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RESEARCH PAPER

Control of plant-parasitic nematodes using cover crops in table grape cultivation in Chile

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Abstract

C. Baginsky, A. Contreras, J.I. Covarrubias, O. Seguel, and E. Aballay. 2013. Control of plant-parasitic nematodes using cover crops in table grape cultivation **in Chile Cien. Inv. Agr. 40(3): 547-557.** A study to evaluate the effect of a three-year rotation with cover crops for the management of plant-parasitic nematodes (PPNs) was performed in two vineyards producing table grapes (*Vitis vinifera* L.) in a semiarid region in the north of Chile. In the first vineyard, located in Copiapó valley (trial I), the crops used in the rotations or monoculture were fava bean (*Vicia faba*) cv. Aguadulce, rape (*Brassica napus var. napus*) cv L-456, forage turnip (*B. rapa var. rapa*) cv. Barkant, barley (*Hordeum vulgare*) cv. Aurora, oat (*Avena sativa*) cv. Urano-INIA, and mustard greens (*Brassica juncea*). At the second vineyard employed in the study, located in Huasco valley (trial II), the same crops and varieties were used in a rotation system with the incorporation of crop/goat manure mixtures or manure only at a rate of 10 Mg ha^{-1}. In both studies, two control treatments were included: a nontreated control and a chemical control in which ethoprop was applied at a rate of 7 kg ha^{-1}. The results from trial I indicated no significant differences between the cover crop rotation treatments and controls for *Xiphinema index* and for the other PPNs populations. In trial II, treatments 4 (manure) and 5 (manure and crop rotation) showed significantly lower values compared to treatment 2 (chemical control), with the highest *X. index* levels, but no differences from the control. No differences were detected for the other PPNs.

Key words: *Vitis vinifera*, crop protection, nematicidal plants, botanical nematicides.

Introduction

Grapevine (*Vitis vinifera* L.) is an economically important crop in Chile, with a cultivated area of approximately 189,000 ha (Guerrero and Gutiérrez, 2012). However, many soil-borne pathogens and pests can damage or completely destroy the new roots that are initiated in spring and after the fruit harvest in late summer. Several genera and species of plant-parasitic nematodes (PPNs) have been reported to cause economic damage to grapevines and are commonly found in vineyards; the most frequently found species are *Xiphinema index, Meloidogyne ethiopica, Mesocriconema xenoplax*, and *Tylenchulus semipenetrans* (Aballay

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et al., 2009). The presence of PPNs continues to be one of the most important problems affecting the root system of grapevines, with damage normally being reflected in lower production and, in some cases, total crop loss. Several studies have estimated that PPNs cause global losses of US$ 78 billion in agriculture, indicating an annual yield loss of 12.5% in table grapes (Smiley, 2005; Sasser and Freckman, 1987). The damage caused by nematodes varies depending on many factors, such as soil type, cultivar, climate, and crop management (Ferris and McKenry, 1974).

Multiples classical methods and strategies are used for the management of PPNs. Currently, the control of PPNs in Chile is based on the use of chemical nematicides, mainly carbamates and organophosphates, applied once or twice per year. Despite these treatments, the nematode population remains almost unchanged (Valenzuela and Aballay, 1996) due to the low residual effect of nematicides, the loss of efficacy with frequent irrigation, and the use of organic amendments, among other soil and application factors. Vineyards affected by PPNs eventually exhibit destroyed roots due to the direct damage by PPNs and/or secondary damage by several fungi associated with the roots systems, resulting in the necessary replacement of the plants before they are 15 years old, *i.e.,* achieving less than 50% of their potential productive life (Pinkerton *et al*., 1999; Montealegre *et al*., 2009). However, even after the affected plants are removed, the soil remains infested for many years due to the long-term persistence of PPNs in the deep soil layers (McKenry, 1999).

Soil fumigants and different rootstocks are alternative approaches under replanting conditions, though these strategies are not extensively used by most growers because the chemicals are fairly expensive and because rootstocks are typically not tolerant to all nematodes, *e.g., X. index* or *M. xenoplax* (Sellés *et al.,* 2012, Aballay *et al.,* 2009).

Although the control of PPNs in Chile is mainly based on the use of agrochemicals, crop rotations and their stubble management have been proposed as alternatives to chemical control (Aballay *et al*., 2004). The use of antagonistic plants has also been evaluated and employed in some vineyards. The modes of action of antagonistic plants have been reported to be the presence of nematotoxic root exudates that can affect PPNs (Bello, 1998) or the stimulation of root growth (Birch *et al.,* 1993). Several plant species have been evaluated for PPN management, including plants belonging the genera *Tagetes, Cosmos, Gaillardia, Zinnia* (Tsay *et al*., 2004), and such Brassicaceae as *Brassica napus* L., *Sinapis alba* L., and *Raphanus sativus* L. (Halbrendt, 1996). The nematicidal effect of 77 plants has been assessed in *in vitro*, in potted plants, and in field experiments in Chile, and it was determined that most were effective against *X. index* and *X. americanum s.l.* (Aballay *et al*., 2001, 2004; Insunza *et al.,* 2000, 2001). The effect of these cover crops depends on the species of plant selected, soil type, nematodes present, and crop management. Nevertheless, the improper selection of a plant may result in adverse effects to the main crop (McLeod, 1994).

Therefore, the aim of this study was to assess the effect of cover crops or intercrops on the nematode populations in two vineyards during three years in a semiarid region in the north of Chile.

Materials and methods

Location and edaphoclimatic characteristics of the vineyards

Two established 8-year-old, own-rooted vineyards located in the north of Chile, the Copiapo vineyard of cv. Flame Seedless (27º 36’ S, 70º 19’ W) in a 1.5 × 3.0-m distribution and the Huasco vineyard of cv. Red Globe (28º 37’ S, 70º 42’ W), Atacama Region, were selected (Figure 1). In the Copiapó vineyard, the soil is of the Amolanas Series (Typic

Haplocambid), consisting on an alluvial terrace, with moderately deep, well-drained soil (CIREN, 2007). The soil texture is mainly sandy loam, with coarse textures in the deeper layers. The Ap horizon is strongly saline, and there is a moderate effervescence with HCl along the soil profile. The climatic conditions (BWI; Juliá *et al*., 2008) are characterized by low winter precipitation (< 20 mm annual), with daily average temperatures fluctuating between 25 °C in summer and 5 °C in winter. In the Huasco vineyard, the soils are of the Cavancha Series (Xerollic Haplargids), are located in an alluvial remnant terrace with a flat topography, and are well drained. The texture is a loamy soil at the upper layers and a clayey loamy texture below 70 cm. The Ap horizon is not saline and does not react with HCl. The area is characterized by high temperatures in January, with an average of 26.5 °C and minimum of 5.7 °C in July without frosts, and precipitation of 25 mm (Osorio *et al*., 1995).

Treatments in the Copiapó vineyard

The trial was established in an eight-year-old grape cv. Flame Seedless vineyard using an overhead trellised system in a frame of 1.5 m × 3.0 m. Thirty-five plots were established and grouped into five completely randomized blocks with seven treatments (Table 1) based on the use of different annual crops in rotation (T3, T4, T7) or monoculture systems (T5, T6). Additionally, a chemical control treatment (ethoprop 7 kg ha^{-1}; T2) and a no treatment control were included.

The crops used in the rotations were as follows: fava bean (*Vicia faba*) cv. Aguadulce; rape (*Brassica napus* var. *napus*) cv. L-456; forage turnip (*Brassica rapa var. rapa*) cv. Barkant; barley (*Hordeum vulgare*) cv. Aurora; oat (*Avena sativa*) cv. Urano-INIA; and mustard greens (*Brassica juncea*). These annual crops were sown by hand on the ridge (1 m wide) of the vine row during the first week of January of each year (mid summer). Fava bean at a rate of 80 kg ha^{-1} was established in seven rows spaced 15 cm apart along the vine row and 15 cm within the row and inoculated with *Rhizobium leguminosarum* sv. *viciae* at a rate of 50 g of inoculants per 100 kg of seed. Forage turnip, oat, and barley were sown in a continuous stream in nine rows spaced 15 cm apart in the vine row at rates of 15, 95, and 70 kg

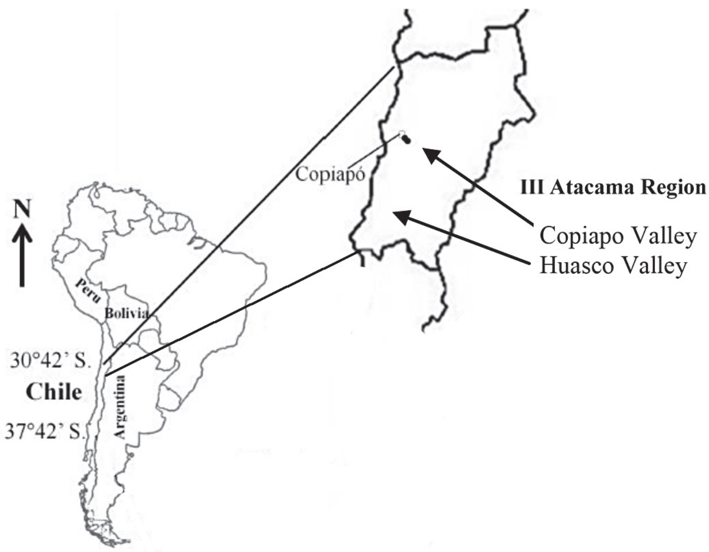

Figure 1. Map of northern Chile. Trial I was conducted in a vineyard located in Copiapo valley (27º 36' S, 70º 19' W), and trial II was conducted in a vineyard located in Huasco valley (28º 37' S, 70º 42' W). Both valleys are located in the Atacama region, Chile.

ha^{-1}, respectively. The plants were manually cut and incorporated into the first 20 cm of the soil just before grapevine bud brake in July of each year. Samples of the cover crops were collected for biomass determinations (data not shown).

A chemical control was applied on September of each year by adding the product directly to the root zone at four points 50 cm from the vine axis and at a depth of 30 cm using an injector. The vineyard had a drip irrigation system with a double line and drippers of 4 L h^{-1} separated by 1 m along the line.

Treatments in the Huasco vineyard

A monocrop system and the incorporation of a mixture of crops and manure were included in this trial. The trial was established in an eight-year-old cv. Red Globe vineyard using an overhead trellised system in a frame of 3.5 m × 3.5 m. In the year prior to establishing this trial, an amendment with manure was performed by incorporating goat manure (68.7% OM, pH 7.3, 1.7% total N, 355 mg kg^{-1} available P, and available K, Ca, and Mg at 36.3, 18.7 and 22.5 cmol kg^{-1}, respectively) at a rate of 10 Mg ha^{-1} to the entire vineyard.

Twenty-five plots were identified and grouped into five completely randomized blocks with four treatments (Table 1): control (T1); chemical nematicide control (ethoprop 7 k ha^{-1}) (T2); crop rotation (fava bean-mustard greens-oat) (T3); and crop rotation (fava bean-barley-mustard greens) established over manure (T4). The cultivars of fava bean, barley, mustard greens, and oat were the same as those used for Trial I, and the managements were essentially the same. These annual crops were sown by hand on and between the vines rows (T3) or on the manure (T5) during autumn (March) of each year. The seeding procedure was similar to that used in Trial I, but the T3 the crops were established in a total of 18 rows at 20-cm spacing. In this case, the seeding rate of fava bean, mustard greens, and oat was 200, 35, and 250 kg

Table 1. Treatments established in the Copiapó and Huasco vineyards during three consecutive seasons using cover crops.

			Season		
Vineyard	Treatment	Description	One	Two	Three
Copiapó	T1	Untreated	*	*	*
	T2	Chemical nematicide	Ethoprop	Ethoprop	Ethoprop
	T3	Rotation 1	Fava bean	Mustard greens	Oat
	T4	Rotation 2	Fava bean	Barley	Rape
	T5	Monoculture 1	Rape	Rape	Rape
	T6	Monoculture 2	Forage turnip	Forage turnip	Forage turnip
	T7	Rotation 3	Rape	Fava bean	Mustard greens
Huasco	T1	Untreated	*	*	*
	T2	Chemical nematicide	Ethoprop	Ethoprop	Ethoprop
	T3	Rotation	Fava bean	Mustard greens	Oat
	T4	Manure	Goat manure	Goat manure	Goat manure
	T5	Manure and rotation	Goat manure + Fava bean	Goat manure + Barley	Goat manure + Mustard greens

*No chemical control, without crop rotation and manure.

ha^{-1}, respectively. In T5, manure was applied on the vine row at a rate of 12.5 Mg ha^{-1} $year^{1}$, and the crops were sown over this amendment. Fava bean was planted in four rows spaced at 20 cm apart, with a plant spacing of 12 cm at a rate of 22 kg ha^{-1}, and barley and mustard were planted in a continuous stream at rates of 20 and 4 kg ha^{-1}, respectively. The vineyard was irrigated through a double irrigation line system with drippers of 4 L h^{-1} separated by 1 m along the line. The plants were manually cut and incorporated into the first 20 cm of the soil just before grapevine flowering in August of each year. Samples of the cover corps were collected for biomass determinations (data not shown). The chemical nematicide ethoprop was applied during the three growing seasons in the month of September (spring), the normal time for the application of these chemicals, following the same method stated above.

Prior to seeding the cover crops, an extra irrigation was applied in both trials but was based on the needs of vine during the season the management (fertilization and irrigation), according to the normal program used by the farmer. Weeds were manually controlled. The cover crops were allowed to develop until the application of a sprouting controller (hydrogen cyanamide) in July-August at which time the plants were manually chopped, distributed on the inter-row, and irrigated to support decomposition and the penetration of exudates into the soil.

Assessments

Soil and root samples were collected prior to the establishment of the trials. Using a shovel, samples were collected to a depth of 25-35 cm within rows where most of the feeder roots are present. Approximately 10 subsamples were collected at random to produce a 1-L sample. The subsamples were mixed, placed in plastic bags, and stored at 8 °C until they were processed approximately two weeks later. Nematodes were extracted from a 250-cm^3 soil volume by decanting/sieving through nested 710-, 250-, 150-, and 45-μm sieves (Southey, 1986). The final suspension was decanted onto filter paper and placed on a Baermann funnel for 48 h. To obtain an optimal recovery of adults and of the fourth juvenile stage of the species of *Xiphinema*, soil samples suspended in water were passed through 750- and 250-μm sieves only and then placed on a nylon sieve of 90-μm on a Baermann funnel for 24 h (Brown and Boag, 1988). Counting and identification were performed using a stereoscopic microscope (Zeizz, Stemi 2000-C, Göttingen, Germany).

Experimental design and statistical analyses

In the first trial (Copiapó vineyard), the experimental unit was a plot of 7.5 m long × 1.5 m wide containing 4 vines with their respective ridge (10 cm height). Nematode sampling was performed for the two central plants in each plot. In the second trial (Huasco vineyard), the experimental unit was a plot of 14 m long × 7 m wide containing 8 vines distributed in two rows of four plants per row. Considering a borderline effect, only the two central plants per row and the respective soil were included in the evaluations.

To evaluate the effect of the different treatments, the reproductive index (R) was calculated, which relates the final population (Pf) with the initial population (Pi) (Oostenbrink, 1966); the final population corresponds to that obtained at the end of the third season of the study. Prior to calculating R and performing an analysis of variance (ANOVA), the nematode population density data were transformed as log (x + 1) for normalization, as suggested for nematode counts that are skewed, with a normally negative binomial distribution (Noe, 1985). When significance at $P \leq 0.05$ was detected, the treatment means were compared according to Tukey's multiple range test.

Results

In the Copiapó vineyard, *X. index* was the major PPN founded, followed by *Meloidogyne* spp., *M. xenoplax, Paratylenchus* sp., *Pratylenchus thornei, Hemicycliophora* sp., and *T. semipenetrans*. The density of nematodes present in 250 cm^3 of soil for each taxon was as follows: *X. index*, 310; *Meloidogyne ethiopica*, 55; *Mesocriconema xenoplax*, 110; *Paratylenchus* sp., 21; and others in lower numbers and only occasionally detected. The effect of the treatments was assessed independently for *X. index* and the other PPNs present.

The effect of cover crops on the densities of *X. index* populations was not different between the chemical treatment and untreated control, though a clear tendency toward R values <1 was observed with some of the cover crops (Figure 2), mainly treatments 3 (rotation 1, fava bean, mustard greens, oat), 5 (monoculture of rape), and 6 (monoculture of forage turnip).

The effect of the treatments on the other PPNs present in trial I (Figure 2) was similar to that found for *X. index*, with no significant differences among the treatments, despite treatments 2 (chemical control), 3 (rotation 1, fava bean, mustard greens, oat), 4 (rotation 2, fava bean, barley, rape), 6 (monoculture of forage turnip), and 7 (rotation 3, rape, fava bean, mustard greens) exhibiting a lower R than the control treatment.

In the Huasco vineyard, the nematode densities detected prior to the establishment of the treatments was also dominated by *X. index* (460 nematodes/250 cm^3 soil), followed by *M. xenoplax* (70 nematodes/250 cm^3 soil), *P. thornei* (35 nematodes/250 cm^3 soil), and *M. ethiopica* (21 nematodes/250 cm^3 soil). When the treatment effects were assessed with regard to the variation of *X. index* populations (Figure 3), the R of treatments 4 (manure) and 5 (manure and crop rotation) showed significantly lower values compared to treatment 2 (chemical control). This result showed the null effect of the nematicide compared to the alternatives, with both presenting values lower than 1. Both treatments 4 and 5 included manure, which was applied during the three years of the study. No differences were detected when these treatments were compared to the control (Figure 3).

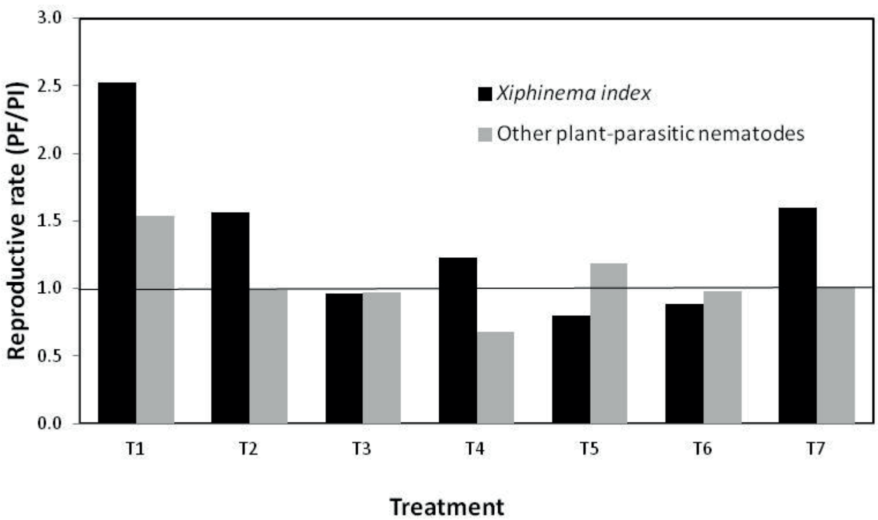

Figure 2. Reproductive index of *Xiphinema index* and other plant-parasitic nematodes in the Copiapó vineyard. T1, control; T2, chemical control (ethoprop); T3, rotation 1 (fava bean, mustard greens, Oat); T4, rotation 2 (fava bean, barley, rape); T5, monoculture 1 (rape); T6, monoculture 2 (forage turnip); T7, rotation 3 (rape, fava bean, barley, mustard greens). No significant differences were detected between the treatments according to an ANOVA test ($P \leq 0.05$)

The effect of the treatments on the other PPNs present in this vineyard showed that the populations remained at similar densities, without significant differences in R (Figure 3). The control exhibited an R value close to 1, revealing no variation in the nematode population over time. However, the chemical control had an R value greater than 1, similar to that observed with *X. index*. Treatments 4 and 5 also showed a tendency to reduce the PPN population densities.

Discussion

The results show that the incorporation of organic matter through the use of cover crops during a period of three years was not able to produce a significant decrease in PPNs under the conditions of these trials. *Xiphinema index* is a nematode that presents a wide distribution in the vineyards from this region; for many years, chemical nematicides have been the control method employed yet can be an environmental contaminant, and few effects have been achieved (Valenzuela and Aballay, 1996). The use of some nematicidal plants was able to reduce *X. index* population densities in potted plant experiments (Aballay *et al.*, 2004), but the size of the roots system in a productive vineyard would indicate that the infested area is much larger and that the impact of cover crops may not be sufficient as a control measure. Indeed, most of the studies using cover crops have been performed in combination with annual crops, with a much smaller root system.

The lack of control is not influenced by the crop used, as most of these nematodes are not able to reproduce on the crops, and only leguminous plants can serve as a host for *M. ethiopica*. Some studies under field conditions have reported promising results with certain plants, such as *Vicia villosa* and *Tagetes minuta*, grown between two successive vine crops, without incorporation into the soil, and without vines (Villate *et al.*, 2012). It is possible that the presence of the vine roots in our study is a permanent stimulus to maintain the growth of nematodes and that the effect of crops is not observed with the same intensity, as also occurred with the action of different cover crops on *X. americanum s.l.* in wine grape vineyards (Aballay *et al.*, 2001). In the Copiapó vineyard, the monocrops rape seed and forage turnip caused a reduction in the *X. index* populations, but it was not different from the treatment chemical

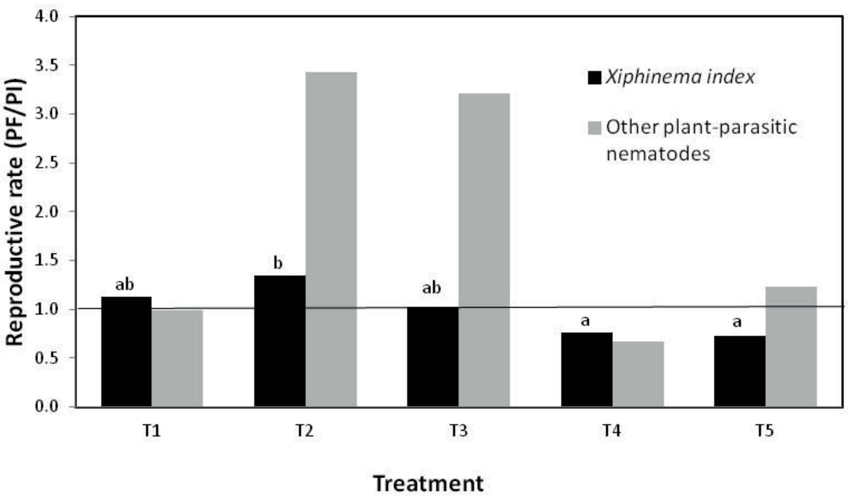

Figure 3. Reproductive index of *Xiphinema index* and other plant-parasitic nematodes in the Huasco vineyard. T1, untreated; T2, nematicide; T3, rotation F-M-O (fava bean, mustard greens, oat); T4, manure; T5, manure and rotation F-M-O. The different letters indicate significant differences according to Tukey's test (P≤0.05).

nematicide. In contrast, some monocrops can increase PPNs populations (Rahman *et al*., 2007). The same trend was observed with the use of goat manure for three years and the mixture of goat manure with fava bean, barley, and mustard green rotations in the Huasco vineyard. Indeed, the use of manure has proven be an efficient alternative for controlling different PPNs in several crops (Rodriguez-Kábana *et al*., 1987) and in grape vines (Rivera and Aballay, 2008). In this study, the observed differences may be due to the application rate, which has not been determined for different manures and conditions of grapevine growth. In our study, 10 Mg ha^{-1} was applied, which may be good for stimulating root growth. However, to have some incidence o nematode control it has been suggested manure amounts over 110 Mg ha^{-1} under complete under broadcast basis, or 30-40 Mg only to the equivalent surface planted (Mian and Rodriguez-Kábana, 2001).

The use of chemical nematicides was not able to decrease the nematode populations, showing that this approach is not different from the use of alternative management practices. In the Huasco vineyard, the reduced activity of ethoprop may have been influenced by the previous use of manure in this field, considering that organophosphate chemicals tend to be fixed in organic matter, thereby losing their efficacy (Bunt, 1987).

The use of manure and cover crops with manure tend to decrease nematodes, with recommendations of the use of crop rotations and crop residue retention for better results (Govaerts *et al*., 2007). The effect of the nutrients provided by the manure could support a better sanitary condition of the vines, decreasing the PPNs populations, as was observed by Rahman *et al.* (2007) for treatments with N fertilization in a long-term field experiment. In our study, due to the pest pressure and soil and weather conditions, there may not have been enough time to improve root growth and decrease PPNs activity.

Previous works with some of these crops have shown that barley is efficient in decreasing populations of *Meloidogyne hapla* (Bowman *et al*., 2000). In other studies performed by Scholte and Lootsma (1998) and Bauer *et al.* (2010), it was determined that the incorporation of oat plants prior to blooming decreased the population densities of *M. xenoplax* associated with potato crops and peaches, respectively. However, including oat was the least effective treatment in our study.

In previous studies with *V. faba* as a cover crop, it was demonstrated that the presence of *Nacobbus aberrans* and *Globodera* spp. in potato crops was decreased between 30% and 42% (Iriarte *et al*., 1998; Pacajes *et al*., 2002) after incorporation. The plants from this family may have some effect on nematodes due to their low C/N ratio, which favors the production of ammonia when the crop residues are incorporated (Mian and Rodriguez-Kábana, 2001), particularly under alkaline conditions, as in both of our study areas, with pH values between 7.3 and 8. In addition, some metabolites with pesticide action are produced, including lectins, rotenone, tephrosin, and deguelin (Bowman *et al*., 2000).

In conclusion, the results of the present study indicate that, under high pressure of PPNs in perennial crops, the use of the cover crops assessed here is not able to reduce the nematode populations and that the possibility of using manure plus crops must be assessed under increasing amounts of manure. Additionally, at least under the soil and weather conditions of our study, chemical control is not an effective alternative to consistently suppress the high nematode populations observed.

Resumen

C. Baginsky, A. Contreras, J.I. Covarrubias, O. Seguel y E. Aballay. 2013. Control de nemátodos fitoparásitos mediante el uso de cultivos de cobertera en parronales de uva de mesa en Chile. Cien. Inv. Agr. 40(3): 547-557. Se realizó un estudio destinado a evaluar el efecto de cultivos de cobertura en rotación durante tres años, sobre el control de nematodos fitoparásitos en dos plantaciones de uva de mesa en una región semiárida del norte de Chile. La primera plantación correspondió a un parronal en Copiapó (ensayo I), donde los cultivos utilizados en rotación o en monocultivo fueron haba (*Vicia faba*) cv. Aguadulce; raps (*Brassica napus var. napus*) cv L-456; rábano forrajero (*B. rapa var. rapa*) cv. Barkant; cebada (*Hordeum vulgare*) cv. Aurora; avena (*Avena sativa*) cv. Urano-INIA y mostacilla (*Brassica juncea*). El segundo parronal utilizado para el estudio está ubicado en la localidad de Huasco (ensayo II), donde se utilizaron los mismos cultivos y variedades, en un sistema de rotación, incorporándose además una mezcla de cultivos sembrados sobre guano de cabra y guano de cabra solo, en dosis de 10 Mg ha^{-1}. En ambos estudios se incluyó un control absoluto y un control químico, ethoprop, en una dosis de 7 kg ha^{-1}. Bajo las condiciones de este estudio, los resultados indican que en el ensayo I, no hubo diferencias significativas en los tratamientos basados en los cultivos de cobertura en rotación y el control de poblaciones de *Xiphinema index* así como en el resto de los fitoparásitos. En el ensayo II, solo se observan diferencias entre los tratamientos en base solo a guano y guano y rotaciones con el tratamiento químico, siendo este último el menos efectivo de todos los tratamientos. En el resto de los fitoparásitos, no hay diferencias después de tres años de evaluaciones.

Palabras clave: Nematicidas botánicos, plantas nematicidas, protección de cultivos, *Vitis vinífera*.

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