PLANT-PARASITIC NEMATODES IN CHILEAN VINEYARDS

E. Aballay1*, P. Persson2, and A. Mårtensson3

1Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile; 2Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden, SE- 750 07 Uppsala, Sweden; 3Department of Soil Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, SE- 750 07 Uppsala, Sweden. *Corresponding author: eaballay@uchile.cl

ABSTRACT


A survey to detect the presence and distribution of plant-parasitic nematodes in Chilean vineyards was undertaken during the years 2006 and 2007 covering an area of 40000 km², over 90% of the surface area cultivated with Vitis vinifera L. Descriptive statistics relative to the distribution observed, number of nematodes per taxa and sample was performed. Correspondence analysis and canonical correspondence analysis were performed to determine relations between nematodes taxa with cultivars and soil type. Twelve nematode genera were extracted from 1818 soil samples, but only four were considered to be highly pathogenic to the crop, Xiphinema spp. (X. index, X. americanum sensu lato), Meloidogyne spp. (M. ethiopica, M. spp.), Mesocriconema (M. xenoplax) and Tylenchulus (T. semipenetrans). The population densities of determined taxa were fairly variable; however, at least one of the species was present in every sample in high density. No correlations between nematode species were observed, nor was there a tendency of two or more nematodes to occur together. The total variance of nematodes was only marginally explained by cultivars and soil with an incidence of 18% and 0.5%, respectively, and an unexplained variation of 81.3%. These data indicated that environmental factors or management practices not measured in the survey greatly influenced nematode populations, and that previous selection of the variables to be evaluated should include some of them, like irrigation, tillage or fertilizers, especially under intensive agriculture.

Key words: Chile, grapes, Meloidogyne ethiopica, Mesocriconema xenoplax, multivariate techniques, plant-parasitic nematodes, survey, Tylenchulus semipenetrans, Xiphinema.

RESUMEN


Durante 2006 y 2007 se realizó una prospección de nematodos fitoparásitos en viñedos en la zona central de Chile, cubriendo una superficie aproximada de 40.000 km², equivalente a más del 90% de la superficie donde se cultiva Vitis vinifera L. Los datos obtenidos se analizaron primeramente mediante estadística descriptiva y luego mediante análisis de correspondencia para determinar posibles relaciones entre taxas y luego a través de un análisis de correspondencia canónica para identificar el efecto de las variables ambientales seleccionadas, cultivares y tipo de suelo, sobre la distribución y frecuencia de poblaciones. Doce géneros de nematodos fitoparásitos fueron detectados en un total de 1818 muestras analizadas, de los cuales sólo cuatro se consideran altamente patogénicos al cultivo, Xiphinema spp. (X. index, X. americanum sensu lato), Meloidogyne spp. (M. ethiopica, M. spp.), Mesocriconema (M. xenoplax) y Tylenchulus (T. semipenetrans). El 100% de la superficie prospectada presenta algún grado de infestación por alguno de estos géneros, normalmente con niveles altos y con una distribución heterogénea entre muestras. No se observaron asociaciones entre los distintos nematodos analizados, mientras que de las variables ambientales analizadas, los cultivares explican el 18% de las variaciones de poblaciones determinadas y el tipo de suelo sólo un 0.5% del total, siendo ambos
INTRODUCTION

Grape (Vitis vinifera L.) is an economically important crop in Chile. The current area of vineyards is about 190,000 ha, however it is expected to grow over 20% within the next 10 years. Wine grape varieties are prevalent, and cover about 65% of the total area, while the remaining area is shared between table grapes and varieties for liquor production (SAG, 2005).

Chile presents ideal conditions for grape cultivation due to optimal environmental factors and minor sanitary problems with common pests such as downy mildew. Most of the cultivated grape varieties are not grafted since Chile is devoid of the grape aphid (Daktilosphaera vitifolii). However, problems with plant-parasitic nematodes are increasing. Crop damage caused by plant-parasitic nematodes, often in association with plant pathogenic fungi and bacteria is common (Valenzuela y Aballay, 1996), and consists of loss in plant vigor, quality, and even plant death in sensitive varieties such as Chardonnay. Therefore, surveys were undertaken to detect the presence of plant-parasitic nematodes, their soil population densities and impact on the crop (Gonzalez, 1984; Valenzuela et al., 1992).

The ectoparasitic nematode Xiphinema index is the most reported nematode in the country, mainly in table grapevine, given its ability to efficiently reproduce and reduce plant growth and also to transmit virus. A similar situation occurs with the X. americana group. All cultivars are sensitive to the genus, however Sultana, Red Globe, Flame Seedless and Superior are more affected, showing a drastic growth reduction when the nematode level is high.

On the other hand, Meloidogyne species are frequently found associated to root systems of grape plants, being especially harmful in wine grape varieties, like Chardonnay, Cabernet Sauvignon, Merlot, or Shiraz. Four Meloidogyne species are reported, but the most frequently found is M. ethiopica (Carneiro et al., 2007). The high population density of these parasites is a permanent problem that farmers are facing. Multiple classical control methods and strategies have been employed for the infested soils including shallow and/or tolerant rootstocks, soil fumigation in replant situations, soil solarisation, nematicides, and others (Gonzalez, 1989).

The information obtained through surveys has served as a guide to develop management programmes aimed at minimizing yield losses (Gonzalez, 1989). However the information achieved from these works is not sufficient to identify the main factors regulating distribution and densities of nematodes in different regions and it is not possible to develop new strategies in addition to using chemical nematicides and amendments. Most of the current information is related to the incidence of soil characteristics, plant hosts, weather conditions, (Wallace, 1983; Cadet et al., 2004) and root distribution (Ferris et al., 1974).
Soil texture has been pointed out as regulating the spatial patterns of soil nematodes, especially at field level (Rossi and Quénéhervé, 1998, Avendaño et al., 2004), resulting in an increase of nematodes under coarse textures, but with variations depending on the genus present (Wallace et al., 1993, Prot et al., 1981). According to Popovici and Ciobanu (2000), some other variables, like soil pH, total nitrogen, humus content and exchangeable bases could explain variations in the composition of nematodes communities, but no single factor could be selected as being of overriding importance. Soil moisture may be one element of major importance under irrigated conditions (Guiran and Demeure, 1978; Towson and Apt, 1983), a normal practice in Chilean vineyards. At regional scale the soil texture influence is probably more diverse, but other gradients are suggested to have a greater influence, such as biogeography, climate or some other edaphic factors (Neher et al., 2005).

A survey covering ample areas involves and relates a large variety of soils, increasing the probability of finding variables that help explain nematode distribution; nevertheless, many other unknown factors may appear and produce greater data dispersion. The main purpose of this work was to determine the presence and densities of nematodes parasitic to grapes in the major grapevine cultivated area in Chile. A second objective was to determine the effect of soil type and cultivars on the nematode populations recovered.

MATERIALS AND METHODS

Soil samples were collected from a number of vineyards that belong to five different climatic regions of Chile (IGM, 2005) from Atacama in the north to Libertador General Bernardo O’Higgins in the south. During 2006 and 2007, soil samples were collected in spring and summer (from October to March). The sampled regions/vineyards are located between latitudes 26°00' and 34°58'S and longitudes 69°31' to 71°09'W and comprise an area of about 40000 km² (Fig. 1), more than 90% of the total grape cultivated area in Chile. Annual rainfall measurements report differences between northern and southern regions, from 2 to 800 mm, respectively, and a dry period that vary between 11 and 6 month for northern and southern regions, respectively, which makes summer irrigation necessary in most of the cultivated area. Maximum and minimum averages temperatures are 36°C and 20°C for north and 33°C and 18°C for south during the summer. The period of plant active growth, previous to enter dormancy also varies between 7 and 9 months for vineyards from northern and southern regions, respectively.

Soil and root samples were taken from productive vineyards at least four years old. Selection of the sampling sites was based on plant age and grape cultivar criteria, excluding those showing serious root problems due to fungi, bacteria, or insect damage. Samples were collected using a shovel to dig 25-35 cm deep, where most of feeder roots are present. About 25 subsamples were taken at random to make a ca. 2-kg sample covering up to four hectares in the same soil series. Each subsample was collected from a different plant, selecting those growing under similar conditions and that represented the average of the sampled crop. These subsamples were mixed, kept in plastic bags and stored at 8°C until they were processed about three weeks later.

Nematodes were extracted from a 250 cm³ soil volume combining the sieving and decanting method with Baermann’s funnel (Southey, 1986; Hooper and Evans, 1993) using sieves of 710 µm, 250 µm, 150 µm,
Fig. 1. Area planted with vineyards under survey (shadowed), equivalent to five regions and a total surface of about 40,000 km².
and 45 μm. The final suspension was decanted on a filter paper during 48 hours. To obtain optimal recovery of adults and of the fourth juvenile stage of *Xiphinema* spp., the soil samples suspended in water were sieved through the 750 and 250 μm sieves only, and then filtered through a nylon sieve of 90 μm for 24 hours (Brown and Boag, 1988).

Numbers of nematodes recovered from 250 cm³ of soil were not corrected for extraction efficiency. Genera and species identification was made using a dissecting microscope.

*Meloidogyne* species were identified using enzyme phenotypes, specifically esterase and malate dehydrogenase (Carneiro *et al*., 2007). Not all the samples infested with *Meloidogyne* were analyzed to identify species, only those with higher populations.

The sampled soils were matched to the soil series description previously determined through soil surveys published by the Chilean government which describe over 350 Series for the area under survey (CIREN 1996a, 1996b). These soil descriptions are according to the international scheme (Soil Survey Division Staff, 1993) and consider the physical and chemical characteristics of soils, using maps constructed on a scale of 1:20,000.

Identified soil types were sorted into three general texture groups, 1 = sandy soils, 2 = loamy soils, and 3 = clayey soils, with original sub divisions or textural classes arranged into these three categories as suggested by Rice (2002) to maximize differences between soils. Mainly due to their origin, Chilean soils are characterized by high variability concerning series, phases, variations and associations. This means that significant differences exist between neighbor farms, and even between different parcels of the same vineyard.

Sampling was restricted to ten cultivars, five of the most commonly cultivated table grapes and five of the most commonly cultivated wine grapes, considering only ungrafted plants since rootstocks are not frequently used in Chile. The management of table grapes is similar along the production areas studied; use of drip irrigation, soil pH varying from 6.6 to 7.8, practice of gibberellin applications to promote growth in seedless fruits, fungicides to control gray mold and powdery mildew, and also some insecticides to control mites and *Pseudococcus* spp.

Unlike table grapes, pests and diseases cause fewer problems in wine grape production therefore most of the pesticides are not necessary and no hormones are used to increase berry size. Also, the volume of water applied for irrigation is about 10% of that used for table grape production.

**Statistical methods**

Descriptive statistics were calculated by nematode per taxa and sample, relative to the distribution and number of nematodes observed. The relation between the numbers of nematodes per taxa was first analyzed by correspondence analysis (CA) to identify associations between them without environmental influences (Leps and Smilauer, 1999; Shi, 1993). Canonical correspondence analysis (CCA) was then performed to determine the distribution of nematode taxa in relation to environmental variables, in this case type of soil and grape cultivars. The samples were arranged according to nematode species composition with explanatory variables as a constraint in a canonical optimization process. The direct gradient procedure was performed with CANOCO software, version 4.5 (Ter Braak and Smilauer, 2002). This kind of analysis provides the possibil-
ity of relating variations in populations of different nematode species to environmental factors (Okland, 1996).

Soil type was considered a quantitative variable ranging from sandy to clayey based on studies that indicate better conditions for nematode movement and reproduction are present as the sand percentage and total porosity increase (Jones et al., 1969). Cultivars were nominal variables, then transformed to values of 0 and 1 (dummy variables) according to their absence or presence, respectively. Relationships between the composition of nematode community and the impact of each on environmental data were investigated by using forward selection of environmental variables in CCA. Environmental variables were added as long as the significance level of the partial Monte Carlo permutation test was below 0.05 (Leps and Smilauer, 1999). To evaluate the effects of both different groups of explanatory variables upon the species data, a variance partitioning procedure was performed to determine the relative contribution (proportion of total variation explained) of each variable set on the total variance and their overlap (Ramette, 2007).

Nematode abundance was transformed as log (x + 1) to normalize data prior to application of multivariate analysis, as suggested for nematode counts that are skewed, normally a negative binomial distribution (Noe, 1985; Neher et al., 2005).

RESULTS

Twelve nematode genera were extracted from 1818 soil samples analyzed, but only 4 were considered be highly pathogenic to Vitis root system in Chile. Those encountered at very low densities were not considered in further analysis, i.e. Pratylenchus spp. (P. thornei, Pratylenchus sp.), Paratylenchus spp., Paratrichodorus spp. (P. minor, P. teres), Hemiciclyophora spp., Helicotylenchus spp., Tylenchorhynchus spp. and Zygolycophora spp. The most frequent genera occurring in large populations were Xiphinema (X. index, X. americanum sensu lato), Meloidogyne (three species, being M. ethiopica the most frequent), Mesocriconema (M. xenoplax) and Tylenchorhynchus (T. semi penetrans). Species of Xiphinema were the most frequent, present in 71% of the sampled area, and all the soil samples contained at least one of the four genera of plant parasitic nematodes (Table 1).

Table 1. Population densities of the main five taxa of plant parasitic nematodes per 250 cm$^3$ of soil associated with Vitis vinifera L. in the major production zones in Chile (n = 1818).

<table>
<thead>
<tr>
<th>Nematode</th>
<th>Infested samples (%)</th>
<th>Mean</th>
<th>S.D.</th>
<th>Range</th>
<th>C.V.</th>
<th>Skewness</th>
<th>Kurtosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xiphinema. index</td>
<td>48</td>
<td>160</td>
<td>380</td>
<td>0-3850</td>
<td>238.5</td>
<td>4.46</td>
<td>26.22</td>
</tr>
<tr>
<td>Xiphinema. americanum s.l.</td>
<td>48</td>
<td>67</td>
<td>213</td>
<td>0-3780</td>
<td>316.5</td>
<td>8.21</td>
<td>105.94</td>
</tr>
<tr>
<td>Meloidogyne spp. (j2)*</td>
<td>45</td>
<td>149</td>
<td>466</td>
<td>0-6816</td>
<td>312.5</td>
<td>6.72</td>
<td>65.76</td>
</tr>
<tr>
<td>Mesocriconema xenoplax</td>
<td>49</td>
<td>49</td>
<td>144</td>
<td>0-1860</td>
<td>293-8</td>
<td>6.48</td>
<td>55.02</td>
</tr>
<tr>
<td>Tylenchorhynchus semi penetrans(j2)</td>
<td>13</td>
<td>266</td>
<td>2224</td>
<td>0-41350</td>
<td>836.2</td>
<td>13.82</td>
<td>216.20</td>
</tr>
</tbody>
</table>

S.D. = standard deviation.
C.V. = coefficient of variation.
*Meloidogyne ethiopica was the most abundant species.
Considering a determined taxon, the population density was not homogeneous, as it is shown by descriptive statistics (Table 1). The frequency distribution of population sizes were highly skewed, with values over zero, meaning a deviation from the normal distribution, a variance much greater than the mean, high coefficient of variation, data that show a negative-binomial distribution and make necessary their log transformation prior to analysis. Plant-parasitic nematode populations of the different taxa analyzed may reach very high densities, according to the maximum levels detected, which in some way identifies the potential infestation feasible to achieve under certain environmental or management conditions. Even, more than two genera per sample at high levels may be present.

Relationships between plant-parasitic nematodes, grape cultivars and soil textures

Before evaluating the influence of the environment on the presence of the most important plant-parasitic nematodes in the vineyards, a correspondence analysis (CA) was performed to verify the existence of some gradient and the relation between samples and nematode species. The length of the longest gradient was 3.5, which showed a unimodal answer justifying the posterior use of constrained methods (Leps and Smilauer, 1999). Sample distribution (Fig. 2) shows the relationship between nematode genera and soil samples, and concludes that presence of the nematode species corresponds to all samples and geographical areas. Picture shows that most of the species appear unrelated each other and also with the samples distribution, being *Xiphinema index* the plant-parasitic nematode species that more frequently appears associated with the other nematodes, also pointing out its broader distribution.

The canonical correspondence analysis (CCA) between cultivars and nematode taxa shows a narrower relations between *Meloidogyne* spp. and wine grape cultivars, mainly with Chardonnay and Cabernet Sauvignon, meanwhile *Xiphinema* spp., *M. xenoplax* and *T. semipenetrans* are more associated with grape cultivars as indicated by their centroids (Fig. 3). Soil texture is an environmental vector, with a low influence over nematode distribution meaning that most of genera show a weak association with it. Sauvignon Blanc tends to be an outlier, but it was kept into the structure because is considered an important crop cultivar in different zones.

The first two axes, relating two cultivars and soil, explained 21 and 3% of the variation respectively, and a total of 27% of the variation was explained by the four axes. Both cultivar and soil variables, being assessed together, represent a 0.265 (Table 2), value corresponding to the sum of all canonical eigenvalues for a total inertia (variance) of 1.42, i.e. 18.0% (Table 3). However, considering each variable’s unique influence, but taking into account the other explanatory variable as a covariate, the canonical values are 0.254 and 0.01 for cultivars and soil respectively, both highly significant (*P* < 0.01) according to a forward selection and the Monte-Carlo permutation test (Table 2).

Soil effects on nematode populations appeared scarce compared to nominal variables, but incidence of each of the latter, ten cultivars plus the quantitative one is presented in Table 2. The five wine cultivars studied presented the major impact on nematode populations, the soil occurring in sixth position being followed by two seedless table grape cultivars. Three cultivars, Flame Seedless, Superior, and Red Globe were not statistically significant on the distribution of the plant-parasitic nematodes (*P* > 0.17).
As a way to quantify the effects and to overlap the explanatory variables upon the species data, a partitioning of the variance was performed (Table 3). Clearly, the nominal variables or cultivars as a whole have more influence than the soil on the nematode distribution, this last being 0.5% of the total variability of the response data and the share proportion is 0.2%. The unexplained variability (81.3%) is high.

**DISCUSSION**

Previous surveys identified the same genera and species, but only small areas were considered and populations detected were much lower (Valenzuela et al., 1992; Gonzalez, 1984). The survey hereby presented covers ample areas, and standard deviations of the nematode populations are much higher than the means, typical of aggregated distributions and for extensive surveys (Noe, 1985; Herve et al., 2005). The presence of plant-parasitic nematodes was not restricted to specific areas, since they were found in all sampled geographical zones (Fig. 2).

Despite the presence of twelve genera of plant-parasitic nematodes associated to

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**Fig. 2.** Detrended correspondence analysis (DCA) of plant-parasitic nematodes graphing the probability of different species occurring together and soil samples distribution around these species. Crosses represent samples (n = 1818). M. xenop = *Mesocriconema xenoplax*; Meloid = *Meloidogyne* spp.; Tyl. sem = *Tylenchulus semipenetrans*; X. am = *Xiphinema americanum* sensu lato; X. index = *Xiphinema index*.
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the root system of grape vines, only four have been reported as more aggressive, and causing economically important damage. It seems that their presence in most of the vineyards is partially the cause of lack of vigor in big part of the surveyed productive area, as a result of high nematode populations and of the frequent association of two or more genera simultaneously affecting different zones of the roots.

Presence of citrus nematode (*Tylenchulus semipenetrans*) had been detected in a previous survey, causing damage in grapes that were cultivated following citrus (Aballay and Navarro, 2005). However, management programs against this species are not frequent and may be due to lack of information about its distribution.

Based on recent studies and reports, most of the *Meloidogyne* species identified correspond to *M. ethiopica* (Carneiro et al., 2003; 2007), which has been often misidentified as *M. incognita*. This misidentification unfortunately led to the planting of grape varieties known to be resistant or tolerant to *M. incognita*. *Mesocrictonema xenoplax* is abundant and its importance in Chile is increasing. Currently, the use of chemical nematicides is the only method to control this nematode, since no rootstock has been reported to be tolerant or resistant to this species.

The genus *Xiphinema* is the main root pest of grapes in Chile. Two members of the genus which are widely present, *X. index* and *Xiphinema americanum sensu lato*, transmit the grape fan leaf virus (GFLV) and tomato ringspot virus (TomRSV) respectively, both viruses present in Chile (Auger et al., 1992). Therefore, a major concern of farmers and plant nurseries relates to the implementation of a reliable program of virus–free plant production. Only 29% of the surveyed fields were free of *Xiphinema* spp. In most of the cases, these fields are new production areas, coming from crops or natural vegetation non suitable for reproduction of this nematode. Several samples with low numbers of *Xiphinema* specimens were collected from areas with previous old vineyards, replanted with different cultivars of *V. vinifera*, and where no nematode population increase or symptoms of replant problem were observed (McKenry, 1999). This is an interesting situation, since it may be the resultant of certain biotic/abiotic factors combinations, and further work should be oriented to study this natural suppressiveness (Kerry, 2000).

No correlation or association between nematode species, or tendency of two or
more nematodes to occur together was observed after removing environmental variables (Fig. 2). The clearest trend is the low probability of *Meloidogyne* spp. and *T. semipenetrans* to be present in the same sample.

The influence of the soil environment on the dynamics of plant feeder is considered as the second most important factor after the host plant (Norton 1989; Cadet et al., 2004). The influence of the soil is the result of the action and interaction of several edaphic factors. There is some agreement in considering soil texture and structure as relevant variables, since they affect the size of soil pores and the existence of stable compound aggregates. Coarse structures and well-structured soils are suitable factors for a faster population growth (Jones et al., 1969; Avendaño et al., 2004).

In this research, we observed only a limited influence of soil texture on nematode populations, since high nematode populations densities were detected in both clayey and sandy soils. Vineyards that contain clayey soil types typically receive applications of CaSO₄ to improve porosity and soil structure, practice that may reduce differences in nematode population densities in different soil types. Activities like tillage also produce a looser soil, increasing pore

Table 2. Contribution of explanatory variables selected according to a forward selection on the presence and density of plant-parasitic nematodes in vineyards, accounted for the variables selected, explaining part of the total variance (0.265).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variability explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar Chardonnay</td>
<td>0.072**</td>
</tr>
<tr>
<td>Cultivar Cabernet Sauvignon</td>
<td>0.073**</td>
</tr>
<tr>
<td>Cultivar Sauvignon Blanc</td>
<td>0.042**</td>
</tr>
<tr>
<td>Cultivar Merlot</td>
<td>0.033**</td>
</tr>
<tr>
<td>Cultivar Carmenere</td>
<td>0.018**</td>
</tr>
<tr>
<td>Soil texture</td>
<td>0.009**</td>
</tr>
<tr>
<td>Cultivar Thompson Seedless</td>
<td>0.008**</td>
</tr>
<tr>
<td>Cultivar Crimson Seedless</td>
<td>0.008**</td>
</tr>
<tr>
<td>Total variance explained by variables selected</td>
<td>0.263</td>
</tr>
</tbody>
</table>

**statistically significant at the 0.01 level in the Monte-Carlo permutation test.

Table 3. Determination of the influence of cultivar and soil type on the size of nematode populations by means of the variance partitioning using total and explained variances.

<table>
<thead>
<tr>
<th>Source</th>
<th>Variance</th>
<th>% of total variance</th>
<th>% of explained variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars</td>
<td>0.255</td>
<td>18.0</td>
<td>96.2</td>
</tr>
<tr>
<td>Soil</td>
<td>0.007</td>
<td>0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Shared</td>
<td>0.003</td>
<td>0.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Residual</td>
<td>1.155</td>
<td>81.3</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>1.420</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
spaces. Clayey soils, with no amendments or mechanical work are not propitious for the development of the four nematode species that affect Chilean grapes.

The relation between soil texture and nematode densities has been reported not to be the same for all genera, since others factors like cations may have a significant influence. Cadet et al. (2004) found that *Xiphinema elongatum* population decreases as manganese (Mn) content increases, and *Criconemella* sp. prefers sandy soils with higher sodium levels and low effective cation exchange capacity (Wallace et al., 1993). Jones et al. (1969) reported that a clayey soil affects negatively the presence of *X. diversicaudatum*, nevertheless the specimens can penetrate and move through cracks or fissures.

All grape cultivars considered in this study are susceptible to the nematodes identified, and this is the most important factor determining high nematode populations. Only slight associations were found between *Meloidogyne* spp. and two wine cultivars, and similarly, between *Xiphinema* spp., *M. xenoplax* and *T. semipenetrans* and table grape cultivars. The cultivars have certain effect on nematode distribution, since wine cultivars are concentrated in the three southern regions, while table grapes are uniformly distributed in all regions. There was no relationship between cultivars and soil (Fig. 3), mainly because the same cultivar is cultivated on a wide range of soil textures, and the roots growth depends mainly on the irrigation system and water management.

Despite the fact that both explanatory variables considered, soil and cultivars, have a direct influence on the response variable, independent of other factors influence, the variance explained by them is considered low (18.5%), if compared to the high percentage of non explained variance (Table 3). These data indicate that the explanatory variables are not able to explain most of the presence of the parasites in the samples, and that other factors seem to be determinant on the presence of plant-parasitic nematodes. The identification of these factors is necessary in order to elaborate new management programs and strategies. Some of these unknown factors may be associated to nematode propagation through nurseries (Herve et al., 2005) and also differences in agronomic practices, such as irrigation.

The use of multivariate techniques to determine relations between plant-parasitic nematodes and also with several environmental variables is an alternative way to determine their effect on nematode dynamics, and on crop damage. Most of these techniques have been applied to large scale investigations on distribution and densities of plant and animals (Ramette, 2007) and in studies about nematode abundance, diversity, plant associations and soil characteristics (Popovici and Ciobanu, 2000; Hanel and Cerevkova, 2006). Their application in agricultural systems may have important implications for monitoring and management of plant-parasitic nematodes (Simmons et al., 2008; Cadet et al., 2004). In this study the use of multivariate analyses clearly demonstrates the relative importance of the variables evaluated and pictures the relationship between the different plant-parasitic nematodes hereby considered.

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