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The nematode *Xiphinema index* is, economically, the major virus vector in viticulture, transmitting specifically the Grapevine Fanleaf Virus (GFLV), the most severe grapevine virus disease worldwide. The management of this disease has long been to use soil fumigation, harmful for both the applicator and the environment. The objective of this study was to evaluate an alternative approach using plants to reduce vector nematode populations between uprooting and replanting. Of thirty botanical species, tested in previous greenhouse trials, the seven best performing plants were evaluated for their capacity to reduce *X. index* populations in soil compared to bare soil in 5 field trials on different soil types in Bordeaux and Burgundy. In most trials, sown fallows reduced the number of *X. index* nematodes more efficiently than bare soil. All plants tested in field, except *Trifolium pratense*, showed their efficacy in field on survival of nematodes *X. index* but this efficiency varied according to species and site. The best results were obtained with *Medicago hybride, Tagetes minuta*, *Avena sativa* and *Vicia villosa*. Over the following years we will be evaluating if a decrease of the populations of the nematode vector does lead to a significant drop or delay of GFLV contamination for the newly planted vines.
Keywords : Fallow, *Xiphinema*, Virus, Grapevine fanleaf, soil type

Introduction

Fanleaf degeneration is a viral disease affecting the vines longevity, the production potential and the quality of wine grapes. Two-third of French Vineyards is affected by this viral disease [1].
The two main viruses responsible for the fanleaf disease are the grapevine fanleaf virus (GFLV) and the arabic mosaic virus (ArMV). They are respectively transmitted by *Xiphinema index* and *Xiphinema diversicaudatum* which often living deep in the soil [2]. Among the virus vector's nematodes, *X. index* has the most significant economic impact. The worldwide distribution of *X. index* is closely related to that of grapevine.
The propagation of GFLV is possible either by plant material or by nematodes from grapevine to grapevine. As the dissemination by propagation material has been reduced by clonal selection and certification for plants, it is now necessary to find strategies to control the propagation of GFLV in vineyard conditions. An effective strategy is needed to reduce nematode populations in the soil. For a long time, winegrowers used chemical products to fumigate the soil between two successive grapevine crops. One of the alternatives to the use of these toxic synthetic products is the use of antagonistic plants during fallow period for their effect on the reduction of nematodes vectors.
Several plants are known to their nematicidal effect on different crops. For instance, *Tagetes spp*. have been identified to their effective control of some species of root-infesting nematodes as *Pratylenchus penetrans* or *Meloidogyne hapla* [3, 4]. *Sorghum vulgare* incorporated as green manure before planting lettuce resulted in lower reproduction of M*eloidogyne hapla* [5].
In *vitro* biotests, *Thymus vulgaris* or *Ruta graveolens* extracts showed a nematicidal activity against *X. index* and *X. americanum* [6] but this effect was not confirmed in field tests. *Chenopodium ambrosioides* extracts have an effect on *X. index* and *X. americanum* in vitro but also against *Meloidogyne spp.* or *Ditylenchus dipsaci* [7, 8]. Aballay showed that all the plants tested in greenhouse trial (*Chenopodium ambrosioides, Ruta graveolens, Thymus vulgaris and Brassica juncea)* were able to significatively reduce *X. index* populations but the final densities were significantly higher than chemical control [9]. Field tests with *Brassicus napus, Cosmos bippinatus, Lupinus albus, Thymus vulgaris, Zinnia elegans, Hordeum vulgare or Calendula officinalis*, showed none of these plants were able to control the population of nematodes during their development or incorporation. Only Brassica significantly decreased populations after incorporation [10].
This study evaluates the use of a few plant species, previously selected through greenhouse screening, for their capacity to reduce *X. index* populations [11, 12] during fallow in the field.

Material and method

Seven plant species able to decrease the nematodes populations by about 50% compared to bare soil (negative control) in controlled conditions [11, 12], were tested in field conditions. Several trials were carried out between 2007 and 2012 with the same plants to take into account the effect of sowing date and pedoclimatic conditions.

- **Experimental field design**

The trials were carried out in the Bordeaux and Burgundy vineyard regions. For each trial, the protocol design encompassed 10 to 20 replicates of each tested plant. In the chessboard-like arrangement, each replicate (3 x 3m plot) was surrounded by several control plots (negative control = bare soil). A total of 98 to 142 plots was required to evaluate 3 to 4 plants per trial. Mechanical weeding was performed on the bare soil plots.

The sowing of the plant plots was carried out at the end of April for the first trial and in September/October for the four others trials with a SEMBONER manual single row seeder.

The plants were grown from 4 to 9 month depending on sowing date and vine planting date. Table 1 shows the characteristics of each trial.

Table 1 : Characteristics of the five trials

Trial	A	B	C	D	E
Site	Margaux (33)	Saint Emilion (33)	Gevrey Chambertin (21)	Saint Emilion (33)	Arsac (33)
Climate	Oceanic	Oceanic	Continental	Oceanic	Oceanic
Soil	Sandy gravel soil	Sandy gravel soil	Clay limestone soil	Clay limestone soil	Sandy gravel soil
Uprooting date	March 2007	March 2009	May 2012	July 2012	March 2012
Plants	*Vicia villosa, Lupinus albus, Tagete minuta, Medicago hybride*	*Avena sativa, Vicia villosa, Medicago hybride, Trifolium pratense*	*Avena sativa, Vicia villosa, Medicago hybride*	*Onobrychis viciifolia, Avena sativa, Medicago hybride*	*Avena sativa, Vicia villosa, Medicago hybride*
Control	Bare soil	Bare soil	Bare soil	Bare soil	Bare soil
Number of replicates	10	12	20	20	20
Samples number	98	142	110	110	114
Sowing	April 2007	September 2009	October 2012	September 2012	October 2012
Sampling date	August 2007	July 2010	June 2013	June 2013	April 2013
Period	4	9	8	9	6

- **Nematodes sampling and extraction**

At each sampling point, a trench (1.5 m long, 0.4 m wide, 1 m deep) was dug with an excavating machine. A 4 liter sample of soil was collected manually from 5-6 points from the undisturbed sides of the trench at depths of 0.3 to 0.8 depending on soil profile.

After mixing, nematodes were extracted in the laboratory from a 2 liter subsample, using the method described by Villate [12]. Adults and juveniles were counted from the final suspension under a dissecting microscope. Maximum observed rooting depth was observed for each plot

- **Data analysis**

For each site, the effect of sown fallows on the rooting depth was analyzed using ANOVA. When a significant effect was found, multiple comparisons were conducted to test differences using Tukey's HSD test. For each site, number of *X. index* on soil and number of *X. index* on bare soil were defined as a matched pair of counts. Effect of plants on *X. index* were analyzed as proportion data using a GLM with binomial errors and logit link and to test the difference to zero. Soil effect was tested with mixed-model. We used fixed effects for plants and soils and a random effect to take in account the site.

Results

- **Vine root system**

To control *X. index*, the first challenge for the plants tested is to reach the deeper soil layers where the nematodes live. So rooting depth was measured for each plant on each plot. The results are presented in figure 1.

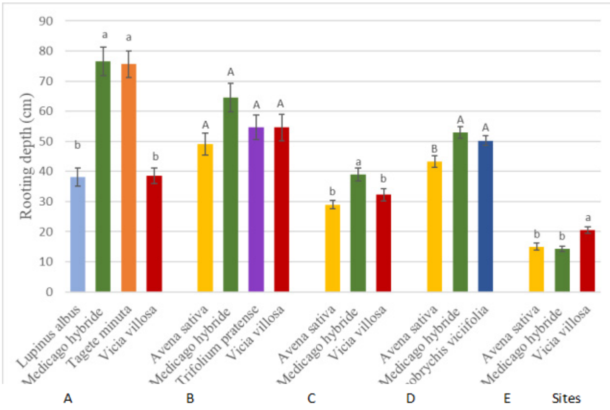

Figure 1: Depth of rooting of each plant on the 5 test sites.

In trial A, *Medicago hybride* and *Tagetes minuta* roots reached 75 cm mean depth whereas those of *Lupinus albus* and *Vicia villosa* reached only 38 cm. In trial B, there was no difference between rooting depths, *Medicago hybride* obtained 65 cm mean depth which was less than in trial A. For the same growing time, roots of *Medicago hybride* were even less developed in trial C and D with respectively 39 cm and 53 cm mean depth compared to the two first trials but *Medicago* was still the deepest rooting plant. In trial C, the difference was significant with *Vicia villosa* (32 cm) and *Avena sativa* (29 cm). In trial D, *Onobrychis viciifolia* obtained results similar to *Medicago hybride* with 50 cm mean depth while roots of *Avena sativa* reached 43 cm . In the last trial E, the plants were poorly developed the day of the observations in April, their roots had still reached 20 cm mean depth for Vicia villosa and around 15 cm mean depth for *Avena sativa* and *Medicago hybride*. Based on these results, it seems that the rooting depth is more dependent on the soil type than the length of the crop cycle (except for test E). For the same species, rooting will be deeper in sandy gravel soils than in clay limestone soils.

- **Antagonistic effect of plant species on *Xiphinema index* numbers**

All tested plant were able to reduce the populations of *X. index* compared to bare soil during at least one trial (one vegetative cycle in field) except *Trifolium pratense* (Figure 2). However, there are differences depending on the test sites.

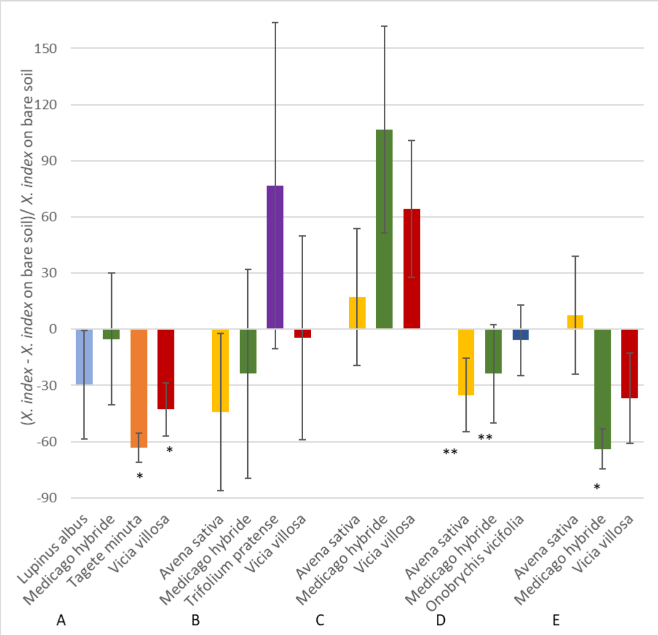

Figure 2: Efficiency of fallow plants on the reduction of *X. index* populations compared to bare soil

In the first trial (Site A), sown in spring and harvested in August, revealed a good efficiency of all 4 plants. The strongest reduction of *X. index* populations was obtained with *Tagetes minuta* that reached more than 60% decrease in *X. index*. A decrease in *X. index* populations of 40% and 30% was observed on plots sown with *Vicia villosa* and *Lupinus albus*, respectively, whereas only 5% reduction on *X. index* populations was obtained for *Medicago hybride* compared to bare soil. But only *Tagetes minuta* and *Vicia villosa* show a significant difference compared to the bare soil. In the second trial (Site B) was sown in autumn and harvested the next spring, which prevented the sowing of (frost sensitive) summer crops *Tagetes* and *Lupinus albus*. Only *Medicago hybride* and *Vicia villosa* species could be evaluated again. *Trifolium pratense* and *Avena sativa* completed the design trial. The best result in this trial was obtained with *Avena sativa* which reached more than 40% decrease of *X. index* compared to bare soil but the difference was not significant. *Vicia villosa* and *Medicago hybride* were less successful with only 24 % decrease in *X. index* numbers for *Medicago hybride* and no significant difference with bare soil. *Trifolium pratense,* that gave very good results in the greenhouse, [11, 12] did not confirm this in the field.
The other three trials were sown in October 2012 and harvested in late spring 2013. The trial on the site E was harvested earlier in April. On site C, the plants fallow did not reduce the populations of nematodes and on the contrary, there was more *X. index* than the bare soil control at the end of the trial (no statistical difference). On site D, all plants showed a decrease of *X. index* populations after 9 month in the same proportion with *Avena sativa* and *Medicago hybride* as in trial site B but the differences were significantes compared to the bare soil on this trial. *Onobrychis viciifolia* did not confirm in the field the results obtained in greenhouse. In site E the plants didn't have time to develop but *Medicago hybride* still allowed a very strong reduction in populations compared to the control with a statistical difference.
The period of plant development does not seem to have a strong impact on the percentage reduction of *X. index* populations. On the other hand, soil type seems to play a larger role in the nematodes population decrease with a stronger decrease on sandy gravel soils than on clay limestone soils.

Discussion

All the plants tested in field conditions had shown reduced nematode populations in greenhouse tests but they did not confirm these results in all field trials. In the field, the highest reduction was obtained with *Medicago sativa* (trials B, D and E) but in trial A this plant showed no effect and in trial C there was more *X. index* on average on the plots of this plant compared to the bare soil plot. Conversely, *Avena sativa* gave the most regular results in the different trials with reduction between 30% and 45% on trials B and D but no effect of this plant compared to bare soil in trial C and E. *Vicia villosa* did not confirm the strong diminution of *X. index* populations compared to the bare soil, obtained in the first trial, in the next trials. The decrease of *X. index* populations were better in greenhouse than those in field for *Tagetes minuta* and a little less for *Lupinus albus* but these two plants have been set up on only one trial [11]. However *Tagetes spp*. is known for its strong nematicidal effect on several species of nematodes and the results obtained in this test confirms once again this property. *Lupinus albus* reduced *X. index* population in our conditions while Aballay and Insunza [10] had not shown efficacy. Likewise, *Trifolium pratense* did not reduce populations of nematodes in the field, but was used in only one trial.
We can not explain why *Vicia villosa, Medicago hybride*, and to a lesser extent *Avena sativa,* have led to an *increased* survival of the populations of nematodes on the site C in Burgundy. The implantation of more trials in this geographic area would have been necessary to conclude. It could be that anaerobic conditions due to waterlogging in the bare soil reduced nematode numbers, while waterlogging was prevented by the plants. The success of one of a plant should be connected with its development and particularly with its rooting depth. However, the best results have not always been obtained with the plants which had the deepest root system.
Many unexplained points persist and the implantation of new trials is necessary to better qualify the link between the pedoclimatic conditions, the implantation of the species and their efficiency to reduce the populations of *X. index.* New experiments will be conducted to better understand the mechanisms of action of the best plants to reduce populations of *X. index* in the field. New trials will be set up to follow the rate of recontaminations of a new vine by the GFLV after two years of fallow with these nematicidal plants.

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