AN ABSTRACT OF THE THESIS OF

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Title: Facilitating Plant-parasitic Nematode Management Decisions for Washington *Vitis vinifera* Growers: Determining the Spatial Distribution of Plant-parasitic Nematodes and the Host Status of *Vitis* spp. to *Meloidogyne hapla*

Abstract approved:

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A major constraint to the production of self-rooted wine grapes (*Vitis vinifera*) in eastern Washington is plant-parasitic nematodes. Plant-parasitic nematodes can impact grape productivity by limiting water and nutrient uptake, educing physiological changes, and extracting plant nutrients from roots, thereby reducing root biomass, plant vigor, and yield. The most commonly encountered plant-parasitic nematodes in Washington *V. vinifera* vineyards are *Meloidogyne hapla*, *Mesocriconema xenoplax*, *Pratylenchus* spp., *Xiphinema americanum*, and *Paratylenchus* sp.; however, little is known about their biology, distribution, and pathogenicity in this production system. Therefore, the objectives of this study were to 1) determine the spatial distribution of plant-parasitic nematodes in eastern Washington *V. vinifera* vineyards, and 2) determine the host status of *V. vinifera* varieties and clones predominantly grown in Washington, and several *Vitis* spp. rootstocks to *M. hapla*. For the first objective, two vineyards in eastern Washington were sampled over a two-year period to determine

the horizontal and vertical distribution of plant-parasitic nematodes. To achieve the second objective, *V. vinifera* varieties and clones and *Vitis* spp. rootstocks were inoculated with *M. hapla*, grown in the greenhouse, and destructively harvested to determine nematode reproduction. Results from the spatial studies showed that, in general, populations of *M. hapla* and *M. xenoplax* were aggregated under irrigation emitters and that population densities of these nematodes decreased with soil depth. While *Pratylenchus* spp. population densities also decreased with depth, populations of these nematodes were concentrated along the alley ways between vine rows. *Paratylenchus* sp. and *X. americanum* were random in both their vertical and horizontal distributions within the vineyards. We also found that soil moisture plays a dominant role in the distribution of fine roots and plant-parasitic nematodes within semi-arid vineyards. Where soil moisture was the highest, fine root biomass and population densities of *M. hapla* and *M. xenoplax* were also the highest. The opposite was true for *Pratylenchus* spp., with higher population densities of this nematode in drier areas of the vineyard. There was no relationship of *X. americanum* and *Paratylenchus* sp. population densities with soil moisture. These results show there is potential to only treat a 60 cm area around the vine row when targeting *M. hapla* and *M. xenoplax*; however, this strategy would not be effective against *X. americanum* or *Paratylenchus* sp. It also appears that *Pratylenchus* spp. are not parasites of *V. vinifera* in this production system and that there may not be a need to consider these nematodes from a management perspective. In our host status trials with *M. hapla*, all of the *V. vinifera* varieties and clones were excellent hosts for *M. hapla*, but the magnitude of increase in population size of *M. hapla* on white (Chardonnay and Riesling) compared to red (Cabernet Sauvignon, Merlot, and Syrah) varieties was significantly greater. White varieties had higher *M. hapla* eggs/g root and an almost 40% higher reproduction factor value than red varieties. All the *Vitis* spp. rootstocks screened (Salt Creek, Freedom, Harmony, St. George, Riparia Gloire, 101-14, 3309C, 110R, and 420A) allowed very little or no *M. hapla* reproduction, and therefore are considered non-hosts. This research will provide Washington grape growers with the knowledge

to select appropriate planting material to minimize the impact of *M. hapla* on grapevines. The results of both studies greatly expand the knowledge of the spatial distribution of plant-parasitic nematodes in semi-arid Washington *V. vinifera* vineyards as well as the host status of commonly planted *V. vinifera* varieties to *M. hapla*.

Facilitating Plant-parasitic Nematode Management Decisions for Washington *Vitis vinifera* Growers: Determining the Spatial Distribution of Plant-parasitic Nematodes and the Host Status of *Vitis* spp. to *Meloidogyne hapla*

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Chapter 1: Introduction

1.1 Overview of the Washington grape industry

Washington is the second largest wine (*Vitis vinifera)* producing region in the United States with 17,401 ha of vineyards cultivating more than 30 different varieties (USDA 2013). White grape varieties are prevalently grown, with the leading varieties being Riesling and Chardonnay. Leading red varieties are Cabernet Sauvignon, Merlot, and Syrah. Washington has been producing wines for the past century and the first commercial-scale vineyard in eastern Washington was established in the 1960s. Production rapidly expanded during the next decade and continued to expand to the thirteen American Viticulture Areas recognized today. In 2012, the Washington grape industry had an economic value of $236 million (USDA 2013), producing predominately premium wines and juice grapes (*Vitis labruscana*). Washington is the largest juice grape producer in the United States, with 10,522 ha of *V. labruscana* (USDA 2013).

1.2 Biology and symptoms of plant-parasitic nematodes found in semi-arid Washington vineyards

A common pest of concern in Washington vineyards are plant-parasitic nematodes. Plant-parasitic nematodes are a global pest and are estimated to cause $100 billion economic loss to agriculture worldwide with $6 billion agricultural loss in the United States (Sasser and Freckman 1987). The damage caused by plant-parasitic nematodes can be extensive and is a result of the nematodes using their stylet, a hypodermic-like mouthpart, to puncture plant cells and remove cell contents when feeding. In a grapevine, nematode feeding can cause the vine to prematurely decline (Anwar and Gundy 1989; Stirling and Cirami 1984; Lider 1960), have reduced vigor (Téliz 2007; Nicol et al. 1999; Ferris 1975), and have increased susceptibility and severity to biotic stresses such as pests (phylloxera (*Daktulosphaira vitifoliae*)), diseases (crown gall disease), and viruses (Grapevine Fanleaf Virus and Tomato

Ringspot Virus), and abiotic stresses such as drought (Esmenjaud and Bouquet 2009; Téliz 2007; Ramsdell 1996; Brown 1993; McKenry 1992). Symptoms also include reduced root and shoot growth (Nicol et al. 1999; Walker 1997; Anwar and Gundy 1989), reduced ability to uptake water and nutrients (Nicol et al. 1999), physiological changes in the roots (Ferris et al. 2012), and reduced yield (Esmenjaud and Bouquet 2009; McKenry et al. 2006; Nicol et al. 1999; Nicol and Heeswijck 1997; McKenry 1992; Lider 1960). The extent of yield loss varies depending on location, variety, species of plant-parasitic nematode present, and the population density of nematodes, but losses can range from 7 to 60% (Téliz 2007; Nicol and Heeswijck 1997).

Surveys were conducted in Washington to determine the plant-parasitic nematodes associated with semi-arid *V. vinifera* vineyards (Zasada et al. 2012). The most commonly encountered plant-parasitic nematodes were *Meloidogyne hapla*, *Paratylenchus* sp., and *Xiphinema* spp. detected in 60%, 50%, and 59% surveyed vineyards, respectively. Other plant-parasitic nematodes found were *Pratylenchus* spp. detected in 45% of surveyed vineyards and *Mesocriconema xenoplax* found in 14% of surveyed vineyards; *Tylenchorynchus* spp. and *Trichodorus* spp. were found in only a few vineyards at very low population densities. In other grape growing regions of the world, *Meloidogyne*, *Xiphinema*, *Mesocriconema*, and *Pratylenchus* spp. have been shown to be the most damaging nematodes to grapevines (Pinkerton et al. 2004; Esnard and Zuckerman 1998; Brown et al. 1993a). Jensen (1961) reported that *Meloidogyne* and *Pratylenchus* spp. are well recognized for injuring high-value irrigated crops in the Pacific Northwest.

1.2.1 Meloidogyne spp.

Meloidogyne spp. are of worldwide concern and are a major production and economic constraint to grapevines (Zasada 2012; Nicol et al. 1999; Arredondo 1992; Jenser et al. 1991; Raski et al. 1973). Cosmopolitan in distribution, these nematodes can invade almost every crop agriculturally grown (Hussey and Janssen 2002), and

have been reported to reduce grapevine yields by up to 60% (Nicol and Heeswijck 1997). Over 50 species of *Meloidogyne* are described with seven species found on grapevines. However, only four species, *M. incognita, M. hapla, M. javanica,* and *M. arenaria,* are considered to be the most damaging (Esmenjaud and Bouquet 2009; Esnard and Zuckerman 1998), accounting for 95% of all *Meloidogyne* spp. infestations in cultivated fields (Hussey and Janssen 2002). *Meloidogyne hapla*, the northern root-knot nematode, is found only in the Pacific Northwest (Zasada et al. 2012) due to its much more temperate distribution compared to the other three major *Meloidogyne* spp., which prefer warmer climates and are found worldwide (Esmenjaud and Bouquet 2009; Esnard and Zuckerman 1998).

Meloidogyne spp. are sedentary endoparasites, remaining stationary inside the roots of a host plant. Newly hatched second-stage juveniles (J2) migrate through the soil to find a host plant root. Second-stage juveniles penetrate the tip of the root just above the root cap and move intercellularly to the vascular cylinder becoming stationary and inducing the formation of specialized 'giant cells' in susceptible hosts. These specialized cells act as the nematode's feeding site in the root. Second-stage juveniles swell and undergo three molts, developing into a third- and fourth-stage juvenile (J3 and J4) before progressing into an adult male or female. Adults are typically female with a spherical body and protruding neck. However, under unfavorable developmental conditions such as stress on the host plant, males can develop. Vermiform males discontinue feeding, and leave the root to move freely into the soil seeking a female. Females lay eggs outside of the root in a gelatinous matrix. A single egg mass of *M. hapla* can contain 400 to 500 eggs. Under optimal conditions, the lifecycle of this nematode can take four to five weeks to complete, producing four to six generations per season. Optimal conditions for *M. hapla* include a thermal temperature of 25°C for egg hatch and 20°C for J2 mobility in soil (Esmenjaud and Bouquet 2009; Hussey and Janssen 2002; Nicol et al. 1999; Brown et al. 1993a; de Guiran and Ritter 1979; Williams 1974; Bird and Wallace 1965).

Infection of a plant by *Meloidogyne* spp. induces the formation of characteristic galls on host roots. Small galls with usually a single nematode occur on young feeder roots and larger galls can be a consequence of multiple infections at the same location. Heavily infested roots of grapevines have significantly reduced root systems and a higher number of galls (Brown et al. 1993a) limiting the plants ability to uptake water and nutrients (Ramsdell et al. 1996). *Meloidogyne* spp. are also known to reduce grapevine yields, result in poor growth (Esnard and Zuckerman 1998), and initiate earlier bud break (Anwar and Gundy 1989) which can lead to frost damage in regions such as the Pacific Northwest.

1.2.2 Xiphinema spp.

Xiphinema spp., dagger nematodes, are migratory ectoparasites moving freely in the soil and feeding on the external surface cells of host plant roots. Similar to *Meloidogyne* spp., *Xiphinema* spp. have a wide host range. The majority of hosts for this nematode are woody plants such as grapes, tree fruits, and forest species. Only *X. americanum*, the American dagger nematode, has been reported in Washington (Zasada et al. 2012). This nematode has a long life cycle of at least one year and hatches as first-stage juveniles (J1) from eggs deposited in the soil. First-stage juveniles undergo three molts, during which the nematode steadily grows and feeds at the root tips of the host plant, before developing into an adult. Male *X. americanum* are rare, and therefore are not necessary for reproduction. *Xiphinema americanum* have a low rate of reproduction. These nematodes can survive up to three years in the soil without a host plant, and survival for long periods of time in frozen soils has been reported, with eggs being the most resistant stage to temperature extremes (Esnard and Zuckerman 1998; Halbrendt and Brown 1992; Ferris and McKenry 1975; Siddiqi 1973; Malek 1969). In grapevines, *X. americanum* can induce the malformation and necrosis of root tips, which stops root growth (Brown et al. 1993a; Anwar and Gundy 1989). This causes significant decline to grapevines and reduces vigor (Esnard and Zuckerman 1998). *Xiphinema* spp. are also known to vector viruses pathogenic to

grape (Pinkerton et al. 1999; Esnard and Zuckerman 1998; Raski and Radewald 1958), including Grapevine fanleaf virus, vectored by *X. index* (Esmenjaud and Bouquet 2009; Téliz 2007; Brown et al. 1993b; Raski 1983), Peach rosette mosaic virus in 'Concord' grapevines (*V. labrusca*) vectored by *X. index* (Bird and Ramsdell 1985), and tomato ringspot virus and tobacco ringspot virus vectored by *X. americanum* (Esnard and Zuckerman 1998; Siddiqi 1973). As a result, *Xiphinema* spp. cause indirect damage to grapevines through transmitting viruses (McKenry and Anwar 2006; Ramsdell et al. 1996; Ferris and McKenry 1975). However, none of these nematode-transmitted viruses have been found in Washington vineyards associated with its nematode vector.

1.2.3 Mesocriconema xenoplax

Mesocriconema xenoplax (= *Criconemella xenoplax*), the ring nematode, is a migratory ectoparasite remaining motile in the soil. This nematode undergoes the first molt in the egg, hatches as a J2, and molts three more times before becoming a mature adult. Reproduction occurs parthenogenetically and adult females singularly deposit three to five eggs per day; males are rarely found. The lifecycle takes 25 to 35 days under optimal thermal conditions of 22 to 26˚C (Seshadri 1964). *Mesocriconema xenoplax* is sensitive to temperature and moisture extremes (Nyczepir and Halbrendt 1993; Seshadri 1964; Thomas 1959). These nematodes have a global distribution and are widely found throughout vineyards in Australia, European countries such as Switzerland and Germany, and in the United States including California, Oregon, and Michigan (Pinkterton et al. 1999). In Californian vineyards, McKenry (1992) found a 10 to 25% reduction in yield when more than 500 *M. xenoplax*/kg soil was present. *Mesocriconema xenoplax* has also been associated with overall unhealthy vineyards (Pinkerton et al. 2004; Pinkerton et al. 1999; Klingler and Gerber 1972; Meagher 1969) and are known to extensively damage roots in greenhouse studies (Santo and Bolander 1977). This nematode can significantly reduce pruning weights, root growth,

yield, and the colonization of roots by arbuscular mycorrhizal fungi (AMF) (Schreiner et al. 2012; Pinkerton et al. 2004).

1.2.4 Pratylenchus spp.

Pratylenchus spp., root-lesion nematodes, are migratory endoparasites. These motile nematodes enter host roots and tunnel through the cells to the cortex where they feed on the cytoplasm of the cortical cells. This can cause considerable damage to the root. *Pratylenchus* spp. can also leave the root and move through the soil seeking other roots to infect. Eggs are laid singularly in the root or in the soil after sexual reproduction or parthenogenesis. These nematodes undergo their first molt in the egg, hatch as J2, and molt three more times to become a mature adult. All stages of *Pratylenchus* spp. are infective. The lifecycle ranges from 45 to 65 days depending upon soil temperature (Agrios 1988). This short lifecycle can result in several generations to occur in a single season. As a result of infection by *Pratylenchus* spp., necrotic lesions can form on the roots, reducing the uptake of water and nutrients by the roots. Wounding of plant roots can also make the root more susceptible to secondary infections (Subbotin et al. 2008; De Waele and Elsen 2002; Walker 1984; Corbett 1973). *Pratylenchus* spp. found in Washington have not been identified to the species level and their impact on Washington grapevines are unknown. Only *P. vulnus* has been shown to cause premature decline in vineyards and prevent the successful establishment of new vines (Anwar and Gundy 1989; Ferris and McKenry 1975; Raski et al. 1973). These widespread plant-parasitic nematodes are also known to cause economic loss in vineyards in Australia (Walker and Morey 2000).

1.2.5 Paratylenchus spp.

Paratylenchus spp., pin nematodes, are migratory ectoparasites that feed on the exterior surfaces of host roots. These nematodes emerge from eggs as J2 and quickly undergo three molts to become an adult. Before their final molt into an adult, *Paratylenchus* spp. develop into J4 or pre-adults, a uniquely resistant stage in which

the nematode lacks a complete stylet and does not feed. Pre-adult pin nematodes can survive for long periods of time in unfavorable conditions such as temperature and moisture extremes and therefore can persist in soils for years (Rhoades and Linford 1959). However, if current environmental conditions are optimal, the J4 will quickly molt into an adult female or male. Female *Paratylenchus* spp. can lay on average one to three eggs per day, and under optimal life cycle temperatures of 18 to 20˚C, complete a generation in 36 to 38 days (Wood 1973). All developmental stages of these nematodes feed on roots (Loof 1975; Wood 1973; Eck 1970), but the damage caused by *Paratylenchus* spp. on grapes is minimal (Pinkerton et al. 1999). The species of *Paratylenchus* present in Washington vineyards has not been determined.

1.3 Nematode management

The most basic way to manage plant-parasitic nematodes is exclusion, or preventing the introduction of plant-parasitic nematodes into noninfected locations. Nematodes are most commonly introduced into fields through flood or irrigation water, windblown dust, and humans, animals, and unclean machinery accidentally transporting infested soil or plant tissues to clean fields. Exclusion procedures include sanitation, such as cleaning machinery, planting certified clean plant material, and using only nematode-free soil or planting media (Bird 1981). In order to effectively manage nematodes, a grower needs to have an understanding of the problem. This is achieved through collecting soil samples to determine which nematode species are present and their population densities. When nematodes are discovered in a field, management practices need to be implemented but controlling plant-parasitic nematodes can be very difficult due to their wide range in hosts and perseverance in soil. Therefore, management practices for plant-parasitic nematodes are typically preventative and aimed at pre-plant control, but post-plant controls do exist; however, most post-plant methods give only inconsistent results. The major management practices of plant-parasitic nematodes in vineyards are chemical, cultural, rootstocks, and plant resistance.

1.3.1 Chemical management

Chemical management practices consist of pre-plant soil fumigation and post-plant nematicide applications. For decades, soil fumigation was the most common practice to control economically important plant-parasitic nematodes in high-value crops. First developed for use in strawberries in California, soil fumigants have been shown to improve root and plant health through controlling soilborne pathogens (Duniway 2002). Recent stricter guidelines on the use of fumigants and nematicides have significantly limited their use and the number of chemicals available. For instance, methyl bromide was one of the top fumigants applied to soil for over 40 years for the control of nematodes and soilborne pathogens. However, in 1993, the U.S. Environmental Protection Agency (EPA) officially classified methyl bromide as a Class I Stratospheric Ozone Depleting Substance under the U.S. Clean Air Act. As a result, methyl bromide has been banned since 2001 in the United States and it is being phased out worldwide (Atkins et al. 2003; Duniway 2002; Ristaino and Thomas 1997; Noling and Becker 1994).

The chemical alternatives currently available are not as completely effectual at combating soilborne pathogens or have the versatility of methyl bromide (Duniway 2002). However, fumigants containing 1,3-dichloropropene (1,3-D), such as Telone® C-35 (Dow AgroSciences, IN), have been shown to be highly effective in controlling plant-parasitic nematodes and is one of the last general-use soil fumigants still used (Zasada et al. 2010; Oka et al. 2000). While a great nematicide, it does not provide any weed control and can have reduced efficacy in fine-textured soils (Zasada et al. 2010). Another effective soil fumigant is metam sodium (Vapam®; AMVAC, Newport Beach, CA) (Qiao 2010). Metam sodium is a broad spectrum biocide that is widely used; however, it has inadequate volatility resulting in poor soil distribution (Zasada et al. 2010). In Washington, 1,3-D is normally applied as a broadcast shank application while metam sodium is applied through the drip line when establishing a new vineyard. All of the fumigants have recently undergone the reregistration process

(U.S. EPA 2013), and further restrictions to the uses of fumigant nematicides are expected. Therefore, effective alternatives to pre-plant soil fumigation need to be explored, especially because there are few proven registered post-plant nematicides available to grape growers in Washington.

1.3.2 Cultural controls

Cultural methods for controlling plant-parasitic nematodes are numerous and include fallow, soil solarization, biofumigation, green manures, crop rotation, and cover crops. Fallow is when a field is left barren usually for a year or two. It is most commonly used as site preparation before planting a field and can reduce plant-parasitic nematodes population levels due to the lack of a host. However, a major component with fallow is that weed control is critical to its success. Grasses and weeds present in fallowed fields or along their border can act as a reservoir for nematodes, such as *X. americanum* (Siddiqi 1973). Another potential problem with fallow is that it can be extremely ineffectual in reducing nematode population levels in vineyards or fields if roots from previous crops are not completely removed. The roots of woody plants, such as grapes, can also serve as a reservoir for plant-parasitic nematodes and help maintain population levels. These roots can remain alive in the soil, providing an alternative host to the nematode and protecting endoparasitic nematodes from potential lethal environmental conditions (Raski et al. 1973).

Another cultural method for managing nematodes, soilborne pathogens, and weeds is soil solarization. This method works through covering moist soil with a plastic tarp to thermally suppress pathogens, nematodes, and weeds. The tarp allows soil temperatures to increase to levels that are detrimental to soilborne pathogens and plant-parasitic nematodes with the highest temperatures near the soil surface (Zasada et al. 2010; Pinkerton et al. 2000; Xue et al. 2000; Stapleton and DeVay 1983). Various studies have investigated the impact of soil solarization for the management of plant-parasitic nematodes with mixed results. Chellemi et al. (1997) investigated

soil solarization on tomatoes in Florida and discovered that soil solarization decreased population densities of *Paratrichodorus minor* and *Criconemella* spp., but had no effect on *M. incognita*. In California, Stapleton and DeVay (1983) showed that soil solarization plus 1,3-D significantly reduced *Meloidogyne* spp., *M. xenoplax*, *Pratylenchus* sp., *Paratylenchus* spp., *Xiphinema* spp., and other plant-parasitic nematodes population levels.

Green manures and biofumigation are another great alternative to chemical fumigation and can be highly effective in eliminating soilborne organisms such as plant-parasitic nematodes. The decomposition of incorporated green manure biomass releases biofumigant compounds such as hydrogen cyanide and isothiocyanates, which are toxic to plant-parasitic nematodes (Zasada et al. 2010; Ploeg 2008; Stirling and Stirling 2003). For example, rapeseed (*Brassica napus*) contains the secondary plant metabolite glucosinolates, a sulfur-containing compound (Stirling and Stirling 2003). During decomposition, the glucosinolates are hydrolyzed by the plant enzyme myrosinase, becoming isothiocyanates which kill nematodes (Rahman and Somers 2005; Mojtahedi et al. 1991). Marigolds (*Tagetes* spp.) can also act as a biofumigant and have been shown to suppress up to 14 different species of nematodes, including *M. hapla* and *P. penetrans* (Wang et al. 2007). Marigolds can be highly toxic to nematodes because they release the chemical compound α-therthienyl, a sulfur-containing compound that acts as a natural nematicide, insecticide, and fungicide. Nematodes that enter the roots of marigolds or contact soil containing α-therthienyl will die (Wang et al. 2007). Biofumigation seems promising, but more research is needed to further understand the biofumigant mode-of-action and which nematode species are susceptible to this management practice.

Crop rotation is where non-host crops are rotated into a field for a sufficient length of time to reduce plant-parasitic nematode population levels. In addition to being a non-host, rotation crops can also be resistant hosts, trap crops, green manure crops, or allelopathic crops (Zasada et al. 2010). While this is a very common method

for managing nematodes in cultivated fields, crop rotation cannot be employed in all agriculture fields because of economic constraints. It can also be ineffectual on certain plant-parasitic nematodes such as *Xiphinema* spp., which can live in the soil for long periods of time without the presence of a host crop, and on *Meloidogyne* spp. due to the wide host range of this nematode (Oka et al. 2000).

The last major cultural management practice is cover crops. Cover crops are very beneficial in agricultural fields and vineyards, and in the last decade, they have become commonly used to help control plant pathogens and plant-parasitic nematodes. Cover crops are used to suppress weeds, increase organic matter, manage pests and pathogens, increase soil fertility, and improve soil structure, soil moisture, and water penetration (Guerra and Steenwerth 2012; Sanguankeo et al. 2009; Ingels et al. 2005; Bordelon and Weller 1997). In vineyards, cover crops help control vigor (Guerra and Steenwerth 2012; Sanguankeo et al. 2009; Ingels et al. 2005; Bordelon and Weller 1997) through providing competition of water and nutrient resources to grapevines. Recent research has discovered that some cover crops, such as mustards (*Brassica* spp.), suppress plant-parasitic nematode population levels through acting as trap crops, preventing nematode reproduction, and acting as biofumigants (Wilmer et al. 2002; Hagan et al. 1998).

A major constraint to the implementation of cultural practices in vineyard production systems is the amount of time and economics of not immediately replanting a new vineyard. In addition, the effectiveness of these practices in semi-arid vineyards in Washington is unknown. Soil solarization could be a viable yet expensive pre-plant alternative because of the hot, dry summers in this region. However, implementation of this management practice would require at least a year out of production since soil solarization can only be executed in the summer, and it would be an arduous task in large vineyards. Cover crops and fallow could also work in this production system as pre-plant management strategies, but this would again require a vineyard to be out of production for at least a year to achieve desired results. A

constraint to the implementation of cover crops and green manures is the availability of water; because of this limitation, other ways to manage plant-parasitic nematodes in Washington vineyards need to be investigated.

1.3.3 Rootstocks

The most widely employed management practice to maintain low plant-parasitic nematode population densities in vineyards is replanting with vines grafted to nematode-resistant rootstocks (Téliz 2007; Hardie and Cirami 1988; Lider 1960). Rootstocks have been used in viticulture since the late 1800s to protect against soil pests, such as phylloxera, root aphids, and plant-parasitic nematodes (Ferris et al. 2012; Reisch et al. 2012; Cousins and Striegler 2005; Nicol et al. 1999). Rootstocks have also been developed for drought and pH tolerance, cold hardiness, increased vigor, and increased production (Cousins and Striegler 2005; Shaffer 2004). Since the 1930s, identification of rootstocks with resistance to plant-parasitic nematodes has been pursued (Lider 1960).

Commonly used nematode resistant rootstocks include Dogridge and Salt Creek (=Ramsey) (*V. champinii*), which are completely resistant to *M. javanica, M. incognita,* and *M. arenaria,* but are susceptible to *X. index, X. americanum,* and *M. xenoplax* (Ferris et al. 2012; McKenry and Anwar 2006; Nicol et al. 1999). Freedom and Harmony (1613 C x *V.* x *champinii*) rootstocks have moderately good resistance to *M. javanica*, *M. incognita* and phylloxera, and were the first formal breeding *Meloidogyne* spp. resistant rootstocks to emerge (Ferris et al. 2012). Freedom is also resistant to *X. index* but is susceptible to *X. americanum, M. hapla,* and *M. xenoplax*; Harmony is susceptible to *M. hapla, X. index*, *X. americanum,* and *M. xenoplax* (Ferris et al. 2012; Esnard and Zuckerman 1998; Stirling and Cirami 1984; Raski 1973). Another rootstock that is commonly used is St. George (*V. rupestris*), but it is highly susceptible to *M. incognita, X. index*, and *M. xenoplax* (Ferris et al. 2012; Cousins and Striegler 2005; Nicol et al. 1999). The rootstock 420A (*V. berlandieri* x *V. riparia*) is

resistant to *M. xenoplax* and phylloxera, is lime tolerant, and is moderately tolerant to *M. incognita,* and *M. arenaria*. However, it is susceptible to *X. index, M. javanica, M. hapla,* and *Pratylenchus* spp. (Cousins and Striegler 2005; Nicol et al. 1999; Lider 1960). Commonly used in California, 110R (*V. berlandieri* x *V. rupestris*) and SO4 (*V. berlandieri* x *V. rupestris*) are both susceptible to *M. hapla, X. index, Pratylenchus* spp., and *M. xenoplax* (Nicol et al. 1999). Little is known about the resistance of other commonly planted rootstocks to plant-parasitic nematodes (Nicol et al. 1999), such as Riparia Gloire (*V. riparia*), 101-14 (*V. riparia* x *V. rupestris*), and 3309C (*V. riparia* x *V. rupestris*), which are the most commonly used rootstocks in Oregon. However, rootstocks are still being evaluated and screened with frequent new releases.

Many nematode-resistant rootstocks exist for a large variety of agricultural crops, but rootstocks differ in their susceptibility to nematodes; for example, *Meloidogyne* spp. resistant rootstocks may be susceptible to *Pratylenchus* spp. (Esnard and Zuckerman 1998). Previous rootstock breeding programs have focused on breeding rootstocks that are generally resistant to a single nematode. Rootstocks labeled as nematode resistant are not resistant to all nematodes or even all species within a genus (Stirling and Cirami 1984). For instance, Freedom and Harmony are resistant to *M. incognita* and *M. javanica* but are susceptible to *M. chitwoodi* (Ferris et al. 2012). Multiple resistances to common plant-parasitic nematodes are not typically available in any one rootstock (Ferris et al. 2012; Nicol et al.1999; Esnard and Zuckerman 1998), but there are rootstocks that offer some resistance to a limited range of nematodes. Recent breeding programs have focused on creating rootstocks that possess resistance to more than one species, such as USDA 10-17A, USDA 10-23B, USDA 6-19B, RS-3 and RS-9, which have been recently released to the grape industry and are resistant to *M. incognita, M. javanica, X. index, M. xenoplax,* and *P. vulnus* (Ferris et al. 2012).

Using rootstocks can be beneficial when replanting a new vineyard, especially because plant-parasitic nematodes have been shown to cause poor establishment and

growth of grapevine replants (Stirling and Cirami 1984). Raski (1954) discovered that nematodes typically infect new grapevines in the first year of growth, and vines fail to establish a healthy and productive root system as a result; resistant rootstocks are an excellent management tool to prevent this. However, rootstocks can cause other pathogens and nematodes to increase and may select for virulent populations. Ferris et al. (2012) reported that strong resistance to *Meloidogyne* spp. and *Xiphinema* spp. in rootstocks may increase pressure from other nematodes on grapevines. There was an emergence of new virulent pathotypes of *M. incognita* and *M. arenaria* from the wide usage of Harmony rootstock (Ferris et al. 2012; Esmenjaud and Bouquet 2009; Anwar and McKenry 2002; McKenry 1992; Cain et al. 1984). Freedom rootstock has also selected for virulent pathotypes of *Meloidogyne* spp. (Ferris et al. 2012). Cain et al. (1984) similarly reported that rootstocks with only partial resistance selected for new damaging pathotypes in nematodes.

Even though there are many benefits to using rootstocks in vineyards, several grape growing regions do not utilize rootstocks. For example, Australian grape growers generally use own-rooted *V. vinifera* vines (Rahman and Somers 2005). Washington is another location that prefers to only use own-rooted *V. vinifera* vines (Harbertson and Keller 2012; Keller et al. 2012), and in Washington, grape growers are unlikely to plant rootstocks in the near future due to cold-tolerance and frost concerns (Keller et al. 2012). Washington's climate of very cold winter temperatures can cause scion dieback and lethal cold injury to trunks; grafted vines cannot be retrained from suckers after this type of injury but it is possible with own-rooted *V. vinifera* vines. The survey of plant-parasitic nematodes in semi-arid vineyards in Washington demonstrated that plant-parasitic nematodes are prevalent and can occur at damaging levels (Zasada et al. 2012). In the future, due to the limitation and lack of nematode management options, growers may need to turn to rootstocks to manage nematodes. Limited knowledge exists about rootstock susceptibility and tolerance to *M. hapla*, the major plant-parasitic nematode threat in Washington.

1.3.4 Plant resistance

Nematode resistance has been variably defined over the past century, but it was recently defined as the ability of a plant to inhibit the reproduction of nematodes. Conversely, plant tolerance to nematodes is defined as the ability of a plant to grow and produce satisfactory yields in the presence of nematodes, even under heavy infestations (Starr et al. 2002; Nicol et al. 1999; Cook and Evans 1987; Stirling and Cirami 1984; Robinson 1969; Wallace 1963). Plant resistance is a more desirable attribute because tolerant plants can still have reduced yields and plant tolerance may not reduce nematode reproduction (Nicol et al.1999). Resistance to plant-parasitic nematodes is a widely used approach to obtain sufficient yields in the presence of high densities of nematodes. Used in combination with other cultural control methods, it is an effective, economical management tool for crop protection and improving yield in nematode infected fields, even when nematode population densities exceed the damage threshold (Starr et al. 2002; Wilmer et al. 2002; Barker and Koenning 1998). Typically, resistant varieties are the same species of the crop and therefore production progresses normally without extra expenses to growers (Wilmer et al. 2002).

Resistance is an important means for crop protection and obtaining normal yields; however, a common concern is that the intensive use of resistant cultivars, similar to resistant rootstocks, can select for more aggressive pathotypes (Wilmer et al. 2002). Development of resistant crops is usually accomplished through selecting plants that have the lowest rates of nematode reproduction. Unfortunately for most agronomic crops, varieties that are resistant to plant-parasitic nematodes, including *V. vinifera*, are unavailable (Wilmer et al. 2002; Young 1998). Research needs to be expanded in this field to potentially find or develop resistant crops.

1.4 Justification and objectives

While plant-parasitic nematodes are commonly found and are widespread in Washington's semi-arid vineyards, very little is known about the pathogenicity and

biology of these nematodes in this production system. Most of the research focusing on these topics occurred in other regions, including Oregon (Pinkterton et al. 2004; Pinkterton et al. 1999), California (McKenry 1992; Anwar and Gundy 1989; Ferris and McKenry 1975), Michigan (Ramsdell et al. 1996; Bird and Ramsdell 1985), and Australia (Quader et al. 2001; Nicol et al. 1999; Stirling and Cirami 1984), all of which have different climates compared to Washington. With very little research focusing on plant-parasitic nematodes in Washington, growers are at a disadvantage in knowing how to best manage plant-parasitic nematodes in their vineyards. This scarcity in information is detrimental to the Washington grape industry. Research needs to be undertaken to provide new knowledge and help Washington grape growers develop better informed plant-parasitic nematode management decisions.

The first objective of this research was to determine the spatial distribution of plant-parasitic nematodes in eastern Washington *V. vinifera* vineyards. With the changing landscape of nematode management, knowledge of how plant-parasitic nematodes are distributed in Washington vineyards will help growers target these production-limiting pests. While the spatial distribution of plant-parasitic nematodes within vineyards has been researched in other parts of the world, this has not yet been investigated in Washington semi-arid vineyards. Ferris and McKenry (1975) examined the spatial distribution of *X. americanum* and four *Meloidogyne* spp. in a vineyard in Selma, California, and discovered *Meloidogyne* spp. population densities were highest in the upper 60 cm of soil in the vine row, but their population levels varied in the upper 15 cm due to extreme environmental changes such as water and temperature fluctuations. Nematode population densities declined with depth, but *Meloidogyne* spp. were still detected to a depth of 120 cm. In the same study, it was also discovered that *Meloidogyne* spp. followed root distribution, which were highest in the vine row. *Xiphinema americanum* were mainly found in the upper 45 cm of undisturbed soil, with highest population densities in the top 15 cm of soil. The distribution of this nematode indicated that other factors, besides root distribution, dictated where this

nematode exists. Ponchilla (1972) showed that *X. americanum* survive best in soils with large pore spaces, indicating these nematodes may have an oxygen requirement. Quader et al. (2001) similarly investigated the distribution of *Meloidogyne* spp. in South Australian vineyards. They discovered that *Meloidogyne* spp. had the highest population densities in the vine rows indicating that the nematodes aggregate in the root zone. Similar to the study in California, the authors discovered nematodes to be located where the majority of roots were distributed. The results of these studies will provide Washington grape growers with the knowledge of where plant-parasitic nematodes are located in their vineyards enabling them to target specific areas within the vineyard for nematode management.

The second objective of this research was to determine the host status of predominately grown *V. vinifera* varieties and clones in Washington to *M. hapla*. In addition, the host status of several rootstocks to *M. hapla* was investigated. While the effect *Meloidogyne* spp. have on grapevines and rootstocks is well researched in other grape producing regions of the world, the effect *M. hapla* has on grapevines in the Pacific Northwest remains unknown. Also, little is known about own-rooted grape variety and clone susceptibility to *M. hapla.* This is of significant importance for the Washington industry where vineyards are planted with own-rooted vines and is a huge oversight considering Zasada et al. (2012) discovered *M. hapla* to be present in 60% of 157 vineyards surveyed in Washington and in 27% of these vineyards population densities of this nematode were above the theoretical threshold of 100 *M. hapla*/250 g soil in Washington (Santo, unpublished data). This research will provide Washington grape growers with the knowledge to select appropriate planting material to minimize the impact of *M. hapla* on vine productivity.

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Chapter 2: The spatial distribution of plant-parasitic nematodes in semi-arid *Vitis vinifera* vineyards in Washington

2.1 Introduction

Common pests of global economic concern in wine grape (*Vitis vinifera*) vineyards are plant-parasitic nematodes. In grapevines, nematode feeding can cause the vine to prematurely decline (Anwar and Gundy 1989; Stirling and Cirami 1984; Lider 1960), have reduced vigor (Téliz 2007; Nicol et al. 1999; Ferris 1975), and have an increased susceptibility and severity to biotic and abiotic stresses such as pests, diseases, viruses, and drought (Esmenjaud and Bouquet 2009; Téliz 2007; Ramsdell 1996; Brown 1993; McKenry 1992). Symptoms in grapes also include reduced root and shoot growth (Nicol et al. 1999; Walker 1997; Anwar and Gundy 1989), reduced water and nutrient uptake (Nicol et al. 1999), and reduced yield (Esmenjaud and Bouquet 2009; McKenry et al. 2006; Nicol et al. 1999; Nicol and Heeswijck 1997; McKenry 1992; Lider 1960). Yield losses can range from 7 to 60% but varies depending on location, grape variety, species of plant-parasitic nematode present, and the population level of nematodes present in a vineyard (Téliz 2007; Nicol and Heeswijck 1997).

Little is known about plant-parasitic nematodes in Washington vineyards even though Washington is the second largest wine grape producing region in the United States. The Washington wine industry has an economic value of $236 million comprising 17,401 ha of vineyards cultivating more than thirty different varieties (USDA 2013). Washington's vineyards are prevalently grown on the eastern side of the state, which features thirteen American Viticulture Areas. Eastern Washington receives approximately 16 hours of sunlight in the summer and has an annual rainfall of 20.3 cm. Due to limited rainfall, vineyards of eastern Washington are semi-arid and rely on drip irrigation for productivity. The majority of the vineyards in Washington are grown strictly as own-rooted *V. vinifera* vines due to potentially damaging winter temperatures (Harbertson and Keller 2012; Keller et al. 2012).

Zasada et al. (2012) conducted surveys in Washington to determine the plant-parasitic nematodes associated with semi-arid *V. vinifera* vineyards. The most commonly encountered plant-parasitic nematodes were *Meloidogyne hapla*, *Paratylenchus* spp., and *Xiphinema* sp. detected in 60%, 50%, and 59% of sampled vineyards, respectively. Other plant-parasitic nematodes found were *Pratylenchus* spp. detected in 45% of sampled vineyards and *Mesocriconema xenoplax* found in 14% of sampled vineyards; *Tylenchorynchus* spp. and *Trichodorus* spp. were found in only a few vineyards at very low population densities. Jensen (1961) reported that *Meloidogyne* and *Pratylenchus* spp. are well recognized for injuring high-value irrigated crops in the Pacific Northwest.

Meloidogyne hapla is a sedentary endoparasite, and for most of its life remains stationary inside the roots of a host plant. This nematode can cause significantly reduced root systems (Brown et al. 1993), limit the plant's ability to take up water and nutrients (Ramsdell et al. 1996), and reduce yield (Esnard and Zuckerman 1998). *Pratylenchus* spp. are migratory endoparasites that enter host roots and tunnel through the cells to the cortex where they feed on the cytoplasm of the cortical cells**.** *Pratylenchus* spp. cause necrotic lesions on the roots reducing water and nutrient uptake and can also make the root more susceptible to secondary infections (Subbotin et al. 2008; De Waele and Elsen 2002; Walker 1984; Corbett 1973). *Xiphinema* spp. are migratory ectoparasites that move freely in soil and feed on the exterior surfaces of host roots. This nematode can induce malformation and necrosis of root tips, which can stop root growth and reduce yield (Brown et al. 1993; Anwar and Gundy 1989). *Xiphinema* spp. can also vector viruses; however, no nematode transmitted viruses have been found in Washington vineyards associated with its nematode vector. *Mesocriconema xenoplax* (= *Criconemella xenoplax*) is another migratory ectoparasite that remains motile in the soil. *Mesocriconema xenoplax* can significantly reduce pruning weights, root growth, yield, and the colonization of roots by arbuscular mycorrhizal fungi (AMF) (Schreiner et al. 2012; Zasada et al. 2012; Pinkerton et al.

2004). *Paratylenchus* spp. are also migratory ectoparasites that can stay in the soil for long periods of time but have a minimal impact on grapes (Pinkerton et al. 1999).

While it has been demonstrated that plant-parasitic nematodes are abundant and widespread in Washington grapes (Zasada et al. 2012), very little is known about the pathogenicity and biology of these nematodes in this production system. With the changing landscape of nematode management, knowledge of how plant-parasitic nematodes are distributed in Washington vineyards will help growers target these production-limiting pests. While the spatial distribution of plant-parasitic nematodes within vineyards has been researched in other parts of the world, this has not yet been investigated in Washington. Ferris and McKenry (1974) examined the spatial distribution of *X. americanum* and four *Meloidogyne* spp. in a vineyard in Selma, California, and discovered that *Meloidogyne* spp. population levels were highest in the upper 60 cm of the soil profile under the vine row and that the population densities of this nematode declined with depth but were still detected 120 cm deep. In this same study, they also discovered that *Meloidogyne* spp. followed root distribution within the vine row and that *X. americanum* were mainly found in the upper 45 cm of undisturbed soil, with highest population densities found in the top 15 cm of soil. Quader et al. (2001) similarly investigated the distribution of *Meloidogyne* spp. in South Australian vineyards. They found *Meloidogyne* spp. had the highest population densities in the vine rows indicating that these nematodes aggregate in the root zone. Similar to the study in California, the authors also reported that *Meloidogyne* spp. were located where the majority of roots were distributed.

With very little research focusing on plant-parasitic nematodes in Washington, growers are at a disadvantage in how to combat plant-parasitic nematodes in their vineyards. This scarcity in information is detrimental to the Washington grape industry. Research needs to be undertaken to fill this void in knowledge and guide Washington grape growers on plant-parasitic nematode management decisions. The objective of this study was to determine the spatial distribution of plant-parasitic

nematodes in eastern Washington *V. vinifera* vineyards. The results of this study will provide Washington grape growers with the knowledge of where plant-parasitic nematodes are located in their vineyards, enabling them to target specific areas within the vineyard for nematode management.

2.2 Materials and methods

2.2.1 Site description

Two vineyards in eastern Washington were sampled. The first vineyard was located in Paterson, WA, geographic coordinates: +45° 57' 26.69", -119° 36' 19.16". The vineyard was a 34-year-old *Vitis vinifera* cv. Chardonnay growing on Hezel loamy fine sand soil with a slope of 0 to 30 degrees. The mean annual precipitation in this area is 15 to 25 cm and the mean annual air temperature is 11 to 12˚C. The vineyard has a frost-free period of 150 to 200 days (USDA 2012). The second location was a 38-year-old *V. vinifera* cv. Riesling vineyard in Mattawa, WA with geographic coordinates +46° 36' 18.40", -119° 48' 18.49". The vineyard is on Warden silt loam soil with 0 to 5 percent slope. At this site, the mean annual precipitation is 15 to 23 cm and the mean annual air temperature is 9 to 11˚C. The area has a frost-free period of 135 to 200 days (USDA 2012). Alley way management in both vineyards consisted of grass cover crops, such as orchardgrass (*Dactylis glomerata*) and crested wheatgrass (*Agropyron cristatum*), and both vineyards were deficit-irrigated for vine productivity during the growing season. The Chardonnay vineyard had a consistent vine spacing of 1.8 m, while the vine spacing in the Riesling vineyard was more variable and was between 1.8 to 2.1 m; row spacing for both vineyards was 2.7 m. Row orientation was north-south. Soil sampling for both experiments was conducted mid-September, during the berry ripening phase of the grapevines.

2.2.2 Horizontal distribution experiment

At each vineyard, five 1.2 x 1.8 m plots were established (Figure 2.1). At the Chardonnay vineyard, this area spanned between two vines and included two drip irrigation emitters while at the Riesling vineyard, the vine spacing was more variable and the area included at least one vine and two to three emitters. A flag was placed every 30 cm within each plot to designate a sampling location; each plot consisted of 35 samples. A soil sample, 5 cm diameter x 45 cm deep, was collected from each location within a plot using a bucket auger (model AMS350.07, ASC Scientific, Carlsbad, CA). Samples were placed in labeled plastic bags and kept cool until processing.

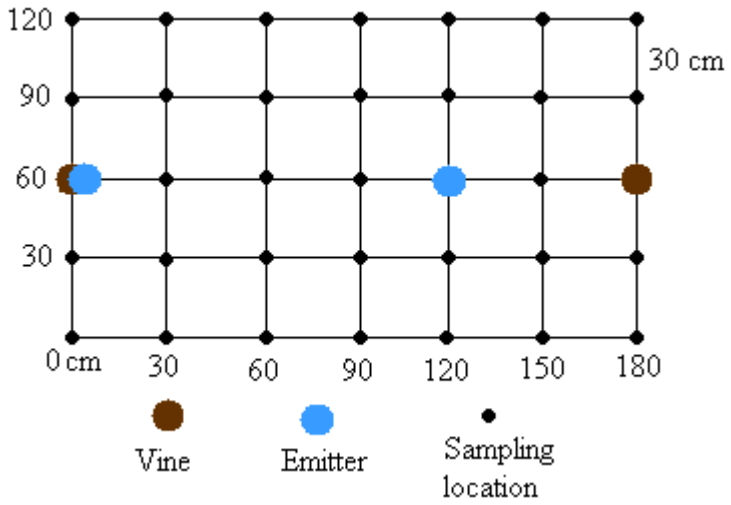

Figure 2.1. Sampling scheme to determine the horizontal distribution of plant-parasitic nematodes in semi-arid *Vitis vinifera* vineyards. Each point represents a sampling location; sampling locations were spaced 30 cm apart.

Initially, each soil sample was passed through a 2.83-µm sieve with roots and debris being retained on the sieve. Roots were picked by hand from the sieve and placed in a 50 ml tube containing water. Roots were further sorted into fine (≤ 2 mm) and coarse (> 2 mm) categories; fine roots were blotted dry, weighed, and stored in AA (acetic acid:alcohol 10%:50% v/v) for up to three months while coarse roots were discarded.

Plant-parasitic nematodes were extracted from a 250 g subsample of soil by the elutriation-sucrose centrifugation process. Using a semiautomatic elutriator, flowing water moves the soil subsample through a series of funnels and sieves to separate large soil particles and debris from the nematodes and finer soil particles. A representative aliquant (1/5) of the nematode and fine soil particle solution was separated into three tubes which fall onto a 38-µm sieve where the nematodes were retained and collected (Byrd et al. 1976) for sucrose floatation and centrifugation. The sucrose floatation and centrifugation procedure is where nematodes are centrifuged in a sucrose solution (454 g of sugar mixed in one liter of warm water), causing the nematodes to be suspended in solution and the soil particles to fall to the bottom of the container. The nematodes in the solution were poured over a 38-µm sieve to be collected in a relatively soil-free solution (Jenkins 1964). Nematodes were then identified based upon morphological characteristics and counted under a stereo-microscope (Leica, Buffalo Grove, IL) at x40 magnification. Nematode extraction and quantification occurred at the Washington State University-Irrigated Agriculture Research and Extension Center, Prosser, WA. In addition, speciation of *Pratylenchus* at both sites was determined using β,1-4 endoglucanse species specific primers at the USDA-ARS Horticultural Crops Research Unit in Corvallis, OR (Peetz, unpublished data). To determine soil moisture content, the oven-drying gravimetric method was used (Schmugge et al. 1980). Each soil sample was dried in an oven at 105˚C for one week and soil moisture was calculated as:

$$\theta = \frac{Wet\ soil\ (g) - \ Dry\ soil\ (g)}{Dry\ soil\ (g)} \times 100$$

2.2.3 Vertical distribution experiment

Similar to the sampling scheme for horizontal distribution, five plots within each vineyard were established for sampling. The sampling plots were evenly distributed across the vineyard block to ensure a full representation of the vineyard

was obtained. The location of plots within a vineyard were based upon an even emitter spacing between vines, a uniform wetting zone around the vine, an absence of large weeds around the trunk, and a level terrain.

From each plot, six soil samples were collected to a depth of up to 90 cm. At some locations, particularly at the Riesling vineyard, a hardpan at approximately 61 cm was present which prohibited the full 90 cm of soil to be collected. Soil was collected to the lowest possible depth at each location. The locations sampled were directly underneath an irrigation emitter, 30 cm north of the emitter, 30 cm south of the emitter, 30 cm west of the emitter, and 30 cm east of the emitter (Figure 2.2).

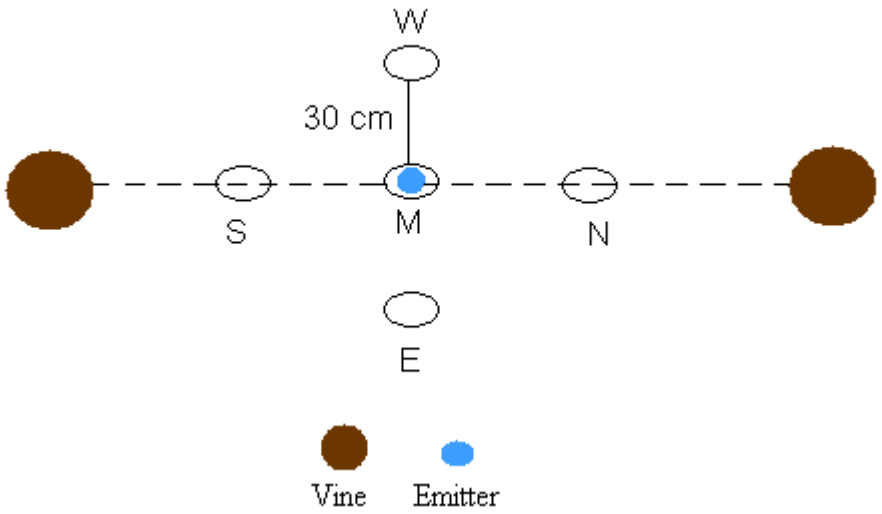

Figure 2.2. Sampling scheme to determine the vertical distribution of plant-parasitic nematodes in semi-arid *Vitis vinifera* vineyards. The middle (M), north (N), south (S), east (E), and west (W) point was sampled; sampling locations were spaced 30 cm apart.

The soil samples were collected using a Bosch SDS-Max Demolition Hammer, (model 11316EVS, Farmington Hills, MI). The hammer was attached to a 5 cm diameter x 1.2 m long stainless steel soil collection tube with a Quick Relief soil tube bit (Giddings Machine, Windsor, CO). The soil core collection tube was lined with a 4.5 cm diameter x 1.2 m long removable polyethylene terephthalate (PTEG) plastic

liner. After the soil collection tube was hammered into the ground to 90 cm, or as deep as possible, a standard hi-lift jack was used to remove the collection tube from the ground. The PTEG plastic liner was then removed from the tube and capped with two vinyl end caps, labeled, and stored in a cooler.

In the laboratory, each soil containing plastic liner tube was sawed into 15 cm increments of 0-15, 16-30, 31-45, 46-60, 61-75, and 76-90 cm using a 30 cm saw (Portland Saw, Portland, OR). Once each segment was separated, the soil was emptied into a bucket, thoroughly mixed, and placed in a labeled plastic bag. A small portion of soil from each sample was placed in a separate bag for determination of soil moisture content as described above. Nematodes were extracted from a 250 g subsample, quantified and identified as described above.

2.2.4 Statistical analysis

Data from the two vineyard sites were analyzed separately ($p > 0.05$). Prior to analysis, plant-parasitic nematode population density data from the horizontal and vertical distribution studies were $\log_{10}(x + 1)$ transformed to meet the normality and equal variance assumptions of the model (JMP 9.0.0, SAS Institute, Cary, NC). Data from both studies were analyzed using a linear mixed model analysis of variance (ANOVA) that allowed for both fixed and random effects; replicate plot was regarded as a random effect. Fixed variables in the horizontal distribution study were distance from emitter, distance from vine, and the interaction of distance from emitter * distance from vine. These fixed variables were analyzed against each plant-parasitic nematode present at the vineyard, soil moisture, and fine root biomass. For the vertical distribution study, the fixed variables were depth, sampling location, and the depth * sampling location interaction. These variables were analyzed against each plant-parasitic nematode present at the vineyard and soil moisture. Means were separated using Tukey's least significant differences ($p < 0.05$) (JMP 9.0.0). In the horizontal distribution study, the relationship between soil moisture and fine root biomass to each

plant-parasitic nematode, as well as the relationship of soil moisture to fine root biomass, was determined with simple linear regression analysis (JMP 9.0.0). In addition, contour plots of the horizontal distribution of plant-parasitic nematodes, soil moisture, and fine root biomass were created for each vineyard (SigmaPlot 12.0, San Jose, CA).

2.3 Results

In both the horizontal and vertical distribution studies, the plant-parasitic nematodes present in the Chardonnay vineyard were *Meloidogyne hapla*, *Mesocriconema xenoplax*, *Pratylenchus neglectus*, *Xiphinema americanum*, and *Paratylenchus* sp. In the Riesling vineyard, *M. hapla*, *X. americanum*, *Paratylenchus* sp., and a mixed population of *Pratylenchus* spp. (*P. neglectus* and *P. thornei*) were found. *Tylenchorhynchus* spp. and *Helicotylenchus* spp. were also found in both vineyards but at very low densities (< 5 nematodes/250 g soil) and were not include in the analyses.

2.3.1 Horizontal distribution experiment

In the Chardonnay vineyard, the average population densities of plant-parasitic nematodes were 191 (± 22) *M. hapla*/250 g soil, 110 (± 14) *Paratylenchus* sp./250 g soil, 33 (± 4) *P. neglectus*/250 g soil, 295 (± 45) *M. xenoplax*/250 g soil, and 50 (± 7) *X. americanum*/250 g soil. *Meloidogyne hapla* was the only nematode for which both distance from vine and distance from emitter, as well as the interaction between these main effects were significant (Table 2.1); population densities of this nematode were concentrated below the emitters within the vine row (Figure 2.3A). *Mesocriconema xenoplax* had a similar horizontal distribution to that of *M. hapla* (Figure 2.3B); however, population densities of this nematode were only influenced by distance from emitter (Table 2.1). Population densities of *P. neglectus* were influenced by both distance from vine and the interaction between the main effects (Table 2.1), with greater densities of this nematode found near the alley ways (Figure 2.3C). The

horizontal distributions of *Paratylenchus* sp. and *X. americanum* were not explained by any of the variables in the model (Table 2.1). *Paratylenchus* sp. was randomly distributed within the sampling area (Figure 2.3D), while *X. americanum* was aggregated around one of the vines (Figure 2.E). Soil moisture was concentrated in a 0.9 m band down the vine row with the wettest locations under the emitters (Figure 2.3F); however, there was no significant influence of distance from vine or emitter on soil moisture. The horizontal distribution of fine roots was influenced by distance from emitters but not by distance from vines (Table 2.1); roots were concentrated under both emitters (Figure 2.3G).

Table 2.1. P-values from a linear mixed model analysis of variance of the effect of distance from vine, distance from emitter, and the interaction of these main effects on plant-parasitic nematode population densities, soil moisture, and fine root biomass in a Chardonnay vineyard, Paterson, Washington.

	Meloidogyne hapla	***Mesocriconema xenoplax***	***Pratylenchus neglectus***	***Paratylenchus* sp.**	***Xiphinema americanum***	**Soil Moisture**	**Fine Roots**
Distance from Vine	*0.015*[a]	0.131	*0.033*	0.174	0.194	0.581	0.141
Distance from Emitter	*< 0.001*	*< 0.001*	0.458	0.229	0.354	0.834	*< 0.001*
Vine*Emitter	*0.036*	0.072	*0.031*	0.852	0.606	0.594	0.782

[a]Plant-parasitic nematode data were $\log_{10}(x + 1)$ transformed prior to analysis ($n = 175$).

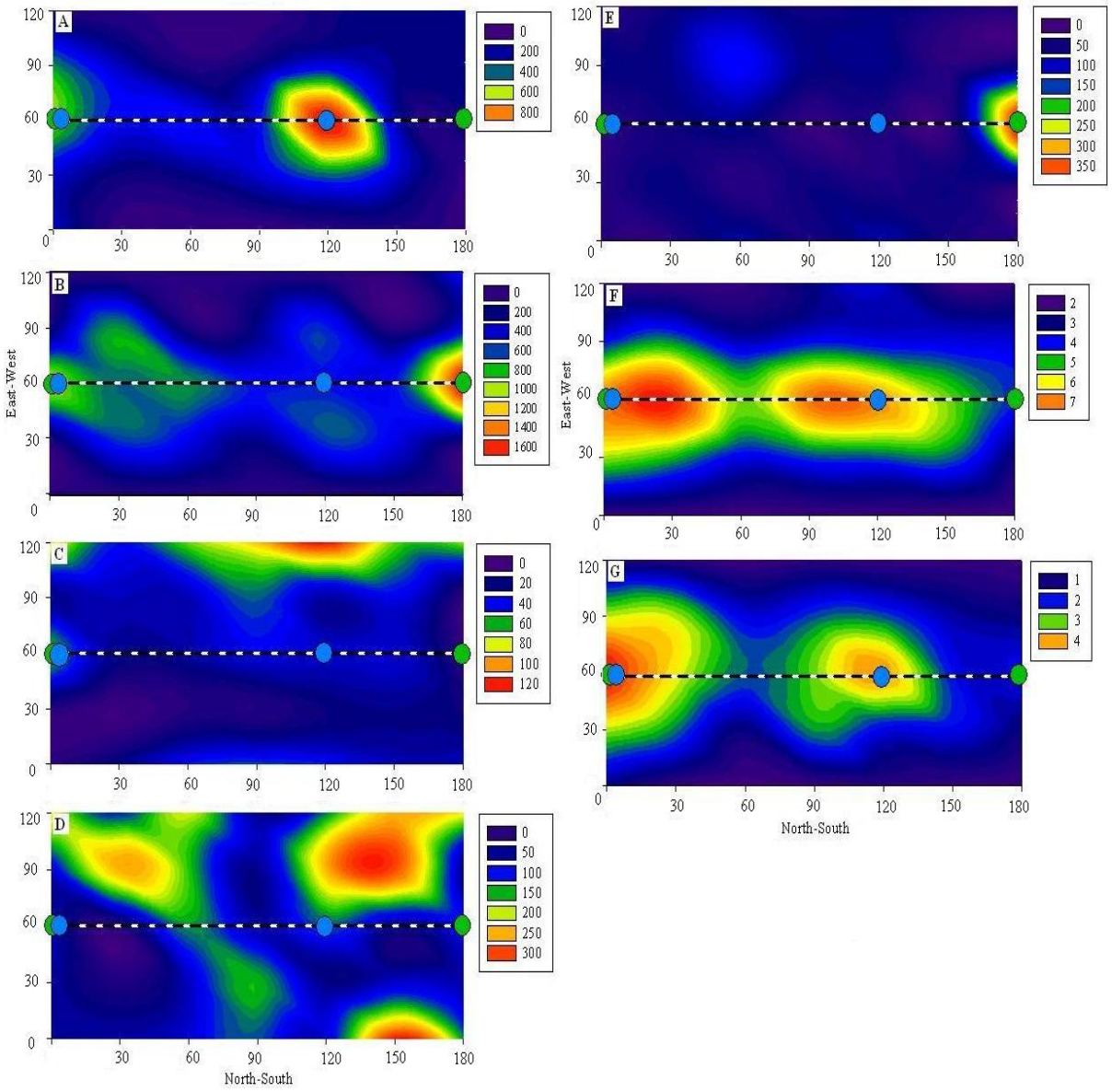

Figure 2.3. Horizontal distribution of *Meloidogyne hapla* (A), *Mesocriconema xenoplax* (B), *Pratylenchus neglectus* (C), *Paratylenchus* sp. (D), *Xiphinema americanum* (E), soil moisture (F), and fine root biomass (G) in a Chardonnay vineyard, Paterson, Washington. Contour plots were generated from the average of five observations. Soil moisture (g/cm^3) was determined gravimetrically. Fine root (< 2 mm) biomass is expressed as grams (g) wet roots. Plant-parasitic nematode population densities are nematodes/250 dry soil. Green circles represent vines and blue circles represent emitters.

In the Riesling vineyard, the average population densities of plant-parasitic nematodes were 1,011 (± 95) *M. hapla*/250 g of soil, 207 (± 34) *Paratylenchus* sp./250 g of soil, 135 (± 19) *Pratylenchus* spp./250 g of soil, and 9 (± 1) *X. americanum*/250 g of soil. The horizontal distribution of *M. hapla* was influenced by distance from emitter (Table 2.2); this nematode was concentrated in a 0.9 m band around the emitters and along the vine row (Figure 2.4A). *Pratylenchus* spp. was the only nematode for which both distance from vine and distance from emitter, as well as the interaction between these main effects was significant (Table 2.2). Similar to the results from the Chardonnay vineyard, population densities of *Pratylenchus* spp. were concentrated near the alley ways (Figure 2.4B). Also similar to results from the Chardonnay vineyard, the horizontal distributions of *Paratylenchus* sp. and *X. americanum* were not explained by any of the variables in the model (Table 2.2). *Paratylenchus* sp. was only located in a small clump in the eastern alley way between the vine and emitter (Figure 2.4C). *Xiphinema americanum* was randomly distributed in this vineyard with highest population densities away from the vine row (Figure 2.4D). Both soil moisture and fine root biomass were only influenced by distance from emitter. Soil moisture was concentrated in a band along the vine row with the wettest areas below the emitters (Figure 2.4E). The majority of the sampling locations in the Riesling vineyard had soil moistures above 7%; this was considerably wetter than the Chardonnay vineyard where soil moisture was greater than 7% in only two sampling locations. The average soil moisture content was 3.98% (± 0.20%) and 5.85% (± 0.17%) at the Chardonnay and Riesling vineyards, respectively; soil moisture between the two vineyards was significantly different ($p \leq 0.001$). Roots were concentrated around the drip line, with the highest density of fine roots right next to the vine and emitter combination (Figure 2.4F). Fine root biomass did not differ across the vineyards ($p \geq 0.05$). The average weight of fine roots at the Chardonnay vineyard was 1.60 g (± 0.15 g) and at the Riesling vineyard was 2.20 g (± 0.15 g).

Table 2.2. P-values from a linear mixed model analysis of variance of the effect of distance from vine, distance from emitter, and the interaction of these main effects on plant-parasitic nematode population densities, soil moisture, and fine root biomass in a Riesling vineyard, Mattawa, Washington.

	Meloidogyne hapla	***Pratylenchus* spp.**	***Paratylenchus* sp.**	***Xiphinema americanum***	**Soil Moisture**	**Fine Roots**
Distance from Vine	0.696[a]	*0.003*	0.568	0.628	0.382	0.412
Distance from Emitter	*< 0.001*	*< 0.001*	0.784	0.776	*< 0.001*	*< 0.001*
Vine*Emitter	0.822	*0.006*	0.375	0.361	0.429	0.618

[a]Plant-parasitic nematode data were $\log_{10}(x + 1)$ transformed prior to analysis ($n = 175$).

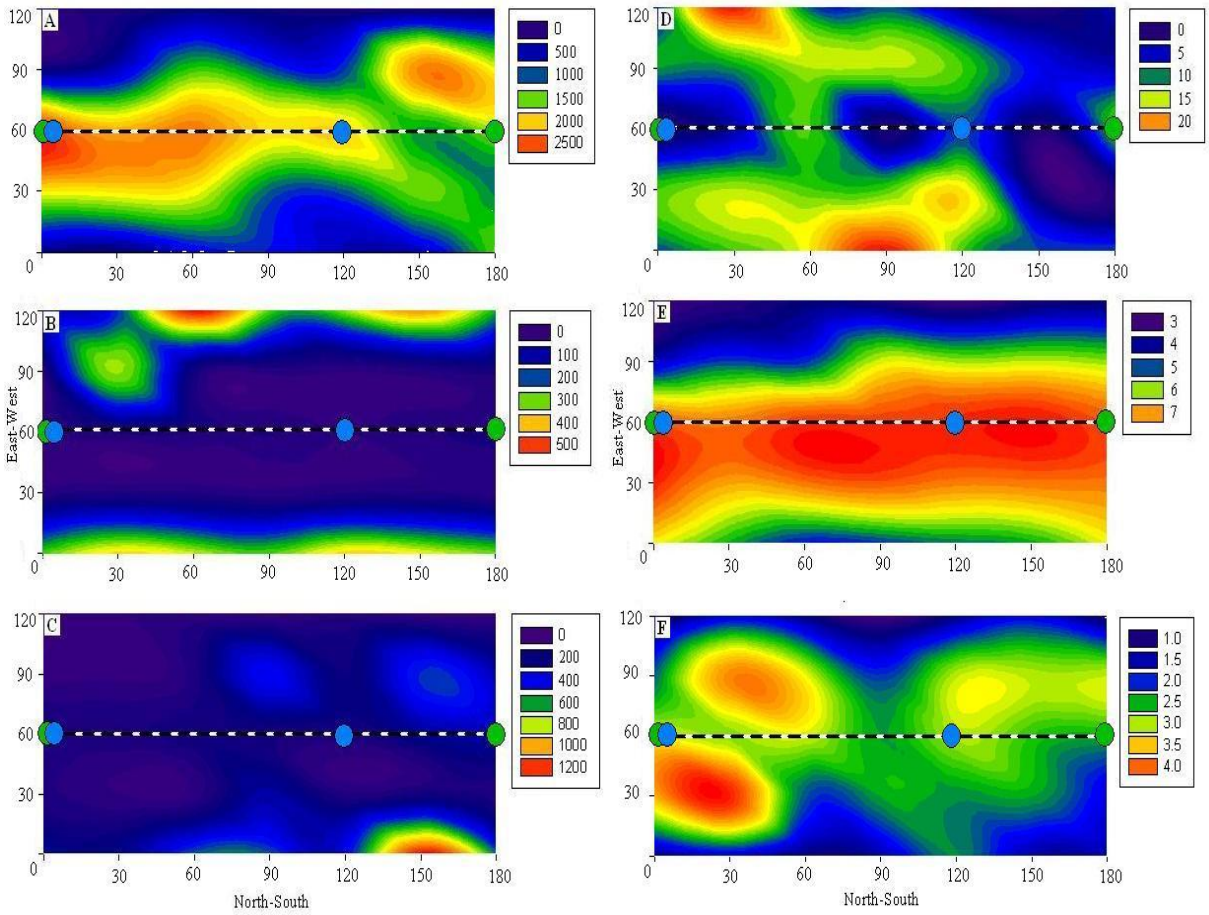

Figure 2.4. Horizontal distribution of *Meloidogyne hapla* (A), *Pratylenchus* spp. (B), *Paratylenchus* sp. (C), *Xiphinema americanum* (D), soil moisture (E), and fine root biomass (F) in a Riesling vineyard, Mattawa, Washington. Contour plots were generated from the average of five observations. Soil moisture (g/cm^3) was determined gravimetrically. Fine root (< 2 mm) biomass is expressed as grams (g) wet roots. Plant-parasitic nematode population densities are nematodes/250 dry soil. Green circles represent vines and blue circles represent emitters.

Meloidogyne hapla, *M. xenoplax,* and *P. neglectus* population densities were related to soil moisture in the Chardonnay vineyard. Population densities of *M. hapla* and *M. xenoplax* were positively correlated with soil moisture while the opposite was found for *P. neglectus* (Figure 2.5). The same trends were observed between these plant-parasitic nematodes and fine root biomass (Figure 2.6). These relationships were weaker for *M. xenoplax* and *P. neglectus* compared to *M. hapla*. Similar to *M. hapla*

and *M. xenoplax*, fine root biomass was positively related to soil moisture (Figure 2.7). Population densities of *Paratylenchus* sp. and *X. americanum* were not related to either soil moisture or fine root biomass.

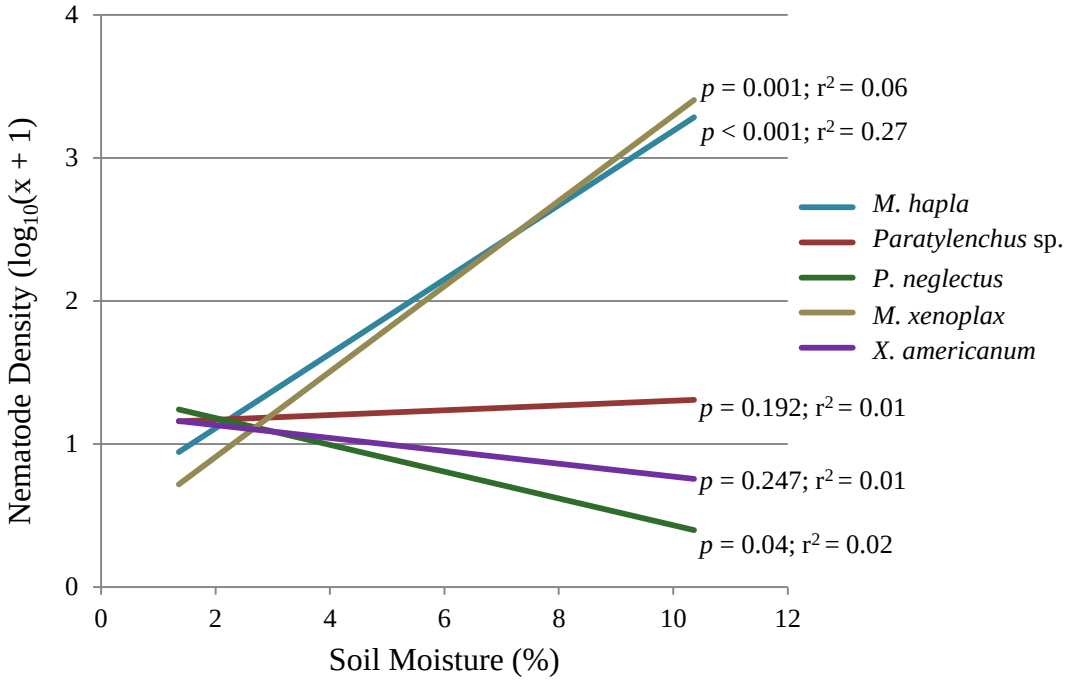

Figure 2.5. Relationships of plant-parasitic nematode population densities and percentage soil moisture in a Chardonnay vineyard, Paterson, Washington. Nematode population data was $\log_{10}$ (x+1) transformed prior to analysis. Lines represent the fitted linear regression analysis (n = 175).

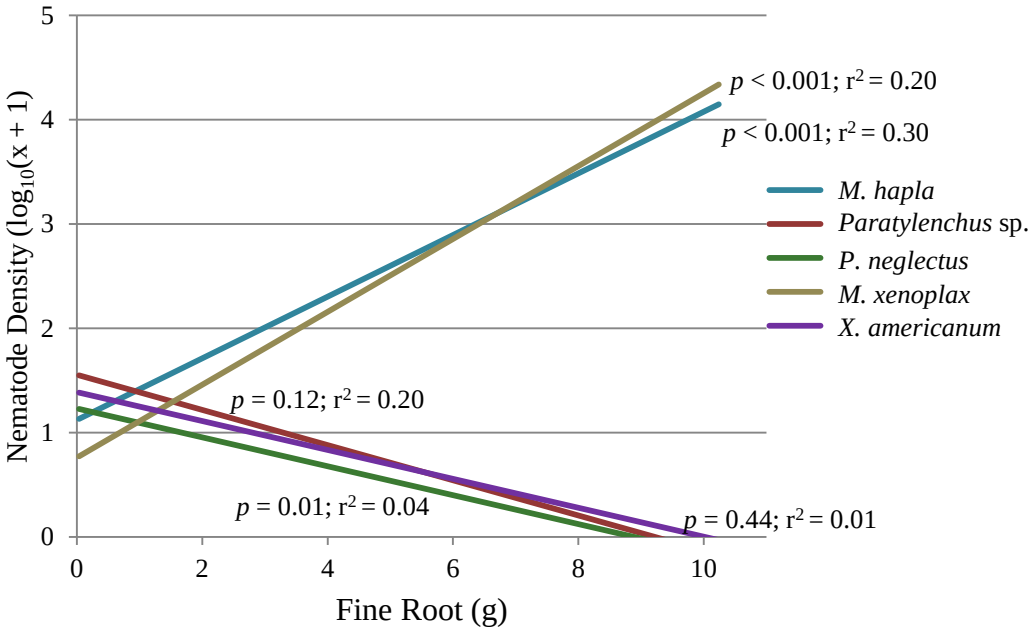

Figure 2.6. Relationships of plant-parasitic nematode population densities and fine root biomass in a Chardonnay vineyard, Paterson, Washington. Nematode population data was $\log_{10}$ (x+1) transformed prior to analysis. Lines represent the fitted linear regression analysis (*n* = 175).

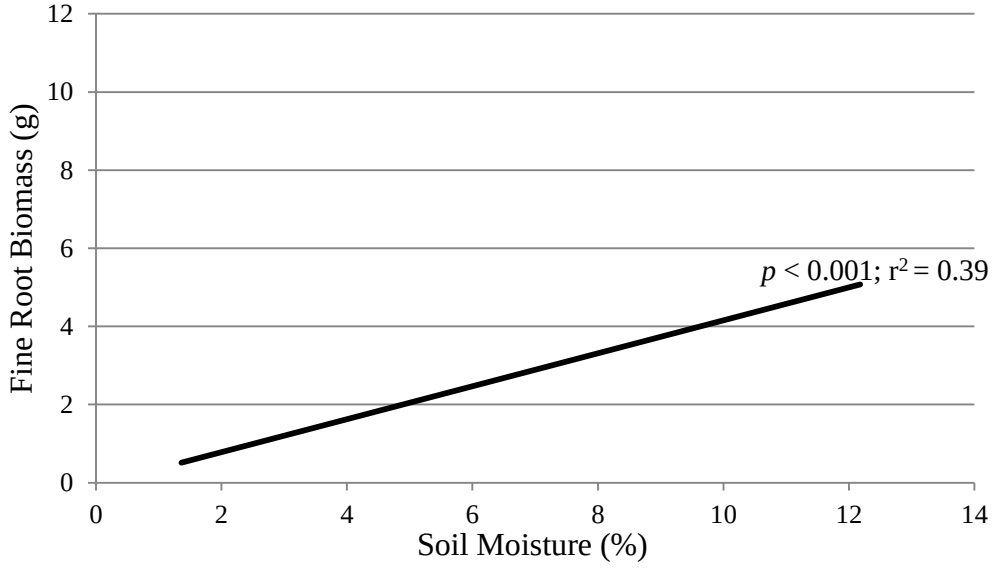

Figure 2.7. Relationships of fine root biomass and percentage soil moisture in a Chardonnay vineyard, Paterson, Washington. The line represents the fitted linear regression analysis (*n* = 175).

In the Riesling vineyard, *M. hapla* and *Pratylenchus* spp. population densities were related to soil moisture (Figure 2.8). Higher soil moisture content was correlated with greater population densities of *M. hapla*; the opposite was true for *Pratylenchus* spp. This relationship was much stronger for *M. hapla* than for *Pratylenchus* spp. The relationship between fine root biomass and plant-parasitic nematodes followed the same trend as soil moisture (Figure 2.9). Fine root biomass was also driven by soil moisture (Figure 2.10). Similar to the Chardonnay vineyard, *Paratylenchus* sp. and *X. americanum* were not related to either soil moisture or distribution of fine roots.

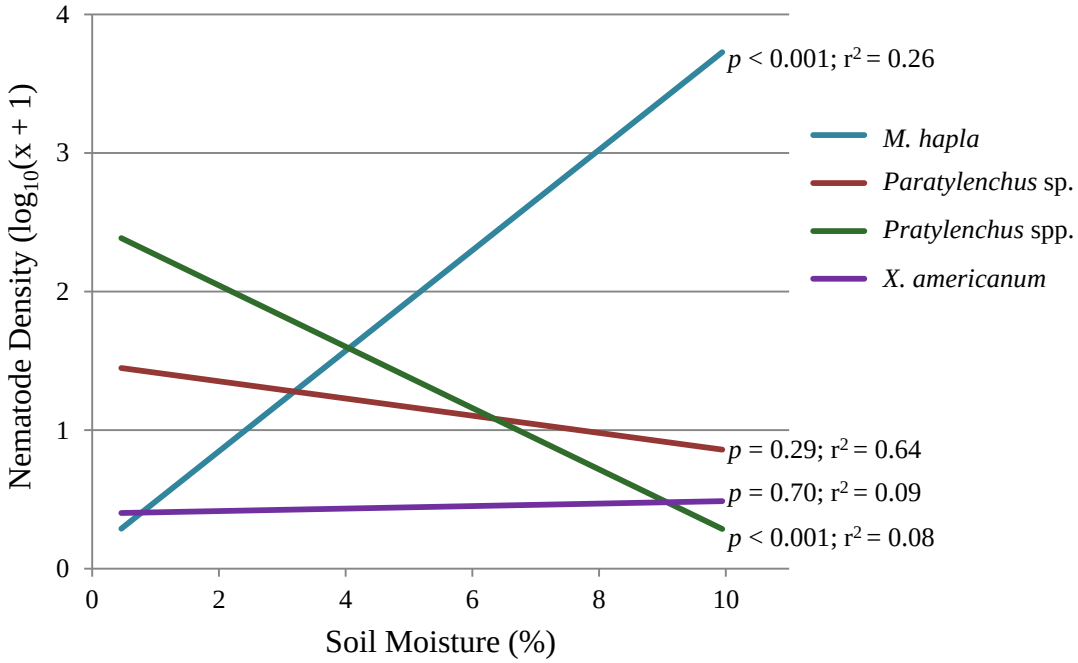

Figure 2.8. Relationships of plant-parasitic nematode population densities and percentage soil moisture in a Riesling vineyard, Mattawa, Washington. Nematode population data was $\log_{10}$ (x+1) transformed prior to analysis. Lines represent the fitted linear regression analysis (n = 175).

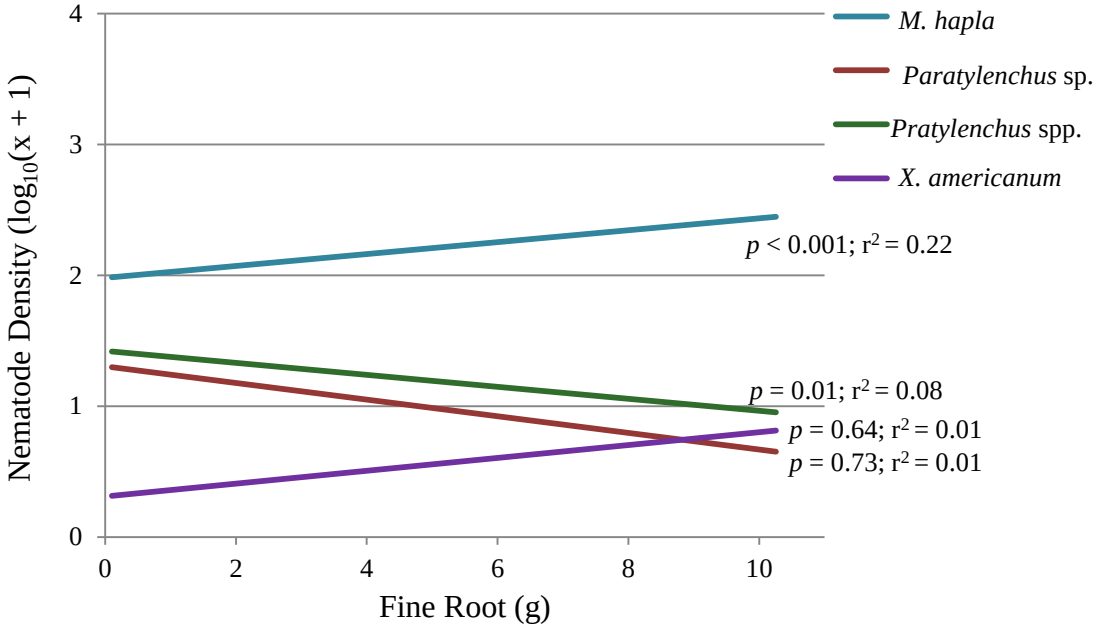

Figure 2.9. Relationships of plant-parasitic nematode population densities and fine root biomass in a Riesling vineyard, Mattawa, Washington. Nematode population data was log_{10} (x+1) transformed prior to analysis. Lines represent the fitted linear regression analysis (*n* = 175).

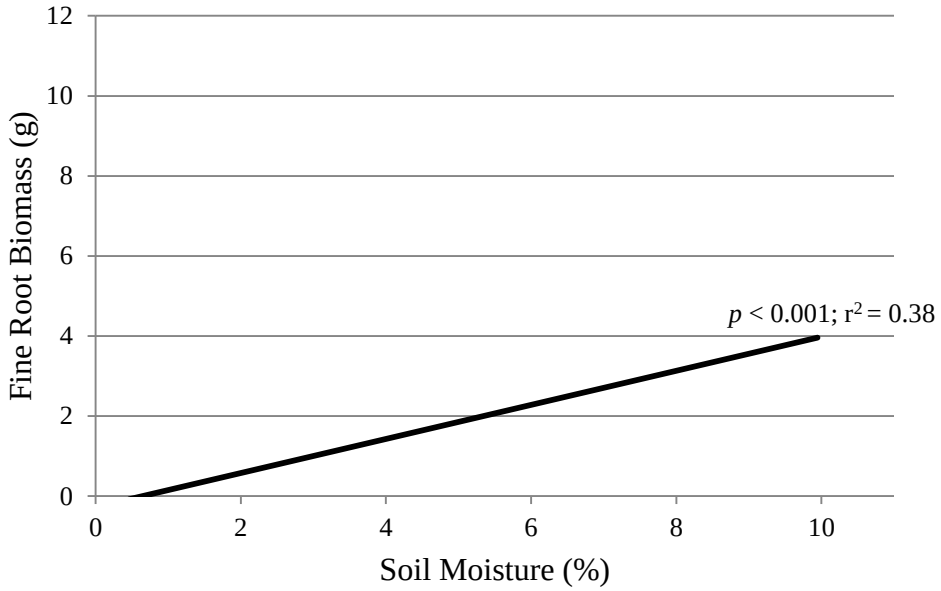

Figure 2.10. Relationships of fine root biomass and percentage soil moisture in a Riesling vineyard, Mattawa, Washington. The line represents the fitted linear regression analysis (*n* = 175).

2.3.2 Vertical distribution experiment

In the Chardonnay vineyard, the average population densities of plant-parasitic nematodes were 106 (± 19) *M. hapla*/250 g of soil, 409 (± 57) *Paratylenchus* sp./250 g of soil, 22 (± 3) *P. neglectus*/250 g of soil, 56 (± 18) *M. xenoplax*/250 g of soil, and 91 (± 10) *X. americanum*/250 g of soil. Depth and the interaction between depth and location were significant for *M. hapla* (Table 2.3). *Meloidogyne hapla* population densities within sampling locations across depths were in general similar except for the north and west locations (Table 2.4). At these sampling locations, there were higher population densities of *M. hapla* at the 0 to 30 cm depths compared to the 61 to 90 cm depths. At several of the locations, there was a tendency for lower *M. hapla* population densities at 0 to15 cm; however, this was not statistically supported. Depth was also significant for *M. xenoplax*, *Paratylenchus* sp., and *P. neglectus*, while location and the interaction between depth and location were not (Table 2.3). Population densities of *P. neglectu*s and *M. xenoplax* both decreased with depth (Figure 2.11). There were significantly fewer *P. neglectus* at greater soil depths ($\geq$ 31 cm) than in the upper 0 to 30 cm of soil. Population densities of *M. xenoplax* were similar across soil depths down to 45 cm, with fewer *M. xenoplax* found deeper in the soil profile (46 to 90 cm) than at shallower depths (0 to 30 cm). In contrast, population densities of *Paratylenchus* sp. increased with soil depth with more nematodes recovered at the 46 to 60 cm soil depth than at 0 to 15 cm. *Xiphinema americanum* was distributed throughout the soil profile, with no significant effect of depth (Table 2.3, Figure 2.11). In addition, the distribution of *X. americanum* did not differ between locations and there was no interaction between depth and location. For soil moisture, there were significant depth and location effects, but no interaction of these effects (Table 2.3). Soil moisture decreased with depth (Table 2.5) with 30% more soil moisture at 0 to 15 cm than at the deeper soil depths. The highest soil moisture content was below the emitter with the lowest moisture content east of the emitter.

Table 2.3. P-values from a linear mixed model analysis of variance of the effect of depth, location in relationship to emitter, and the interaction of these main effects on plant-parasitic nematode population densities and soil moisture in a Chardonnay vineyard, Paterson, Washington.

	Meloidogyne hapla	***Mesocriconema xenoplax***	***Pratylenchus neglectus***	***Paratylenchus* sp.**	***Xiphinema americanum***	**Soil Moisture**
Depth	*< 0.001*[a]	*< 0.001*	*< 0.001*	*< 0.001*	0.054	*< 0.001*
Location	0.738	0.167	0.960	0.109	0.393	*< 0.001*
Location*Depth	*0.014*	0.721	0.113	0.900	0.873	0.157

[a]Plant-parasitic nematode data were $\log_{10}(x + 1)$ transformed prior to analysis (n = 175).

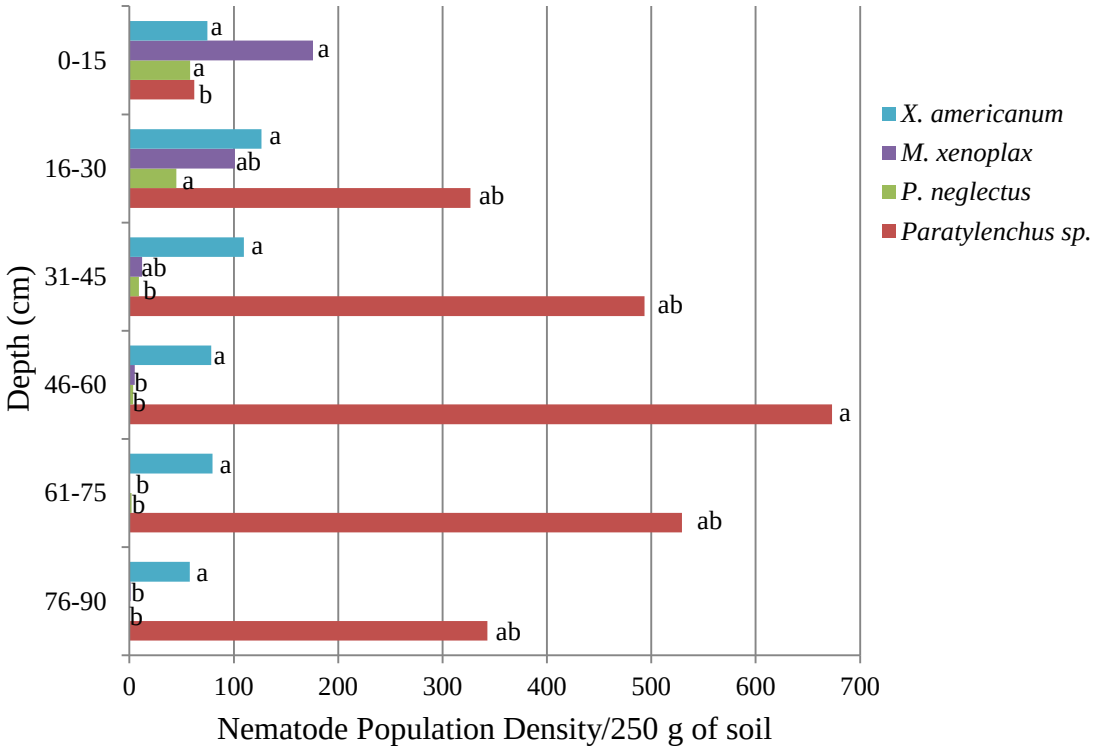

Figure 2.11. Plant-parasitic nematode population densities at different soil depths in a Chardonnay vineyard, Paterson, Washington. Nematode densities are expressed as back-transformed means from logarithmic transformations used to normalize the analysis of variance. Means separation within a nematode species with the same letter are not significantly different ($p < 0.05$). Values are the mean of 25 cores collected either directly under the emitter or within 30 cm of an emitter.

Table 2.4. The interaction of soil depth and location in relation to *Meloidogyne hapla* population densities in a Chardonnay vineyard, Paterson, Washington.

Depth (cm)	**Location**[a]					**Total**
	M	**N**	**S**	**W**	**E**	
0-15	8 bcde[b]	318 ab	39 abcde	332 abcd	137 abcde	834
16-30	106 abcde	396 a	196 ab	310 abcde	278 abc	1286
31-45	88 abcde	136 abcde	50 abcde	262 abcde	46 abcde	582
46-60	111 abcde	13 bcde	32 abcde	11 bcde	3 de	170
61-75	84 abcde	0 e	8 bcde	4 de	10 bcde	106
76-90	47 abcde	0 cde	0 bcde	0 de	7 abcde	54
Total	444	863	325	919	481	

[a]Locations sampled were directly under the emitter (M) and 30 cm North (N), South (S), West (W), and East (E) of the emitter.
[b]Values are the means of 180 observations. Nematode populations are shown as population density/250 g of soil. Back transformed means are presented. Means followed by the same letter are not significantly different according to Tukey's honestly significant difference test ($p < 0.05$).

Table 2.5. Soil moisture content at different depths and locations relative to an emitter in a Chardonnay vineyard, Paterson, Washington.

Soil Moisture Content (g/cm^3)			
Depth (cm)	$\bar{x}$	**Location**	$\bar{x}$
0-15	17.20 a[a]	M	12.58 a
16-30	15.16 a	N	9.23 ab
31-45	9.99 b	S	10.99 ab
46-60	7.98 bc	W	9.90 ab
61-75	6.12 c	E	8.61 b
76-90	5.12 c		

[a]Values are the means of 180 observations. Back transformed means are presented. Means followed by the same letter are not significantly different according to Tukey's honestly significant difference test ($p < 0.05$).

In the Riesling vineyard, the average population densities of plant-parasitic nematodes were 566 (± 100) *M. hapla*/250 g of soil, 26 (± 10) *Paratylenchus* sp./250 g of soil, 9 (± 3) *Pratylenchus* spp./250 g of soil, and 14 (± 3) *X. americanum*/250 g of soil. The vertical distribution of plant-parasitic nematodes and soil moisture in the Riesling vineyard were similar to those observed in the Chardonnay vineyard. Depth

was significant for *M. hapla, Paratylenchus* sp., and *Pratylenchus* spp. but not for *X. americanum* (Table 2.6). Population densities of *M. hapla* decreased with depth, with more nematodes recovered at shallower (0 to 30 cm) than deeper (31 to 90 cm) depths (Figure 2.12). The same trend was observed for *Pratylenchus* spp.; however, similar densities were detected down to 60 cm for this nematode with no *Pratylenchus* spp. found lower in the soil profile (Figure 2.12). *Paratylenchus* sp. decreased with depth until 60 cm as well and no *Paratylenchus* sp. were found at the lower depths in the soil profile. Among the plant-parasitic nematodes, location relative to the emitter was significant only for *M. hapla*. The highest population density of *M. hapla* was observed at the northern location (Table 2.7). The middle, southern, and eastern locations had similar population densities while the western location had a significantly lower population of *M. hapla* compared to the other locations. The distribution of *X. americanum* was not influenced by any variable.

Table 2.6. P-values from a linear mixed model analysis of variance of the effect of depth, location in relationship to emitter, and the interaction of these main effects on plant-parasitic nematode population densities and soil moisture in a Riesling vineyard, Mattawa, Washington.

	Meloidogyne hapla	***Pratylenchus* spp.**	***Paratylenchus* sp.**	***Xiphinema americanum***	**Soil Moisture**
Depth	*< 0.001*[a]	*0.001*	*0.014*	0.073	*< 0.001*
Location	*< 0.001*	0.153	0.910	0.675	*< 0.001*
Location*Depth	0.110	0.862	0.940	0.567	*< 0.001*

[a]Plant-parasitic nematode data were $\log_{10}(x + 1)$ transformed prior to analysis (n = 175).

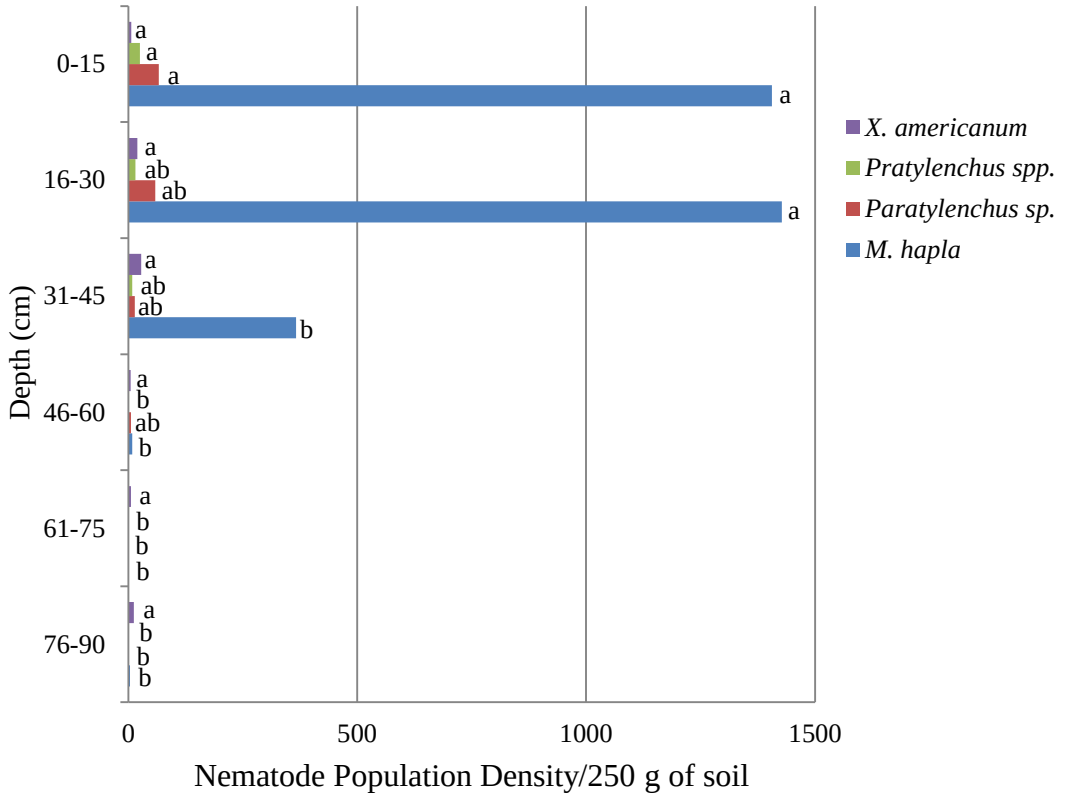

Figure 2.12. Plant-parasitic nematode population densities at different soil depths in a Riesling vineyard, Mattawa, Washington. Nematode densities are expressed as back-transformed means from logarithmic transformations used to normalize the analysis of variance. Means separation bars within a nematode species with the same letter are not significantly different ($p < 0.05$). Values are the mean of 25 cores collected either directly under the emitter or within 30 cm of an emitter.

Table 2.7. *Meloidogyne hapla* population densities in relation to location in a Riesling vineyard, Mattawa, Washington.

Location[a]	$\bar{x}$
N	1094.90 a[b]
M	555.45 ab
S	503.29 ab
E	539.00 ab
W	82.22 b

[a]Locations sampled were directly under the emitter (M) and 30 cm North (N), South (S), West (W), and East (E) of the emitter.
[b]Values are the means of 180 observations. Nematode populations are shown as population density/250 g of soil. Back transformed means are presented. Means followed by the same letter are not significantly different according to Tukey's honestly significant difference test ($p < 0.05$).

All of the factors in the model were significant for soil moisture in the Riesling vineyard (Table 2.6). For the locations directly under the emitter and 30 cm to the north, south, and west of the emitter, soil moisture decreased with depth (Table 2.8). At the eastern location of the emitter, there was no significant difference in soil moisture throughout the soil profile. Soil moisture in the upper 45 cm of soil was lowest east of the emitter compared to soil moistures at these depths at other locations. Below 46 cm, no differences in soil moisture were detected among the sampling locations around or under the emitter.

Table 2.8. The interaction of soil depth in relation to soil moisture (g/cm^3) in a Riesling vineyard, Mattawa, Washington.

Depth (cm)	**Location**[a]					**Total**
	M	**N**	**S**	**W**	**E**	
0-15	21.00 a[b]	15.68 abcd	14.70 abcde	20.19 a	11.68 cdef	83.25
16-30	19.64 ab	12.87 bcdef	15.68 abcd	17.78 abc	8.95 def	74.92
31-45	14.27 abcde	11.62 cdef	11.97 cdef	14.37 abcde	9.60 def	61.83
46-60	11.49 cdef	10.04 def	10.47 def	9.19 def	9.41 def	50.60
61-75	9.01 def	6.67 f	8.60 def	8.23 ef	8.27 ef	40.78
76-90	6.83 f	6.86 f	6.94 f	6.56 f	6.33 f	33.52
Total	82.24	63.74	68.36	76.32	54.24	

[a]Locations sampled were directly under the emitter (M) and 30 cm North (N), South (S), West (W), and East (E) of the emitter.
[b]Values are the means of 180 observations. Back transformed means are presented. Means followed by the same letter are not significantly different according to Tukey's honestly significant difference test ($p < 0.05$).

2.4 Discussion

The horizontal and vertical distributions of plant-parasitic nematodes in semi-arid, drip-irrigated Washington wine grape vineyards were consistent across the geographic locations considered in this study. In general, *M. hapla* and *M. xenoplax* followed soil moisture and population densities of these nematodes decreased with depth; *Pratylenchus* spp. decreased with depth but population densities were concentrated along the alley ways; and, *Paratylenchus* sp. and *X. americanum* were random in distribution and influenced by very few of the measured parameters. Differences between the two sampled vineyards, including grape variety and soil type, make this dataset and the results obtained robust. With data gathered from two different vineyards, our findings are applicable to other semi-arid wine grape vineyards in eastern Washington.

A noticeable difference that was observed between the two vineyards was soil moisture; the Riesling vineyard was wetter than the Chardonnay vineyard. The differing water dynamics between the two vineyards in the short-term was due to different irrigation schedules at the vineyards and in the long-term due to different soil types. For both the horizontal and vertical studies, sampling at the Riesling vineyard was conducted the day after irrigation while sampling in the Chardonnay vineyard occurred four days after irrigation. This explains why soil moisture at the Riesling vineyard was evenly distributed across the vine row while soil moisture was predominately located under the emitters in the Chardonnay vineyard. The long-term wetting dynamics within the vineyards is more readily explained by the distribution of fine roots within the vineyards. Fine roots establish in areas where there is consistent moisture and nutrients. The contour plots of fine roots showed defined pockets of roots directly below emitters in the Chardonnay vineyard while roots were more dispersed in the Riesling vineyards along the vine row. This distribution reflects the ability of the soils in these two vineyards to retain moisture. The silt loam soil in the Riesling vineyard has a higher water holding capacity of 30 g/cm^3 than the sandy loam soil in the Chardonnay vineyard which has an available water capacity of 23 g/cm^3 (NRCS 2014). Due to the larger pore spaces, a low

surface area in the sandy loam soil, and the differing water holding capacities, water drains more readily through the sandy loam soil as opposed to silt loam soil which retains water for longer periods of time.

It is important to note that the soil moisture content observed from our studies was a singular snapshot in time and soil moisture distribution is not static in the vineyard; however, the distribution of fine root biomass and plant-parasitic nematodes are fixed in the soil profile. At both sites, soil moisture decreased with depth with the highest soil moisture content in the upper 30 cm; this was true for all locations except for east of the emitter where soil moisture content was similar throughout depth. Right below the emitter and the locations along the vine row had the highest soil moisture content since they directly received water. In both vineyards, fine root biomass was located in the vine rows. Hunter (1998) confirms that in vineyards, the majority of the roots exist within the vine row. Due to soil moisture controlling fine root biomass and distribution, soil moisture was the driving force in these semi-arid vineyards. Fine root biomass and plant-parasitic nematode distribution follow soil moisture.

The distribution of *M. hapla* was positively related to soil moisture content and fine root biomass. *Meloidogyne hapla* population densities were concentrated in a 0.9 m band around the vine row clustered below the emitters. Similarly, their highest population densities in the vertical study were also discovered in the vine row indicating that *M. hapla* aggregates in the root zone. These results conform to the biology of *Meloidogyne* spp.; fine roots are the preferred site for entry of second-stage juveniles, which invade right behind the root tip (Anwar and McKenry 2002). Population densities of *M. hapla* also decreased with depth. Numerically, higher population densities were recovered at shallower (0 to 45 cm) depths compared to deeper (46 to 90 cm) depths in both vineyards, although this data was not always statistically supported. Our results for *M. hapla* are similar to results from other spatial studies evaluating the distribution of *Meloidogyne* spp. in vineyards. *Meloidogyne* spp. population densities were highest in the upper 60 cm of soil in the vine row and declined with depth (Ferris and McKenry 1974).

In this same study, *Meloidogyne* spp. population densities were variable in the upper 15 cm of soil due to extreme environmental changes such was water and temperature fluctuations. This is similar to our findings where we observed fluctuations in the population densities of *M. hapla* in the upper 15 cm of soil, although again, this was not statistically supported. Ferris and McKenry (1974) also found that *Meloidogyne* spp. population densities followed root distribution, which they found were highest in the vine row. Quader et al. (2001) investigated the distribution of *Meloidogyne* spp. in South Australian vineyards and similarly found that *Meloidogyne* spp. had the highest population densities in the vine rows where the majority of roots were distributed.

Similar to *M. hapla,* population densities of *M. xenoplax* were positively related to soil moisture and fine root biomass. The majority of the *M. xenoplax* population was located in the wetting zone in the vine row with the highest densities directly under the vines suggesting that *M. xenoplax* follows fine root distribution. Our results additionally indicated that *M. xenoplax* decreased with depth in the soil profile. While not statistically supported, the highest population densities of *M. xenoplax* were found 0 to 30 cm in the soil, with the highest population in the upper 15 cm of soil. These results are similar to those of Smolik and Dodd (1983) where *M. xenoplax* decreased with soil depth in shortgrass prairie and the highest population densities of *M. xenoplax* were found in the upper 20 cm. While *M. xenoplax* was present in the Chardonnay vineyard, it was not found in the Riesling vineyard. This discrepancy could be due to differences in cropping histories at the two vineyards. The Chardonnay vineyard was established in an old pivot irrigation area that was previously cropped with annual crops such as potato, wheat, alfalfa, and mint, with both wheat and mint hosts to *M. xenoplax* (Hafez et al. 2010; Nyczepir and Bertrand 1990). Due to the intensive crop production of this area, it is likely that *M. xenoplax* was introduced into this field through infected planting material or machinery. In contrast, the Riesling vineyard was planted into virgin soil removed from native vegetation such as rabbitbrush (*Chrysothamnus nauseosus*), Cheatgrass (*Bromus tectorum*), and sagebrush (*Artemisia* spp.) (Daubenmire 1975;

Weaver 1917). In addition, due to the isolation of this vineyard there is less likelihood of accidental contamination with plant-parasitic nematodes. *Mesocriconema xenoplax* was present in only 14% of the 157 sampled vineyards in Washington (Zasada et al. 2012), showing that while *M. xenoplax* is present in Washington vineyards, it does not have a widespread distribution.

Pratylenchus spp. had a similar distribution pattern in both vineyards. Population densities of this nematode were aggregated in the vineyard alley ways. Quader et al. (2003) found *Pratylenchus* spp. to be distributed evenly across the vineyard. Their results suggest both the grapevine and cover crop planted between the rows were hosts, while in our studies it appears plants other than the grapevine are hosts for this nematode. To further support this finding, *Pratylenchus* spp. was negatively related to grape fine root biomass. This lack of association with grape roots, along with its horizontal distribution, again implies that grapes are not the main host for this nematode in semi-arid Washington vineyards. Walker and Morey (2000) proposed that *Pratylenchus* spp. could reproduce on nearby susceptible cover crops then reinvade grape roots even if the grapes are a poor host; however, this idea was not supported by our data. We also found that *Pratylenchus* spp. population densities declined with soil depth. Likewise, Quader et al. (2003) reported that *Pratylenchus* spp. decreased with depth, with higher numbers in the upper 30 cm of soil. This further supports the idea that shallow rooted plants are the preferred host for *Pratylenchus* spp. in this production system. While our molecular identification indicated that the *Pratylenchus* species present in the Chardonnay vineyard was *P. neglectus* and in the Riesling vineyard *P. neglectus* and *P. thornei* were present, only ten species of *Pratylenchus* from each vineyard were identified and we are uncertain whether additional species of *Pratylenchus* are present in eastern Washington vineyards. Smiley et al. (2004) reported *P. neglectus* had a widespread distribution in dryland fields of the Pacific Northwest, and Smiley et al. (2013) reported this nematode was present in 90% of semi-arid fields in south-central Washington. While various species of *Pratylenchus* have

been discovered in vineyards worldwide, only *P. vulnus* has been shown to cause damage to grape (Quader et el. 2003; Pinochet et al. 1976; Raski 1954).

The distribution of *X. americanum* both horizontally and vertically was random in both vineyards. In addition, population densities of this nematode were not related to any of the measured variables in either study. Other researchers have reported this random distribution of *Xiphinema* spp. in vineyards (Quader et al. 2003; Ferris and McKenry 1974; Ponchillia 1972). While not statistically supported, population densities of *X. americanum* were concentrated in the 15 to 45 cm soil depth in our study. Similarly, Ferris and McKenry (1974) found that population densities of *X. americanum* were higher in the upper 45 cm of undisturbed soil in the vine row. Contrary to our results, Quader et al. (2003) reported that the highest densities of *X. americanum* occurred in the top 15 cm of soil. It was observed that population densities were numerically lowest in the upper 15 cm in our study. This is most likely due to fluctuations in environmental conditions (wetting and drying) at this soil depth; *Xiphinema* spp. are classified as a colonizer-persister-5, meaning these nematodes are sensitive to environmental disturbances (Bongers and Bongers 1998). Population densities of *X. americanum* were higher in the Chardonnay vineyard than in the Riesling vineyard. Average population densities in the Chardonnay vineyard in the horizontal and vertical distribution studies were 50 *X. americanum*/250 g of soil and 91 *X. americanum*/250 g of soil, respectively; the Riesling vineyard had an average of 9 *X. americanum*/250 g of soil in the horizontal study and an average of 14 *X. americanum*/250 g of soil in the vertical study. Variables other than depth, soil moisture, or fine root biomass may be influencing the distribution of this nematode. *Xiphinema americanum* survive best in soils with large pore spaces, indicating these nematodes may have an oxygen requirement (Ponchilla 1972). The sandy soil type, with larger pore spaces, in the Chardonnay vineyard may have been more conducive for survival of this nematodes compared to the finer textured silt loam soil in the Riesling vineyard.

Similar to *X. americanum, Paratylenchus* sp. had an inconsistent horizontal distribution and was not influenced by any of the measured variables. There was also no apparent consistent pattern of *Paratylenchus* sp. distribution between the two sampled vineyards. Ferris and McKenry (1976) reported that *Paratylenchus* spp. had a random distribution, and that this nematode had the most inconsistent distribution of all the plant-parasitic nematodes found. In our study, population densities of *Paratylenchus* sp. were influenced by depth in both vineyards. In the Riesling vineyard, *Paratylenchus* sp. decreased with depth with few nematodes detected below 61 cm. Verschoor et al. (2001) studied the spatial distribution of nematodes in grasslands and also discovered *Paratylenchus* sp. population densities decreased with depth. Additionally, in a shortgrass prairie (*Bouteloua gracilis*, *Opuntia polycantha,* and *Artemisia frigida*), *Paratylenchus* sp. decreased with depth (Smolik and Dodd 1983). While both these studies correspond with our results from the Riesling vineyard, they are in contrast to our findings in the Chardonnay vineyard where *Paratylenchus* sp. increased with soil depth to 60 cm. This may be explained by the fact that this nematode has been shown to follow the distribution of roots (Verschoor et al. 2001). It is possible in the Chardonnay vineyard that grapevine roots extend further into the soil profile due to a deeper extent of soil moisture and the larger pore spaces of the sandy soil. Although *Paratylenchus* sp. had high population densities in both sampled vineyards, the effect that *Paratylenchus* sp. has on grapevines is minimal (Pinkerton et al. 1999).

Despite slight differences in plant-parasitic nematode population densities, soil moisture, and fine root biomass between vineyards, the overall trends of these variables were similar. In Washington semi-arid, drip irrigated wine grape vineyards, soil moisture drives fine root biomass and plant-parasitic nematode distribution. In general, plant-parasitic nematode populations were concentrated in the upper 45 cm of soil at both vineyards. These findings suggest that when targeting plant-parasitic nematodes, grape growers should concentrate the majority of their management tools in a 1 m horizontal band around the vine row to a depth of 45 cm. This would significantly reduce the area of

chemical application and the cost. However, this strategy might not be effective against *Pratylenchus* spp. due its congregation around the alley ways and *X. americanum* due to its widespread distribution.

This information fills the void in knowledge of the spatial distribution of plant-parasitic nematodes in the second-largest grape production state in the United States. However, more research is needed to understand the impact that plant-parasitic nematodes have on vine productivity in this region. Future research should expand sampling to additional vineyards, including vineyards planted with red grape varieties, to determine if the same trends are observed. Seasonal studies on the population dynamics of plant-parasitic nematodes, particularly *M. hapla*, will help growers target nematodes when susceptible stages (second-stage juveniles) are present in soil. Finally, additional studies of the distribution of nematodes under different vine and emitter spacings, as well as under different irrigation regimes would be interesting. With the changing landscape of nematode management, knowledge of how plant-parasitic nematodes are distributed in Washington vineyards will help growers better target these production-limiting pests. The results of this study will provide Washington grape growers with the knowledge of where plant-parasitic nematodes are located in their vineyards enabling them to target specific areas within the vineyard for nematode management.

2.5 Literature cited

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Chapter 3: Host status of own-rooted *Vitis vinifera* and *Vitis* spp. rootstocks to *Meloidogyne hapla*

3.1 Introduction

Washington is the second largest wine (*Vitis vinifera)* producing region in the United States with an economic value of $236 million in 2012 (USDA 2013). Cultivating more than thirty different varieties, the 17,401 hectares of vineyards in Washington (USDA 2013) are mostly located on the eastern side of the state. White grape varieties are prevalently grown, with the leading varieties being Riesling and Chardonnay. Leading red varieties are Cabernet Sauvignon, Merlot, and Syrah. Due to low rainfall and a photoperiod of approximately 16 hours during the summer, vineyards in eastern Washington are semi-arid and drip irrigated. Washington vineyards are planted with own-rooted grapevines as opposed to grapevines grafted onto rootstocks. While rootstocks can provide a host of benefits to vineyards, Washington grape growers are unlikely to plant rootstocks in the near future due to cold-tolerance and frost concerns (Keller et al. 2012).

A major concern that Washington grape growers face is plant-parasitic nematodes. Plant-parasitic nematodes are a global pest, estimated to cause $100 billion economic loss to agriculture worldwide with $6 billion agricultural loss in the United States (Sasser and Freckman 1987). Nematodes can cause extensive damage through feeding on plants roots, and in grapevines, nematode feeding can cause the vine to prematurely decline (Anwar and Gundy 1989; Stirling and Cirami 1984; Lider 1960), have reduced vigor (Téliz 2007; Nicol et al. 1999; Ferris 1975), and have an increased susceptibility and severity to abiotic and biotic stresses such as pests, diseases, viruses, and drought (Esmenjaud and Bouquet 2009; Téliz 2007; Ramsdell 1996; Brown 1993; McKenry 1992). Symptoms that occur in grapevines also include reduced root and shoot growth (Nicol et al. 1999; Walker 1997; Anwar and Gundy 1989), reduced ability to uptake water and nutrients (Nicol et al. 1999), physiological changes in the root systems (Ferris et al. 2012), and reduced yield (Esmenjaud and Bouquet 2009; McKenry et al. 2006; Nicol et al. 1999; Nicol and Heeswijck 1997; McKenry 1992; Lider 1960). The

extent of yield loss varies depending on location, grape variety, species of plant-parasitic nematode present, and density of nematodes, but losses can range from 7 to 60% (Téliz 2007; Nicol and Heeswijck 1997).

Surveys conducted by Zasada et al. (2012) in eastern Washington to determine the plant-parasitic nematodes associated with semi-arid vineyards found *Meloidogyne hapla* to be one of the most abundant nematode present in the surveyed vineyards; it was discovered in 60% of the vineyards. *Meloidogyne* spp., or root-knot nematodes, are a major production and economic constraint to grapevines worldwide. They have been reported to reduce grapevine yields by up to 60% (Nicol et al. 1999; Nicol and Heeswijck 1997; Arredondo 1992; Jenser et al. 1991; Raski et al. 1973). Over 50 species of *Meloidogyne* are described with seven species found on grapevines; however, only four species, *M. incognita, M. hapla, M. javanica,* and *M. arenaria,* are considered to be the most damaging (Esmenjaud and Bouquet 2009; Esnard and Zuckerman 1998), accounting for 95% of all *Meloidogyne* infestations in cultivated crops (Hussey and Janssen 2002). Of the four major *Meloidogyne* species, only *M. hapla*, the northern root-knot nematode, is found in the Pacific Northwest because this nematode has a more temperate distribution compared to the other three major *Meloidogyne* spp., which prefer warmer climates and are found worldwide (Esmenjaud and Bouquet 2009; Esnard and Zuckerman 1998).

Meloidogyne spp. are sedentary endoparasites remaining stationary inside the roots of a host plant. Newly hatched second-stage juveniles (J2) migrate through the soil searching for a host plant roots. Penetrating at the tip of the root, J2 move intercellularly to the vascular cylinder where they induce the formation of specialized ‘giant cells’; the nematode then resides there for the duration of their life. Adult females lay their eggs outside of the root in a gelatinous matrix. A single egg mass of *M. hapla* can contain 400 to 500 eggs. Under optimal conditions, the lifecycle of this nematode can take four to five weeks to complete, producing four to six generations per season (Esmenjaud and Bouquet 2009; Hussey and Janssen 2002; Nicol et al. 1999; Brown et al. 1993; de Guiran

and Ritter 1979; Williams 1974; Bird and Wallace 1965). *Meloidogyne* spp. can cause significantly reduced root systems (Brown et al. 1993), limit the plants ability to uptake water and nutrients (Ramsdell et al. 1996), reduce yield (Esnard and Zuckerman 1998) and initiate earlier bud break (Anwar and Gundy 1989) which can lead to frost damage in regions such as the Pacific Northwest.

While *M. hapla* is abundant and widespread in Washington semi-arid vineyards, very little is known about the pathogenicity and host status of commonly planted *V. vinifera* varieties to *M. hapla* in this viticulture region. The effect *Meloidogyne* spp. have on grapevines and rootstocks is well researched in other grape producing regions such as Oregon (Pinkterton et al. 2004; Pinkterton et al. 1999), California (McKenry 1992; Anwar and Gundy 1989; Ferris and McKenry 1975), and Australia (Quader et al. 2001; Nicol et al. 1999; Stirling and Cirami 1984), but all of these locations have different climates and species of *Meloidogyne* compared to Washington. Very little research has been conducted on the biology and pathogenicity of *M. hapla* in grapevines in Washington or other temperate grape growing regions. Therefore, the effect *M. hapla* has on grapevines in Washington remains unknown. Also, little is known if own-rooted clones vary in their susceptibility to *M. hapla.* This is a huge 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 objective of this study was to determine the host status of predominately grown *V. vinifera* varieties and clones grown in Washington to *M. hapla*. In addition, the host status of several *Vitis* spp. rootstocks to *M. hapla* was investigated. This research will provide Washington grape growers with the knowledge to select appropriate planting material to minimize the impact of *M. hapla* on vine productivity.

3.2 Materials and methods

3.2.1 Own-rooted Vitis vinifera experiments

Dormant grape cuttings were obtained from Ste. Michelle Wine Estates (Paterson, WA) in 2012 and from Inland Desert Nurseries (Benton City, WA) in 2013. The varieties and clones evaluated were: Merlot 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.

Cuttings of each clone were categorized into similar sizes to ensure uniformity. Using pruning shears, 15 cuttings of each clone were cut into three node segments with the bottom node cut diagonally and rooted in a perlite and vermiculite mixture (Santo and Hackney 1980). In 2013, the bottom node of each cutting was dipped in rooting hormone (Dip'N Grow, Clackamas, OR) to ensure root growth. The cuttings were then placed on a mist bench with a heating pad for two months and were misted with water every 30 minutes. In April of each year, the grapes were removed from the mist bench and placed in a greenhouse under a shade cloth to be hardened off. A week later, 12 established grape cuttings of each clone with uniform root systems were transplanted into 3.7 L pots containing a steam pasteurized 1:1 sand:Willamette loam soil. The vines were single-shooted and the inflorescences removed to promote root growth. The grapevines were initially fertilized with a 9-45-15 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 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 under lights to give a photoperiod of 16 hour days; temperatures were set to 25˚C during the day and 20˚C at night.

In late May, eight plants of each clone were selected based on uniformity in size for inoculation with *M. hapla*. In 2012, some grapevines did not become fully established

and three clones had less than eight plants. These were Syrah Phelps with only six vines, Syrah 07 with five vines, and Merlot 03 with seven vines. The pots were arranged in a randomized block design with eight blocks in the greenhouse. The grapevines were randomly sorted in each block using Excel (Microsoft, Redmond, WA). *Meloidogyne hapla* originally collected from a *V. vinifera* vineyard in Veneta, OR, and reared on tomatoes (*Lycopersicon esculentum* Mill. cv. Rutgers) was 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 minutes (Hussey and Barker 1973). The egg solution 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 solution was placed on a counting slide to determine the number of eggs per ml; the solution was diluted until there was the desired number of eggs per ml. In early June of each year, the grapevines were inoculated with a density of 3 *M. hapla* eggs/gram of soil which was equivalent to 9,000 eggs/3.7 L pot. The inoculum was applied by pipetting 2 ml of inoculum into six holes 4 cm deep around the base of the vine. The holes were closed and plants were watered regularly the next day.

Parameters measured to evaluate plant growth were leaf area and shoot length. Leaf area was measured by comparing each leaf to concentric circles of known sizes and adding the values from a vine together; shoot length was recorded using a flexible tape measure (Schreiner et al. 2012). Soil temperatures and ambient temperatures were also recorded daily every 30 minutes (WatchDog Data Loggers, Model 125; Spectrum Technologies, Inc., Aurora, IL). Soil temperatures probes were buried in pots containing solely soil which received water when the grapes were watered; ambient temperature probes were hung above the benches in the shade. Soil temperature data was used to determine nematode degree-days (NDD) accumulated by *M. hapla* using a base temperature of 10ºC (Charchar and Santo 2009). Degree days were determined with the formula:

$$NDD = \left(\frac{T_{max} + T_{min}}{2}\right) - 50$$

In October of 2012 and September of 2013, the experiments were destructively harvested. For each plant, the shoot was removed, placed in a paper bag, dried at 70ºC for five days, and weighed. Soil was shaken free of the roots and a 50 g subsample of soil from each pot was collected to extract *M. hapla* second-stage juveniles (J2) using the Baermann funnel method (Ingham 1994). After 5 days, the J2 were collected from the bottom of the funnel in a 50 ml tube. The volume in the tube was adjusted to 10 ml and the number of J2 in 5 ml of the solution was determined using a Leica compound microscope (Wetzlar, Germany). Roots were then gently rinsed free of soil. Rinsed roots were placed in a 500 ml covered container with a 10% bleach (Clorox, Oakland, CA) solution and shaken at 300 rpm for 3 minutes 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-µm and 25-µm sieves and rinsed for 1 minute. The roots were removed from the 90-µm sieve, then rolled in a paper towel, placed in an oven, dried for five days at 70ºC, and weighed to obtain dry weight. The eggs retained on the 25-µm sieve were back washed into a 50 ml polyethylene tube. Prior to counting, the eggs were stained by adding four drops of a 0.35% acid fuchsin and 25% lactic acid solution to the egg solution and boiling for 1 minute (Byrd et al. 1972). In 2013, the number of eggs in 1 ml of the 50 ml egg solution was determined using a Leica inverted microscope. However, in 2012, the egg solution was diluted by adding 1 ml of the 50 ml solution into 9 ml to get a 10 ml dilution with 1 ml of this dilution used to determine the number of eggs.

3.2.2 Vitis spp. rootstock experiments

Nine rootstocks (Sunridge Nurseries, Inc., Bakersfield, CA) were evaluated for *M. hapla* host status: Salt Creek, Freedom, Harmony, St. George, Riparia Gloire, 101-14, 3309C, 110R, and 420A (Table 3.1). Dormant cuttings of each rootstock were treated in the same manner as the 2013 *V. vinifera* variety/clone trials described above. Two

independent trials were conducted in 2013. The first trial was integrated into the 2013 variety/clone trial, with each rootstock replicated eight times. Replications of each rootstock were randomized within the *V. vinifera* variety/clone trial with one replication of each rootstock added to every block. The second trial was conducted in a separate greenhouse with *V. vinifera* White Riesling Neustad 90 as a control. This trial was also replicated eight times and arranged in a randomized block design with eight blocks. All of the rootstocks, regardless of trial, were inoculated with 3 *M. hapla* eggs/g soil and maintained in the same manner as vines in the *V. vinifera* variety/clone trials described above. The first rootstock trial was harvested at the same time as the *V. vinifera* variety/clone trial while the second rootstock trial was destructively harvested one week later. Plants were harvested as described above. The number of eggs in 1 ml of the 50 ml egg solution was determined using a Leica inverted microscope.

Table 3.1. Parentage of the *Vitis* rootstocks evaluated for host status to *Meloidogyne hapla*.

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

3.2.3 Statistical analysis

Meloidogyne hapla data is presented as eggs/g root, J2/250 g soil, and total *M. hapla*/pot (eggs + J2). In addition, reproduction factor values, RF= final nematode population/initial nematode population (Oostenbrink 1966) were calculated. A RF value > 1 indicates that the plant is a good host while a RF value < 1 indicates a poor host. Prior

to analysis, nematode and plant data was $\log_{10}$ transformed to fulfill normality and equal variance assumptions of the model (R Studio v0.98, Boston, MA). After transformations, the data was analyzed using a function that performed a one-way analysis of variance (ANOVA) for Randomized Block Design (RBD), with a treatment value of 15 and a block value of 8 for the *V. vinifera* variety/clone trials. Data from the 2012 and 2013 trials were analyzed separately because the trials were significantly different from each other ($p = 0.001$). Using the RBD function, clones, varieties (Chardonnay, Riesling, Cabernet Sauvignon, Merlot, Syrah), and types of grape (red, white) were analyzed separately to determine if the treatments were significantly different. Block was shown to not have an effect on the experiment ($p = 0.05$ in 2012; $p = 0.40$ in 2013). Mean separations were performed using Tukey's Honestly Difference test (HSD.test); differences were considered significant at $p < 0.05$. Data from the rootstock trials were analyzed similarly to the *V. vinifera* variety/clone trials. However, the two rootstock trials were combined and analyzed together because they were shown not to be significantly different from each other ($p = 0.54$). The combined data was $\log_{10}$ transformed and then analyzed using the RBD function, with a treatment value of 10 and a block value of 8. Blocks were again shown to not have an effect ($p = 0.12$). Means separations were performed using Tukey's HSD.test with significant differences at $p < 0.05$.

3.3 Results

3.3.1 Own-rooted Vitis vinifera experiments

Across clones in 2012, the number of *M. hapla*/pot, the number of *M. hapla* eggs/g of root, as well as the RF values were greater than in 2013 ($p = 0.001$). In addition, the root systems of the grapes in 2012 were significantly larger than in 2013 ($p = 0.003$). However, shoot dry weights in 2013 were significantly greater than the shoot dry weights in 2012 ($p < 0.001$). In 2012, root and shoot biomass data were more variable compared to 2013 which had more consistent weights. Lastly, the NDD between years were

different; 2012 had 25% more NDD than 2013. The NDD in the 2012 were 2460 and in 2013 were 1949.

In 2012, shoot and root dry weights varied significantly among clones with Merlot 15 having the highest shoot biomass and Riesling 198 having the lowest shoot biomass (Table 3.2). Similarly, Riesling 198 also had one of the lowest root biomasses and Merlot 15 had one of the highest. Cabernet Sauvignon 04 had the actual highest root dry weight and Syrah Phelps had the lowest. In 2013, clone shoot and root dry weights were also significantly different from each other (Table 3.3). Syrah Phelps had the highest shoot biomass and Chardonnay 15 had the lowest. Both the Chardonnays had the lowest shoot biomasses but their root biomasses were near the median root dry weight. Merlot 06 had the highest root biomass and Shiraz 07 had the lowest.

Significant differences were observed in *M. hapla* measurements among the clones in both years. In 2012, both the Chardonnay clones had the highest number of *M. hapla* eggs/g of root, followed by all three Riesling clones (Table 3.2). Merlot 06, followed by Merlot 03, had the lowest *M. hapla* eggs/g of root. A similar trend was observed in 2013 (Table 3.3); all three Merlot clones had the lowest *M. hapla* eggs/g of root and Chardonnay 06 followed by Riesling 239 and Chardonnay 15 had the highest number of *M. hapla* eggs/g of root. In 2012 (Table 3.2), both Chardonnay clones had the highest total number of *M. hapla*/pot, followed by the Riesling clones, while the Syrah/Shiraz clones had the lowest total number of *M. hapla*/pot. In 2013 (Table 3.3), Chardonnay 06 followed by Riesling 239 and Chardonnay 15 had the highest total number of *M. hapla*/pot; all three Merlot clones had the lowest number of total number of *M. hapla*/pot. In both years, Chardonnay 06 had the highest number of *M. hapla* eggs/g of root and total number of *M. hapla*/pot. The number of *M. hapla* J2/250 g of soil in the grape clones in 2012 was not significantly different (Table 3.2), but in 2013, they were significantly different (Table 3.3). Cabernet Sauvignon 21 had the highest number of *M. hapla* J2/250 g of soil in 2013 with Merlot 03 and Syrah 07 both having the lowest number of *M. hapla* J2/250 g of soil. The RF values of the grape clones in 2012 were

significantly different from each other (Table 3.2). Both Chardonnay 06 and Chardonnay 15 had the highest RF values followed by all three Riesling clones; Syrah Phelps had the lowest RF value. The same trend was observed in 2013 (Table 3.3), with Chardonnay 06 and Chardonnay 15 having the highest RF values followed by the three Riesling clones. However, the Merlot clones in 2013 had the lowest RF values. Even though there were subtle differences among clones, the overall trend was similar, with Chardonnay and Riesling clones typically having the highest number of *M. hapla*/g root and total *M. hapla*/pot as well as RF values, Cabernet Sauvignon clones being intermediate, and Merlot and Syrah clones with the lowest.

Table 3.2. *Vitis vinifera* variety and clone shoot and root dry weights, number of *Meloidogyne hapla* eggs/g root, *M. hapla* second-stage juveniles (J2)/250 g of soil, total *M. hapla* recovered from inoculated plants, and reproduction factor (RF) values of *M. hapla* in 2012.

Variety/Clone	**Shoot Dry Weight (g)**	**Root Dry Weight (g)**	***M. hapla* eggs/g of root**	**J2/250 g of soil**	**Total *M. hapla***	**RF[b]**
Cabernet Sauvignon 02	11.42 ab[a]	17.14 abc	10,540 e	509 ab	187,000 bcd	20.1 bcd
Cabernet Sauvignon 04	12.94 ab	18.01 ab	11,530 e	952 a	214,000 bcd	22.5 bcd
Cabernet Sauvignon 06	8.78 ab	12.93 bcdef	14,410 cde	758 a	190,600 bcd	20.2 bcd
Cabernet Sauvignon 21	11.30 ab	16.39 abcd	10,890 e	664 a	179,100 cd	19.0 cd
Chardonnay 06	7.99 ab	9.34 fg	50,620 a	1,256 a	528,100 a	57.0 a
Chardonnay 15	12.06 ab	12.62 cdefg	32,830 ab	943 a	411,800 ab	44.5 ab
Merlot 03	11.00 ab	12.78 bcdef	5,301 e	288 b	69,960 d	7.4 d
Merlot 06	13.12 ab	18.28 a	5,044 e	788 a	102,700 d	10.4 d
Merlot 15	13.64 a	16.12 abcde	8,040 e	1,491 a	146,500 cd	14.3 cd
Syrah 07	9.60 ab	8.12 fg	12,550 de	292 b	66,300 d	7.0 d
Syrah Phelps	9.85 ab	7.02 g	6,936 e	308 b	51,030 d	5.3 d
Shiraz 07	10.08 ab	8.88 fg	12,750 de	510 ab	123,200 d	13.0 d
Riesling 90	11.87 ab	11.67 defg	31,290 bc	663 a	351,100 abc	38.1 abc
Riesling 198	7.47 b	8.58 fg	30,670 bcd	637 a	278,900 bcd	30.1 bcd
Riesling 239	8.95 ab	10.94 efg	22,160 bcde	1,149 a	238,000 bcd	25.0 bcd
***p-values*[c]**	*0.003*	*< 0.001*	*< 0.001*	0.26	*< 0.001*	*< 0.001*

[a]Values are the means of eight observations. Nematode data was $\log_{10}$ 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 ($p < 0.05$).

[b]Reproduction factor (RF) values calculated as final nematode population density/initial nematode population density.

[c]*p-values* were generated using a linear mixed model.

Table 3.3. *Vitis vinifera* variety and clone shoot and root dry weights, number of *Meloidogyne hapla* eggs/g root, *M. hapla* second-stage juveniles (J2)/250 g of soil, total *M. hapla* recovered from inoculated plants, and reproduction factor (RF) values of *M. hapla* in 2013.

Variety/Clone	Shoot Dry Weight (g)	Root Dry Weight (g)	*M. hapla* eggs/g of root	J2/250 g of soil	Total *M. hapla*	RF[b]
Cabernet Sauvignon 02	14.93 a[a]	9.42 a	8,356 d	375 ab	79,760 de	8.4 de
Cabernet Sauvignon 04	15.51 a	9.17 a	10,360 cd	388 ab	93,490 cde	9.9 cde
Cabernet Sauvignon 06	15.18 a	7.80 ab	12,300 bcd	294 ab	92,310 cde	9.9 cde
Cabernet Sauvignon 21	15.30 a	9.32 a	11,060 bcd	2,912 a	128,600 cde	10.4 cde
Chardonnay 06	12.28 b	9.10 a	34,820 a	781 ab	304,600 a	32.8 a
Chardonnay 15	12.04 b	8.92 a	23,530 abc	619 ab	199,400 abc	21.3 abc
Merlot 03	13.41 ab	9.54 a	4,103 d	88 b	35,910 e	3.9 e
Merlot 06	14.19 a	10.94 a	5,281 d	213 b	59,040 de	6.3 de
Merlot 15	13.20 ab	8.23 ab	5,417 d	613 ab	49,480 de	4.7 de
Syrah 07	14.35 a	7.96 ab	17,950 bcd	88 b	107,000 cde	11.8 cde
Syrah Phelps	15.79 a	9.04 a	7,666 d	156 b	74,320 de	8.1 de
Shiraz 07	15.26 a	7.11 ab	14,150 bcd	500 ab	104,400 cde	10.9 cde
Riesling 90	15.33 a	9.72 a	16,650 bcd	963 ab	163,600 bcd	16.9 bcd
Riesling 198	14.80 a	10.38 a	13,610 bcd	275 b	135,400 cde	14.7 cde
Riesling 239	14.66 a	10.76 a	25,620 ab	1,519 ab	267,600 ab	27.7 ab
p-values[c]	*0.014*	0.09	*< 0.001*	*0.036*	*< 0.001*	*< 0.001*

[a]Values are the means of eight observations. Nematode data was $\log_{10}$ 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 ($p < 0.05$).

[b]Reproduction factor (RF) values calculated as final nematode population density/initial nematode population density.

[c]*p-values* were generated using a linear mixed model.

The trends that were observed among the *V. vinifera* clones were similar to those found when clones within varieties were combined for analysis. In both years, Chardonnay had the highest number of *M. hapla* eggs/g of root and RF values (Table 3.4). While Chardonnay had a similar number of *M. hapla* eggs/g of root to that of Riesling, RF values in both years were significantly higher for Chardonnay than Riesling ($p < 0.001$). Chardonnay and Riesling always had a higher number of *M. hapla* eggs/g root and RF values than any of the red varieties. Each year, Merlot had the lowest number of *M. hapla* eggs/g of root, but Syrah had the lowest RF values. In 2012, the number of *M. hapla* eggs/g of root in Chardonnay was almost seven times the number of *M. hapla* eggs/g of root in Merlot, and the RF value for Chardonnay was more than six times higher the RF value for Syrah. Likewise, in 2013, Chardonnay had almost six times more *M. hapla* eggs/g of root than Merlot, and an almost six times higher RF value than Syrah.

Table 3.4. *Meloidogyne hapla* eggs/g root and reproduction factor (RF) values on *Vitis vinifera* varieties in 2012 and 2013.

Variety/Type	***M. hapla* eggs/g root**	**RF[b]**	***M. hapla* eggs/g root**	**RF[b]**
	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
***p-values*[c]**	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

[a]Values are the means of eight observations. Nematode data was $\log_{10}$ 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 ($p < 0.05$).
[b]Reproduction factor (RF) values calculated as final nematode population density/initial nematode population density.
[c]*p-values* were generated using a linear mixed model.

When red (Cabernet Sauvignon, Syrah, and Merlot) and white (Chardonnay and Riesling) varieties were combined for analysis, the white varieties were significantly better hosts for *M. hapla* in all measured parameters compared to the red varieties (Table 3.5). White varieties had four times the *M. hapla* eggs/g of root than red varieties in 2012. A similar trend was observed in 2013 with white varieties having 2.5 times the *M. hapla* eggs/g of root than red varieties. Red varieties also had significantly lower RF values in both years than white varieties. White varieties had 36 and 37% higher RF values than red varieties in 2012 and 2013, respectively.

Table 3.5. Number of *Meloidogyne hapla* eggs/g root and reproduction factor (RF) values on *Vitis vinifera* white (Chardonnay and Riesling) and red (Cabernet Sauvignon, Syrah, and Merlot) varieties in 2012 and 2013.

Variety/Type	***M. hapla* eggs/g root**	**RF[b]**	***M. hapla* eggs/g root**	**RF[b]**
	2012		**2013**	
White	38,730 a[a]	36.4 a	21,110 a	23.4 a
Red	9,881 b	11.9 b	8,820 b	8.3 b
***p-values*[c]**	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

[a]Values are the means of eight observations. Nematode data was $\log_{10}$ 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 ($p < 0.05$).
[b]Reproduction factor (RF) values calculated as final nematode population density/initial nematode population density.
[c]*p-values* were generated using a linear mixed model.

3.3.2 Vitis spp. rootstock experiments

The NDD in the two respective experiments were similar, with 1949 and 2149 NDD recorded in trial 1 and 2, respectively. Differences were observed among the rootstocks in above and belowground biomass (Table 3.6). Freedom had the lowest shoot dry weight, but the third highest root dry weight. Salt Creek had the highest shoot biomass but the second lowest root biomass. The rootstock 3309C had the

lowest root dry weight, and Riparia Gloire had the highest. The positive control, Riesling 90, had significantly higher number of *M. hapla* eggs/g of root, *M. hapla* J2/250 g of soil, total *M. hapla*/pot, and RF value compared to the rootstocks (Table 3.6). Among the rootstocks, there were no differences in the measured *M. hapla* parameters ($p > 0.05$), with all of the rootstocks being considered poor (RF > 1) or nonhosts (RF = 0) for *M. hapla*.

Table 3.6. *Vitis* spp. rootstocks shoot and root dry weights, number of *M. hapla* eggs/g of root, *M. hapla* second-stage juveniles (J2)/250 g of soil, total *M. hapla* recovered from inoculated vines, and reproduction factor (RF) values.

Rootstock	**Shoot Dry Weight (g)**	**Root Dry Weight (g)**	***M. hapla* eggs/g of root**	**J2/250 g of soil**	**Total *M. hapla***	**RF[b]**
Salt Creek	23.02 ab[a]	9.05 d	21 b	19 b	338 b	0.0 b
Freedom	15.50 e	15.27 ab	18 b	22 b	433 b	0.0 b
Harmony	18.86 cde	9.26 d	12 b	22 b	332 b	0.0 b
St. George	15.62 e	10.19 cd	8 b	19 b	272 b	0.0 b
Riparia Gloire	19.68 bcd	17.46 a	470 b	84 b	6,138 b	0.6 b
101-14	21.46 abc	16.68 ab	547 b	109 b	7,006 b	0.6 b
3309C	15.95 de	8.23 d	13 b	3 b	102 b	0.0 b
110R	21.18 abc	10.33 cd	17 b	38 b	553 b	0.0 b
420A	24.11 a	13.83 abc	14 b	25 b	419 b	0.0 b
Riesling 90[c]	20.47 abc	13.46 bc	22,302 a	1,400 a	202,700 a	20.7 a
***p-values*[d]**	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*

[a]Values are the means of eight observations. Nematode data was $\log_{10}$ 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 ($p < 0.05$).

[b]Reproduction factor (RF) values calculated as final nematode population density/initial nematode population density.

[c]Own-rooted Riesling 90 was used as a positive control.

[d]*p-values* were generated using a linear mixed model.

3.4 Discussion

To our knowledge, this is the first report of the difference in host status of *V. vinifera* grape varieties to *M. hapla*. While all of the *V. vinifera* varieties evaluated in the experiments would be considered very good hosts to *M. hapla* (RF > 1), the magnitude of increase in population size of *M. hapla* on white (Chardonnay and Riesling) compared to red (Cabernet Sauvignon, Merlot, and Syrah) varieties was significantly greater. White varieties had higher *M. hapla* eggs/g root and an almost 40% higher RF value than red varieties. In addition, these experiments demonstrated that a range of commercially-available rootstocks are resistant to *M. hapla*.

While there were differences in the host status of grape varieties to *M. hapla,* clones within a variety did not differ in host status. *Vitis vinifera* clones originate from a slight genetic mutation from the variety during asexual propagation (Hartmann et al. 1990), and therefore, clones of the same variety are very similar in their genetic makeup. Conversely, *V. vinifera* varieties are much more genetically diverse (Martinez et al. 2006; Herrera et al. 2002), which 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 rhizosphere compounds, or genetics. In our studies, it was observed that the root system of red varieties differed from those of white varieties (Figure 3.1). The root systems of the white varieties (Figure 3.1A) appeared to have a much greater abundance of fine roots compared to the red varieties (Figure 3.1B). In general, fine roots are the preferred site for entry of second-stage juveniles, which invade right behind the root tip (Anwar and McKenry 2002). 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, and therefore a greater distance

between root tips, would result in a lower *M. hapla* invasion than white varieties which have an abundance of fine roots. Differences in infection rates among grape varieties by *Meloidogyne* spp. have been reported (Ferris et al. 2012). For example, egg production and fecundity in *V. vinifera* French Colombard, Ruby Cabernet, and Thompson Seedless varied significantly from each other (Ferris et al. 1984). It is important to note that no research has investigated the relationship between fine root biomass and *Meloidogyne* spp. infectivity in grapes.

Figure 3.1. Example of the appearance of a white *Vitis vinifera* variety (Riesling 198; A) and a red variety (Merlot 06; B) root systems at experiment termination. Rooted vines were inoculated with 9,000 *Meloidogyne hapla*/g soil, allowed to grow for approximately six months in the greenhouse, and then harvested. Roots were bleached with 10% NaClO during the nematode extraction process.

Another potential explanation for the difference in host status of white and red *V. vinifera* varieties to *M. hapla* may be rhizosphere exudates. The rhizosphere, located about 0 to 2 mm from the root surface, plays a significant role in root exudates production and secretion, and is known to greatly influence soil ecology (Bertin et al. 2003). Root exudates consist of many compounds such as sugars, amino acids, fungal stimulators, inhibitors, attractants, toxins, and waste products (Uren 2000; Rovira 1969); these root exudates drastically change the chemical and biological characteristics in the rhizosphere (Bertin et al. 2003). A major source of exudates in the rhizosphere is the part of the root right behind the root tip; this region is strongly

attractive to *Meloidogyne* spp. as it is the location where J2 invade. Due to the production of root exudates in this region of the root, these exudates have the potential to attract and/or repel *Meloidogyne* spp. (Anwar and McKenry 2000; Huang 1985). Different plant species secrete different exudates (Rovira 1969), and it is possible that different varieties of the same plant could also secrete different exudates. For instance, Haynes and Jones (1976) found that cucumber plants that carry the dominant bitter gene (*Bi*) repelled *Meloidogyne* spp. while cucumbers without the gene were attractive to *Meloidogyne* spp. This could be true for *V. vinifera* as well; red varieties could secrete root exudates into the rhizosphere that are slightly less attractive or detrimental to *M. hapla*, while white varieties could secrete root exudates that are slightly more attractive to *M. hapla* making these varieties more susceptible than red varieties. 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. Another possible explanation is that root exudates can initiate egg hatch (Rovira 1969), and white varieties could possibly release exudates that initiate faster egg hatch than red varieties. On the micro scale, nematodes are also thought to increase root exudation where they invade the root (Rovira 1969), and it is possible that white varieties could initiate an increased production of exudates. This increased production could create a gradient of attractive compounds that make it easier for *M. hapla* to find the roots or make those roots more attractive, thereby increasing infection. However, differing root exudates in different *V. vinifera* varieties has not yet been evaluated but could possibly explain why white varieties had significantly higher RF values than red varieties.

Another possibility is that red and white *V. vinifera* varieties differ in their post-infection resistance mechanisms. A form of post-infection resistance is the release of compounds toxic to *Meloidogyne* spp. For instance, phenolics are believed to be responsible for disease resistance and have been shown to increase resistance to *Meloidogyne* spp. (Huang 1985). Singh and Choudhury (1973) found that tomato varieties with the highest phenolic content were immune to *Meloidogyne* spp., while

susceptible tomato cultivars had the lowest phenolic content. Basha et al. (2005) reported that the wines produced from red and white *V. vinifera* varieties differ in phenolic composition, with some white wines having no phenolic content; this could explain why red varieties, which have a higher phenolic content than white varieties, are poorer hosts and have a less conducive environment for *M. hapla*.

While the same trend in host status of the *V. vinifera* varieties was observed in both years, across *V. vinifera* varieties there were 150% higher reproduction in 2012 compared to 2013. This may be explained by several aspects. First, 2012 was a considerably hotter year than 2013, with 511 more NDD. The higher number of degree days in 2012 would lead to faster development of *M. hapla* (de Guiran and Ritter 1979) allowing the nematodes to complete its lifecycle more quickly and allowing more generations to occur; *Meloidogyne* spp. require 6,500-8,000 heat units to complete its lifecycle (de Guiran and Ritter 1979). Second, as a result of the hot summer in 2012, the grapes were struck with heat stress in late July leaving about one-third the grapes significantly stunted, from which they did not fully recover. With the grapes already heat-stressed resulting in a weakened defense system, it is possible that they were more susceptible to higher invasion rates of later generations of *M. hapla* (Rahman et al. 2012). Another reason that could explain why the RF values differed is that in 2013, the fertigation system malfunctioned soon after nematode inoculation occurred and the grapes were not properly fertilized the first month. This resulted in smaller root systems compared to 2012 (Tables 3.2 and 3.3) with fewer potential infection sites for *M. hapla* to invade; potentially allowing fewer nematodes to invade the roots. Finally, due to the grapes starting to senescence early in 2013, that trial was taken down one month earlier than the 2012 trial. This shortened the time for subsequent generations of *M. hapla* J2 to invade the roots and complete their lifecycle. The same trends were observed for both years despite differences in experimental environments, making the results more robust.

Vitis rootstocks evaluated as part of this research provides additional information on the relative susceptibility to plant-parasitic nematodes (Table 3.7;

Ferris et al. 2012). There are few studies that have evaluated the host status of *Vitis* rootstocks to *M. hapla* (Ramsdell et al. 1996; Stirling and Cirami 1984; Lider 1960). Therefore, this data is very important in broadening rootstock resistance information for this nematode. Our results indicate that only Riparia Gloire and 101-14 supported *M. hapla* reproduction; however, these rootstocks would still be considered poor hosts (RF < 1) for *M. hapla*. Salt Creek, Freedom, Harmony, St. George, 3309C, 110R, 420A supported no nematode reproduction (RF = 0) and therefore would be considered non-hosts and resistant to *M. hapla*. Similarly, Lider (1960) also 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 Cuain (1976) and Ramsdell et al. (1996) found Riparia Gloire and 3309C to be susceptible to *M. hapla*, respectively.

Table 3.7. Resistance and susceptibility of *Vitis* rootstocks to plant-parasitic nematodes commonly encountered in vineyards. Table adapted from Ferris et al. (2012).

Genotype	*M. incognita Race 3*	*M. javanica*	*Meloidogyne pathotypes Harmony A&C*	*M. chitwoodi*	*M. hapla*	*X. index*	*M. xenoplax*	*P. vulnus*	*T. semipenetrans*	*X. ameriacanum*	*Para. hamatus*
101-14Mgt			R		R	S	S	MR			S
110Richter			MR		R	S	S	S			S
3309Couderc	S	S	S		R	MS	S	S	S	S	S
420A			R		R	S	S	MS			S
Dog Ridge	R	R	S		R	S	S		MR	MR	MS
Freedom	R	R	S	S?	R	R	MS	MS	S	MS	MR
Harmony	R	R	S	S	R	MS	S	S	S	S	S
Ramsey	R	R	S	S?	R	MR	S	MS	MS	S	S
Riparia Gloire			R		R	R	S	MR			S
St. George	S		S		R	S	S	MS			MS

R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.

While the majority of the *Vitis* rootstocks evaluated in this trial would be considered non-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. 2012; Anwar and McKenry 2002; Anwar and McKenry 2000; Ferris et al. 1984). For example, Ferris et al. (2012) reported that in Harmony, there is a hypersensitivity reaction 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.

Even though a grape is known to be resistant to a certain nematode, that resistance can be overcome and the widespread use of rootstocks can result in the

emergence of new virulent pathotypes. For example, there was an emergence of new virulent pathotypes of *M. incognita* and *M. arenaria* from the wide usage of Harmony (Ferris et al. 2012; Esmenjaud and Bouquet 2009; Anwar and McKenry 2002; McKenry 1992; Cain et al. 1984). Also, the overuse of Freedom selected for virulent pathotypes of *Meloidogyne* spp. (Ferris et al. 2012), and Cain et al. (1984) similarly reported that rootstocks with only partial resistance select for new damaging nematode pathotypes. It is also important to note that natural variability exists in nematode populations. For instance, Santo and Hackney (1980) discovered that different populations of *M. hapla* recovered from three different crops vary in chromosome number, pathogenicity, and susceptibility to crops. In this study, *M. hapla* populations from alfalfa (*Medicago sativa*) and red currants (*Ribes rubrum*) had differing reproduction on Concord grapes compared to populations recovered from Concord grapes. This demonstrates that there is genetic diversity among *M. hapla* populations, and may explain why other researchers reported contrary resistance/susceptibility results to our findings. Only one population of *M. hapla* (from Veneta, Oregon) was used in our experiments to examine the host status of commonly grown *V. vinifera* and *Vitis* rootstocks, and as indicated by Santo and Hackney (1980), it may be possible different populations of *M. hapla* have differing resistance and susceptibility.

This research greatly expands the knowledge of the host status of several *V. vinifera* varieties and *Vitis* rootstocks to *M. hapla*. It provides Washington grape growers with valuable information to select appropriate planting material to minimize the impact of *M. hapla* on vine productivity. This will play a crucial role when establishing a new or replanting a vineyard with *V. vinifera* own-rooted vines in areas with high *M. hapla* population densities. It will also help wine grape growers in the Pacific Northwest select the proper rootstock to plant in *M. hapla* infested fields. However, future research should expand *Vitis* rootstock screening to include additional populations of *M. hapla* to make the non-host status findings of these rootstocks more robust. 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 *V. vinifera* varieties and *Vitis* rootstocks to determine if similar results are obtained and to begin to determine the pathogenicity of *M. hapla* to grapevines in a field setting. More research is also needed to understand the mechanisms of resistance and susceptibility of *Vitis* planting materials to *M. hapla*, since very little research has investigated the mechanism of nematode resistance in grape (Lider 1954). Finally, the mechanism behind why white *V. vinifera* varieties are better host than red varieties should be elucidated by characterizing root exudates, root morphology, and what part of the *M. hapla* life cycle is susceptible to these difference. It would also be interesting to study if this phenomenon applies to other *Meloidogyne* spp. in grapevines as well.

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Chapter 4: Conclusions

A major concern that Washington grape growers face is plant-parasitic nematodes. Plant-parasitic nematodes are a global pest, estimated to annually cause $100 billion economic loss in agriculture worldwide with $6 billion loss in the United States (Sasser and Freckman 1987). Unfortunately, little is known about the biology and impact of plant-parasitic nematodes in Washington *Vitis vinifera* vineyards, the second largest wine producing region in the United States. Zasada et al. (2012) conducted surveys in Washington to determine the plant-parasitic nematodes associated with semi-arid *V. vinifera* vineyards. The most commonly encountered plant-parasitic nematodes were *Meloidogyne hapla*, *Paratylenchus* sp., and *Xiphinema americanum* detected in more than half of the sampled vineyards; *Pratylenchus* sp., and *Mesocriconema xenoplax* were also discovered. Of these sampled vineyards, 27% contained damaging population densities of *M. hapla*, making *M. hapla* the most important plant-parasitic nematode in Washington vineyards. The limited availability of effective nematode management options in addition to the Washington grape industry's perception that plant-parasitic nematodes limit production makes plant-parasitic nematodes a threat to the long-term productivity of grapevines. Knowledge of how these plant-parasitic nematodes are distributed in Washington vineyards and the host status of commonly planted *V. vinifera* vines in Washington to *M. hapla* will help growers better manage these production-limiting pests.

As a result from our studies, the spatial distributions of *M. hapla*, *Paratylenchus* sp., *X. americanum*, *Pratylenchus* spp., and *M. xenoplax*, and the factors that influence their distribution in semi-arid eastern Washington vineyards are now known. Soil moisture was shown to be the driving force in these vineyards, influencing both fine root biomass and population densities of most of the plant-parasitic nematodes. Soil moisture declined with depth and was located predominately under the irrigation emitters in the vine row. The majority of fine roots were also located in the vine rows. *Meloidogyne hapla* and *M. xenoplax* had similar distributions

in the vineyards with higher population densities detected in the vine row indicating these nematodes aggregate in the root zone where soil moisture and fine roots are abundant. *Meloidogyne hapla* and *M. xenoplax* were also influenced by depth with the majority of these nematodes recovered in the upper 45 cm and 30 cm of the soil profile, respectively. The fact that *Pratylenchus* spp. population densities were higher near the alley ways implies that grapes are not the main host for this nematode in semi-arid Washington vineyards. This is further supported by the shallow distribution of this nematode, with most *Pratylenchus* spp. found in the upper 45 cm of the soil indicating that shallow rooted plants, such as weeds and cover crops, are the preferred host for this nematode. *Xiphinema americanum* and *Paratylenchus* sp. had no consistent distribution in both vineyards. However, both *Paratylenchus* sp. and *X. americanum* (although not significantly supported) were influenced by depth with the majority of these nematodes located in the upper 45 cm of soil. Although *Paratylenchus* sp. had high population densities in both our sampled vineyards, the effect that *Paratylenchus* sp. has on grapevines is minimal (Pinkerton et al. 1999).

When targeting these plant-parasitic nematodes, grape growers should concentrate their management tools in a 1.0 m horizontal band around the vine row and in the upper 45 cm of the soil profile, where the majority of fine roots, soil moisture, and two economically important plant-parasitic nematodes, *M. hapla* and *M. xenoplax*, are located. Although this method has not been implemented before in vineyards, results from this research indicate this unique management tool has promise and will reduce the economics of soil fumigation. Other novel management methods that could be implemented due to the results of this research include off-set planting when replanting a vineyard, or planting new grapevines in the old alley ways as opposed to the old vine rows. This permits the vines to grow in relatively nematode-free soil, allowing the grapevine to become successfully established and better able to withstand high pest pressures. Also, changing the emitter spacing in vineyards with sandy soils is a possibility. *Meloidogyne hapla* and *M. xenoplax* were concentrated

directly under the emitters in the Chardonnay vineyard; changing the location of where the emitters release water will result in a new spring of root growth under the new emitter location where no to low population densities of plant-parasitic nematodes exist. However, this is not applicable in the Riesling vineyard due its soil type and soil moisture's and the plant-parasitic nematode's widespread distribution in the vine row; therefore, knowledge of the soil type is important. A novel post-planting technique is using nematicides only where concentrations are known to be the highest in the vineyard; for example, applying nematicides, such as Enzone (sodium tetrathiocarbonate) and Cordon (1,3-dicholoropropene), through the drip line to specifically target nematodes in the vine row. This research also directs away from certain cultural controls when managing plant-parasitic nematodes, such as cover cropping. Cover cropping in this production region would be ineffective due to the fact that most of the plant-parasitic nematodes present in these vineyards are not located in the alley way, and *Pratylenchus* spp., located in the alley ways, do not have grape as their primary host.

All of the *V. vinifera* varieties/clones screened in the greenhouse experiments would be considered excellent hosts for *M. hapla*; clones within a variety did not differ in host status. The level of susceptibility of *V. vinifera* varieties to *M. hapla* in decreasing order was: Chardonnay, Riesling, Cabernet Sauvignon, Syrah, and Merlot. The magnitude of increase in population size of *M. hapla* on white (Chardonnay and Riesling) compared to red (Cabernet Sauvignon, Merlot, and Syrah) varieties was significantly greater. White varieties had higher *M. hapla* eggs/g root and an almost 40% higher reproduction factor (RF) value than red varieties. These results indicate that white varieties are at a greater risk of supporting damaging population densities of *M. hapla* than red varieties. Of the *Vitis* rootstocks screened, only Riparia Gloire and 101-14 supported *M. hapla* reproduction; however, these rootstocks would still be considered poor hosts for *M. hapla*. The rootstocks Salt Creek, Freedom, Harmony, St. George, 3309C, 110R, and 420A supported no *M. hapla* reproduction and therefore

would be considered non-hosts and resistant. These results are very valuable to Washington grape growers. Even though the majority of the vineyards in Washington grow strictly own-rooted *V. vinifera* vines, as nematode pressures increase and the climate shifts, Washington grape growers may need to switch to rootstocks to maintain productivity. Our combined *V. vinifera* and *Vitis* spp. rootstock data will provide Washington grape growers with the knowledge to select appropriate planting material to minimize the impact of *M. hapla* on grapevines in semi-arid Washington vineyards.

Future research should be implemented to make these findings more robust. For the host status trials, additional populations of *M. hapla* should be screened to confirm the accuracy of our results and field evaluations in Washington of *V. vinifera* varieties and *Vitis* rootstocks should be conducted to determine the pathogenicity of *M. hapla* to grapevines in a field setting. Also, mechanisms of resistance and susceptibility of *Vitis* planting materials to *M. hapla* should be elucidated. For the spatial distribution studies, future research should expand sampling to additional vineyards, especially areas planted with red varieties. In addition, knowledge of the seasonal population dynamics of plant-parasitic nematodes will help growers know when to best manage nematodes in Washington. In conclusion, our results provide Washington grape growers with the knowledge of where plant-parasitic nematodes are located in their vineyards enabling them to target specific areas within the vineyard for nematode management and allow them to select the best *Vitis* varieties to reduce the impact of *M. hapla* in infested fields. The results of this research greatly expand the knowledge of the biology, host status, and spatial distribution of plant-parasitic nematodes in semi-arid Washington vineyards.

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