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Impact of Grapevine (*Vitis vinifera*) Varieties on Reproduction of the Northern Root-Knot Nematode (*Meloidogyne hapla*)

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Abstract: One of the most commonly encountered plant-parasitic nematodes in eastern Washington *Vitis vinifera* vineyards is *Meloidogyne hapla*; however, limited research exists on the impact of this nematode on *V. vinifera*. The objectives of this research were to determine the impact of *M. hapla* on Chardonnay and Cabernet Sauvignon vine establishment and to determine the host status of *V. vinifera* varieties/clones predominantly grown in Washington to *M. hapla*. In a microplot experiment, Chardonnay and Cabernet Sauvignon vines were planted into soil inoculated with different densities of *M. hapla*; population dynamics of *M. hapla* and vine performance were monitored over 3 yr. In greenhouse experiments, several clones representing five *V. vinifera* varieties, Chardonnay, Riesling, Cabernet Sauvignon, Merlot, and Syrah, were evaluated as hosts for *M. hapla*. In both microplot and greenhouse experiments, white varieties were significantly better hosts than red varieties. In the greenhouse experiments, Chardonnay and Riesling had 40% higher reproduction factor values than Syrah and Merlot, however, all varieties/clones screened were good hosts for *M. hapla* (reproduction factors > 3). In the microplot experiment, *M. hapla* eggs/g root were 4.5 times greater in Chardonnay compared to Cabernet Sauvignon 3 yr after planting but there was no evident impact of *M. hapla* on vine establishment.

Key words: host-parasite interaction, host status, *M. hapla*, microplots, own-rooted, Washington.

Washington is the second largest wine-producing state in the United States with an economic value of $233 million (USDA, 2014). More than 30 different varieties of wine grapes are cultivated on the 18,211 ha of vineyards in Washington (USDA, 2014) mostly located on the eastern side of the state. Predominantly white grape varieties are grown, with Chardonnay and Riesling leading production. Leading red varieties are Cabernet Sauvignon, Merlot, and Syrah. Due to a low mean annual rainfall of 20 cm and a photoperiod of approximately 16 hr during the summer, vineyards in eastern Washington are semi-arid and drip irrigated to maintain productivity. Washington vineyards are primarily planted with own-rooted grapevines (*V. vinifera*) as opposed to grapevines grafted onto rootstocks due to cold tolerance and frost concerns (Keller et al., 2012).

Plant-parasitic nematodes, a global pest estimated to cause $100 billion economic loss to agriculture worldwide (Sasser and Freckman, 1987), are commonly found in Washington vineyards. Surveys of vineyards were conducted in eastern Washington by Zasada et al. (2012) who found *Meloidogyne hapla*, the northern root-knot nematode, to be the most abundant nematode present; it was found in 60% of the surveyed vineyards. *Meloidogyne* spp., or root-knot nematodes, are a significant production and economic constraint to grapevines worldwide. A sedentary endoparasite, these nematodes remain stationary inside the roots of a host plant for most of their lives. Adult females lay their eggs outside the roots in a gelatinous matrix; a single egg mass can contain up to 1,000 eggs (de Guiran and Ritter, 1979). Under optimal conditions, the lifecycle of this nematode can take 5 wk to complete, producing several generations per season (de Guiran and Ritter, 1979; Nicol et al., 1999; Esmenjaud and Bouquet, 2009). 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 to be damaging (Esnard and Zuckerman, 1998; Esmenjaud and Bouquet, 2009); in Washington, only *M. hapla* has been found (Zasada et al., 2012).

Although *M. hapla* is abundant and widespread in Washington's semi-arid vineyards, very little is known about the pathogenicity of *M. hapla* to commonly planted *V. vinifera* varieties used in this region and the host status of these varieties to the nematode. Although the effect *Meloidogyne* spp. have on grapevines is well researched in other grape-producing regions, such as California (Lider, 1960; Ferris and McKenry, 1975; Anwar and Gundy, 1989) and Australia (Stirling and Cirami, 1984; Nicol et al., 1999), these locations have different climates and species of *Meloidogyne* compared to Washington. Therefore, the effect *M. hapla* has on grapevines in Washington remains unknown. This is an oversight considering 27% of vineyards in Washington had *M. hapla* population densities above 100 *M. hapla*/250 g soil (Zasada et al., 2012), the theoretical threshold for this nematode (Santo, unpublished data). The objectives of this research were to (i) evaluate the impact of *M. hapla*, as well as population dynamics of this nematode, on own-rooted Chardonnay and Cabernet Sauvignon, and (ii) determine the host status of *V. vinifera* varieties and clones commonly grown in Washington to *M. hapla*.

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Materials and Methods

Microplot experiment: A 4 × 2 factorial experiment with four different population densities of *M. hapla* (0, 50, 150, and 300 eggs/250 g soil) and with two different own-rooted *V. vinifera* varieties (Chardonnay and Cabernet Sauvignon) was established in 114 liters pot-in-pot (Parkerson, 1990) microplots (Nursery Supplies Inc., McMinnville, OR). Each treatment combination was replicated seven times in a randomized complete block design for 56 experimental units (microplots). The experiment was conducted at the Washington State University, Irrigated Agriculture Research & Extension Center, Prosser, WA.

The experiment was conducted in a field with a Warden silt loam soil. The area was deep ripped to 45 cm after which Telone II (1,3-dichloropropene) was shanked into the soil to a depth of 30 cm at a rate of 185 liters/ha in October 2006. The microplots were arranged on a 1.5 × 3.0 m spacing and placed into 60 cm deep × 60 cm in diameter augered holes. Prior to the placement of microplots, a 5-cm-deep layer of gravel was placed in the bottom of each hole. The pots were then filled with soil and additionally fumigated with metam sodium at a rate of 589 liters/ha as a drench in 2 liters of water. The following spring, May 2007, prior to planting, a population of *M. hapla* originally isolated from an alfalfa (*Medicago sativa* L.) field in Washington, and maintained on pepper (*Piper nigrum* L. cv. California Wonder) in a greenhouse, was added to appropriate microplots depending upon the inoculation density required. *M. hapla* eggs were extracted from pepper roots by shaking roots in 0.05% NaOCl solution for 3 min (Hussey and Barker, 1973). The egg solution was then poured over nested 250- and 25-µm sieves with eggs being retained on the 25-µm sieve. Eggs were collected in water and adjusted to achieve desired inoculation densities. At inoculation, the eggs for each pot were first added to 1 liter of moistened pasteurized soil and gently mixed. At each microplot, including the noninoculated microplots, two-thirds of the soil was removed from the microplot and one-third of the inoculum was added to the soil surface and mixed into the soil and then covered with more soil. This was repeated two more times to completely fill the microplots. Care was taken to avoid contamination of the surrounding soil and equipment.

Rooted cuttings of Chardonnay clone 02 or Cabernet Sauvignon clone 04 (Inland Desert Nursery, Benton City, WA) were planted into microplots immediately after inoculation. The single shoot was staked and grown without manipulation throughout 2007. Due to poor initial vine growth in 2007 resulting from transplant shock, vines were two budded during the dormant period in 2008 and a single shoot was again retained on all vines for the 2008 growing season. In 2009 and 2010, two buds were retained per vine and both main shoots were trained vertically on a single stake up to a height of 2 m. Lateral shoots that developed in 2009 and 2010 were retained, but were not further manipulated producing a bush-type canopy. All fruit clusters produced on vines in 2009 and 2010 were also retained. Microplots were kept well-watered during the growing season by applying irrigation water two to three times per wk by hand as dictated by weather conditions. All microplots were watered to field capacity. Microplots were fertilized in the spring of each year ~2 wk after budburst. Each microplot received 50 g of a complete fertilizer (20-10-20 with micronutrients; Peters Professional, Scott Chemical Co., St Louis, MO) broadcast over the soil surface. Fungicides to control powdery mildew and bunch rot were applied as per normal practices in the region.

M. hapla population dynamics in soil were monitored in the spring (April or May) and fall (October) of each year, beginning in October 2007. Nematodes were extracted from two soil cores (1.5-cm diameter × 45-cm deep) collected from each microplot; core holes were filled with fumigated soil after each sampling. Nematodes were extracted from the entire soil sample (after obtaining total wet weight) by elutriation (Byrd et al., 1976) and centrifugation methods (Jenkins, 1964) and enumerated under a stereoscope. *M. hapla* egg population densities in roots were also determined in spring and fall of 2009 and fall of 2010. In 2009, before processing the soil sample for vermiform *M. hapla*, roots contained in the cores were handpicked from soil, rinsed, and placed in a 50-ml polyethylene tube with a 0.25% NaOCl solution and shaken at 300 rpm for 3 min on a Lab Companion SK-600 Benchtop Shaker (Jeio Tech, Seoul, Korea) to free *M. hapla* eggs from the gelatinous matrix attached to the root. After the roots were shaken, the solution was poured over nested 90- and 25-µm sieves. The roots were removed from the 90-µm sieve, placed in an oven, dried for 5 d at 105°C, and weighed to obtain root dry weight. The eggs retained on the 25-µm sieve were back-washed into a 50-ml polyethylene tube. In fall 2010, after destructively harvesting the vines (see below), a subsample of fine roots were collected and processed similarly to that described above. Number of *M. hapla* eggs in a sample were enumerated under an inverted microscope.

At the termination of the experiment in October 2010, the aboveground portion of the vine was removed and partitioned into new wood (canes) and old wood (trunks and cordons). Tissues were air dried, then placed in a 105°C oven for 24 hr and weighed to obtain dry weights. Pots were then removed from the ground with a front-end loader, the contents of the pot placed on a screen supported by saw horses, and the roots were washed free of soil. A subsample of fine roots was removed from the root system to quantify *M. hapla* eggs in roots (see above) and the remainder of the root system was air dried, placed in a 105°C oven for 24 h, and then weighed.

Data were analyzed for effects of *M. hapla* inoculation density, variety, and inoculation density × variety interactions using a repeated measure mixed linear model

analysis of variance (ANOVA) with inoculation density and variety as fixed factors and block as a random factor. No *M. hapla* J2 or eggs were found in the noninoculated controls (0 eggs/g soil) and these data were excluded from the analysis. Soil and root data for *M. hapla* were initially transformed by $\log_{10}$ (x + 1) to correct for heteroscedasticity. Treatment means in all analyses were separated using Tukey's Honestly Significant Differences (HSD) test ($P \leq 0.05$). All analyses were performed using JMP 9.1 (SAS Institute Inc., Cary, NC).

Variety/clone greenhouse experiment: The varieties and clones evaluated included: Merlot clone 03, Merlot 06, Merlot 15, Cabernet Sauvignon 02, Cabernet Sauvignon 04, Cabernet Sauvignon 06, Cabernet Sauvignon 21, Syrah Shiraz 07, Syrah 07, Syrah Phelps, Chardonnay 06, Chardonnay 15, White Riesling Neustad 90, White Riesling GM 198, and White Riesling GM 239. Dormant grape cuttings were obtained late winter of 2012 and 2013 (Ste. Michelle Wine Estates, Prosser, WA). Cuttings of each clone were categorized into similar sizes to ensure uniformity. Fifteen cuttings of each clone were cut into three node segments with the bottom node cut diagonally and rooted in perlite and vermiculite potting media (Santo and Hackney, 1980). The cuttings were then placed on a mist bench with a heating pad for 2 mon. In April of each year, the grapes were removed from the mist bench and placed in a greenhouse under shade cloth to harden off. A week later, eight established grape cuttings of each clone were transplanted into 3.7-liter pots containing steam pasteurized 1:1 sand:Willamette loam soil. Pots were arranged in a randomized block design with eight blocks in the greenhouse. The vines were thinned to a single shoot and inflorescences removed to promote root growth. The grapevines were fertigated biweekly with 20-20-20 NPK (20N-8.8P-16.6K; Scotts, Marysville, OH) throughout the duration of the experiment. The grapevines were grown in a greenhouse with a 16 hr/d photoperiod; temperatures were set to 25°C during the day and 20°C at night.

M. hapla originally collected from a *V. vinifera* vineyard in Veneta, OR, and reared on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers), was used as inoculum. Inoculum was obtained by destructively harvesting tomato plants and collecting eggs from washed roots as described above. In early June of each year, the grapevines were inoculated with a density of three *M. hapla* eggs/g of soil which was equivalent to 9,000 eggs/3.7 liter pot. The inoculum was applied by pipetting 2 ml of inoculum into six holes approximately 4-cm deep around the base of the vine.

Plants were destructively harvested in the fall of each year. For each vine, the shoot was removed, placed in a paper bag, dried at 105°C for 5 d, and weighed. Roots were shaken free of soil and a 50 g subsample of soil from each pot was collected to extract *M. hapla* 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 solution was determined using an inverted microscope. The remaining roots were oven dried as per shoots.

M. hapla data are presented as eggs/g root and total/pot (eggs + J2). In addition, reproduction factor values, RF= final nematode population/initial nematode population (Oostenbrink, 1966) were calculated. Before analysis, nematode and plant data were $\log_{10}$ (x + 1) transformed to fulfill normality and equal variance assumptions of the model (R Studio v0.98, Boston, MA). After transformation, data were analyzed using a one-way ANOVA with first clone and then variety (excluding Shiraz since only one clone was included in the experiment) as main effects and block as a random effect. Data from the 2012 and 2013 trials were analyzed separately because the trials were significantly different from each other ($P \leq 0.001$). Mean separations were performed using Tukey's HSD test ($P \leq 0.05$).

Results

Microplot experiment: In the analysis of *M. hapla* J2/250 g soil, inoculation density and the variety × inoculation density interaction were not significant ($P > 0.01$), therefore, *M. hapla* J2 population densities across inoculation densities were averaged for each variety within a sampling date (Fig. 1). Population densities of *M. hapla* J2/250 g soil were greater in Chardonnay than in Cabernet Sauvignon starting in fall of 2008, 1.5 years after planting ($P \leq 0.001$) (Fig. 1). By the fall of 2010, *M. hapla* J2 population densities were 29 times higher in Chardonnay than in Cabernet Sauvignon. In the analysis of *M. hapla* eggs/g root, there were significant difference over time for both variety and inoculation density ($P \leq 0.001$), but not for the interaction ($P > 0.1$) (Table 1).

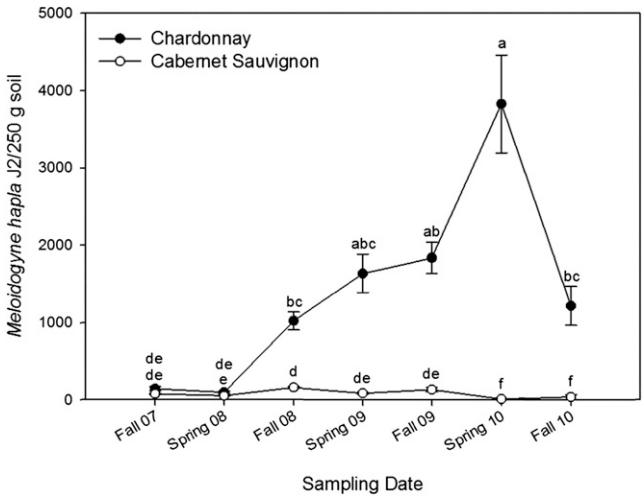

Fig. 1. Effect of *Vitis vinifera* varieties Chardonnay and Cabernet Sauvignon, on population densities of *Meloidogyne hapla* second-stage juveniles (J2) in a microplot experiment over time. Data was $\log_{10}$(x + 1) transformed prior to analysis; nontransformed means ± standard error are presented ($n = 21$). Letters above symbols indicate significant differences according to Tukey's honestly significant difference test ($P \leq 0.05$).

TABLE 1. Effects of own-rooted *Vitis vinifera* varieties Chardonnay and Cabernet Sauvignon and inoculation densities of *Meloidogyne hapla* on subsequent *M. hapla* egg population densities in a microplot experiment.

Effect	Treatment	Spring 2009	Fall 2009	Fall 2010
		M. hapla eggs/g dry root		
Variety	Chardonnay	23 b[a]	14 b	408 c
	Cabernet Sauvignon	1 a	4 a	90 b
Initial density[b]	Zero[c]	0	0	0
	Low	19 ab	7 a	152 bc
	Medium	9 a	10 ab	324 cd
	High	23 ab	18 ab	557 d

[a] Values are the means of seven observations. Data were $\log_{10}(x + 1)$ transformed prior to analysis; nontransformed means are presented. Means followed by the same letter within an effect are not significantly different according to Tukey's honestly significant difference test ($P \leq 0.05$).

[b] Zero = no *M. hapla*; Low = 50 *M. hapla* eggs/250 g soil; Medium = 150 *M. hapla* eggs/250 g soil; and High = 300 *M. hapla* eggs/250 g soil.

[c] Data from the noninoculated control (zero) were not included in the analyses.

Across all sampling dates, population densities of *M. hapla* eggs were always higher in Chardonnay than in Cabernet Sauvignon. At the final sampling date (fall of 2010), there were 4.5 times as many *M. hapla* eggs in roots of Chardonnay than in roots of Cabernet Sauvignon. When inoculation density was considered, only the densities at the final sampling date (fall 2010) differed from the densities at the other sampling dates ($P \leq 0.001$). At that time, there were 3.7 more *M. hapla* eggs in roots of vines inoculated with high (300 eggs/250 g soil) vs. low (50 eggs/250 g soil) initial densities. There were no differences in pruning weights (2008 and 2009) or any vine tissues from the final destructive harvest in 2010 (new wood, old wood, and roots) among the treatments ($P \geq 0.05$) (data not shown).

Variety/clone greenhouse experiment: Differences were observed in *M. hapla* J2, egg, and total population densities among the clones in both years. In 2012, both of the Chardonnay clones had the highest densities of *M. hapla* eggs/g of root, followed by all three Riesling clones (Table 2); Merlot 06 and 03 had the fewest *M. hapla* eggs/g of root. The number of *M. hapla* J2/250 g of soil between the grape clones did not differ (data not shown). When total *M. hapla* population densities (eggs + J2) was considered, both Chardonnay clones had the greatest densities of *M. hapla*, followed by the Riesling clones, while the Syrah/Shiraz and Merlot clones had the lowest total number of *M. hapla* (Table 2). Similar trends were observed in 2013; all three Merlot clones had the lowest *M. hapla* eggs/g of root. Chardonnay 06 followed by Riesling 239 and Chardonnay 15 had the greatest densities of *M. hapla* eggs/g of root (Table 2). *M. hapla* J2/250 g in soil differed among clones ($P = 0.036$), with the Syrah and Merlot clones having the lowest *M. hapla* J2/250 g of soil (data not shown). Chardonnay 06 followed by Riesling 239 and Chardonnay 15 had the greatest densities of total *M. hapla*; all three Merlot clones had the lowest total number of *M. hapla* (Table 2). In both years, Chardonnay 06 had the greatest density of *M. hapla* eggs/g of root and total number of *M. hapla*/pot. The grapes with the highest RF values in 2012 and 2013 were both the Chardonnay clones and the three Riesling clones. Syrah Phelps had the lowest RF value in 2012 while the Merlot clones had the lowest RF values in 2013 (Table 2). In both years, shoot dry weight ranged from 7.47 to 15.79 g and root dry weight ranged from 7.02 to 18.28 g (data not shown).

For comparison across varieties, clones within a variety were combined for analysis. In both years, Chardonnay had the greatest density of *M. hapla* eggs/g of root and RF values (Table 3). Although Chardonnay had a similar number of *M. hapla* eggs/g of root to that of Riesling, RF values in both years were significantly

TABLE 2. *Meloidogyne hapla* eggs/g root and total *M. hapla* (eggs + J2) recovered from inoculated vines, and reproduction factor (RF) values of *M. hapla* in a greenhouse trial evaluating the host status of *Vitis vinifera* varieties and clones in 2 yr.

Variety/Clone	*M. hapla* eggs/g of root	Total *M. hapla*	RF[b]	*M. hapla*s/g of root	Total *M. hapla*	RF
	2012			2013		
Cabernet Sauvignon 02	10,540 e[a]	187,000 bcd	20.1 bcd	8,356 d	79,760 de	8.4 de
Cabernet Sauvignon 04	11,530 e	214,000 bcd	22.5 bcd	10,360 cd	93,490 cde	9.9 cde
Cabernet Sauvignon 06	14,410 cde	190,600 bcd	20.2 bcd	12,300 bcd	92,310 cde	9.9 cde
Cabernet Sauvignon 21	10,890 e	179,100 cd	19.0 cd	11,060 bcd	128,600 cde	10.4 cde
Chardonnay 06	50,620 a	528,100 a	57.0 a	34,820 a	304,600 a	32.8 a
Chardonnay 15	32,830 ab	411,800 ab	44.5 ab	23,530 abc	199,400 abc	21.3 abc
Merlot 03	5,301 e	69,960 d	7.4 d	4,103 d	35,910 e	3.9 e
Merlot 06	5,044 e	102,700 d	10.4 d	5,281 d	59,040 de	6.3 de
Merlot 15	8,040 e	146,500 cd	14.3 cd	5,417 d	49,480 de	4.7 de
Syrah 07	12,550 de	66,300 d	7.0 d	17,950 bcd	107,000 cde	11.8 cde
Syrah Phelps	6,936 e	51,030 d	5.3 d	7,666 d	74,320 de	8.1 de
Shiraz 07	12,750 de	123,200 d	13.0 d	14,150 bcd	104,400 cde	10.9 cde
Riesling 90	31,290 bc	351,100 abc	38.1 abc	16,650 bcd	163,600 bcd	16.9 bcd
Riesling 198	30,670 bcd	278,900 bcd	30.1 bcd	13,610 bcd	135,400 cde	14.7 cde
Riesling 239	22,160 bcde	238,000 bcd	25.0 bcd	25,620 ab	267,600 ab	27.7 ab

[a] Values are the means of eight observations. Nematode data were $\log_{10}(x + 1)$ 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 ($P \leq 0.05$).

[b] RF values calculated as final nematode population density/initial nematode population density.

TABLE 3. *Meloidogyne hapla* eggs/g root and reproduction factor (RF) values on *Vitis vinifera* varieties in greenhouse experiments.

	M. hapla eggs/g root	RF[b]	*M. hapla* eggs/g root	RF[b]
Variety	2012		2013	
Chardonnay	45,786 a[a]	45.1 a	26,990 a	27.1 a
Riesling	33,759 a	27.6 b	17,180 ab	19.8 b
Cabernet Sauvignon	19,885 b	18.2 bc	9,776 bc	9.6 bc
Syrah	8,507 b	7.9 c	11,720 bc	10.3 c
Merlot	13,166 b	9.5 c	4,645 c	4.9 c

[a] Values are the means of eight observations. Nematode data were $\log_{10}(x + 1)$ 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 ($P \leq 0.05$).

[b] RF values calculated as final nematode population density/initial nematode population density.

higher for Chardonnay than Riesling ($P \leq 0.001$). Chardonnay and Riesling always had greater densities of *M. hapla* eggs/g root and RF values than the other varieties. Syrah had the fewest *M. hapla* eggs/g of root and RF value in 2012 while Merlot had the fewest *M. hapla* eggs/g of root and RF value in 2013. In 2012, the number of *M. hapla* eggs/g of root and RF value in Chardonnay were 6 to 7 times greater than the corresponding measurements in Syrah. Likewise, in 2013, Chardonnay had six times more *M. hapla* eggs/g of root and RF value than Merlot.

Discussion

To our knowledge, this is the first report of differences in host status of *V. vinifera* grape varieties to *M. hapla*. This difference was first observed in the microplot experiment when population densities of *M. hapla* J2 were 6.5 times greater in Chardonnay than in Cabernet Sauvignon 1.5 years after inoculation and planting. This trend continued throughout the life of the microplot experiment with the magnitude of this difference becoming greater over the 3.5-year period. This result was further supported in greenhouse experiments evaluating a wider range of *V. vinifera* varieties and clones as hosts for *M. hapla*. While all of the *V. vinifera* varieties evaluated in the greenhouse experiments are considered good ($10 > RF > 1$) to excellent ($RF > 10$) hosts (Ferris et al., 1993) for *M. hapla*, the magnitude of increase in population size of *M. hapla* on the two white varieties (Chardonnay and Riesling) was much greater than two of the red varieties (Merlot, and Syrah), with Cabernet Sauvignon being intermediate between them. Differences in host status of *V. vinifera* varieties for *Meloidogyne arenaria* have been previously documented (Ferris et al., 1982). The *V. vinifera* varieties Carignane, Barbera, French Colombard, Ruby Cabernet, Cabernet Sauvignon, and Zinfandel were all susceptible to *M. arenaria* while Tokay, Thompson Seedless, and Perlette were moderately resistant. Unlike our results with *M. hapla*, the trend for white varieties to be consistently better hosts than red varieties was not observed for *M. arenaria*.

Although there were differences in the host status of grape varieties to *M. hapla*, clones within a variety did not differ. *V. vinifera* clones originate from slight genetic mutations during asexual propagation (Hartmann et al., 1990), and therefore, clones of the same variety are very similar in their genetic makeup. Conversely, the genetic makeup of different *V. vinifera* varieties is more diverse, and this may explain why the *V. vinifera* varieties evaluated in these trials varied in host status to *M. hapla*.

The finding that white *V. vinifera* varieties are better hosts for *M. hapla* may be due to differences in rooting behavior, production of root or rhizosphere metabolites, or other factors. In our greenhouse experiment, the root systems of the white varieties appeared to have a much greater abundance of fine feeder roots compared to the red varieties, although this was not directly quantified. Fine roots are the preferred entry site for juveniles, which invade right behind the root tip (de Guiran and Ritter, 1979). Therefore, with more fine roots, there are more potential sites for nematode invasion which could lead to a higher rate of infection. Also, McKenry and Anwar (2006) reported that grapes with widespread root systems have root tips far apart from each other, reducing penetration and success of *Meloidogyne* spp. This could help explain why red varieties with fewer fine roots would result in a lower *M. hapla* invasion than white varieties, which have an abundance of fine roots.

Another potential explanation for the difference in host status of white and red *V. vinifera* varieties to *M. hapla* may be related to the quantity and/or quality of root exudates that influence initial contact with nematode juveniles. The rhizosphere, located about 0 to 2 mm from the root surface, plays a significant role in root exudate production and secretion and is known to greatly influence soil ecology (Bertin et al., 2003). A major source of exudates in the rhizosphere is right behind the root tip; the production of root exudates in this region may allow exudates to attract and/or repel *Meloidogyne* spp. due to their preference to this region of root (Huang, 1985; Anwar and McKenry, 2000). Different plant species and different varieties within a species secrete different exudates (Rovira, 1969). This could be true for *V. vinifera* as well; red varieties may secrete root exudates that are less attractive or detrimental to *M. hapla*, while white varieties may secrete root exudates that are more attractive to *M. hapla*. This idea is supported by Huang (1985) who reported that roots of susceptible and resistant plants, even within a genus, can vary in their attractiveness to *Meloidogyne* spp., although the exudates responsible for attraction or repulsion are unknown.

In the microplot experiment, *M. hapla* did not impact vine establishment or productivity based on seasonal prune weights or whole plant biomass at the final

harvest, despite the nematode reaching high densities in soil. The population densities of *M. hapla* found in Chardonnay were well above those reported for *Meloidogyne* spp. to cause damage to *V. vinifera* grapevines (Nicol et al., 1999). The microplots were a very conducive environment for *M. hapla* population increase in Chardonnay, similar to what was observed in microplot studies with *Mesocriconema xenoplax* where greater fine root abundance provided easier access to fine roots and resulted in higher nematode abundance than in field soils (Schreiner et al., 2012b). However, the artificial environment of microplots may have also negated any impact of *M. hapla* on vine growth. For example, the vines in this study received more water than would normally be applied to vineyards in eastern Washington, as wine grapes in this region are grown under regulated deficit irrigation to reduce vegetative growth and encourage fruit development (Schreiner et al., 2007). The effects of nematode parasitism may not become apparent until vines are under additional stressors, as indicated by the finding that high-vigor vines are better able to tolerate nematode parasitism (Ferris and McKenry, 1975). *Mesocriconema xenoplax* had a greater impact on vine productivity when additional stressors, low light or partial defoliation, were introduced (Schreiner et al., 2012a). This impact was attributed to nematode parasitism reducing carbohydrate reserves in the roots and wood needed to support growth in future years.

The goal of adding different densities of *M. hapla* to the microplots was to ascertain if a particular threshold population of *M. hapla* at planting time could be identified that causes damage to newly planted own-rooted *V. vinifera*. However, we did not observe a difference in vine growth as a function of inoculation density or even a consistent difference in *M. hapla* population densities in soil or roots. Defining the impact between initial population densities of plant-parasitic nematodes on vine growth and yield has been elusive for many researchers (Nicol et al., 1999). Understanding the relationship of plant-parasitic nematode densities and response of perennials is complex due to numerous factors including, but not limited to, reintroduction of nematodes, presence of non-dectectable densities, survival in soil, and decline in nematode populations after damage to the perennial host (Barker and Olthof, 1976). Population densities of other *Meloidogyne* spp. reported to cause damage to *V. vinifera* are 150 eggs/g root (Sauer, 1977) and 50 J2/250 g soil (McKenry, 1992). Population densities in excess of these proposed thresholds were consistently observed for *M. hapla* in this study. It is difficult to relate thresholds proposed in other regions to the eastern Washington wine grape growing region because of differences in climate and *Meloidogyne* spp.

This research expands the knowledge of the host status of several *V. vinifera* varieties to *M. hapla*. Our results indicate that own-rooted white varieties (Chardonnay and Riesling) are significantly better hosts to *M. hapla* than the red varieties (Syrah, and Merlot). While the red varieties will still support *M. hapla* reproduction, the population build up in the soil might be slower compared to white varieties, potentially allowing better grapevine establishment and tolerance to *M. hapla* parasitism. Additional work is still required to understand the effects of *M. hapla* on vine establishment and productivity in eastern Washington to help guide management decisions.

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