MANAGEMENT OF GRAPEVINE FANLEAF VIRUS VECTORS

GESTION DU REPOS DU SOL DANS LA LUTTE CONTRE LE COURT-NOUE DE LA VIGNE

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SUMMARY

Grapevine Fanleaf Virus (GFLV) is the main virus disease on grapevine. Yield and vigour decrease quickly due to viral diseases which considerably reduces the lifespan of a vineyard plot. Such situations can be avoided by applying some precautions in the period between uprooting and replanting, based on the latest research findings.

As fanleaf disease is due to viruses transmitted by nematodes, surviving vector nematode populations should be eliminated before replanting a plot. A fallow period of 5 to 7 years is often recommended, but is economically difficult to apply. Using systemic herbicides to kill the old vines before uprooting is a first step in reducing food resources for the remaining nematodes. We have developed a nematode sampling system to quantify vector nematode populations. The results show that nematode populations are highly variable, and allow modulation of the fallow period in relation to the recontamination risk.

Research is now focusing on plants that reduce nematode populations faster than by simply leaving the soil bare during the fallow period. After initial screening under greenhouse conditions, a national French project was started recently to assess, under field conditions (various soil and climatic conditions), the effect of different selected plants on the nematode populations. Equally, the rate of GFLV recontamination of the plots after replanting is being assessed over a long period of time.

Other improvements for managing plant-parasitic nematodes could be integrated, such as using resistant rootstock.

RÉSUMÉ

Le *Grapevine Fanleaf virus* (GFLV) est la principale virose sur la vigne. Le rendement et la vigueur diminuent rapidement sur les parcelles virosées réduisant par conséquent leur durée de vie. Des problèmes de recontamination et donc d'arrachages précoces pourraient être évités en suivant un itinéraire cultural adapté.

Le court-noué est dû à des virus transmis par les nématodes du sol du genre *Xiphinema*, vecteurs qui devraient être éliminées avant de replanter une parcelle de vigne. Pour cela, une période de jachère de 5 à 7 ans est recommandée mais économiquement difficile à appliquer. La dévitalisation par des herbicides systémiques est la première étape pour éliminer les ressources alimentaires des nématodes présents sur la parcelle. Suite à des observations sur de nombreuses parcelles nous avons développé un diagnostic afin de quantifier les populations de nématodes vecteurs. Les résultats montrent que les quantités de nématodes sont très variables selon les parcelles et que la modulation de la période de jachère est possible en fonction du risque.

Nos recherches se concentrent maintenant sur des plantes capable de réduire les populations de nématodes plus rapidement qu'en laissant le sol sans couvert végétal pendant la période de jachère. Après un premier tri en conditions contrôlées sous serre, un projet national français vient de débuter afin d'évaluer, dans des conditions de terrain (divers types de sols et de conditions climatiques), l'effet des plantes sélectionnées sur les populations de nématodes. Le taux de recontamination des parcelles par le GFLV après replantation sera également évalué sur une longue période.

D'autres améliorations pour la gestion des nématodes phytoparasites pourrait être intégrées, comme l'utilisation de porte-greffes résistance.

Keywords: Monitoring, Virus, Fallow period, *Xiphinema*

Mots clés : Biosurveillance, Virus, Repos du sol, *Xiphinema*.

INTRODUCTION

The nematode *Xiphinema index* is, economically, the major virus vector in viticulture, transmitting specifically the *Grapevine fanleaf virus* (GFLV) (Hewitt *et al,*. 1958), the most severe grapevine virus disease worldwide (Andret *et al.*, 2004). In France, a second virus, *Arabis Mosaic Virus* (ArMV),

transmitted by the nematode vector *X. diversicaudatum* is present in vineyards (Jha and Posnette, 1959; Harrison and Cadman, 1959). Virus infestation affects yield quality and quantity. Decrease of yield can be sufficiently large to cause uprooting of vines and thus reduce their lifespan. Figure 1 shows an example of a decrease in yield on a plot showing clear symptoms of fanleaf. Fifteen years after planting, its yield was less than 30 hl/ha and it had to be uprooted after only 22 years (Van Leeuwen, personal communication). Indeed, plot renewal is the only solution when a plot is fully infested. Nematodes are capable of surviving in the soil for several years; experiments have described survival for at least 4 years in laboratory conditions, without any food (root residues) in the stored soil (Demangeat *et al.* 2005). Devitalisation followed by a fallow period of seven years is recommended to remediate soils but these techniques are not always applied. In the absence of devitalisation the root remnants in the deeper soil layers will persist for several years, and these can provide nutritional resources for nematodes. Herbicide devitalisation of the vine plants allows killing off all root residues. Magnien (1998) showed that the percentage of recontaminations after 10 years was strongly reduced when using systemic herbicides before uprooting (72% recontaminations without devitalisation versus 10% with).

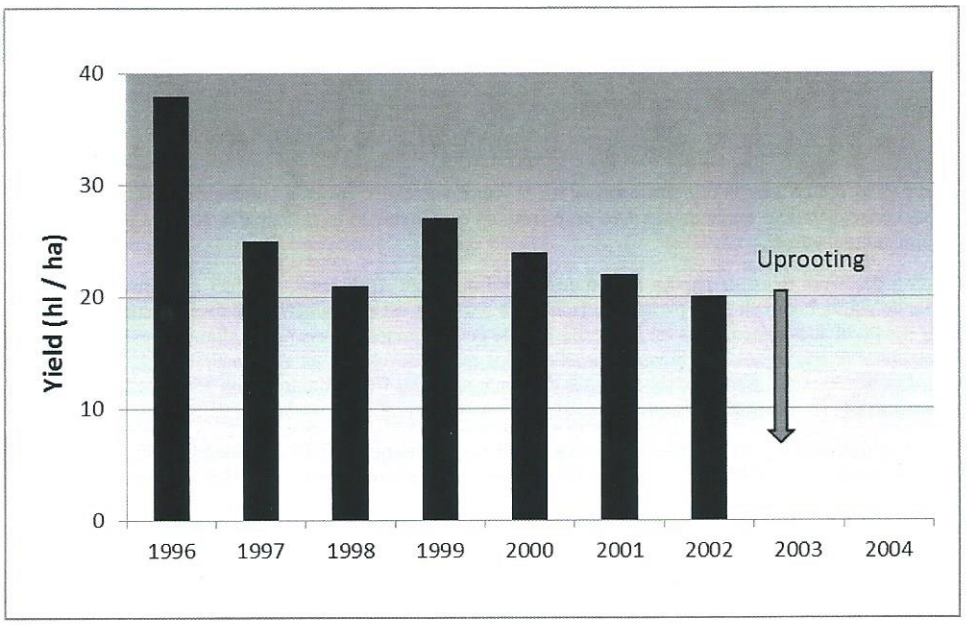

Figure 1: Evolution of yield in a GFLV infested plot planted in 1982 with Merlot.
Evolution du rendement sur une parcelle de Merlot plantée en 1982 et contaminée par le GFLV.

Dissemination of the virus by plant material has been reduced over the past by implementing rigorous certification schemes and establishing quarantine facilities but it is necessary to plant in soil free of infected nematode vector to control GFLV in vineyards. Villate (2008) showed, using microsatellite markers that transfer of nematodes from one plot to another is rare. Plot populations had clearly different genetic structure, showing that infestations are historic, and maintained on the plot.

During the fallow period, sowing of an annual crop for soil decompaction or as green fertilizer is often recommended to farmers. Since 2007, we study the impact of some crops on populations of *X. index*. Different plant species belonging to different botanical families have been identified for nematicidal effects. But nematodes species and their interactions with host plants vary widely. Therefore plants used to control nematodes on other crops could be inefficient on *X. index*. Moreover agronomic characteristics of vineyard soils are not favourable to the installation of other crops (water stress, low in organic matter or nitrogen, and high copper content). Taking into account all these parameters, we screened plants species against *X. index*. We present in this paper some new approaches for the management of diseased and uprooted plots to ensure longevity of re-planting through monitoring of nematode populations and the screening technique used to select plants to suppress nematodes during fallow.

MATERIAL AND METHODS

Nematode sampling and extraction

Nematode sampling was performed by digging 1 to 1.5 meter deep trenches using an excavating machine. Then samples were collected using a hand spade on 3 to 5 points of the sides of the trench in the 'undisturbed' areas of the rooting zone, most often at 0.6 to 1 meter deep. Total sample size was 5 litres. Nematodes were extracted as described by

Villate (2008) from 2 litre sub-samples. Nematodes were identified under a stereo microscope.

Monitoring of nematodes on uprooted plots

A total of 108 uprooted plots were sampled. The number of samples on each plot was very variable (2 to 50 according to plot size and sampling objectives). Most often, we did around 10 samples per hectare. In total 711 samples were collected and extracted.

Screening of plant efficiency

Plant species (figure 2) were sown in 3L of autoclaved vineyard soil. 200 *X. index* were inoculated in each pot (10 replicates per plant species). The greenhouse temperature was maintained at 20 °C and the plants watered automatically by drip irrigation. After a crop cycle, nematodes remaining in the 3L soil were extracted as described by Villate (2008) and counted.

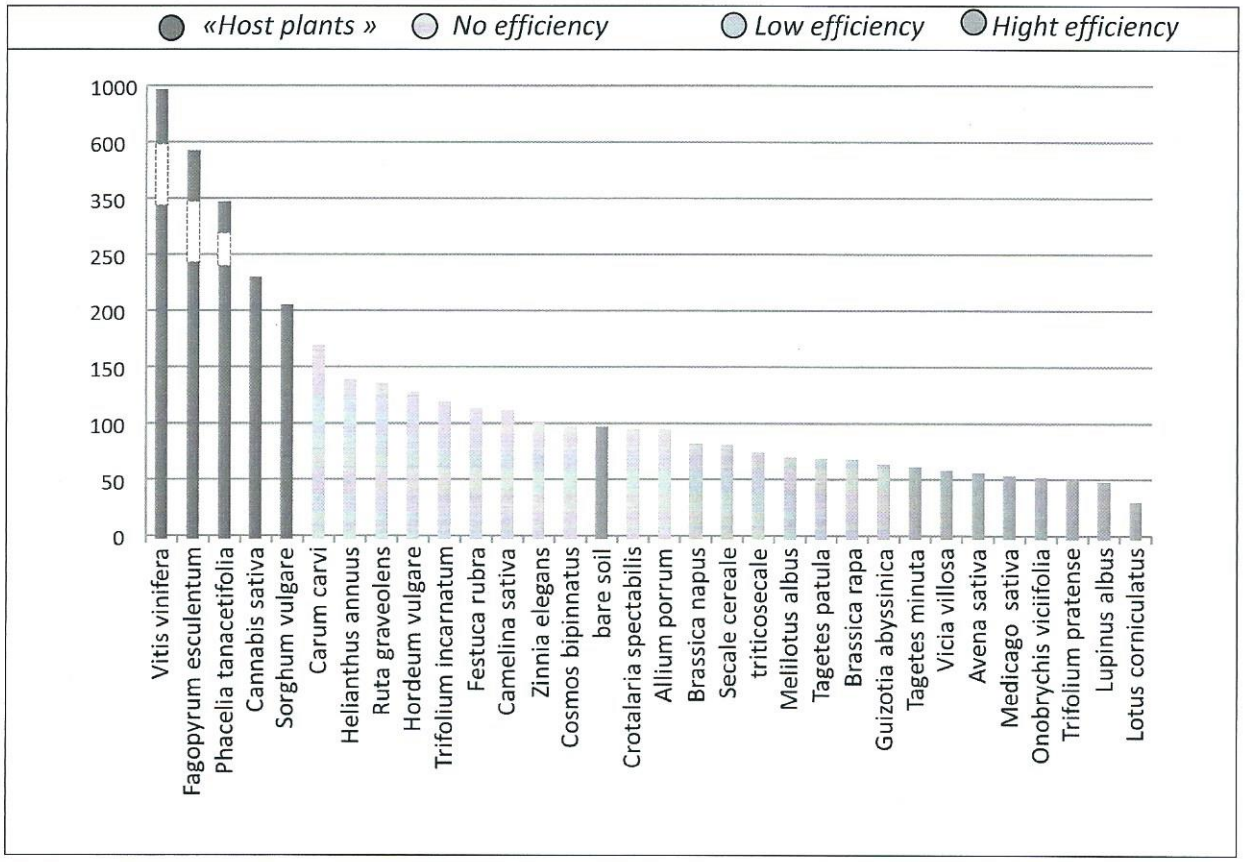

Figure 2: Efficiency of crop in controlled conditions (% remaining nematodes compared to bare soil); mean value on 10 replicates.

Efficacité des plantes testées en conditions contrôlées (% de nématodes restants par rapport au sol nu); valeur moyenne sur 10 répétitions

RESULTS AND DISCUSSION

Nematode populations on uprooted plots

Table I resumes the results of sampling of uprooted plots. *X. index* is more present than *X. diversicaudatum*, on all criteria: *X. index* was found on 79 plots against 39 for *X. diversicaudatum*. We observed *X. index* in 44% of the 711 samples against only 11% showing *X. diversicaudatum*. On average, the maximum number of individuals observed on any of the trenches of a given 'positive' plot is 39 *X. index* against 10 *X. diversicaudatum*. This clearly confirms that the GFLV vector *X. index* is indeed more present than *X. diversicaudatum*.

On a 'positive plot', the percentage of 'positive' samples was only 54% for *X. index* and 35% for *X. diversicaudatum*. This confirms that nematode populations show a clearly aggregated distribution as reported by Villate (2008).

From these results, it seems that adaptation of the fallow period according to vector population could be considered. Based on the 'maximum' of any of the two species observed on a plot we propose a decision rule for the optimisation of the duration of the fallow period (Table II).

Table II shows that on 24 out of 108 plots nematode vectors were not detected. In this case fallow is not necessary to reduce nematode populations. When maximum nematode numbers do not reach 6 nematodes per litre of soil we advise a fallow period of 18 months (30% of the plots). For the

TABLE I

Sampling results of 108 uprooted plots in Bordeaux for *Xiphinema index* and *X. diversicaudatum* nematodes in a 2 litre soil sample (711 samples). "Maximum Number" is the highest value of *Xiphinema* in these samples on a given plot.

Nombre de nématodes dans deux litres de sol, Xiphinema index et X. diversicaudatum, sur 108 parcelles arrachées en Bordelais (711 échantillons). « Le nombre maximum » correspond à l'effectif de nématode le plus élevé trouvé sur une parcelle donnée.

		X. index	*X. diversicaudatum*
All plots	Number of positive plot (%)	79 (73%)	39 (36%)
	Number of positive samples (%)	310 (44%)	81 (11%)
Positive plots	Mean "maximum number of nematode" / sample	39	10
	Percentage of positive samples	54	35

TABLE II

Classification of plots according to maximum numbers of nematodes for each species and for both species combined.
Classement des parcelles en fonction du nombre maximum de nématodes dénombrés pour chacune des espèces connues et pour les deux espèces regroupées.

Max number of *Xiphinema*	Number of plot with *X. index*	Number of plot with *X. diversicaudatum*	Number of plot with both species combined
0	29	70	24
1 to 6	35	26	30
6 to 20	20	4	23
> 20	24	8	31
Total	108	108	108

other 50% of the plots there seems a high risk of recontamination with such short fallow periods. With populations under 20 nematodes / litre a fallow period of 3 years would be sufficient (20% of the plots). Finally 30% of the plots are 'highly' contaminated with nematode vectors fanleaf virus and would require a long fallow of at least 4 years. However, it could be argued that the frequency of detection should also be considered, for a plot that shows nematodes in most samples, fallow period should be increased.

During our sampling, we noted that high nematode populations were often associated with the presence of living roots, especially important in absence of devitalisation. Therefore the proposed approach of fallow period reduction should always be associated to efficient devitalisation (just after the last harvest). Devitalisation of vines and fallow duration modulation based on knowledge of *Xiphinema* populations should permit to optimize the management of infested plots between uprooting and replanting of new vines.

Choice of fallow crops

Thirty plant species were tested in the greenhouse for effectiveness on populations of *X. index* under controlled conditions as a first selection. The results are shown figure 2. The efficiency of every plant is compared with 'bare soil' as a control. Four plant species were found to be (so far unknown) host-plants on which the *X. index* multiply. Several of these plants are even regularly implemented (alone or in mixtures) during the fallow period (sorghum, buckwheat, phacelia). Phacelia has been reported having a nematicidal effect on beet cyst nematode (*Heterodera schachtii*) but is ineffective or even harmful to reduce populations of *X. index*, clearly illustrating that a 'nematode species adapted' choice is needed. Not surprisingly a large number of plants are ineffective or show an insufficient effect in our setup to reduce populations of *X. index*. Barley is one of those plants but was excluded since it has been shown that it is a host plant of *X. diversicaudatum*. Finally, eight species were particularly effective in reducing populations of *X. index* with an efficiency of at least 30% higher compared to bare soil.

One of the additional criteria in the selection of plant species is the fact that these should not be host plants for GFLV. Therefore an additional test was carried out under controlled conditions with 10 repetitions for these 8 species exposing them to

viruliferous *X. index* for more than 6 months. ELISA tests performed on the roots and leaves of each plant gave all negative results.

After these greenhouse tests, a second step, testing these plants under natural conditions is essential. A French National project (France AgriMer Project) has already started to continue the evaluation of the "nematicidal" effect of these plants using spring or winter planting at the field scale. The objective is to confirm the tests conducted in greenhouse and assess their adaptability to soil and climatic conditions.

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