were calculated as (eggs on roots + second-stage juveniles in soil)/initial nematode population density (9000 eggs).

[b]Values are the means of 16 observations. Nematode data was $\log_{10}$ (x + 1) transformed prior to analysis; nontransformed means are presented. Means followed by the same letter are not significantly different according to Tukey's honest significant difference test with significance level at $p < 0.05$.

In the second trial, similar to the first trial, the root system of the own-rooted Chardonnay was the smallest under *M. hapla* Alderdale parasitism; however, this was only significantly different from the largest root system parasitized by the *M. hapla* Prosser population (Figure 1C). While the highest density of eggs/g root and RF value were again observed in the *M. hapla* Paterson population in the second trial, this density and value were not significantly different from the next highest density or two next highest RF values, respectively (Figure 1D). Again, in the second trial, *M. hapla* Veneta had the numerically lowest eggs/g root and lowest RF value.

Experiment 3: Comparing host status of *M. hapla* versus *M. chitwoodi*. Growth of the rootstock Matador differed from that of own-rooted Chardonnay ($p < 0.001$; Table 3); Matador had ~52% more shoot biomass. The opposite was observed for root biomass. The root system of Matador was 64% smaller than own-rooted Chardonnay. Neither *Meloidogyne* species impacted shoot or root biomass of Matador or

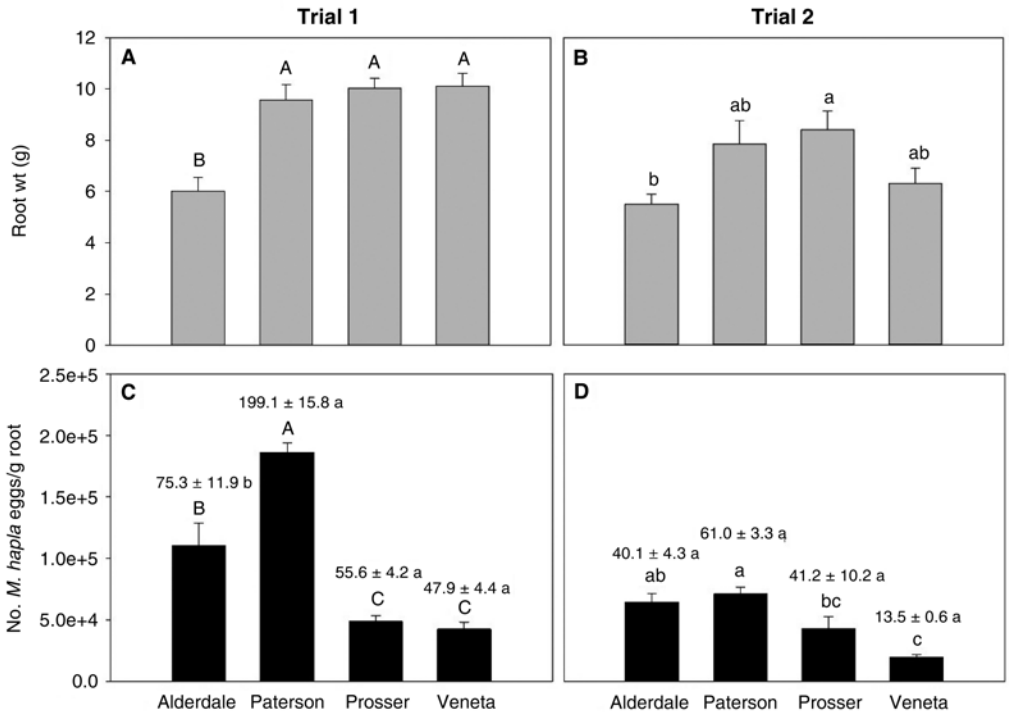

Figure 1 Reproduction of *Meloidogyne hapla* populations collected from Washington (Alderdale, Paterson, and Prosser) and Oregon (Veneta) on own-rooted *Vitis vinifera* Chardonnay in Experiment 2. Reproduction factor (eggs on roots + second-stage juveniles in soil)/initial nematode population density (9000 eggs) values are shown at the top of graphs **C** and **D**. Values presented numerically and as columns are the mean ± standard error of five observations. Nematode data was $\log_{10}$ (x + 1) transformed prior to analysis; nontransformed means are presented. Mean or columns within a graph panel followed by the same letter are not significantly different according to Tukey's honest significant difference test with significance at $p < 0.05$.

own-rooted Chardonnay ($p > 0.05$). Matador was not a good host for either *M. chitwoodi* or *M. hapla* Alderdale with RF values < 0.03 (Table 3). On own-rooted Chardonnay, the final population density was 6000 times greater than the final population density of *M. chitwoodi* ($p < 0.001$; Table 3).

Discussion

Our data provides additional information on the relative susceptibility of commercially available rootstocks to plant-parasitic nematodes (Ferris et al. 2012); specifically, those that are present in the Pacific Northwest. Few studies have evaluated the host status of *Vitis* rootstocks to *M. hapla* or *M. chitwoodi* (Lider 1954, Stirling and Cirami 1984, Ramsdell et al. 1996). Therefore, these data are very important for broadening knowledge of the host status of rootstocks for this nematode. Our results indicate that all the rootstocks considered, Riparia Gloire, 101-14 Mgt, Salt Creek, Freedom, Harmony, St. George, 3309C, 110R, 420A, and Matador, are poor hosts for *M. hapla*. Salt Creek was reported to be resistant to *M. hapla* (Lider 1954) and another report found both Salt Creek and Freedom to be resistant to *M. hapla* (Stirling and Cirami 1984). Contradictory to our findings, two studies found Riparia Gloire (Dalmasso and Cuani 1976) and 3309C (Ramsdell et al. 1996) to be susceptible to *M. hapla*.

Most of these rootstocks have been evaluated for host status to other *Meloidogyne* spp., including *M. incognita*, *M. javanica*, and *M. arenaria*. Widespread use of Harmony and Freedom rootstocks has resulted in aggressive pathotypes of *Meloidogyne* spp. that are capable of feeding on N-allele grapevine rootstocks (Cousins 2011); many rootstocks resistant to other populations of *Meloidogyne* are susceptible to these pathotypes, designated as *M. arenaria* Harmony A and *M. incognita* Harmony C (Cain et al. 1984, Anwar et al. 1999). The rootstocks 3309C and St. George are considered susceptible to *M. incognita* Race 3, *M. javanica*, *M. arenaria*, *M. arenaria* Harmony A, and *M. incognita* Harmony C (Nicol et al. 1999, Cousins and Walker 2002, McKenry and Anwar 2006, Ferris et al. 2012). Freedom and Harmony are resistant to most populations of *M. incognita*, *M. javanica*, and *M. arenaria*, except for the ones stated previously (Chitambar and Raski 1984, McKenry et al. 2001). Salt Creek (also known as Ramsey) was found to be a non-host to a mixed population of *M. incognita, M. arenaria*, and *M. javanica*, but is a host to *M. arenaria* Harmony (McKenry et al. 2001). The Matador rootstock was developed to be resistant to an *M. arenaria* Harmony A, but there is little other information on host status for other nematodes for this rootstock (Cousins 2011). Riparia Gloire is considered resistant to *M. arenaria* Harmony A and *M. incognita* Harmony C, but is susceptible to *M. incognita* Race 3, as is St. George (Cousins and Walker 2002, Ferris et al. 2012). 101-14 Mgt is resistant to *M. arenaria* Harmony A, *M. incognita* Harmony C, *M. incognita*, *M. arenaria*, and *M. javanica* (Sauer 1967, Nicol et al. 1999, Ferris et al. 2012). Both 110R and 420A are resistant to *M. arenaria* Harmony A and *M. incognita* Harmony C (Ferris et al. 2012), but 420A is susceptible to *M. javanica*, and 110R is reported susceptible to field populations of *M. incognita*, *M. javanica*, *and M. arenaria* in Spain (Sauer 1967, Téliz et al. 2007).

While the majority of the *Vitis* rootstocks evaluated in this trial are poor hosts for *M. hapla*, the mechanism of resistance may differ among rootstocks. Resistance mechanisms in grapevines may occur at nematode penetration, feeding, development, or reproduction (Ferris et al. 1982, Anwar and McKenry 2000, McKenry and Anwar 2006, Ferris et al. 2012). In Harmony, a hypersensitive response in the grape to *Meloidogyne* spp. prevents development (Ferris et al. 2012). Due to Salt Creek's widespread root system, there is reduced penetration and success of *Meloidogyne* spp (McKenry and Anwar 2006).

The major grapegrowing region of Washington State, east of the Cascade Mountains, is marked by hot, dry summers and cold winters. A major concern with rootstocks for this region is tolerance to cold, both directly for the rootstock and indirectly on the scion. High-vigor rootstocks may delay cold-acclimation of the scion and result in vines that are more susceptible to fall cold events (Cousins 2005). In one of the few rootstock evaluations in Washington State, the rootstock 99R (*V. berlandieri* × *V. rupestris*) performed poorly over winter, which was attributed to its long growing period and late cold acclimation (Keller et al. 2012). This may indicate that 110R (*V. berlandieri* × *V. rupestris*) or 420A (*V. berlandieri* × *V. riparia*) may also fare poorly under Washington conditions. Rootstocks with *V. berlandieri* heritage, which is native to the southern United States, may be less cold tolerant and have delayed maturity. Very high-vigor rootstocks, such as St. George, Salt Creek, and Freedom, may also have delayed cold-acclimation in fall and may be less cold-hardy as a result. Generally, rootstocks with *V. riparia* heritage are more likely to be cold-tolerant, but are less drought-tolerant (Pongrácz 1983). Rootstocks with *V. champinii* heritage, which is from central Texas, like Freedom, Harmony, and Salt Creek, may not be particularly cold-hardy. Rootstocks with *V. rupestris* heritage, including

Table 3 Reproduction of *Meloidogyne hapla* and *M. chitwoodi* on own-rooted *Vitis vinifera* Chardonnay and the *Vitis* rootstock Matador in Experiment 3.

Vine type/ nematode	Shoot wt (g)	Root wt (g)	Eggs/ g root	RF[a]
Chardonnay				
M. hapla	17.5 ± 0.9 a[b]	24.5 ± 2.2 a	45,069 ± 7450 a	118.4 ± 20.7 a
M. chitwoodi	17.3 ± 0.8 a	25.6 ± 1.1 a	10 ± 3 b	0.02 ± 0.01 b
Matador				
M. hapla	34.1 ± 1.7 b	18.2 ± 0.8 b	4 ± 2 b	0.0 b
M. chitwoodi	37.9 ± 1.7 b	± 0.7 b	3 ± b	0.01 ± 0.0 b
p values	0.001	0.001	0.001	0.001

[a]Reproduction factor (RF) values were calculated as final total nematode population density (eggs on roots + second-stage juveniles in soil)/initial nematode population density (9000 eggs).

[b]Values are the mean standard error of 12 observations. Nematode data was $\log_{10}$ (x + 1) transformed prior to analysis; nontransformed means are presented. Means followed by the same letter are not significantly different according to Tukey's honest significant difference test with significance at $p < 0.05$.

St. George (*V. rupestris*) and 110R (*V. berlandieri* × *V. rupestris*), have high drought tolerance (Carbonneau 1985, Serra et al. 2014). Riparia Gloire and 101-14 Mgt are considered to have low drought tolerance, 3309C and 420A have low to medium drought tolerance, and Salt Creek (= Ramsey) has medium to high drought tolerance (Carbonneau 1985, Serra et al. 2014). Matador, a cross of 101-14 Mgt and *V. mustangensis* and *V. rupestris* parents, has not been evaluated for cold hardiness or drought tolerance.

To further explore the poor host status of the rootstocks for *M. hapla* observed in Experiment 1, we challenged a subset of the rootstocks to three additional populations of *M. hapla* collected from Washington and Oregon. There is evidence in the literature that races or pathotypes of *M. hapla* are present in Washington (Ogbuji and Jensen 1972, 1974, Santo and Hackney 1980). Nematode species can be differentiated into pathotypes and races on the basis of host range, pathogenicity or virulence, mode of reproduction, or genetic differences. Two proposed races of *M. hapla* were differentiated by chromosome number: Race A, which reproduces by facultative meiotic parthenogenesis, and Race B, which is pentaploid parthenogenetic (Triantaphyllou 1966). In the Pacific Northwest, five pathotypes of *M. hapla* were identified based upon their varying ability to reproduce on a range of hosts (Ogbuji and Jensen 1972). In Concord grape (*V. labruscana*), the presence of *M. hapla* pathotypes was considered after the observation that an *M. hapla* population collected from alfalfa (*Medicago sativa)* was a poor host on Concord grape, contrary to field observations where *M. hapla* was associated with vines exhibiting poor growth (Santo and Hackney 1980). To determine if *M. hapla* populations vary in virulence and reproduction on Concord grape, three populations of *M. hapla*, all identified as Race A based upon chromosome number, were collected from alfalfa, currant (*Ribes* sp.), and Concord grape in Washington (Santo and Hackney 1980). When inoculated onto Concord grape, the *M. hapla* populations varied in reproduction rate, with higher final population densities of the currant and Concord grape *M. hapla* populations than of the alfalfa *M. hapla* population (Santo and Hackney 1980). Additionally, the *M. hapla* population from Concord grape reduced root biomass compared to that observed for the alfalfa and grape *M. hapla* populations.

Our study again demonstrates that the reproductive potential of *M. hapla* populations varies. The *M. hapla* Paterson population consistently had the numerically greatest reproduction (eggs/g root) of the *M. hapla* populations evaluated. In one trial, the final RF value for this population was more than two times higher than the other *M. hapla* populations. However, it is important to note that all of the *M. hapla* populations would be considered successful parasites on *V. vinifera*, with >13-fold increase in population densities over a six-month period. We also observed consistent trends in root biomass outcomes with the different *M. hapla* populations. The *M. hapla* Alderdale population is potentially more virulent on *V. vinifera* than other *M. hapla* populations. This demonstrates that there is reproductive and virulence diversity among *M. hapla* populations in Washington as previously observed (Santo and Hackney 1980), and may explain why other researchers reported resistance/susceptibility results for *Vitis* rootstocks that were contrary to our findings (Dalmasso and Cuani 1976, Ramsdell et al. 1996).

Due to the potential for expanding winegrape vineyards to fields once cropped with agronomic hosts of *M. chitwoodi* like potato, small grains, or corn, an understanding of the ability of *M. chitwoodi* to parasitize *V. vinifera* and *Vitis* rootstocks is required to guide vine selection. *M. hapla* and *M. chitwoodi* are commonly found in mixed populations in the Pacific Northwest. Across the region, *M. chitwoodi* was more commonly detected in diagnostic samples from 2012 to 2016, with 60% occurrence compared to 25% for *M. hapla* when present (Zasada et al. in press). When root and soil samples from potato were analyzed (Nyczepir et al. 1982), the dominant species in the region was *M. chitwoodi* (56 to 93% incidence), with *M. hapla* present at an incidence of 0 to 39%. The greater incidence of *M. chitwoodi* was attributed to a cool growing season and increased acreage of small grain rotation crops, which are better hosts for *M. chitwoodi* than *M. hapla*. Plants in the Vitaceae are moderate to poor hosts for *M. chitwoodi* (EPPO 1991). *M. chitwoodi* did not produce high densities of eggs/g root on own-rooted *V. vinifera* Cabernet Sauvignon compared to the densities observed for *M. arenaria* Harmony A and *M. incognita* on the same host (Anwar et al. 2002); however, abundant *M. chitwoodi* second-stage juveniles were found in soil surrounding roots of own-rooted Cabernet Sauvignon. In this same study, the host status of nine rootstocks for *M. chitwoodi* was considered. Some of these rootstocks were poor hosts for *M. chitwoodi* (USDA 6-19B, 10-23B, and 10-17A, and RS-2, RS-3, and Harmony) and some were moderate hosts (Ramsey, Teleki 5C, Freedom, and Harmony). From a Washington viticulture perspective, it appears that own-rooted Chardonnay is a poor host for *M. chitwoodi*, indicating that there should be minimal risk to planting new *V. vinifera* own-rooted vineyards into areas where *M. chitwoodi* is present. However, if rootstocks are deployed, *M. chitwoodi* may be able to increase in population density, depending upon rootstock selection. The impact of *M. chitwoodi* on vine productivity is unknown.

Conclusions

This is the first comprehensive greenhouse evaluation of the host status of many commercially available *Vitis* rootstocks for *M. hapla*. Our results indicate that many rootstocks are poor hosts for *M. hapla*. These results were confirmed when *Vitis* rootstocks were challenged with four different populations of *M. hapla* collected from vineyards in Oregon and Washington. It was also found that *M. hapla* populations varied in reproductive potential and virulence on *V. vinifera*, and that own-rooted Chardonnay and the rootstock Matador were not hosts for *M. chitwoodi*. While rootstocks resistant to *Meloidogyne* spp. in greenhouse experiments also showed resistance in the field (Stirling and Cirami 1984), the next step in this research is to establish field evaluations of *Vitis* rootstocks in Washington to determine if similar results are obtained to those reported here.

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