EVALUATION OF FIVE NEMATODE-ANTAGONISTIC PLANTS USED AS GREEN MANURE TO CONTROL *XIPHINEMA INDEX* THORNE ET ALLEN ON *VITIS VINIFERA* L.

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ABSTRACT

Aballay, E., R. Sepúlveda and V. Insunza. 2004. Evaluation of five nematode-antagonistic plants used as green manure to control *Xiphinema index* Thorne et Allen on *Vitis vinifera* L. Nematropica 34:45-51.

The ectoparasitic nematode *Xiphinema index* is economically important on grapevine (*Vitis vinifera* L.) in Chile, as a root parasite as well as a vector of Grapevine Fan Leaf Virus (GFLV). The objective of this study was to evaluate the effect of five nematode-antagonistic plants incorporated as green manure in greenhouse pot cultures on *X. index* maintained on grapevine, cv. Thompson Seedless. The antagonistic plants were: rapeseed (*Brassica napus* L. cv. Rangi), wormseed (*Chenopodium ambrosioides* L.); rue (*Ruta graveolens* L.), brown mustard (*Brassica juncea* Czern. & Coss. cv. Nemfix) and thyme (*Thymus vulgaris* L.). Their effects were compared with the following four treatments: barley (*Hordeum vulgare* L.); fenamiphos at 0.04%; grapevine plants inoculated with *X. index* as control; and grapevine with no further treatment. The incorporation of *B. juncea* cv. Nemfix reduced the nematode population by nearly 65% compared to the more than an 80% reduction with fenamiphos, with both treatments significantly different from all other treatments. The fenamiphos treatment resulted in the lowest root damage but caused a significant reduction of the total fresh weight of the grapevine plants, possibly due to phitotoxicity. At the rates of incorporation (2% W/V), brown mustard, thyme, wormseed and rue appeared to be well suited as potential green manure crops for control of *X. index* in grapevine.

Key words: allelopathy, *Brassica juncea*, *Brassica napus*, *Chenopodium ambrosioides*, fenamiphos, grapevine, grapevine fan leaf virus (GFLV), nematicidal plants, *Ruta graveolens*, *Thymus vulgaris*.

RESUMEN

Aballay, E., R. Sepúlveda y V. Insunza. 2004. Evaluación de cinco plantas con acción nematicida usadas como abono verde para el control de *Xiphinema index* Thorne et Allen en *Vitis vinifera* L. Nematropica 34:45-51.

Xiphinema index es un ectoparásito económicamente importante en la vid (*Vitis vinifera* L.) en Chile, tanto por su daño directo al sistema radical, como por ser vector del virus de la hoja en abanico de la vid (GFLV). El objetivo de este estudio fue evaluar el efecto sobre *X. index* de cinco plantas antagónicas a los nematodos, incorporadas como abono verde en vides cv. Thompson Seedless, cultivadas en macetas y bajo condiciones de invernadero. Las plantas antagónicas fueron: raps (*Brassica napus* L. cv. Rangi), paico (*Chenopodium ambrosioides* L.); ruda (*Ruta graveolens* L.), mostaza (*Brassica juncea* Czern. & Coss. cv. Nemfix) y tomillo (*Thymus vulgaris* L.). Sus efectos se compararon con cuatro tratamientos: cebada (*Hordeum vulgare* L.), fenamiphos al 0,04%, vides inoculadas con *X. index*, y vides sin tratamiento. La incorporación de *B. juncea* cv. Nemfix redujo las poblaciones del nematodo en casi 65% al compararla con mas de 80% de reducción con fenamiphos., siendo ambos tratamientos significativamente diferentes con los otros tratamientos. El tratamiento con fenamiphos causó el menor daño radical y de poblaciones de *X. index*, pero también redujo significativamente el peso total de las vides, probablemente por fitotoxicidad. En las dosis incorporadas (2% W/V), la mostaza, tomillo, paico y ruda, parecen ser potencialmente aptas para ser usados como abonos verde en el control de *X. index* en vides.

Palabras claves: alelopatía, *Brassica juncea*, *Brassica napus*, *Chenopodium ambrosioides*, fenamiphos, grape fanleaf virus (GFLV), plantas nematicidas, *Ruta graveolens*, *Thymus vulgaris*, vid.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops in Chile, covering an area of about 146,600 ha, of which 50,800 ha are intended for the export of fresh grapes, with Thompson Seedless as the most important cultivar.

Among the pests and pathogens associated with grapevine in Chile, plant parasitic nematodes in the genus *Xiphinema* spp. are of great significance, because of the direct and indirect damage they cause. *X. index* Thorne et Allen is considered the most harmful species in grapevine, especially in varieties for fresh consumption, because it reduces plant growth and is the vector of grapevine fan leaf virus (GFLV). In the Metropolitan Region of central Chile, soil cropped with grapevine, cv. Thompson Seedless, has an average infestation of 350 *X. index*/250 cm^3 of soil. Such densities are considered to be high, and in the northern regions of the country the levels are higher. Surveys carried out by González (1993) showed that *X. index* is present in at least 85% of the plantations between the central-north and central-south areas of the country and is commonly associated with the grapevine cultivars Sultanina and Flame Seedless.

Chemical nematicides, are widely used in Chile for nematode control. However, non-chemical alternatives have also been studied in Chile, such as the utilization of endemic and introduced plants with nematicidal, or nematode-antagonistic effects (Contreras, 2000; Insunza *et al.*, 2001a, 2001b). These researches were based upon the allelopathic activity of several types of plants, that release compounds into the microenvironment through root exudation, or tissue disruption, leaching from plants or residues and decomposition of residues. Various plant species, belonging to diverse botanical families, have been reported to contain or produce nematicidal compounds (Gommers and Bakker, 1988; Alam *et al.*, 1990; Chitwood, 2002). In agriculture it is feasible to use some of these antagonistic plants to control nematodes, whether in crop rotations, intercropping, as cover crops or as green manures (Rodríguez-Kabana, 1992).

Thymus vulgaris contains terpenoids of the thymol, geraniol and phenolic acid type. It is known as a medicinal plant (Rojas, 1999), probably due to thymol, that is slowly released in moist soils (Alonso, 1998). Thymol, with anthelminthic, antiseptic and antifungal properties, is a volatile, phenolic monoterpene present in the essential oil of thyme. Adding thymol to the soil drastically reduced final populations of *Meloidogyne arenaria*, *Heterodera glycines*, *Paratrichodorus minor* and dorylaimid nematodes, as well as the disease incidence of the endoparasites (Soler-Serratosa *et al.*, 1996). In in vitro biotests, *T. vulgaris* extracts showed nematicidal activity against *Xiphinema index* and *X. americanum* (Insunza *et al.*, 2001a, b). In field tests performed in Chile, however, the growing of thyme and its subsequent incorporation into the soil did not reduce *X. index* populations in table grapevines (Aballay and Insunza 2002), nor *X. americanum s.l.* density in wine grapevines (Aballay *et al.*, 2001). The diverging results between laboratory and field experiments should be considered in relation to the various methods of application.

Chenopodium ambrosioides is recognized as an anthelminthic plant and contains substances that can be antiviral, antifungal, antibiotic, nematicidal, insecticidal or allelopathic. The most active principle is an oil, consisting of up to 86% ascaridol, a terpenoid peroxide (Quarles, 1992; Munoz *et al.*, 2001). There have been reports documenting the in vitro nematicidal activity of wormseed against several plant parasitic nematodes, including *X. index* and *X. americanum s.l.* (Insunza *et al.*, 2001a, b), as well as against *Meloidogyne* spp. in potted soil (Garcia Espinoza, 1980). It was also effective in controlling *Ditylenchus dipsaci* in garlic bulbs (Insunza and Valenzuela, 1995).

Ruta graveolens is a medicinal plant with known anthelminthic properties, traced to its content of rutoside acid and alkaloids. Extracts from *R. graveolens* leaves showed nematicidal activity in vitro during the hatching of eggs of *Meloidogyne* spp. and *Heterodera schachtii* (Sasanelli, 1997) and against adult females of *Xiphinema index* (Sasanelli, 1992; Insunza *et al.*, 2001a) and *X. americanum s.l.* (Insunza *et al.*, 2001b). The chemical compounds responsible for the nematicidal activity of *R. graveolens* are still unknown. Testing some of them, including juglone, rutin and xanthotoxin, gave no satisfactory results (Sasanelli and Adabbo, 1995; Sasanelli 1997). However, extracts from this plant effectively controlled *Ditylenchus dipsaci* in garlic bulbs (Insunza and Valenzuela, 1995). When used as green manure on sunflower, rue leaves reduced the final population level of *Meloidogyne javanica* (Sasanelli and Adabbo, 1993).

There are few reports of *X. index* control by means of antagonistic plants under field conditions. However, data from in vitro tests showed that extracts of a number of plants proved to be toxic to this nematode (Dechet, 1991; Sasanelli, 1992; Contreras, 2000; Insunza *et al.*, 2001a). In a field experiment, eight plants were evaluated as cover crops and as green manures for table grapes and only *Brassica napus* significantly reduced the *X. index* population (Aballay and Insunza, 2002). These data indicate the potential of natural allelochemicals to control *X. index.*

The objective of this research, was to assess the effect of five green manure treatments on *Xiphinema index* and any subsequent positive or negative effect on the growth of young grape plants.

MATERIAL AND METHODS

The study was carried out in a greenhouse, with temperatures ranging from 28 to 32°C during the day and 18-22°C at night and watered to field capacity every two days. The plants selected for the study included *Chenopodium ambrosioides* L. (wormseed), *Ruta graveolens* L. (rue), *Thymus vulgaris* L. (thyme) and the Brassicaceous crops, *Brassica juncea* Czern. and Coss. cv. Nemfix (brown mustard) and *Brassica napus* L. cv. Rangi (rapeseed) and all had shown nematicidal properties in vitro against *X. index* in earlier studies (Contreras, 2000; Insunza *et al.*, 2001a).

Additional treatments included barley (*Hordeum vulgare* L.) with no known nematicidal background, and fenamiphos at 0.04% (0.1% V/V Nemacur 400 EC) as a chemical treatment. Furthermore, grapevine plants inoculated with *X. index* as control, and grapevine with no further treatments were used for comparison.

The substratum used was a mixture of equal parts of sand, humus and arable soil (18,3% sand, 41,3% silt, 40,4% clay), with pH final of 6.2 and 5.2% organic matter. The plant species to be evaluated were grown in the field. At blooming stage, they were harvested and the aerial parts were chopped and mixed with the substratum at a 2% W/V concentration, and placed in 6 L pots. A one-year old rooted grapevine plant cv. Thompson Seedless was planted in each pot.

Ten days after planting, each pot was inoculated with a population of ca. 1000 juvenile and adult *X. index*. The inoculum was extracted from soil planted with *Ficus carica*, and placed in 4 holes, 10 cm deep at 5 cm distance from the plant. Fenamiphos was applied the day after the inoculation with nematodes, irrigating each pot with a 2 L solution, equivalent to 0.8 cc/pot.

Parameters evaluated 6 months after nematode inoculation were: final population density of *X. index*, number of knots or apical nodulations in 10 g of roots (a typical feeding symptom on new roots), and fresh weights of both, the grapevine shoots and roots. The nematode analyses were registered as the averages of two 250-cm^3 soil samples per pot. Nematodes were extracted by the Cobb's sieving and decanting method. The suspension of nematodes caught on the 180 µm sieve was further freed from debris by a sieve with a layer of 150 µm nylon gauze, for approximately 24 hours (Southey, 1986).

A completely randomized experimental design was used, with 9 treatments and 8 replicates per treatment. Data was subjected to analysis of variance and means were compared using Duncan's Multiple Range Test ($P < 0.05$).

RESULTS AND DISCUSSION

The final *X. index* populations did not differ significantly ($P < 0.05$) between the plant-based treatments though all differed significantly from the inoculated control, which had the highest population level (Table 1). However, with the exception of *Brassica juncea*, the final nematode densities were significantly higher than the chemical control, which showed the lowest nematode numbers in inoculated treatments.

All the amendments in the present study resulted in a significant reduction of the *X. index* populations. *Brassica juncea* cv. Nemfix was the most effective while *B. napus* cv. Rangi was less effective, even though both cultivars have been selected for their high tissue glucosinolate content and high biomass. However the biocidal activity of glucosinolates may depend on

Table 1. Effect of treatments on final population densities of *Xiphinema index*, number of damaged roots and total fresh plant weights (n = 8).

Treatments	Final population of *X. index*[1]	Number of damaged roots (apical knots)/10 g of roots	Fresh plant weight (g)
Brassica juncea var. Nemfix	363.0 bc	204.0 ab	94.0 ab
Brassica napus var. Rangi	717.0 b	220.0 ab	91.2 ab
Chenopodium ambrosioides	662.0 b	185.0 b	95.4 ab
Hordeum vulgare	700.0 b	268.0 a	93.5 ab
Ruta graveolens	552.0 b	255.0 a	103.1 a
Thymus vulgaris	702.0 b	157.0 b	102.1 a
Fenamiphos (Nemacur 400EC)	18.0 c	79.0 c	76.0 c
Only nematodes (control)	1245.0 a	247.0 a	84.9 bc
No nematodes nor amendments	0.0 c	0.0 d	89.6 bc

Figures with the same letters within each column do not differ significantly from each other ($P < 0.05$) according to Duncan's Multiple Range Test.

[1]Number of nematodes per 250 cm^3 of soil. Pi = 1000 nematodes per pot/plant.

their type and concentration. Nearly 100 different forms of glucosinolate have been identified from Brassicaceae plants. Differences in toxicity also exist between cultivars and other factors, such as plant age and plant parts (Halbrendt and Jing, 1994). Cell disruption, which maximizes glucosinolate hydrolysis and isothiocyanates release, seems to be one of the most important factors for suppressing soil-borne pathogens (Morra and Kirkegaard, 2002).

Root damage, assessed as knots or apical nodulation appeared to be a good indicator for assessing the treatment effect on nematode populations, because it incorporates nematicidal as well as the nematostatic effects. The two non-chemical treatments with and without nematodes (no amendments) revealed the extent of root damage caused by the nematodes (Table 1).

The fenamiphos treatment showed the least root damage by *X. index* and resulted in 50% less root damage compared with the best plant treatment (*Thymus vulgaris*), although it did not fully protect the plants. The plant-based treatments with *C. ambrosioides* and *T. vulgaris* showed less root damage than *R. graveolens*, *H. vulgare* and the inoculated control. However, though the level of *X. index* population reduction with all green manures was greater than the inoculated control, this was not reflected in a less root damage in the treatments. This was especially true in the case of *B. juncea* which showed the largest population decrease.

Only the *T. vulgaris* and *C. ambrosioides* treatments significantly reduced root damage caused by *X. index* indicating that an important part of the effect of these two species might be nematicidal or nemostatic in nature.

The incorporation of organic matter—independent of plant species—had a positive effect on plant growth, regardless of its possible effects on nematode control (Table 1). *Hordeum vulgare* is not known for nematicidal activity but has been grown as cover crop and green manure (Altieri, 1990). It was included here to test for a possible nematicidal effect due to the incorporation of organic matter. No differences were found among the other tested plants, but *R. graveolens* and *T. vulgaris* were significantly different from the chemical treatment and the two treatments with grapevine plants without plant material incorporation. Various organic amendments have a suppression effect on nematode populations, however their effectiveness might be due to factors other than the release of toxic compounds. Often the increase in predacious or parasitic activity of soil biota, or ammonification during degradation are mentioned in this context (Rodriguez-Kabana *et al.*, 1987).

The grapevines to which brown mustard was added showed some symptoms of leaf spot, supposed due to phytotoxicity. With the 400 ppm fenamiphos treatment, the low total plant weight may be attributed in part to phytotoxicity, a situation observed in previous works with fenamiphos over 200 ppm concentration in different crops (Bunt, 1975).

Among the plant amendments, nematode suppression by the incorporation of *Brassica juncea* was most effective, and was not significantly different from the strong control showed by fenamiphos. This plant, as with other Brassicaceae, produces glucosinolates that upon enzymatic hydrolysation by mirosinase results in a range of toxic products, mainly isothiocyanates, thiocyanates, and nitriles. Several studies have demonstrated the broad biocidal, including nematicidal activity of these substances e.g., against *Xiphinema americanum* (Halbrendt, 1996).

From the experiment results, it is concluded that *Brassica juncea* cv. Nemfix, *Chenopodium ambrosioides*, *Ruta graveolens* and

Thymus vulgaris when used as green manures offer possibilities as non-chemical alternatives in the control of *Xiphinema index*. It remains to make sure that these alternatives can be recommended under field conditions through conventional or slightly modified agronomical practices.

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