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Research Article

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

Inga A. Zasada,[1]* Amanda D. Howland,[2] Amy B. Peetz,[1] Katherine East,[3] and Michelle Moyer[3]

[1]USDA-ARS-Horticultural Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330; [2]Division of Plant Sciences and Bond Life Sciences Center, University of Missouri-Columbia, Columbia, MO 65211; and [3]Washington State University – Irrigated Agriculture Research and Extension Center, Prosser, WA 99350.

*Corresponding author (inga.zasada@ars.usda.gov; tel: 541-738-4051; fax: 541-738-4025)

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Abstract: The majority of wine grape (*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. To use rootstocks for *M. hapla* management or for 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 another root-knot nematode, *M. chitwoodi,* on rootstock varieties and own-rooted *V. vinifera* 'Chardonnay' was evaluated. The rootstocks 'Salt

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Creek', 'Freedom', 'Harmony', 'St. George', 'Riparia Gloire', '101-14 Mtg', '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. A *M. hapla* population collected from a *V. vinifera* vineyard in Paterson, WA had 33 to 78% greater reproduction than the other *M. hapla* populations. A *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, semi-arid, virulence, *Vitis vinifera*

Introduction

More than thirty different winegrape varieties are cultivated on approximately 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 these type of weather events occurred in what is referred to the "Halloween Freeze" (October 31) of 2002, and the "Thanksgiving Freeze" (November 24) of 2010, when temperatures dropped to -11.5°C and -17.3°C, respectively (AgWeatherNet; weather.wsu.edu). When vines are own-rooted, vineyards can be readily retrained the season immediately following cold damage, allowing for only a 1-year loss in crop (Moyer et al. 2011). However, when cold damage occurs to vines that are grafted onto a rootstock variety, the

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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 2 to 3 years following a damaging cold event.

The modern Washington wine grape 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, there are vineyards that are either past, or approaching, the end of their productive lifespans, and many vineyards 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 by Zasada et al. (2012) found *Meloidogyne hapla*, the northern root-knot nematode, to be the most abundant nematode present, found in 60% of the surveyed vineyards. The proposed threshold is 100 *M. hapla*/250 g soil (Santo unpublished data 2000), which was a density exceeded in 26% of surveyed wine grape vineyards in Washington. While *M. hapla* is the predominant species found in the region, *M. chitwoodi*, another other common *Meloidogyne* species in the Pacific Northwest, is also widespread in other crop production systems (Zasada et al. *in prep*). Own-rooted *V. vinifera* varieties have been shown to be good hosts for *M. hapla* (Howland et al. 2015). Unfortunately, with the preference for own-rooted vines in 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 (Arredondo 1992; Jenser et al. 1991; Nicol et al. 1999). As sedentary endoparasites, these nematodes remain stationary inside the roots of a host plant for

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the majority of their lifespan. Adult females lay their eggs outside the roots in a gelatinous matrix; a single egg mass can contain up to 400-500 eggs. The infective stage is the second-stage juvenile which hatches from eggs, 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 U.S., *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 to be damaging (Esnard and Zuckerman 1998, Esmenjaud and Bouquet 2009).

Most wine grape 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,b), and Matador, Minotaur, and Kingfisher (Cousins 2011). In a summary of the literature on nematode-resistant rootstocks by Ferris et al. (2012), *M. hapla* is not included. In fact, very little is known about the response and host status of rootstocks to *M. hapla*, and certainly there are no breeding programs focused on the development of rootstocks with resistance to *M. hapla*.

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The host status of rootstocks for the industry-prevalent *M. hapla* must be known in order for the Washington wine industry to effectively deploy rootstocks for nematode management as well as for other horticulturally-desired characteristics. The research presented here is the first step in this direction. The objectives of this research were to: 1) Determine the host status of *Vitis* rootstocks for *M. hapla*; 2) Determine if *M. hapla* populations from Washington and Oregon differ in virulence on *Vitis* rootstocks and own-rooted *V. vinifera* 'Chardonnay'; and 3) Compare the ability of *M. hapla* to that of *M. chitwoodi* to parasitize own-rooted Chardonnay and the rootstock Matador.

Materials and Methods

Experiment 1 – Determining host status of rootstocks for *M. hapla*. Nine rootstocks, Salt Creek, Freedom, Harmony, St. George, Riparia Gloire, 101-14, 3309C, 110R, and 420A (Sunridge Nurseries, Inc., Bakersfield, CA) (Table 1), were evaluated for host status to a single population of *M. hapla*. The 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, Clackamas, OR) to stimulate root growth. Cuttings were inserted in a perlite and vermiculite mixture (Santo and Hackney 1980), placed on a bench with a heating pad for two months, and 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

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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 initially fertilized with a 9-45-15 NPK starter fertilizer (Jack's Professional, Allentown, PA) at a rate of 4 g/L, delivering 336 ppm 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 ppm N; vines were fertigated biweekly though the duration of the experiment. The grapevines were grown in a greenhouse at a16 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 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-µm- 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 9,000 eggs/3.7 L pot, or a density of 3 *Meloidogyne*

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eggs/gram of soil. The inoculum was applied to each grapevine by pipetting 5 ml of 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 70ºC 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. *Meloidogyne 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 as per 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 (designated Paterson and Alderdale, respectively) and the third collected from a *V. 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

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vine uniformity and rooted as described above. The same experimental methodologies as 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* vs. *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 a *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 methodologies as described 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.

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Data analysis. *Meloidogyne* data are presented as eggs/g root. In addition, reproduction factor values, RF= final nematode population (eggs + J2)/initial nematode population (9,000 eggs/pot) were calculated. A RF value > 1 indicates that the plant is a good host while a 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., Cary, NC). In all analysis, trial was considered as a random factor while all other treatments were fixed factors. When the trial x treatment interaction was significant ($P < 0.001$) then the trials were analyzed separately. To meet analysis of variance assumptions, nematode data were $\log_{10}$ (x+1) transformed prior to analysis. Statistically significant differences among treatments were computed by Tukey's honestly significant difference test with significance level 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, 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 compared to the rootstocks (Table 2). Among the rootstocks, there were no differences in the measured *M. hapla* parameters, with all the rootstocks being considered poor hosts (RF < 1; less-then-replacement reproductive rate) for *M. hapla*.

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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 independent of the other rootstock varieties. The results from the trial repetitions were significantly different ($P = 0.001$), therefore, they were analyzed separately (Fig. 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 compared to the other *M. hapla* populations (Fig 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 of the RF values for the other *M. hapla* populations (Fig 1B). While *M. hapla* Alderdale produced more eggs/g root than *M. hapla* Prosser and Veneta, the RF values were similar. 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 to the largest root system parasitized by the *M. hapla* Prosser population (Fig 1C). While the highest density of eggs/g root and RF value was again observed in the *M. hapla* Paterson population in the second trial, this density and value were not significantly different to the next highest or two highest densities or values, respectively (Fig. 1D). Again, in the second trial, *M. hapla* Veneta had the numerically lowest eggs/g root and RF value.

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Experiment 3- Comparing host status of *M. hapla* vs. *M. chitwoodi*. Growth of the rootstock Matador differed from that of own-rooted Chardonnay ($P < 0.001$; Table 3); Matador had approximately 52% more shoot biomass. The opposite was observed for root biomass. The root system of Matador was 64% smaller than that of own-rooted Chardonnay. Neither of the *Meloidogyne* species impacted shoot nor root biomass of Matador or 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 6,000 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. There are few studies that have evaluated the host status of *Vitis* rootstocks to *M. hapla* or *M. chitwoodi* (Lider 1960, 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 Mtg, Salt Creek, Freedom, Harmony, St. George, 3309C, 110R, 420A, and Matador would be considered poor hosts for *M. hapla*. Lider (1960) found Salt Creek to be resistant to *M. hapla*, and Stirling and Cirami (1984) found Salt Creek and Freedom to be resistant to *M. hapla*. Contradictory to our findings, Dalmasso and Cuani (1976) and Ramsdell et al. (1996) found Riparia Gloire and 3309C to be susceptible to *M. hapla*, respectively.

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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 have resulted in aggressive pathotypes of *Meloidogyne* spp., which are capable of feeding on N-allele grapevine rootstocks (Cousins 2011), and many rootstocks resistant to other populations of *Meloidogyne* are susceptible to these pathotypes (Cain et al. 1984, Anwar et al. 1999); these are designated as *M. arenaria* Harmony A and *M. incognita* Harmony C. 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 and Anwar 2001, 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 a *M. arenaria* Harmony A, but there is little other information on host status for this rootstock for other nematodes (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, along with St. George (Cousins and Walker 2002, Ferris et al. 2012). 101-14 Mtg is resistant to *M. arenaria* Harmony A and *M. incognita* Harmony C, as well as *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

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has been reported to be 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 would be considered 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, Anwar and McKenry 2002, Ferris et al. 2012). For example, Ferris et al. (2012) reported that in Harmony, there is a hypersensitive response in the grape to *Meloidogyne* spp. which prevents development. McKenry and Anwar (2006) speculated that due to Salt Creek's widespread root-system, there is a reduction in penetration and success of *Meloidogyne* spp.

The major grape-growing region of Washington State, east of the Cascade Mountains, is marked by hot, dry summers and cold winters. One of the major concerns with rootstocks for this region is tolerance to cold, both directly for the rootstock and indirectly on the scion. High vigor rootstocks may result in later 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* x *V. rupestris*) performed poorly over winter which was attributed to its long growing period and late cold acclimation (Keller et al. 2011). This may indicate that 110R (*V. berlandieri* x *V. rupestris*) or 420A (*V. berlandieri* x *V. riparia*) may also fare poorly under Washington conditions. Rootstocks with *V. berlandieri* heritage, which is native to southern USA, 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*

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heritage are more likely to be cold-tolerant, but 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 St. George (*V. rupestris*) and 110R (*V. berlandieri* x *V. rupestris*), are considered to 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) have 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, and mode of reproduction as well as genetic differences. Triantaphyllou (1966) proposed two races of *M. hapla* differentiated by chromosome number: Race A which reproduces by facultative meiotic parthenogenesis and Race B which is pentaploid parthenogenetic. 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 a *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

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growth (Santo and Hackney 1980). To determine if *M. hapla* populations vary in virulence and reproduction on Concord grape, Santo and Hackney (1980) collected three populations of *M. hapla*, all identified as Race A based upon chromosome number, from alfalfa, currant (*Ribes* sp.), and Concord grape in Washington. 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 compared to that 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 do vary. The *M. hapla* Paterson population consistently had the numerically greatest reproduction (eggs/g root) of the *M. hapla* populations evaluated. In one of the trials, 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 contrary resistance/susceptibility results for *Vitis* rootstocks to our findings (Dalmasso and Cuani 1976; Ramsdell et al. 1996).

Due to the potential for expansion of wine grape vineyards to fields once cropped with agronomic hosts (e.g., potato, small grains, corn) of *M. chitwoodi*, an understanding of the ability

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of *M. chitwoodi* to parasitize *V. vinifera* and *Vitis* rootstocks is required to guide vine selection. *Meloidogyne 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 unpublished data). 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% in the samples. 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 have been reported as moderate to poor host for *M. chitwoodi* (EPPO 1991). *Meloidogyne chitwoodi* did not produce high densities of eggs/g root on own-rooted *V. vinifera* 'Cabernet Sauvignon' compared to the density observed for *M. arenaria* Harmony A and for *M. incognita* on the same host (Anwar and McKenry, 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. These rootstocks ranged in host status for *M. chitwoodi* from poor host (USDA 6-19B, 10-23B, and 10-17A, and RS-2, RS-3 and Harmony) to 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 of 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.

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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 vary in reproductive potential and virulence on *V. vinifera,* and that own-rooted Chardonnay and the rootstock Matador are not hosts for *M. chitwoodi*. While Stirling and Cirami (1984) found that rootstocks resistant to *Meloidogyne* spp. in greenhouse experiments also showed resistance in the field, the next step in this research is to establish field evaluations in Washington of *Vitis* rootstocks to determine if similar results are obtained to those reported here.

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

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

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

Rootstock	Shoot Dry Weight (g)	Root Dry Weight (g)	*M. hapla* eggs/g of root	RF[a]
Salt Creek	23.0 ab[a]	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 Mtg	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 calculated as (eggs on roots + second-stage juveniles in soil)/initial nematode population density (9,000 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 honestly significant difference test with significance level at $p < 0.05$.

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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 ± 7,450 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	18.1 ± 0.7 b	3 ± 1 b	0.01 ± 0.0 b
p values		0.001	0.001	0.001	0.001

[a]Reproduction factor (RF) values calculated as final total nematode population density (eggs on roots + second-stage juveniles in soil)/initial nematode population density (9,000 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 honestly significant difference test with significance level at $p < 0.05$.

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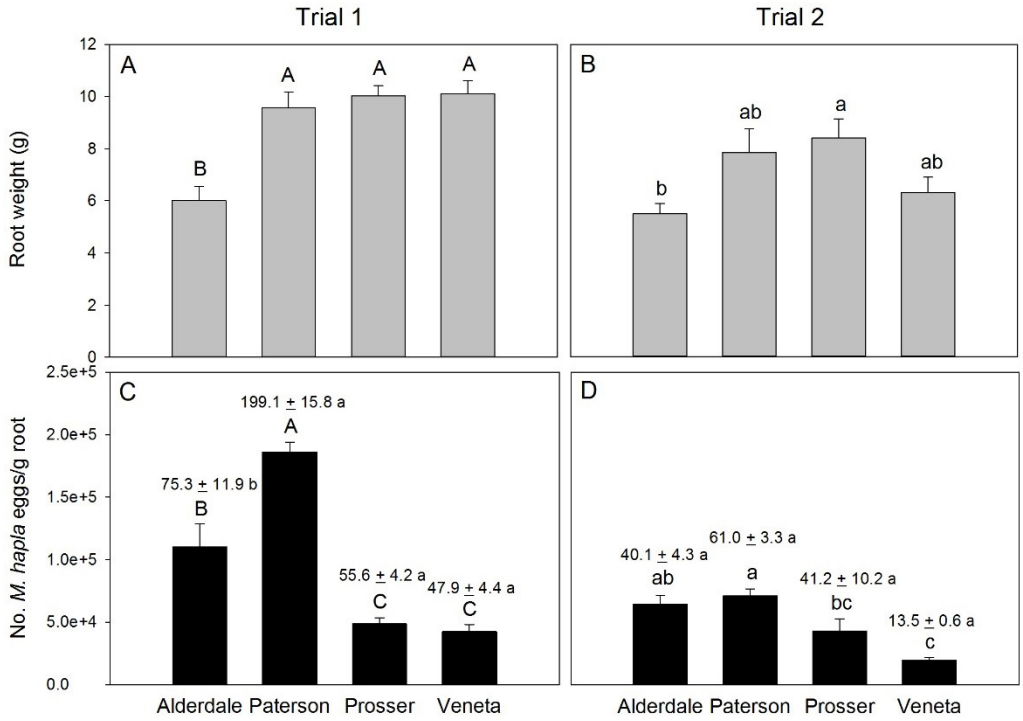

Figure 1 Reproduction of *Meloidogyne hapla* populations collected from Washington (Alderdale, Paterson, and Prosser) and Oregon (Veneta) on own-rooted *Vitis vinifera* 'Chardonnay' in Experiment 2. Reproduction factor (eggs on roots + second-stage juveniles in soil)/initial nematode population density (9,000 eggs)) values are shown at the top of the 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 honestly significant difference test with significance level at $p < 0.05$.