Control of *Xiphinema index* Populations by Fallow Plants Under Greenhouse and Field Conditions

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ABSTRACT

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The dagger nematode *Xiphinema index* has a high economic impact in vineyards by direct pathogenicity and above all by transmitting the *Grapevine fanleaf virus* (GFLV). Agrochemicals have been largely employed to restrict the spread of GFLV by reducing *X. index* populations but are now banned. As an alternative to nematicides, the use of fallow plants between two successive vine crops was assessed. We selected plant species adapted to vineyard soils and exhibiting negative impact on nematodes and we evaluated their antagonistic effect on *X. index* in greenhouse using artificially infested soil, and in naturally infested vineyard conditions. The screening was conducted with plants belonging

to the families *Asteraceae* (sunflower, marigold, zinnia, and nyjer), *Poaceae* (sorghum and rye), *Fabaceae* (white lupin, white melilot, hairy vetch, and alfalfa), *Brassicaceae* (rapeseed and camelina), and *Boraginaceae* (phacelia). In the greenhouse controlled assay, white lupin, nyjer, and marigold significantly reduced *X. index* populations compared with that of bare soil. The vineyard assay, designed to take into account the aggregative pattern of *X. index* distribution, revealed that marigold and hairy vetch are good candidates as cover crops to reduce *X. index* populations in vineyard. Moreover, this original experimental design could be applied to manage other soilborne pathogens.

Additional keywords: allelopathy, nematicidal plants, sampling design, *Vitis vinifera*.

The dagger nematode *Xiphinema index* is economically the major virus vector in viticulture. It transmits exclusively the *Grapevine fanleaf virus* (GFLV), the most detrimental grapevine virus disease worldwide, responsible for the fanleaf disease (6,9,22). GFLV is transmitted from grapevine to grapevine while *X. index* feeds on growing root tips. GFLV particles are retained over long periods at specific sites within its feeding apparatus (12,36). Fanleaf disease has a sustained impact in vineyards where it is established because of the survival of *X. index* between two successive grapevine crops (6,12). Indeed, complete virus control is very difficult as a few surviving viruliferous *X. index* may be sufficient to infect grapevine plants and reinitiate a new GFLV outbreak (12,35). So far, control strategies relied mainly on soil disinfection to reduce *X. index* numbers between two successive grapevine crops (28,35). However, chemical treatments are effective only in the upper layers (0 to 40 cm) of the soil (37), whereas the highest densities of *X. index* usually occur deeper (40 to 110 cm depth), where many thin grape roots are present in nonirrigated vineyards (14,15,39). Moreover, nematodes can survive and retain GFLV in unfavorable conditions for many years (12). Therefore, nematicides have limited efficiency against *X. index*. Moreover, the use of nematicides is being prohibited in an

increasing number of countries, because of their adverse environmental effects and health risks (5). Devitalization using systemic herbicides can be used on GFLV-infected grapevines before uprooting them to destroy roots which could serve as virus and nematode reservoirs, thus delaying contamination of replants (13). Nowadays, the only alternative method to effectively reduce *X. index* numbers is a careful removal of root debris followed by a prolonged fallow period (at least 7 years) (12,14). But due to the limits of these cultural practices, developing alternative control strategies is essential. Among them, plants with an antagonistic effect on the vector *X. index* may be a suited alternative before replanting vines in naturally fanleaf-infested grapevine plots.

The antagonistic effect of some plant species against nematodes aroused an increasing interest during the last decades (2,7,19,20,34). Numerous plant species release compounds that can be biocide or interfere with the life cycle of the nematode (10,18,33), whereas others are simply nonhosts (30). Various plant species, belonging to diverse botanical families, have been reported to contain or release nematicidal compounds (10,17,18). In agriculture, some of these antagonistic plants are used to control nematodes, either in crop rotations, intercropping, as cover crops or as green manures (30). Most of the plants with a nematicidal effect belong to the botanical families *Asteraceae* (marigold [*Tagetes minuta* L.], cosmos [*Cosmos bipinnatus* L.], firewheel [*Gaillardia picta* L.], and zinnia [*Zinnia elegans* L.] among others) (8,16) and *Brassicaceae* (like rapeseed [*Brassica napus* L.], white mustard [*Sinapis alba* L.], and radish [*Raphanus sativus* L.]) (19).

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The main plant species previously evaluated for their antagonistic effect against *X. index* are listed in Table 1. The nematicidal activity of cosmos and white lupin (*Lupinus albus* L.), used as green manure, was also demonstrated against *X. americanum* (1). Those studies revealed a large number of plant species showing a potential antagonistic effect against *X. index* in controlled or in semifield conditions. Confirming the effect of these and new plants in field conditions is indispensable. Indeed, in nonirrigated vineyards, the main challenge of alternative methods for *X. index* control is to reach the deep soil layers where most of the nematodes live. Moreover, vineyard soils often present adverse agronomic characteristics (high sensitivity to water stress, low organic matter and nitrogen content, and high copper content) making them poorly suited for the development of most plants other than grapevine. This observation underlines the need to assess the antagonistic effect of plants against *X. index* under both controlled and field conditions.

Usually, the antagonistic effect of a plant species against a nematode pest, or more widely the effect of a control method against a pest or a pathogen, can be evaluated by comparing final toward initial population levels. However, *X. index* populations display a significant aggregative pattern in vineyards, with patches of 6 to 8 m diameter, together with a significant neighborhood structure of nematode densities (39). Therefore, sampling to accurately estimate nematode densities within fields needs to be performed on a very dense grid and at a depth of 50 to 80 cm (within soil layers that most often contain the highest density of thin growing grapevine roots) and is thus very labor and time consuming. Moreover, it requires heavy machinery (excavating machine) and risks profoundly disturbing the existing nematode populations. Consequently, a preliminary sampling before a field test could interfere with the conclusions of any further field plant evaluation. So, testing antagonistic plants against *X. index* needs an adequate protocol and a design minimizing the cost of sampling and maximizing information about nematode numbers.

The aim of this paper is to (i) evaluate 11 plant species for their antagonistic effect against *X. index* under artificial inoculation in controlled greenhouse conditions, and (ii) assess the antagonistic effect of four plant species under grapevine field conditions with a novel design taking into account the constraint of the aggre-

gative spatial pattern of the nematodes, as well as to assess the ability of these four plants to develop roots deep enough to reach nematodes.

MATERIALS AND METHODS

Identification of the selected plants. We chose plant species from families and species previously evaluated (Table 1) and/or considered as potentially adapted to pedoclimatic constraints of the Bordeaux vineyard. Plant seeds were provided by Jouffray-Drillaud Seeds Company (Cissé, France) except for *Tagetes minuta* provided by Phytosem (Gap, France).

The two trials included 13 selected plant species (Table 2) belonging to five botanical families. Among the plants evaluated in the assay conducted in the greenhouse, four plant species belong to the family *Asteraceae*: zinnia (*Zinnia elegans* Jacq.), for which Insunza et al. (23) showed an in vitro mortality of *X. index* with aqueous extracts from roots; marigold (*Tagetes minuta* L.), which exhibits a nematicidal effect against several nematode species and particularly root-lesion (17,29) and root-knot (26) nematodes; sunflower (*Helianthus annuus* L. 'Impario') for its abundant root system; and nyjer (*Guizotia abyssinica* (L.f.) Cass. Given the significant antagonistic effect of many *Brassicaceae* species against *X. index* observed by Aballay et al. (2–4, Table 1), using extracts or green manure, we chose to include rapeseed (*Brassica napus* L. 'Parapluie') and camelina (*Camelina sativa* L.). Phacelia (*Phacelia tanacetifolia* Benth.), a plant from the family *Boraginaceae*, already reported for its nematicidal effect against the beet cyst nematode *Heterodera schachtii* (21), was also included. We furthermore tested two *Fabaceae* species: the white lupin (*Lupinus albus* L. 'Clovis'), chosen for its significant antagonistic effect as cover crop against *X. americanum*, another nepovirus vector (1) and white melilot (*Melilotus albus* Medik). Lastly, two *Poaceae* species were also evaluated: sorghum (*Sorghum vulgare* Pers. 'Vercors') for its abundant root system and resistance to water stress and rye (*Secale cereale* L. 'Tonus'). The experiment also included the grapevine (*Vitis vinifera* L. 'Cabernet Sauvignon') as a positive control and the bare soil as a reference control. Grapevine plants were micropropagated in vitro and acclimatized in a greenhouse (38).

TABLE 1. Plant species previously evaluated for their antagonistic effect against *Xiphinema index*

Family	Common name	Species	Conditions	Application form	Effect	Reference
<i>Asteraceae</i>	Chamomile	<i>Chamomilla recutita</i>	In vitro	Aqueous extracts from flowers	Significant negative	23
	Cosmos	<i>Cosmos bipinnatus</i>	In vitro	Aqueous extracts (tops)	Significant negative	23
	Cosmos	<i>Cosmos bipinnatus</i>	Field	Green manure	Nonsignificant	2
	Firewheel	<i>Gaillardia picta</i>	Field	Green manure	Nonsignificant	2
	Pot marigold	<i>Calendula officinalis</i>	Field	Green manure	Nonsignificant	2
	Marigold	<i>Tagetes patula</i>	Field	Green manure	Nonsignificant	2
	Zinnia	<i>Zinnia elegans</i>	In vitro	Aqueous extracts (roots)	Significant negative	23
	Zinnia	<i>Zinnia elegans</i>	Field	Green manure	Nonsignificant	2
	Brown mustard	<i>Brassica juncea</i>	Greenhouse	Green manure	Significant negative	4
<i>Brassicaceae</i>	Rapeseed	<i>Brassica napus</i>	Greenhouse	Green manure	Nonsignificant	4
	Rapeseed	<i>Brassica napus</i>	Field	Green manure	Significant negative	2
	Radish	<i>Raphanus sativus</i>	Field	Extracts	Significant negative	3
<i>Chenopodiaceae</i>	Wormseed	<i>Chenopodium ambrosioides</i>	In vitro	Aqueous extracts (tops and roots)	Significant negative	23
	Wormseed	<i>Chenopodium ambrosioides</i>	Greenhouse	Green manure	Nonsignificant	4
<i>Elaeocarpaceae</i>	Maquei	<i>Aristotelia chilensis</i>	Field	Extracts	Significant negative	3
<i>Fabaceae</i>	White lupin	<i>Lupinus albus</i>	Field	Green manure	Nonsignificant	2
<i>Lamiaceae</i>	Lemon balm	<i>Melissa officinalis</i>	Field	Extracts	Significant negative	3
	Pennyroyal	<i>Mentha pulegium</i>	Field	Extracts	Significant negative	3
	Thyme	<i>Thymus vulgaris</i>	Field	Green manure	Nonsignificant	2
	Thyme	<i>Thymus vulgaris</i>	Greenhouse	Green manure	Nonsignificant	4
<i>Oxalidaceae</i>	Wood sorrel	<i>Oxalis rosea</i>	In vitro	Aqueous extracts (whole plant)	Significant negative	23
<i>Plantaginaceae</i>	Greater plantain	<i>Plantago major</i>	Field	Extracts	Significant negative	3
<i>Rutaceae</i>	Common rue	<i>Ruta graveolens</i>	In vitro	Aqueous extracts (leaves)	Significant negative	31
	Rue	<i>Ruta graveolens</i>	Greenhouse	Green manure	Nonsignificant	4
<i>Solanaceae</i>	Pepper	<i>Capsicum annuum</i>	In vitro	Aqueous extracts (pods)	Significant negative	32
	Vestia	<i>Vestia lycioides</i>	In vitro	Aqueous extracts (leaves)	Significant negative	23

For the vineyard assay, we chose two species within plants showing an antagonistic effect in controlled conditions: marigold (*T. minuta* L.) and white lupin (*L. albus* 'Clovis'). We also added two other *Fabaceae* crops, alfalfa (*Medicago sativa* L. 'Timbale') and hairy vetch (*Vicia villosa* L. 'Savane'), because of their ability to develop in vineyard soils.

Production of nematodes. Fig (*Ficus carica*) plants were used to establish rearings of aviruliferous *X. index* in the greenhouse. Fig plants were produced from green cuttings cultivated in a climatic chamber under controlled temperature (26°C) and light (16 h) conditions. About 500 aviruliferous *X. index* were used to initiate rearings with a rooted fig cutting in 10-liter pots containing loess (1/3) and sand (2/3, wt/wt) as artificial substrate. Fig plants were cultivated under controlled conditions (20 to 25°C and 16 h daylight) in the greenhouse.

Greenhouse assay. The assay in controlled conditions was carried out in a greenhouse with temperatures ranging from 15 to 25°C. Additional light (6 a.m. to 8 p.m.) was provided by SONT lamps. We performed the sowing on 28 August 2006 in 3-liter containers (10 replicates per plant species) filled with steam-sterilized soil taken from a Bordeaux vineyard plot. This soil was previously sterilized (30 min at 120°C) to kill any endogenous nematodes. Plant containers were randomly distributed in a single-block design and drip irrigated automatically every day. Finally, on 5 September 2006, we inoculated 200 *X. index* individuals per container into a 5-cm deep hole. Plants were in the seedling stage.

Vineyard assay. This assay was performed in a naturally highly GFLV-infected commercial vineyard located in the Bordeaux region (France). This plot was uprooted in March 2007

TABLE 2. Plants used for the two experimental procedures

Family	Common name	Species	Evaluation condition in the present study		Selection criteria	Reference
Asteraceae	Marigold	<i>Tagetes minuta</i> L.	Greenhouse	Vineyard	Nematicidal effect against a lot of nematode species	16,25,28
	Nijer	<i>Guizotia abyssinica</i> L.f.	Greenhouse		Ability to develop in vineyard soils	nd
	Sunflower	<i>Helianthus annuus</i> L. 'Impario'	Greenhouse		Abundant root system	nd
	Zinnia	<i>Zinnia elegans</i> Jacq.	Greenhouse		Negative effect on <i>X. index</i>	22
Brassicaceae	Rapeseed	<i>Brassica napus</i> L. 'Parapluie'	Greenhouse		Negative effect of many <i>Brassicaceae</i> species against <i>X. index</i> (extracts or green mature)	2,3,4
	Camelina	<i>Camelina sativa</i> L.	Greenhouse			
Boraginaceae	Phacelia	<i>Phacelia tanacetifolia</i> Benth.	Greenhouse		Nematicidal effect against cyst nematodes	20
Fabaceae	White lupine	<i>Lupinus albus</i> L. 'Clovis'	Greenhouse	Vineyard	Negative effect against <i>X. americanum</i> (cover crop)	1
	White melilot	<i>Melilotus albus</i> Medik.	Greenhouse		Ability to develop in vineyard soils	nd
	Alfalfa	<i>Medicago sativa</i> L. 'Timbale'		Vineyard	Ability to develop in vineyard soils	nd
	Hairy vetch	<i>Vicia villosa</i> Brot. 'Savane'		Vineyard	Ability to develop in vineyard soils	nd
Poaceae	Sorghum	<i>Sorghum vulgare</i> Pers. 'Vercors'	Greenhouse		Abundant root system and resistance to water stress	nd
	Rye	<i>Secale cereale</i> L. 'Tonus'	Greenhouse		Abundant root system and resistance to water stress	nd
Vitaceae	Grapevine	<i>Vitis vinifera</i> L. 'Cabernet Sauvignon'	Greenhouse		Positive control

Fig. 1. Experimental design for the field evaluation of antagonistic plants against *Xiphinema index*. Each square (4 × 4 m) supports a crop or bare soil (reference control). The five squares framed in bold represent a unity of generalized estimating equation analysis (here, the example of one replicate of *Medicago sativa* with the four surrounding reference control plots).

after a devitalization with Glyphosate (Roundup) in autumn 2006. No nematicidal treatment had been applied. The upper soil layer was sandy gravel with low organic matter (< 0.5%) over a layer of white sand. Four preexploratory 2-liter samples revealed the presence of 0, 2, 8, and 14 *X. index* individuals per sample, respectively.

We designed a protocol taking into account the maximum cost associated with the sampling (from soil sampling in the field to nematodes extraction and counts in the lab), estimated at 100 sampling points. The protocol design included 10 replicates of each tested plant. In the chessboard-like arrangement, each replicate (4 × 4 m plot) was surrounded with four 4 × 4 m control plots (bare soil). The experimental site encompassed a total of 98 plots and measured 2,112 m² (Fig. 1). We carried out the sowing at the end of April 2007. We made the tested plants grow over 4 months, without irrigation in order to be as close as possible to the real grapevine-growing conditions. Mechanical weeding was done in the bare soil plots all along the experiment.

Nematode sampling, extraction, and count and root measurements. *Greenhouse assay.* After 4 months of cultivation, we extracted nematodes from the total content of each pot as described in Villate et al. (39). Because we could not extract nematodes from all of the 130 pots on the same date, each pot was randomly taken for extraction to avoid any temporal effect on final nematode numbers over a period of 2 weeks.

Vineyard assay. We carried out the final sampling on 27 August 2007. In the center of each of the 98 plots, we dug a 1.2-m-deep and 0.5-m-wide trench with an excavating machine and recovered a 5-liter sample of soil by collecting soil clods from four to six points of the undisturbed opposite sides of the trench, at 0.5 to 0.8 m depth. We also measured the maximum depth reached by the roots of each plant species in each plot on the sides of the trench using a tape measure. Soil samples were stored at 20°C in

the dark until nematode extraction. We gently mixed each 5-liter soil sample and randomly took a 2-liter subsample for nematode extraction.

In both assays, nematodes were isolated from soil samples by the Cobb's sieving and decanting method, as described in Villate et al. (39). Adults and juveniles were counted from the final suspension under a dissecting microscope.

Data analysis. *Greenhouse assay.* To test the plant effect on nematodes, we tested the correlation between the final nematode numbers and the modality (each plant species, grapevine or bare soil) by computing a multi-analysis of variance (ANOVA). The difference between pairs of modalities was tested using the Tukey test (95% confidence limits). All computations and analyses were done using R software package 2.6.1. (27). We first built a model with the modalities grapevine, bare soil and each of the 10 plant species to test whether, as expected, nematode densities were higher in grapevine. Then, we removed the grapevine modality from our model for two reasons. First, we wanted to test whether nematode densities were affected by a treatment different from bare soil, the reference control treatment. Second, the regression curve was hence not influenced by extreme values from this modality and the model gained power in predicting expected values from the other modalities.

Vineyard assay. Analyses aimed here to test whether nematode numbers differed between bare soil plots and sown plots. Nematode numbers were analyzed against the modality (i.e., *T. minuta*, *V. villosa*, *M. sativa*, *L. albus*, or reference control) in a generalized estimating equation (GEE) model to take into account correlated data. Indeed, (i) control plots (bare soil) are shared between four modalities in our experimental design and (ii) because nematode numbers show a neighborhood structure in the soil (39), nematode number in a plot depends on the numbers in the surrounding plots. If this correlation were not taken into account,

Fig. 2. Mean nematode numbers, with standard errors, observed on each modality in the 2-liter pots of the greenhouse assay (10 replicates).

the standard errors of the parameter estimates would not be valid (for details see 24). Each of the 10 replicates of a given plant species was associated with the four surrounding control plots (Fig. 1). We used a $\log(x + 1)$ transformation to normalize the data and the model assumed a Gaussian error (family = Gaussian). The variance-to-mean ratios (dispersal indices = DI) were computed to test whether nematodes were uniformly (uniform distribution; $DI < 1$), randomly (Poisson distribution; $DI \approx 1$), or patchily (aggregated distribution; $DI > 1$) distributed in bare soil plots (24). All the computations were performed with the R software package, version 2.6.1. (27).

RESULTS

Antagonistic effect of plants evaluated in controlled conditions. Four months (1 cropping cycle) after the inoculation of 200 *X. index*, surviving nematode numbers were determined. Figure 2 shows the final numbers, with standard errors, of *X. index* recovered on each plant modality, the grapevine as positive control and bare soil as reference. The ANOVA revealed a significant effect of the modality on nematode final numbers ($F = 984.15$; $df = 12$; $P < 0.001$). The contrast analysis performed by the Tukey test indicated that nematode numbers in grapevine were significantly higher than in each of the other modalities (estimate < 0 and $P < 0.001$); all plant species and bare soil had a significant negative effect on *X. index* numbers compared with grapevine. The high nematode numbers found in grapevine tended to mask the effect of other modalities. So, because we aimed to find plants with a significant negative effect on nematode numbers compared with bare soil, we excluded the grapevine modality from the next analysis (see Materials & Methods). Without the grapevine modality, we still revealed a significant effect of the modality (each of the 10 plant species and bare soil) ($F = 37.608$; $df = 11$;

$P < 0.001$). The Tukey test showed that final nematode numbers were significantly lower in *L. albus*, *M. albus*, *G. abyssinica*, and *T. minuta* compared with the reference control bare soil (estimates < 0 and $P < 0.001$). On the other hand, the final nematode numbers in *H. annuus*, *S. vulgare*, and *P. tanacetifolia* were significantly higher than those of the bare soil (estimates > 0 and $P < 0.05$), and nematode numbers in *M. sativa*, *Z. elegans*, *B. napus*, and *S. cereale* were not significantly different from bare soil. Even when excluding the three plant species showing a positive effect compared with bare soil (*H. annuus*, *S. vulgare*, and *P. tanacetifolia*), we still revealed a significant effect of the modality (plant species or reference control) ($F = 3.8488$; $df = 8$; $P < 0.001$) but only *L. albus*, *G. abyssinica*, and *T. minuta* showed final nematode numbers significantly lower than the bare soil (estimates < 0 , $P < 0.001$ for *L. albus* and $P < 0.05$ for *G. abyssinica* and *T. minuta*).

Antagonistic effect and rooting depth evaluated in vineyard conditions. After 4 months of cultivation, sampling of the 96 squares was performed and the numbers of *X. index* were evaluated in the field experiment. Nematode numbers in bare soil plots were significantly higher than those counted in sown plots

TABLE 3. Generalized estimating equations showing the effects of the plant species on nematode numbers^a

	β	SE	P
Intercept	4.0258	0.1302	0.0000
<i>Lupinus albus</i>	-0.4413	0.2813	0.1167
<i>Medicago sativa</i>	-0.1211	0.3453	0.7257
<i>Tagetes minuta</i>	-0.8082	0.2971	0.0065
<i>Vicia vilosa</i>	-0.6271	0.3133	0.0453

^a β , estimated regression coefficient; SE, standard error of β ; and P, significance of β .

Fig. 3. Mean nematode numbers, with standard errors, sampled in the soil sown with each plant species or in bare soil from the vineyard assay (10 replicates).

(test = GEE, $\chi^2 = 14.76$, $df = 4$, $P < 0.05$). GEE indicated a significant negative effect of *T. minuta* and *V. villosa* on nematode numbers, whereas *L. albus* and *M. sativa* showed a negative but nonsignificant effect (Fig. 3, Table 3). We observed a high variance of nematode numbers in plots planted with *M. sativa* (Figs. 3 and 4). Finally, we observed an aggregative spatial pattern of nematode density when taking into account only bare soil plots (Fig. 4), with DI value of 257.7 ($P < 0.001$). Observations of the root systems showed that *M. sativa* and *T. minuta* roots reached

70 to 80 cm mean depth, whereas those of *L. albus* and *V. villosa* only reached approximately half of this depth (35 to 40 cm).

DISCUSSION

The present study aimed mainly at identifying plants that enable reducing the fallow period between two successive grapevine plantations by accelerating nematode decrease compared with bare soil. To address this issue, a panel of plant species, selected

Fig. 4. Horizontal distribution of *Xiphinema index* numbers in the vineyard assay. Values within circles indicate, for each plot, the numbers recovered per 2-liter soil sample.

on their putative antagonistic effect or from previous studies (2–4,23,31,32), was evaluated both in controlled conditions and in a naturally GFLV and *X. index* infested vineyard. Experiments in controlled conditions allowed an initial screening of potential antagonistic plants against *X. index* (the nematodes are exposed to a high root density), whereas assays in vineyard conditions permitted to test two of the greenhouse antagonistic plants in natural conditions together with two plants already known as agronomically well adapted under vineyard conditions. To our knowledge, the present study is the first report evaluating plants in both controlled and field conditions.

In the controlled greenhouse experiment, our study showed that *M. albus*, *L. albus*, *T. minuta*, and *G. abyssinica* significantly reduced nematode numbers compared with bare soil. The final *X. index* numbers in presence of *H. annuus*, *S. vulgare*, and *P. tanacetifolia* were significantly higher than those of the bare soil, even though they were lower than in grapevine, the natural host of *X. index*. *Medicago sativa*, *Z. elegans*, *B. napus*, and *S. cereale* displayed no significant change compared with bare soil. In the novel experimental design conducted in vineyard, 4 months of marigold (*T. minuta*) and hairy vetch (*V. villosa*) cultivation significantly reduced nematode densities compared with bare soil.

Previous studies already revealed an antagonistic effect of some plant species against *X. index*. They showed both significant and nonsignificant negative effects of *Z. elegans* and *B. napus*, depending on the type of application (Table 1). *Z. elegans* reduced nematode numbers when applied as aqueous extracts (23) but not as green manure in a field experiment (2), whereas *B. napus* showed an antagonistic effect as green manure in the field (2) but not in greenhouse (4). In our greenhouse assay, neither *Z. elegans* nor *B. napus* showed a significant antagonistic effect compared with bare soil. However, both species seem moderate hosts as nematode numbers recovered from both species are significantly lower than in grapevine, the natural host of *X. index*. On the other hand, we revealed an antagonistic effect of *T. minuta*, *L. albus*, and *G. abyssinica* in controlled conditions, whereas these plants did not show any significant effect on nematode numbers when applied as green manure in previous field studies (2).

In the vineyard assay, marigold *T. minuta* also significantly reduced *X. index* numbers compared with bare soil. This result is consistent with the well-known nematocidal effect of marigold against various nematode species (17,26,29). Marigold showed good growth vigor and produced abundant roots reaching deep layers where highest nematode numbers are mostly located. By contrast, the hairy vetch, which also showed a significant antagonistic effect in vineyard, rooted significantly less deeply (35 to 40 cm) and thus appeared unable to reach the soil layers where most of the nematodes are located. These elements indicate the importance of unraveling the mechanisms of the antagonistic activity of the plants to *X. index*. Our experimental approach conducted in vineyards clearly showed that, used as a cover crop, both plants significantly reduce nematode densities compared with bare soil.

Globally, a negative effect of a plant may be due to a direct action towards the nematodes through root compounds or exudates which could be biocidal or interfere in their life cycle (10,17,18,33). Nevertheless other hypotheses may be proposed. Among them a negative physical effect of the plant cannot be excluded. The plant roots absorb water, drastically decreasing soil humidity, and thus disfavor nematode survival, even though a part of the individuals may enter in a quiescent status as observed by Dalmasso (11) in the sympatric *X. mediterraneum* species. As *X. index* occurs deep in the soil, deep-rooting plants used as cover crops appear the most appropriate application form (but *V. villosa* showed significant effects in spite of shallow rooting). By contrast, antagonistic plants used as green manure, even though they may release active compounds incorporated into the soil, are

less likely to have an effect as, after incorporation, they will still not reach the deep nematode individuals.

The spatial distribution pattern of *X. index* has been studied previously in naturally infested vineyard (39). We confirmed this pattern in the present study. When taking into account only bare soil plots, we revealed a significant aggregative pattern. The aggregative pattern and the neighborhood structure of nematode densities are an important constraint that must be considered in vineyard experiment designs to propose appropriate protocol and sampling designs (39). Moreover, sampling *Xiphinema* nematodes in grapevine fields often requires an excavating machine, is very time consuming, and may disturb the soil structure of the field. Nevertheless, field experiments, whatever their objectives, have to fit with these limiting factors and to consider the maximum sampling cost that they can support. Taking into account all these constraints, we have proposed here a novel design that allowed, completed by a GEE analysis, to overcome these major difficulties and to get a reliable evaluation of the antagonistic effect of plant species. This novel and original design, as well as the statistical analysis we proposed here, could be applied to other soil pests and pathogens, in particular other nematodes and fungi.

This design might still be improved given that reference control plots were disposed regularly whereas plants were disposed randomly. By keeping the same maximum sampling cost (100), we could set randomly both reference control and plant species plots. This procedure should permit to calculate data all together and thus will reduce the number of reference control plots and will increase the possible number of replicates per plant species or the number of species tested in a single experiment.

Most studies on the antagonistic effect of plants against nematodes, and particularly *X. index*, were limited to in vitro or greenhouse experiments. Such experiments are useful as initial screening but cannot be conclusive on the effect in vineyard field conditions. Our experimental design successfully accounted for the complexity of testing such plants in vineyard conditions, because of the field constraints related to a soil dwelling pest and unfavorable pedoclimatic conditions. Our results clearly showed that marigold and hairy vetch had a negative effect on the survival of the vector nematode *X. index* under field conditions and are promising candidates in the context of biological control of the nematode vector of GFLV. Hairy vetch is already used as cover crop since it grows well on these soils and fixes nitrogen between two successive grapevines and is adapted to a wide range of soil types. Moreover, its cultivation could be still optimized by autumn sowing to allow the plant to root deeper.

Given the complexity of this system, it clearly appears useful to test these and other plants (preselected in pot experiments) on various types of soil as well as in different vine-growing conditions.

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