BIOFUMIGATION, FALLOW, AND NEMATODE MANAGEMENT IN VINEYARD REPLANT

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ABSTRACT

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The epidemiology of Grapevine fanleaf virus (GFLV) and its nematode vector, *Xiphinema index*, was analyzed by studying the efficacy of biofumigation, fallow, and solarization as nematode control alternatives. The study was carried out in the southeastern Iberian Peninsula, at two sites in the Jumilla area (Murcia, Spain), which has a continental Mediterranean climate, a sand/loamy based soil which had been in fallow for one year and on another loam/clay/sandy soil under 10-year fallow. An absence of *X. index* and live grapevine roots was observed in the loam/clay/sandy soil in the 10-year fallow site *X. index* was not found either in the loam/sandy, biofumigated soil after one year in fallow, although there were live roots. *X. index* and live roots were found in soils after one year in fallow and especially in solarized soils, therefore solarization and one year in fallow are not considered effective control alternatives. It is concluded that biofumigation may be an alternative for *X. index* control, which can also increase soil biodiversity.

Key words: Xiphinema index, Grapevine fanleaf virus, manure, solarization, epidemiology.

RESUMEN

Bello, A., M. Arias, J. A. López-Pérez, A. García-Álvarez, J. Fresno, M. Escuer, S. C. Arcos, A. Lacasa, R. Sanz, P. Gómez, M. A. Díez-Rojo, A. Piedra Buena, C. Goitia, J. L. de la Horra and C. Martínez. 2004. Biofumigación, barbecho y manejo de nematodos en la replantación de viñedos. Nematrópica 34:53-64.

Se analiza la epidemiología del "grapevine fanleaf virus" (GFLV) y su nematodo vector *Xiphinema index* en vid, estudiando la eficacia de la biofumigación, el barbecho y la solarización como alternativas de control. Se realiza el estudio en la comarca de Jumilla (Murcia, España), en el Sureste de la Península Ibérica, con clima mediterráneo continental, en un suelo arenoso-franco con un año de barbecho, y en otro franco-arcillo-arenoso, con 10 años de barbecho. Se observó la ausencia de *X. index* y raíces de vid vivas en el suelo franco-arcillo-arenoso con 10 años de barbecho, tampoco se encontró el nematodo en el suelo arenoso-franco con un año de barbecho y biofumigado, aunque aparecieron raíces vivas. Se encontró *X. index* y raíces vivas en los suelos con un año de barbecho y sobre todo en los solarizados, por lo que no se consideran alternativas eficaces de control. Se concluye que la biofumigación puede ser una alternativa para el control de *X. index*, que además incrementa la biodiversidad del suelo.

Palabras claves: Xiphinema index, virus del entrenudo corto, estiércol, solarización, epidemiología.

INTRODUCTION

Over 300 species of nematodes have been associated with grapevine. Root-knot nematodes (*Meloidogyne* spp.) and virus vectors (*Xiphinema* spp.) are the most important, especially *X. index* Thorne & Allen, 1950 as the Grapevine fanleaf virus (GFLV) vector (Arias *et al.*, 1997b). It has been experimentally proven that *X. italiae* Meyl, 1953 can function as a GFLV vector (Martelli, 1978) although it is not effective in the field. *X. index* is mainly associated with grapevine, although it has also been found on rose, fig, mulberry and other fruit trees, being less frequent in herbaceous plants and natural environments (Arias *et al.*, 1985; Siddiqi, 1986). It has been demonstrated that populations of *X. index* higher than 50 individuals per kg of soil affect grapevine yield (Lamberti and Melillo, 1991). Plants inoculated with 500 individuals of *X. index*, cultivated at 26.6°C, undergo a 23% loss of leaves in the first year and a 65% loss in the second year, with a 38% weight reduction of the aerial part and root, 60% less inflorescences and 89% loss in production (Kirkpatrick *et al.*, 1965).

X. index can retain the virus for over eight months in the absence of a host plant and for several years in the presence of live grapevine root remains; therefore, replacement of virotic stock is ineffective. Current solutions include maintaining the vineyard while production is economically acceptable, and then uprooting, maintaining the soil on bare fallow, ploughing deeply, or rotating with an alternative crop with the objective of eliminating live grapevine roots and weeds. A five-year fallow minimum has been established in California (Raski *et al.*, 1965), while in France, in a more humid environment, six or seven years are considered necessary, or three years if nematicides are applied (Dalmasso, 1970). In irrigated vineyards, an alternative is drying the soil, although flooding is more effective (Weischer, 1975). The application of systemic herbicides has been recommended, previous to uprooting the vineyard, to eliminate root remains, followed by treatment with nematicides (Boubals, 1994).

The use of certified virus-free material, clean machinery, and good agricultural practices, especially fallow and organic amendments, stand out as alternatives to control *X. index*. Resistant plants, biological control agents and physical methods, such as solarization, steaming and thermotherapy have also been used, especially for disinfecting substrates and controlling GFLV. Among chemical products, fumigants, such as 1,3-D + chloropicrin, dazomet, metham sodium, and non-volatile compounds like organophosphate nematicides (ethoprophos and fenamiphos) and carbamate (aldicarb and oxamil) are emphasized. Methyl bromide has also been used (Arias *et al.*, 1997a). Among non-chemical alternatives, biofumigation, based on the use of gasses resulting from the decomposition of organic matter, has demonstrated great efficacy as an alternative to the use of methyl bromide, whose ban is imminent due to its environmental impact (Bello 1998; Bello *et al.* 2003).

This paper aims to make available an alternative control methodology to the use of soil fumigants, since these products, besides involving additional expenditures, can have a negative impact on health and the environment. This study shows the efficacy of biofumigation, fallow and solarization to control phytoparasitic nematodes in the Jumilla area (Murcia, Spain) where problems produced by *X. index* and GFLV have been detected.

MATERIAL AND METHODS

This study was carried out at two areas, "La Jimena" and "La Elia" (Jumilla, Mur-

cia, Spain), which have a continental Mediterranean climate. An average rainfall of 300 mm/year, maximum precipitation in spring and at the end of autumn, with minimums in winter and summer. Winters are cold, reaching -4°C, and go into summer without any transition, with temperatures over 40°C, while autumn is mild. Because of its environmental characteristics, the area is a good site for the epidemiological studies of soil borne plant diseases (Bello *et al.*, 1996).

In the selected plots, rows with a separation of three meters were established for planting vines where alleys (area between rows) had been located in the previous vineyard. Biofumigation was carried out in one-meter wide bands on the rows, where biofumigant organic matter was incorporated. After a hard rain, which saved watering, they were covered with a 0.05 mm thick, 120 cm wide, transparent plastic sheet. Non treated areas were left in bare fallow as a control (Fig. 1).

At "La Jimena" area, the plot studied occupies a surface of 2.5 ha. It is located on the shady side of a hill, and at the time of treatment had been under bare fallow for one year. The soil has a sandy/loam upper horizon (85% sand and 6-7% clay), 0.2-0.3% organic matter content, 10% carbonates, between pH 7.7-8.1, and was classified as Haplic Arenosol (FAO, 1988). Biofumigation was carried out on August 2, 2002, applying 10 kg m^{-2} of a mixture of sheep and chicken manure in a 7:3 ratio, which was incorporated into the soil by means of a rotovator (Bello *et al.*, 2003). Part of the plot underwent solarization, without incorporating organic matter. On November 20, 2002, 96 samples were taken at eight points, at 0-20, 20-40, and 40-60 cm in depth, 24 samples from each treatment (biofumigated, solarized and in each of the fallow controls). Sampling was done following a W trajectory, every 10 rows in the biofumigated area and its control, while in the solarized area and its control, samples were taken along a transect. Eight soil samples were also taken around root systems in an adjoining vineyard for comparison.

At "La Elia" area, located in a fertile plain, the soil has an upper horizon of loam/clay/sandy texture (52-56% sand and 19-25% clay), 0:2-1.7% organic matter content, 29.7-58.4% carbonates and pH 7.7-8.1. Two plots were chosen, with soils classified as Calcaric Regosol and Petric Calcisol in plot (I) and Haplic Calcisol and Petric Calcisol in plot (II) (FAO, 1988). Both plots had been under 10-year bare fallow. Biofumigation carried out on February 15, 2002, on a surface area of 25 ha, by using sheep manure at a dose of 13 kg m^{-2}, which was mixed into the soil with the help of a subsoiler. On November 20, 2002, 65 samples were taken at eight points, 40 from plot (I) according to a diagonal transect every 10 rows, 24 from biofumigated soil at depths of 0-20, 20-40 and 40-60 cm, and 16 from the control at 0-20 and 20-40 cm. The 25 remaining samples belong to plot (II) and were taken at five points, following a W trajectory, every five rows, 15 from biofumigated soil at 0-20, 20-40 and 40-60 cm and the other 10 from fallow at 0-20 and 20-40 cm. At the same time, soil samples on almond, fig and medlar roots from established trees were also taken at this site to determine possible foci of *X. index*.

The first centimeters of the upper part of the soil were eliminated, and approximately 500 g of soil was collected with a hoe between 0-20 cm deep. The rest of the sample was taken with a cylindrical drill, 8 cm in interior diameter and 25 cm long to a depth of 20-40 cm and, avoiding contamination, with the same drill from 40-60 cm. Small-sized nematode species with little mobility (criconematids, tilenchids, and rhab-

ditids), were extracted by sugar centrifugation methods (Nombela and Bello, 1983) The modified Flegg method (Flegg, 1967) was used for those of greater mobility and size (*Xiphinema*, other dorilaimids and enchytraeids). The presence of GFLV was detected through the ELISA-DAS method (Barbara *et al.*, 1978). The influence of biofumigation, fallow and solarization on saprophagous nematodes (Rhabditids) was treated by the one-way variance analysis, as well as to the LSD test for comparison of means.

RESULTS

Results of nematological analyses are expressed as the number of individuals per 200 cc of soil (Tables 1 and 2). Samples are grouped by treatments and each treatment is broken down according to the depth of the sample (0-20, 20-40 and 40-60 cm). Average values of nematodes are rounded off to whole numbers.

"La Jimena" Area (Table 1)

Biofumigated soil was very dry at a depth of 0-20 cm, semi-moist at 20-40 cm and moist at 40-60 cm. The absence of *X. index* and *X. italiae* is notable, with *X. pachtaicum* appearing in only one 20-40 cm sample and *X. rivesi* Dalmasso, 1969 in another one at 40-60 cm. Among phytoparasites, *Macroposthonia xenoplax* (Raski 1952) De Grisse & Loof, 1965 was found only along the edges of the plot in a 20-40 cm sample and in two 40-60 cm samples, the majority being dead, and *Quinisulcius capitatus (*Allen, 1955) Siddiqi, 1971 was found in one 20-40 cm sample. Dorilaimid populations presented averages of five individuals at 0-20 cm; others, five at 20-40 cm and four at 40-60 cm, belonged to *Aporcelaimellus obtusicaudatus* (Bastian, 1965) Alther, 1968. Rhabditids appeared in almost all the samples with averages of 63 individuals at 0-20 cm, 113 at 20-40 cm and one hundred and 15 at 40-60 cm. There were enchytraeids in two 20-40 cm samples and in another two at 40-60 cm. Live roots appeared in two 20-40 cm samples and in another two at 40-60 cm, where the presence of GFLV was detected. Organic matter was distributed uniformly, in some cases reaching 60 cm in depth.

In the control of biofumigation (fallow), soils were moist at all depths. *X. index* appeared in one 40-60 cm sample at the upper edge of the plot and *X. pachtaicum* in two 20-40 cm samples and in another two at 40-60 cm. Among tilenchids, *Criconemoides informis* (Micoletzki, 1922) Taylor, 1936 appeared, and *M. xenoplax* was found in a 0-20 cm sample, in two at 20-40 cm and in four at 40-60 cm; *Q. capitatus* was found in two at 0-20 cm and one at 20-40 cm; and *Paratylenchus microdorus* Andrassy, 1959 appeared in one at 40-60 cm. Dorilaimids presented average populations of 15 individuals at 0-20 cm, 14 at 20-40 cm and 12 at 40-60 cm. These species were found: *A. obtusicaudatus*, *Discolaimoides bulbiferus* (Cobb, 1908) Heyns, 1963; *D. filiformis* Das, Khan & Loof, 1969 and *Nygolaimus brachyuris* (De Man, 1880) Thorne, 1930. Rhabditids presented averages of 28 individuals at 0-20 cm, 21 at 20-40 cm and 14 at 40-60 cm. Enchytraeids also appeared in one 20-40 cm sample and three at 40-60 cm. Live roots were found infected by GFLV in four, eight and seven samples at the depths studied.

Solarized soil was semi-moist between 0-40 cm and moist at 40-60 cm. *X. index* appeared in two 0-20 cm samples, in another two at 20-40 cm and in five at 40-60 cm. *X. italiae* was also found in two 20-40 cm samples and *X. pachtaicum* in one at 0-20 cm, four at 20-40 cm and six at 40-60 cm. No criconematids were found. *P. microdorus* only appeared in one 40-60 cm sam-

Table 1. Biofumigation, fallow and solarization effects on vineyard soil at "La Jimena" (Jumilla, Murcia) (nematodes 200 cc^{-1} soil).[1]

Treatment and depth (cm)	*X. index*[2]			*X. italiae*[2]			*X. pachtaicum*[2]			Criconematids[3]			Dorilaimids[2]			Rhabditids[3]			Enchytraeids[2]			LR[4]	Others
	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M		
Biofumigation																							
0-20	0	0	0	0	0	0	0	0	0	0	0	0	5	0-14	5	7	0-248	63	0	0	0	0	
20-40	0	0	0	0	0	0	1	0-2	1	1	0-4	1	6	0-16	5	7	0-524	113	2	0-8	2	2	*Quini.* (4 indv.)[3]
40-60	0	0	0	0	0	0	0	0	0	2	0-16	2	4	0-8	2	6	0-680	115	4	0-8	2	2	*X. rivesi* (2 indiv.)[2]
Total	0	0	0	0	0	0	1	0-2	1	3	0-16	1	15	0-16	4	20	0-680	97	6	0-8	1	4	
Solarization																							
0-20	2	0-3	1	0	0	0	1	0-1	1	0	0	0	7	0-8	6	4	0-60	10	0	0	0	8	
20-40	2	0-4	1	2	0-6	1	4	0-8	2	0	0	0	7	0-24	12	5	0-12	5	1	0-4	1	8	
40-60	5	0-8	2	0	0	0	6	0-16	4	0	0	0	8	3-16	7	5	0-20	7	0	0	0	5	*Para.* (12 indv.)[3]; Mon. (2 indv.)[2]
Total	9	0-8	1	2	0-6	1	11	0-16	2	0	0	0	22	3-24	11	14	0-60	7	1	0-4	1	21	
Control (Biofumigation)																							
0-20	0	0	0	0	0	0	0	0	0	1	0-4	1	8	4-32	15	8	4-72	28	0	0	0	4	*Quini.* (12 + 12 indv.)[3]
20-40	0	0	0	0	0	0	2	0-8	1	2	0-12	2	8	4-28	14	8	4-68	21	1	0-8	1	8	*Quini.* (4 indv.)[3]
40-60	1	0-5	1	0	0	0	2	0-5	1	4	0-16	4	8	2-18	12	6	0-28	14	3	0-4	1	7	*Para.* (2 indv.)[3]
Total	1	0-5	1	0	0	0	4	0-8	1	7	0-16	2	24	2-32	14	22	0-72	21	4	0-8	1	19	
Control (Solarization)																							
0-20	1	0-1	1	0	0	0	4	0-4	2	0	0	0	8	4-30	12	8	16-76	39	2	0-4	1	8	
20-40	3	0-6	1	0	0	0	4	0-7	2	0	0	0	8	4-38	12	4	0-48	13	3	0-1	1	6	*Para.* (8 indv.)[3]; Mon. (4 indv.)[2]
40-60	1	0-4	1	0	0	0	1	0-8	1	1	0-4	1	8	1-42	10	7	0-140	24	1	0-4	1	6	*Para.* (4 + 40 indv.)[3]
Total	5	0-6	1	0	0	0	9	0-8	2	1	0-4	1	24	1-42	12	19	0-140	25	6	0-4	1	20	
Vineyard (Control)	8	8-154	40	7	0-11	4	8	4-144	34	1	0-12	2	8	10-112	46	8	8-88	35	4	0-8	2	8	

[1]Mon. = Mononchids, *Para.* = *Paratylenchus*, *Quini.* = *Quinisulcius*, P = Presence, A = Minimal-maximal number of individuals, M = Mean.
[2]Extraction by Flegg's method (Flegg, 1967).
[3]Extraction by centrifugation (Nombela & Bello, 1983).
[4]LR = Living roots.

ple. Dorilaimid populations presented averages of six individuals at 0-20 cm, 12 at 20-40 cm and six at 40-60 cm, belonging to *A. obtusicaudatus*; *Carcharodiscus eximius* Peña Santiago & Liebana, 1994; *D. filiformis*, and *Ecumenicus monohystera* (De Man, 1980) Thorne, 1974. Rhabditids presented averages of 10 individuals at 0-20 cm, five at 20-40 cm and seven at 40-60 cm. Enchytraeids also appeared in a 20-40 cm sample and mononchids in a 40-60 cm sample. Live roots infected with GFLV appeared in all 0-40 cm samples and in five 40-60 cm samples.

In the control of solarization (fallow rows), located at the upper part of the plot, soil was moist, with *X. index* appearing in one 0-20 cm sample, in three at 20-40 cm and in one at 40-60 cm. *X. pachtaicum* was found in four 0-20 cm samples, in another four at 20-40 cm, and in one at 40-60 cm. *M. xenoplax* only appeared in one sample at 40-60 cm and *P. microdorus* in one at 20-40 cm and in two at 40-60 cm. Dorilaimids presented averages of 12 individuals at 0-20, another 12 at 20-40 cm and 10 at 40-60 cm from the species *A. obtusicaudatus*, *C. eximius*, *D. filiformis*, *Discolaimus agricolus* Sauer & Anneells, 1985; *E. monohystera* and *Labronema pulchrum* Vinciguerra & Zullini, 1980. Rhabditids showed averages of 39 individuals at 0-20 cm, 13 at 20-40 cm and 24 at 40-60 cm. Enchytraeids also appeared in two 0-20 cm samples, in three at 20-40 cm and in one at 40-60 cm and mononchids in one 20-40 cm sample. Live roots with GFLV appeared in eight, six and six samples, respectively, for the depths studied.

The soil was wet in the vineyard control near the plot studied and *X. index* appeared in all samples, with an average of 40 individuals and a maximum value of 154. *X. italiae*, appeared in seven samples with an average of four and a maximum of 11 individuals, and *X. pachtaicum* which was present in all samples with an average of 34 and a maximum of 154 individuals. *M. xenoplax* only appeared in one sample with 12 individuals. Dorilaimids were in all samples with an average of 46 and a maximum of 112, represented by the species *A. obtusicaudatus* and *D. filiformis*.

The largest populations of rhabditids in sandy/loamy soils were in the biofumigated treatment, increasing with depth, reaching an average of 115 individuals at 40-60 cm, while in fallow and in the vineyard control they did not surpass average values of 39 individuals and in solarization the maximum average was ten individuals. Statistically significant differences were obtained for the rhabditid population in the biofumigated treatment, with respect to solarization and the controls (fallow and vineyard). The highest populations at 20-40 cm (average of 1259 individuals) and at 0-20 cm (average of 584 individuals) were found in loam/clay/sandy, biofumigated soils (Fig. 2). The presence of enchytraeids, small lumbricids that intervene in the decomposition of organic matter, stand out especially in biofumigated soils and in fallow at 20-40 cm, while in solarized soils they only appeared once.

"La Elia" Area (Table 2)

In biofumigated soils, which were semimoist at the time of sampling, *X. italiae* appeared in plots (I) and (II) in each 40-60 cm sample, *X. pachtaicum* was found in plot (I) in one 40-60 cm sample and in another one from plot (II) at 20-40 cm. *P. microdorus* was found in plot (I) in two 0-20 cm samples, in one at 20-40 cm and in another one at 40-60 cm and in plot (II) in one sample only at 40-0 cm. *Pratylenchus thornei* Sher & Allen, 1953 appeared in plot (II) in a 40-60 cm sample. Dorilaimids were in all plots and at all depths, in plot (I) with averages of 29 individuals at 0-20

Table 2. Biofumigation, fallow and solarization effects on vineyard soil at "La Elia" (Jumilla, Murcia) (nematodos 200 cc^{-1} soil).[1]

Treatment and depth (cm)	*X. italiae*[2]			*X. pachtaicum*[2]			*Paratylenchus*[3]			Dorilaimids[2]			Rhabditids[3]			Enchytraeids[2]			Others
	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	
PLOT (I)																			
Biofumigation																			
0-20	0	0	0	0	0	0	2	0-4	1	8	3-144	29	8	40-1876	584	3	0-4	2	
20-40	0	0	0	0	0	0	1	0-4	1	6	0-80	14	8	136-2520	1259	6	0-44	13	
40-60	1	0-1	1	1	0-8	1	1	0-4	1	6	0-28	8	7	0-276	81	2	0-20	3	
Total	1	0-1	1	1	0-8	1	4	0-4	1	20	0-144	17	23	0-2520	641	11	0-44	6	
Fallow (Control)																			
0-20	0	0	0	0	0	0	0	0	0	8	6-23	12	8	8-88	33	1	0-4	1	
20-40	2	0-1	1	2	0-4	1	3	0-12	4	8	3-19	11	7	0-64	18	0	0	0	Mon. (1 + 1 indv.)[2]; *Merl.* (16 indv.)[3]
Total	2	0-1	1	2	0-4	1	3	0-12	2	16	3-23	11	15	0-88	26	1	0-4	1	
PLOT (II)																			
Biofumigation																			
0-20	0	0	0	0	0	0	0	0	0	3	0-4	2	5	12-64	39	0	0	0	
20-40	0	0	0	1	0-1	1	0	0	0	5	1-24	9	5	4-128	48	0	0	0	
40-60	1	0-1	1	0	0	0	1	0-4	1	4	0-8	4	5	12-132	65	2	0-8	2	*Prat.* (12 indv.)[3]
Total	1	0-1	1	1	0-1	1	1	0-4	1	12	0-24	5	15	4-132	51	2	0-8	1	
Fallow (Control)																			
0-20	0	0	0	0	0	0	1	0-8	2	5	1-4	3	4	8-28	10	1	0-2	1	*Bol.* (8 indv.)[3]
20-40	1	0-1	1	2	0-3	1	3	0-32	9	5	5-12	8	5	12-64	32	0	0	0	*Bol.* (12+4 indv.)[3]; *Prat.* (8+4 indv.)[3]
Total	1	0-1	1	2	0-3	1	4	0-32	5	10	1-12	6	9	8-64	21	1	0-2	1	

[1]*Bol.* = *Boleodorus*, *Merl.* = *Merlinius alboranensis*, Mon. = Monochids, *Prat.* = *Pratylenchus*, P = Presence, A = Minimal-maximal number of individuals, M = Mean.
[2]Extraction by Flegg's method (Flegg, 1967).
[3]Extraction by centrifugation (Nombela & Bello, 1983).

cm, 14 at 20-40 cm and eight at 40-60 cm, the species being *A. obtusicaudatus, C. eximius*, *D. bulbiferus* and *Discolaimus major* Thorne, 1939, and in plot (II) with averages of two individuals at 0-20 cm, nine at 20-40 cm and four at 40-60 cm from *A. obtusicaudatus* and *D. filiformis*. Rhabditids presented averages of 584, 1259 and 81 individuals in plot (I) and of 39, 48 and 65 in plot (II) at depths of 0-20, 20-40 and 40-60 cm, respectively. Enchytraeids appeared in three 0-20 cm samples, in six at 20-40 cm and in two at 40-60 cm in plot (I), and in only two 40-60 cm samples in plot (II). The greatest quantity of organic matter was found between 20-40 cm. Live grapevine roots were not seen.

In the biofumigation control (fallow), with wet soil, *X. italiae* appeared in two samples in plot (I) and in one in plot (II) at 20-40 cm. *X. pachtaicum* was found in two samples in each of the two plots at 20-40 cm. The *Paratylenchus* genus was found in three 20-40 cm samples in plot (I), and in plot (II) in one at 0-20 cm and three at 20-40 cm, belonging to the species *Paratylenchus dianthus* Jenkins & Taylor, 1956 and *P. microdorus*. Dorilaimids were in all samples, with average values of 12 individuals at 0-20 cm and 11 at 20-40 cm in plot (I), and in plot (II) in five 0-20 cm samples with averages of three individuals and in another five 40-60 cm samples with an average of eight individuals, which indicates that populations of these nematodes increased eight months after applying biofumigation, with *A. obtusicaudatus* and *D. filiformis* appearing in both plots. Rhabditids in plot (I) presented averages of 33 individuals at 0-20 cm and 18 at 20-40 cm; and in plot (II), averages of 10 at 0-20 cm and 32 at 20-40 cm were detected. These are much lower values than those observed in biofumigated soils. Enchytraeids only appeared in one 0-20 cm sample from plot (I) and in another from plot (II) with populations lower than those found in biofumigated soils. The presence of *P. thornei* is emphasized in plot (II) in two 20-40 cm samples, *Boleodorus thylactus* Thorne, 1941 in one 0-20 cm sample and in two 20-40 cm samples and *Merlinius alboranensis* (Tobar-Jiménez, 1970) Tarjan, 1973 in another one from plot (I) at 20-40 cm. Mononchids also appeared in two 20-40 cm samples, which belong to the *Iotonchus rotundicaudatus* Peña-Santiago & Jiménez-Guirado, 1990. *X. index* did not appear in almond, fig and medlar samples, but *X. pachtaicum* was found with a maximum value of 480 individuals in almond.

DISCUSSION

Biofumigation using a mixture of sheep and chicken manure, at a proportion of 7:3, respectively and a dose of 10 kg m^{-2}, was effective for controlling *X. index* in loam/sandy soils between 0-60 cm in depth, were the roots normally develop in the beginning of replant. This also is the area with the greatest root density in the old vineyard. A reduction in the populations of this nematode is also observed in one-year fallow, considering that the efficacy of this control alternative depends mainly on the elimination of *X. index*. The risk of infection exists from alleys in one-year fallow, when biofumigation is carried out in bands, since roots can last for up to four and a half years in vineyard soil. *X. index* retains the virus for at least eight months in the absence of a host plant and several years in the presence of live grapevine root remains, although it only survives from nine to ten months in sterile soil (Raski and Hewitt, 1960; Raski *et al.*, 1965). Besides it has been observed that some 10% of the *X. index* population survives for more than 60 days in the absence of a host plant, depending on conditions of humidity, survival being very low in saturated as well as in dry soil (Sultan and Ferris,

1991). It must also be taken into consideration that all phases of nematode development have an infective capacity, which they lose during moulting, when changing the oesophagus cuticle (Raski and Hewitt, 1963). Therefore, by eliminating live roots, the duration of fallow could be reduced, although replanting should be done only when the absence of infective *X. index* individuals has been verified (Arias *et al.*, 1997b). The efficacy of biofumigation in combination with fallow in controlling nematodes is confirmed for loam/clay/sandy soils, where only *X. italiae* and *X. pachtaicum* appeared in two 20-60 cm samples.

The study of the efficacy of *X. italiae* as a GFLV vector would be very interesting, since Martelli (1978) pointed out that it is not effective in field conditions. On the other hand, Arias *et al.* (1997b) confirmed that *X. pachtaicum* is not a GFLV transmitter. Although it is a species of great value as a bio-indicator to determine the efficacy of biofumigation and the duration of fallow, since in almond, fig and other nearby crops this species reached a maximum population of 480 individuals. *X. pachtaicum* appeared in all treatments, with greater frequency in loamy/sandy, solarized soils, where it was found in 11 samples, followed by nine in the solarization control, four in the biofumigation control and it was only present in one sample in biofumigated soils. Lastly *X. rivesi* appears in one biofumigated sample. All this contrasts with the high frequency of *X. index, X. italiae* and *X. pachtaicum* in the control vineyard, where they appear in all samples and where *X. index* populations reach 154 individuals and an average of 40. According to these results, solarization is not considered effective for controlling *X. index*, since they move towards deep horizons.

X. index was not found in loam/clay/sandy soil subjected to 10-year fallow. It did not appear in almond, fig or medlar trees either, so that it would be very interesting to conduct a study of its epidemiology. In this sense, Weischer (1975) indicated that the variation of *X. index* populations is greater in sandy soils than in clay ones, being abundant between pH 6.5-8.2, the interval that includes the soils studied in the Jumilla area. Results obtained coincide with those of Arias *et al.* (1997b) where *X. index* is found with greater frequency in sandy/loamy soils and loam/clay/sandy soils. The largest populations are found in soils with percentages of sand between 59-70%, values which represent the soils studied, and when organic matter content is between 2.5-5.5%, which is quite higher than the values we found. Nematodes diminish as the carbonate content increases, although they withstand up to 54%, a value which is surpassed in the loam/clay/sandy soils studied.

From the viewpoint of epidemiology, it must be taken into account that the area of study has a continental Mediterranean climate with extremely cold winters that goes directly from winter to summer, which is dry and has elevated temperatures. And as Weischer (1975) indicated, *X. index* activity is limited under 16°C. Arias *et al.* (1997b) verified that in continental Mediterranean climatic conditions it produces only one generation per year, between March and May, which allows us to indicate the importance of fallow to control its populations.

With relation to phytoparasitic nematodes, interest in the presence of *M. xenoplax* should be considered, although its populations did not surpass 16 individuals, the majority of them dead in biofumigated soils. Their frequency was low in nearby vineyards, so it is thought that this species does not pose serious problems in the plots studied. *C. informis* was also found, but it does not produce problems in grapevine. The low frequency of species from the *Boleodorus*, *Merlinius*, *Paratylenchus* and

Quinisulcius genera seems to indicate that they belong to the nematofauna associated with the natural vegetation. The presence of *P. thornei* must also be indicated in loam/clay/sandy, biofumigated as well as fallow soils.

Dorilaimids, which are very interesting due to their value as bio-indicators, appeared in sandy/loamy soils with averages that did not surpass 15 individuals, values much lower than those which appeared in the nearby vineyard (46 individuals). The lowest population was found in biofumigated soils (average of four individuals) and in solarized soils (average of 11 individuals). On the contrary, fallow presented averages of 12 and 14 individuals. These nematodes are more abundant in loam/clay/sandy biofumigated soils, with averages of 29 individuals at 0-20 cm, 14 at 20-40 cm and eight at 40-60 cm. It was observed that after eight months the loam/clay/sandy biofumigated soils have dorilaimid populations greater than fallow, which presented averages of six and 11 individuals. Besides, mononchids were found in four samples between 20-60 cm in fallow and in solarized soil, represented in sand/loamy soils by *I. rotundicaudatus*.

Among the dorilaimid species *A. obtusicaudatus*, which appeared in all treatments, stands out in the first place, being the only species that was present in biofumigated sandy/loamy soils. The second species in frequency was *D. filiformis*, which appeared in all treatments except the biofumigated ones. *C. eximius* appeared in all treatments, in sand/loamy soil as well as in loam/clay/sandy soil. *D. bulbiferus* is a cosmopolitan species that appeared in biofumigated and fallow soils, but not in those solarized. *C. agricolus, E. monohystera, I. rotundicaudatus* and *L. pulchrum* appeared also in the wettest area of the plot in sand/loamy soil. *N. brachyuris* only appeared in fallow of the sand/loamy soil; it is a cosmopolitan species. *D. major* appeared only in loam/clay/sandy, biofumigated soil. *A. obtusicaudatus, D. filiformis, D. bulbiferus* and *E. monohystera* are frequent in vineyards of Castilla-La Mancha (Arias *et al.*, 1997b), while *C. eximius, D. agricolus* and *N. brachyuris* have been cited for the first time in vineyards in Spain.

Under the biofumigation conditions the number of rhabditids and enchytraeids rose due to the increase in available food source. It is thought that biofumigation, when applied together with bare fallow, whose duration should be established according to the environmental characteristics of each area, can be an effective alternative for controlling *X. index*. When nematode problems exist, biofumigation should be carried out immediately after uprooting the vineyard, to prevent their movement towards deep soil horizons. The establishment of optimum biofumigant doses must be obtained and a search for alternatives to the use of plastic. Solarization and one-year fallow are not effective control alternatives.

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