Survival of *Xiphinema index* in Vineyard Soil and Retention of Grapevine fanleaf virus Over Extended Time in the Absence of Host Plants

Grard Demangeat, Roger Voisin, Jean-Claude Minot, Nathalie Bosselut, Marc Fuchs, and Daniel Esmenjaud

First and fifth authors: Laboratoire de Virologie, Institut National de la Recherche Agronomique, Unit Mixte de Recherche INRA-Universit Louis Pasteur 'Sant de la Vigne et Qualit du Vin', 28 rue de Herrlisheim, 68021 Colmar, France; and second, third, fourth, and sixth authors: Laboratoire de Nmatologie, Institut National de la Recherche Agronomique, Unit Mixte de Recherche Interaction Plantes-Microorganismes et Sant Vgtale, 400 route des Chappes, 06903 Sophia Antipolis, France.

Current address of M. Fuchs: Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

Accepted for publication 23 May 2005.

ABSTRACT

Demangeat, G., Voisin, R., Minot, J.-C., Bosselut, N., Fuchs, M., and Esmenjaud, D. 2005. Survival of *Xiphinema index* in vineyard soil and retention of Grapevine fanleaf virus over extended time in the absence of host plants. *Phytopathology* 95:1151-1156.

Grapevine fanleaf virus (GFLV) is transmitted specifically from grapevine to grapevine by the ectoparasitic root-feeding nematode *Xiphinema index*. Limited information is available on the survival of *X. index* in vineyard soil and on the retention of GFLV by *X. index* over extended periods of time. We addressed these two issues by quantifying the numbers of living *X. index* recovered from soil samples that were collected in

three naturally GFLV-infected vineyards in France and subsequently stored at 7 or 20C in the absence of host plants. Our data indicated a two- to eightfold decrease in *X. index* numbers but the recovery of 8 to 10 living fourth-stage juveniles (J4) and adults per kilogram of soil after 4 years of storage regardless of temperature. In addition, GFLV was detected readily in all groups of 20 isolated *X. index* adults and J4 (except for J4 that were kept 4 years at 20C) by reverse transcription-polymerase chain reaction using total nematode RNAs and a primer set located in conserved regions at the 3' end of viral genomic RNA 2. Our findings on the long-term survival of viruliferous *X. index* under adverse conditions emphasize the need for new control strategies against GFLV.

Grapevine fanleaf virus (GFLV) is responsible for the most severe viral disease of grapevines worldwide. It reduces crop yield up to 80% and affects fruit quality (2,21). GFLV is transmitted specifically from grapevine to grapevine by the ectoparasitic dagger nematode, *Xiphinema index* (2). The nematode acquires and transmits GFLV while feeding on root tips of actively growing grapevines (40).

The reproduction of *X. index* is parthenogenetic, males are scarce, and a single juvenile is capable of establishing a population (9). Eggs laid in the spring hatch a few days later and mature to adults through four juvenile stages, denoted J1 to J4; each stage is separated by a molt. The complete life cycle of *X. index* requires 2 to 14 months under controlled conditions, depending on biotic and abiotic factors (30,40), but can require more than 14 months in the field where temperatures are more variable (39). Under adverse conditions, including low moisture, low temperature, and the absence of host plants, *X. index* undergoes a quiescent phase during which physiological functions are limited and development is interrupted (4).

Transmission of GFLV by *X. index* is noncirculative and semi-persistent (6,22,29). GFLV can be acquired from infected plants and inoculated to recipient plants within 1 to 10 min (40). GFLV does not replicate within the nematode and does not influence the reproduction of *X. index* (10). Virions are retained by *X. index* up to 9 months but do not pass from females to eggs or juveniles (30). Indeed, juveniles lose their ability to transmit the virus after

molting because the lining of the cuticle in the odontophore and the oesophagus, where GFLV particles are retained at specific sites (29), is shed. Therefore, after molting, the nematode must feed again on roots of GFLV-infected grapevines to acquire the virus and become viruliferous. Adults and all juvenile stages are able to acquire and transmit GFLV to healthy plants (30). The transmission is highly specific because *X. index* is the only natural vector of GFLV (2,6). Recently, Andret-Link et al. (3) showed that the coat protein (CP) is the sole viral determinant responsible for this specificity.

Control strategies for GFLV in infected vineyards essentially are directed to the eradication of *X. index*. GFLV-infected grapevines are uprooted, soils disinfested with nematicides, and vineyards fallowed (18,26,29,33). These measures are of limited efficacy because nematicides have a poor penetration potential, especially in clay soils, and *X. index* can be present in deep layers up to 4 m (18). Furthermore, registered nematicides have a high acute toxicity and a detrimental effect on beneficial nontarget soil organisms (1). Finally, the recommended 10-year fallow (34) is highly impractical and requires that viruliferous nematodes die and virus reservoirs disappear after uprooting.

Limited information is available on the ability of *X. index* to survive in soil and to retain GFLV over extended periods of time. *X. index* has been reported to survive for 4.5 years in a fallowed vineyard, although remaining grapevine roots were suspected to have maintained nematode viability by providing feeding sources (27). *X. index* also remains viruliferous for up to 9 months in moist soil in the absence of host plants (30). A better knowledge of *X. index* survival and GFLV retention over extended time would provide useful information about the time interval required to eliminate viruliferous nematodes between two successive grapevine plantings.

Corresponding author: G. Demangeat; E-mail address: demangea@colmar.inra.fr

DOI: 10.1094/PHYTO-95-1151

 2005 The American Phytopathological Society

In this article, we quantified the survival of *X. index* and persistence of GFLV in viruliferous *X. index* in soil collected from three vineyards in France and subsequently stored at 7 or 20°C in the absence of host plants. We report that living, viruliferous nematodes were detected after 4 years of storage regardless of temperature. This finding on the long-term survival of viruliferous *X. index* under adverse conditions stresses the need for new control strategies against GFLV.

MATERIALS AND METHODS

GFLV reference strain F13. GFLV strain F13 (35) was isolated from infected grapevines and propagated in *Chenopodium quinoa*, a systemic herbaceous host. Virus particles were extracted and purified from infected *C. quinoa* plants as described (25). The sequence of RNA 1 and RNA 2 of GFLV strain F13 (28) is available in GenBank as accession nos. D00915 and X16907, respectively.

Nematode reference material. Aviruliferous *X. index* were reared on figs, a good host to *X. index* but a nonhost to GFLV (13,30), in 10-liter pots containing loess (one-third) and sand (two-thirds) (wt/wt). Fig plants were produced by green cuttings and maintained in the greenhouse at 20 to 25°C and 16 h of daylight at INRA in Colmar, France. Other *Xiphinema* spp., including *X. diversicaudatum*, *X. vuittenezi*, and *X. italiae*, were used as reference for nematode identification. Populations of these nematode species were kept at INRA in Antibes, France.

GFLV-infected vineyard sites. Three commercial vineyards naturally infected with GFLV were chosen for this study (Table 1). Two vineyards (Mesnil-sur-Oger and Hautvilliers) were located in the Champagne region in the northeast of France and the third vineyard (Fréjus) was located in the Provence region in the south of France. The vineyards in Mesnil-sur-Oger and Hautvilliers consisted of *Vitis vinifera* cv. Chardonnay grafted onto rootstock 41B and the vineyard in Fréjus consisted of *V. vinifera* cv. Cinsault grafted onto rootstock 44-53 M (Table 1). The three vineyards were planted 20 to 30 years ago. Heavy-textured clay and limestone soil was found in the two Chardonnay vineyards, whereas light-textured sandy soil was found in the Cinsault vineyard. The presence of GFLV was tested in randomly selected vines in the three vineyards by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific GFLV immunoglobulins (36).

Soil sampling and storage. Soil samples of ≈5 kg were hand collected with picks, hoes, and shovels near the roots of GFLV-infected plants after removing the top 20 cm of soil. These initial soil samples were stored in plastic bags at 4°C and used to assess the presence of viruliferous *X. index* (13). Upon successful recovery of viruliferous nematodes from these soil samples, 0.4 to 1.0 metric tons (t) of soil was similarly collected from the Mesnil-sur-Oger vineyard in mid-June and from the Hautvilliers and Fréjus vineyards in late August. Although soil water content was not determined, soil was relatively moist. Soil samples were distributed randomly on site into 35-liter ice chests lined with plastic bags. At each site, there were 15 to 40 ice chests with 30 to 35 kg of soil per chest. Roots (>2 mm in diameter) were carefully

hand discarded during sampling to eliminate potential nematode feeding sources during long-term storage. Ice chests containing soil samples were closed on site to avoid evaporation and then stored at 7 or 20°C in laboratory facilities at INRA in Antibes, France. These two storage conditions were chosen because they cover the optimal temperature range (5 to 20°C) for *X. index* survival (16). They also correspond to the extreme temperatures to which *X. index* are exposed in vineyard soils in the Mediterranean area, including in the Provence region in France (8).

Nematode isolation from vineyard soil samples. Nematodes were extracted from the initial 5 kg and from the 30- to 35-kg soil samples as described (14). The full content of an ice chest was divided into 15 to 17 subsamples of 2 kg for a time course experiment. Nematodes were recovered from subsamples that periodically were removed from randomly selected ice chests. During nematode extraction, stones, gravel, plant debris, and other large soil components were removed with a 2-mm sieve. The residues retained on the 2-mm openings were weighed and the filtrate that passed through the 2-mm openings of the sieve was added to an Oostenbrink elutriator. Nematodes were collected on a 63-μm sieve and deposited onto a modified Baermann funnel that consisted of a moist cellulose filter sitting on a coarse screen in a plastic tray filled with water. This permitted separation of living nematodes from dead nematodes; the dead nematodes remained on the surface of the filter while the living nematodes moved through the filter into the water (17). After 5 days at room temperature, the living nematodes were collected on a 5-μm mesh sieve and counted. The total population was expressed relative to 1 kg of soil (the initial mass of the subsample minus the mass of stones, gravel, plant debris, and large soil components retained on the 2-mm sieve). *X. index* J4 and adults were hand picked under a light microscope by using a micromanipulator wire and stored as samples of 10 individuals at -80°C in microfuge tubes containing distilled water.

Nematodes recovered from the initial soil samples were examined with a light microscope and identified to species based on morphological characteristics (20,32). Single adults or J4 were further characterized by a multiplex polymerase chain reaction (PCR) using specific primers (A-ITS1 and I27) to ribosomal genes and nematode DNA extracts as described (38).

Total RNA extraction from *X. index* and GFLV detection by reverse transcription-PCR. Total RNAs were isolated from 20 randomly selected *X. index* J4 and adults recovered from soil subsamples. An automated shaker (Retsch MM 200, Haan, Germany) was used to disrupt nematodes before RNA extraction using the RNeasy mini-plant kit (Qiagen S.A., Courtaboeuf, France) as described (11). Total nematode RNAs were recovered in 40 μl of RNase-free water and used in reverse transcription (RT)-PCR experiments with reverse primer EV100N1 (5' ³⁵⁷⁴GA-CTATCTAGACACATATATACACTTGGGTCTTTTAA³⁶⁰⁰ 3', with an additional underlined *Xba*I site) and forward primer CPS (5' ³⁰⁴⁵TTGTGCGCCCAGATCTCTCTTTACCA³⁰⁵⁸ 3') (11). These two primers were designed to amplify a 555-bp fragment of the CP gene encoded by RNA 2 (nucleotides 3045 to 3600; nucleotide positions refer to GenBank accession no. D00915). Positive and negative controls for the RT-PCR assays were obtained from 5 μg of purified GFLV strain F13 particles and total RNAs extracted from aviruliferous *X. index*. The RT-PCR products were analyzed under UV illumination following electrophoresis on 1% agarose gels and ethidium bromide staining.

RESULTS

Initial identification of *Xiphinema* spp. and GFLV in the vineyards. The three vineyards selected for this study (Table 1) showed a patchy distribution of fanleaf-diseased vines with marked leaf deformation or stunted growth. DAS-ELISA confirmed the presence of GFLV but not *Arabis mosaic virus* (ArMV),

TABLE 1. Characteristics of three vineyards selected for this study

Location	Viticultural region	Grapevine rootstock, cultivar	No. of nematodes ^a
Hautvilliers	Champagne	41 B, Chardonnay	12 ± 5
Mesnil-sur-Oger	Champagne	41 B, Chardonnay	11 ± 10
Fréjus	Provence	44-53 M, Cinsault	15 ± 8

^a Number of *Xiphinema index* adults and stage 4 juveniles per kilogram of soil. Values are the means ± standard deviation of five randomly selected samples in each vineyard. The number of nematodes is expressed relative to 1 kg of soil that passed through a 2-mm sieve.

another nepovirus often detected in vineyards in France (data not shown).

X. index, the natural vector of GFLV (2), and *X. pachtaicum*, which is commonly found in French vineyards but does not vector viruses (8,14), were identified in the Fréjus vineyard. Only *X. index* was found in the Mesnil-sur-Oger and Hautvilliers vineyards. *X. index* was identified easily based on its elongated, cylindrical body (≈ 3.0 mm long for adults), vulva location at $\approx 40\%$ of body length, and convex-conoid tail with a distinct finger-like protuberance (20,32). *X. pachtaicum* was identified based on its strongly curved body (≈ 1.7 mm long for adults), vulva location at $\approx 50\%$ of the body length, and acute tail (19).

X. index J2, J3, J4, and adults were found in the three vineyards. The numbers of nematodes recovered from the initial soil samples averaged 11 to 15 adults and J4 per kilogram of soil (Table 1). These values are similar to those in previous reports from other GFLV-infected vineyards (29,33).

X. index adults and J4 were further selected for this study. Their identification was confirmed by a multiplex PCR using a cocktail of primers (38). A specific *X. index* fragment of 340 bp was amplified from DNA extracts of randomly selected *X. index* adults or J4 (Fig. 1, lanes 1 to 6), corroborating earlier identification based on morphological features. None of the nematodes recovered from vineyard soil showed typical DNA amplicons of *X. diversicaudatum* (813 bp), *X. italiae* (414 bp), or *X. vuittenzei* (591 bp). However, DNA fragments of the expected sizes were obtained when each of the four *Xiphinema* spp. was used as reference (Fig. 1, lanes 8 to 11) or when individuals of the four species were mixed (Fig. 1, lane 12). These results confirmed that all the nematodes isolated from the soil samples and subsequently hand picked were exclusively *X. index*.

Samples of *X. index* (30 from each vineyard) showed prominent DAS-ELISA optical density at 405 nm (OD_{405nm}) readings (0.84 to 1.42 ± 0.42 after 1 h of substrate hydrolysis) with specific anti-GFLV γ -globulins compared with low OD_{405nm} readings for aviruliferous *X. index* from fig plants (0.2 ± 0.06). Based on these data, we concluded that the three vineyard soils were suitable for examining the survival of *X. index* and the capacity of *X. index* to retain GFLV over extended periods of time in the absence of host plants.

Survival of *X. index* in the absence of host plants. Nematodes were recovered from soil subsamples (2 kg) that were taken at random from randomly selected ice chests. In the soil samples from the Fréjus vineyard, the number of *X. index* declined over time but living adults and J4 were recovered after 48 months at both storage temperatures (Fig. 2A and B). At 20°C , approximately one-third of the initial nematode population was recovered after 48 months. Interestingly, the decrease in adults was linear, whereas that of J4 was highly variable (Fig. 2A). At 7°C , the nematode population was more or less stable up to 30 months and then dropped by twofold after 48 months (Fig. 2B). In the soil samples from the Hautvilliers vineyard, the number of living *X. index* declined very rapidly, regardless of the storage temperature (Fig. 2C and D). Only one-fourth to one-eighth of the initial population was recovered after 2 years of storage. Although the initial number of living *X. index* was unexpectedly small in soil samples from the Mesnil-sur-Oger vineyard (Fig. 2E and F), living adults and J4 were detected after 2 years of storage. These low counts likely were due to an uneven distribution in the field because nematode populations can vary widely across relatively small areas (5,15). Based on the low nematode counts in soil samples from the Hautvilliers and Mesnil-sur-Oger vineyards, the time course experiment with those soils was ended after 2 years.

Only roots less than 2.0 mm in diameter were recovered from soil samples stored in ice chests. Such remaining roots were fully decayed with 12 months.

GFLV retention by *X. index* over extended time in the absence of host plants. GFLV was detected in *X. index* by RT-PCR using total nematode RNAs extracted from randomly

selected samples of 20 *X. index* adults or J4 (11). A specific DNA fragment of the expected size for GFLV was obtained for all *X. index* adults (Fig. 3A) and J4 (Fig. 3B) recovered from the Fréjus vineyard, except for J4 extracted after 48 months of storage at 20°C . The RT-PCR signal was substantially more intense for *X. index* individuals stored at 7°C than for those stored at 20°C . Also, the intensity of the DNA amplicons decreased progressively over time for J4, regardless of temperature, and for adult nematodes stored at 20°C . For *X. index* adults stored at 7°C , the intensity of the DNA amplicons dropped only after 36 months.

A 555-bp DNA amplicon also was obtained for all *X. index* adults (Fig. 4A) and J4 (Fig. 4B) recovered from soil samples of the Mesnil-sur-Oger vineyard. The intensity of RT-PCR product decreased over time for J4, regardless of temperature, and for adults at 20°C . For *X. index* adults stored at 7°C , the decrease was less pronounced. As in the Fréjus vineyard, a more intense DNA band was obtained for nematodes kept in soil at 7°C than for those kept at 20°C .

A 555-bp DNA amplicon also was obtained for all *X. index* adults and J4 recovered from soil samples of the Hautvilliers vineyard stored at 7 and 20°C for 6, 12, 18, and 24 months (data not shown).

The RT-PCR amplicons were not sequenced.

DISCUSSION

This study shows that *X. index* can survive and retain GFLV for at least 4 years in vineyard soil stored at 7 to 20°C in the absence of host plants. Although numbers of *X. index* declined, living nematodes were found throughout the study, and a GFLV CP fragment was amplified by RT-PCR from all *X. index* tested, except from J4 stored 4 years at 20°C . To our knowledge, this is the first in-depth characterization of the survival of *X. index* in the absence of potential feeding sources, including old grapevine root pieces. Although the possibility that viruliferous nematodes might have fed on remaining small roots cannot be ruled out, small pieces of roots present in storage soil were unlikely to support feeding by *X. index* over time because they were dark brown and very crumbly and did not actively grow. Our study also provides the first molecular documentation of the long-term persistence of GFLV, and of a nepovirus in general, within its nematode vector.

Bioassays previously showed that *X. index* held in moist soil without host plants retained GFLV for up to 9 months (30). Also, 5 years of fallow failed to eliminate GFLV from a naturally infested vineyards site (34). In the case of other nematodes and viruses, *X. diversicaudatum* kept in sterile soil in the absence of host plants retained ArMV for 4 months (31), whereas *Tobacco ringspot virus* (TRSV) was transmitted by *X. americanum* to bait plants after 9 months of storage at 8°C in the absence of host plants (23). Furthermore, Bitterlin and Gonsalves (5) reported that *X. rivesi* transmitted *Tomato ringspot virus* after a 2-year storage in natural soil. Although our experiment clearly showed the

Fig. 1. Agarose gel analysis of DNA amplicons obtained by multiplex polymerase chain reaction (PCR) from single *Xiphinema index* extracted from three vineyards in France. *X. index* adults (lanes 1, 3, and 5) or stage 4 juveniles (lanes 2, 4, and 6) were from the Fréjus vineyard (lanes 1 and 2), the Hautvilliers vineyard (lanes 3 and 4), or the Mesnil-sur-Oger vineyard (lanes 5 and 6). Lanes 8 to 11 were amplicons of single *X. index*, *X. italiae*, *X. vuittenzei*, and *X. diversicaudatum* adults, respectively. Lane 12 corresponds to amplicons of an artificial mixture of single *X. diversicaudatum* (813 bp), *X. index* (340 bp), *X. italiae* (414 bp), and *X. vuittenzei* (591 bp) adults. Lane 7: PCR with no DNA template. Lane M: DNA molecular marker (100 bp).

retention of GFLV by *X. index* adults and J4 for up to 4 years, it would be informative to pursue similar experiments beyond 4 years by testing nematodes recovered from fallowed vineyards.

GFLV virions acquired by *X. index* from infected grapevines are retained by adhering to specific sites on the cuticular lining of the oesophageal tract in the anterior part of the nematode odontophore (22,29). Virus particles then are released from the retention sites and subsequently transmitted to a recipient plant.

The release of GFLV is suspected to occur during feeding when salivary gland secretions are released through the oesophagus and stylet into punctured cells (40). Interestingly, when viruliferous *X. index* are exposed to host plants that do not replicate GFLV, the virus is released from the retention sites and nematodes are no longer viruliferous after 12 weeks (29). A similar situation applies to *X. americanum* and TRSV, but after only 3 weeks (37). These data suggest that nepoviruses are released gradually, likely as a

Fig. 2. Survival of *Xiphinema index* in soil samples from **A and B**, the Fréjus vineyard, **C and D**, the Hautvilliers vineyard, and **E and F**, the Mesnil-sur-Oger vineyard. Soil samples were stored without host plants at 20 (**A, C, and E**) or 7°C (**B, D, and F**). Living *X. index* adults and stage 4 juveniles were extracted and counted every 6 months for 4 (**A and B**) or 2 years (**C to F**). Numbers of living nematodes were expressed relative to the soil mass that passed through a 2-mm sieve. No nematode extraction was performed from Fréjus samples stored less than 12 months at 7°C (**B**).

Fig. 3. Agarose gel analysis of Grapevine fanleaf virus (GFLV) coat protein amplicons obtained by reverse transcription-polymerase chain reaction (RT-PCR) from *Xiphinema index* recovered from the Fréjus vineyard. RT-PCR products were from **A**, 20 *X. index* adults and **B**, stage 4 juveniles extracted from soil samples stored at 20 or 7°C. DNA amplicons were from *X. index* isolated immediately after soil sampling in the field (0) and after 6, 12, 18, 30, 36, 42, and 48 months of storage. Nematodes recovered after 24 months of storage were not tested because they were accidentally lost. Lanes C and H correspond to the RT-PCR products obtained from purified GFLV strain F13 particles and from total RNAs of 20 aviruliferous *X. index* cultured on fig plants, respectively.

result of salivary gland activities, when nematodes have access to host plants. In contrast, in the absence of host plants, for instance in fallowed soils, *X. index* individuals apparently enter a quiescent phase with no feeding activity and no reproduction (4,9), and likely with no or reduced salivary gland activity. Therefore, few or no virus particles are released from the retention sites. This biological process could explain the long-term persistence of GFLV within *X. index*.

Raski et al. (27) hypothesized that nematodes can survive by feeding on surviving roots in fallowed soils. In our study, the main roots from which rootlets potentially could grow (27) were discarded when the soil was collected in the GFLV-infected vineyards. Therefore, *X. index* had no access to any food source during storage. Our findings suggest that roots left in a fallowed vineyard soil are not the only explanation for the long-term survival of nematodes. A "survival factor" was hypothesized to explain the long-term persistence of *X. diversicaudatum* in fallowed soil (24). This factor could simply correspond to the inherent potential of nematodes to survive under adverse conditions. *X. index* adults seemed to survive better than J4 (Fig. 2). Perhaps adults contain more food reserves than juveniles. Our RT-PCR assays, however, indicated that J4 retained GFLV as efficiently as adults.

Our RT-PCR time course experiments showed that the intensity of GFLV-specific DNA amplicons decreased substantially over time. Equal numbers of randomly selected nematodes were assessed for all soil samples tested and the RT-PCR assays were repeatedly performed using the same experimental protocol; therefore, the decline in amplicon intensity could correspond to a decline in virus content within nematodes. This decline could result from a degradation of virus particles upon release from retention sites or destabilization of the virion-receptor complex. However, because no strict quantitative experiments were conducted in this study, more work is needed to test these hypotheses.

The effect of temperature on the life cycle and survival of *X. index* is well documented (7,15,16,29). The optimal temperature for reproduction is 24°C, and the life cycle is interrupted below 16°C (7). However, *X. index* can survive in soil at extreme temperatures, including -11°C for 62 days and 37°C for 32 days, but not -22 or 45°C (16). Our RT-PCR assays indicated a stronger signal of the DNA amplicons corresponding to the GFLV CP gene for *X. index* stored in soil at 7°C than for those kept at 20°C. This suggests that virion stability or retention may be greater at 7 than at 20°C.

We did not determine whether the *X. index* that retained GFLV carried viable virions, nor did we determine whether the viruliferous *X. index* were able to transmit GFLV to bait plants after 4 years of storage in vineyard soil in the absence of host plants. Although direct experimental evidence is missing, a number of

observations and arguments suggest that surviving viruliferous *X. index* individuals should be able to transmit GFLV. First, living *X. index* were recovered in all soil samples tested. Second, a single viruliferous nematode is capable of causing a GFLV-infection in grapevine (D. Esmenjaud, unpublished data). Third, it is possible to establish a *X. index* populations on fig plants with 15 individuals recovered from the Fréjus vineyard soil stored over 36 months (D. Esmenjaud, unpublished data).

Our initial intention was to quantify the number of living viruliferous *X. index* in soil samples from the three vineyards over the longest possible time period. Because the initial counts for the Mesnil-sur-Oger and Hautvilliers vineyards were unexpectedly small, recovering enough nematodes for a long-term time course experiment was challenging for these two vineyards. Therefore, our quantification experiments were stopped after 2 years, whereas they could be pursued over 4 years with the soil samples from the Fréjus vineyard.

To prevent GFLV contamination of newly replanted vineyards, eradication of viruliferous nematodes would be ideal. Eradication can be attempted by combined soil treatments with nematicides and extended periods of fallow (18). In most instances, however, nematicides do not efficiently control viruliferous nematode populations, especially in clay soil, because *X. index* are not easily accessible in heavy and deep layers. Applying a systemic herbicide before uprooting the old vines can delay the contamination of new grapevines by destroying feeding sources of surviving viruliferous nematodes (12). A 10-year fallow, which is recommended for eradication of *X. index* and, hence, control of GFLV (34), is difficult to implement for economic reasons, especially in premium vineyard sites. Thus, determining the infectious potential of *X. index* could help vineyard managers decide on the time interval required to eliminate viruliferous nematodes between two successive plantings. Our study demonstrates that viruliferous *X. index* can survive for years in the absence of host plants and helps to explain the limited efficacy of current GFLV control measures. Based on the long-term persistence of viruliferous *X. index*, there is little doubt that research is needed to find new control strategies against GFLV. Research should focus on the development of GFLV- or *X. index*-resistant grapevines. Resistant material can be obtained either by genetic engineering in the case of GFLV or *X. index* or by conventional approaches in the case of *X. index*. Such programs are ongoing in several laboratories.

ACKNOWLEDGMENTS

This work was partially supported by a competitive grant from the European Commission (QLK3-CT-2002-02140), the Comité Interprofessionnel du Vin de Champagne (CIVC), and the Département Santé des Plantes et Environnement of the Institut National de la Recherche Agronomique. We thank P. Cornuet for providing us with purified GFLV preparations; J. Paquette (Domaine de Curebécasse, Fréjus, France), Moët & Chandon (Epernay, France), and the CIVC (Epernay, France) for making some of their GFLV-infected vineyards available to us; and L. M. Yepes and G. Abawi for critically reading the manuscript. This article is dedicated to the memory of René Legin.

LITERATURE CITED

1. Abawi, G. S., and Widmer, T. L. 2000. Impact of soil health management practices on soilborne pathogens, nematodes, and root diseases of vegetable crops. *Appl. Soil Ecol.* 15:37-47.
2. Andret-Link, P., Laporte, C., Valat, L., Ritzenthaler, C., Demangeat, G., Vigne, E., Laval, V., Pfeiffer, P., Stussi-Garaud, C., and Fuchs, M. 2004. *Grapevine fanleaf virus*: Still a major threat to the grapevine industry. *J. Plant Pathol.* 86:183-195.
3. Andret-Link, P., Schmitt-Keichinger, C., Demangeat, G., Komar, V., and Fuchs, M. 2004. The specific transmission of *Grapevine fanleaf virus* by its nematode vector *Xiphinema index* is solely determined by the viral coat protein. *Virology* 320:12-22.
4. Antoniou, M. 1989. Arrested development in plant parasitic nematodes. (*Abstr.*) *Helminthology Ser. B* 58:1-19.

Fig. 4. Agarose gel analysis of *Grapevine fanleaf virus* (GFLV) coat protein amplicons obtained by reverse transcription-polymerase chain reaction (RT-PCR) from *Xiphinema index* recovered from the Mesnil-sur-Oger vineyard. RT-PCR products were from **A**, 20 *X. index* adults and **B**, stage 4 juveniles isolated from soil samples stored at 20 or 7°C. DNA amplicons were from *X. index* extracted immediately after soil sampling in the field (0) and after 6, 12, 18, and 24 months of storage. Lanes C and H correspond to the RT-PCR products obtained from purified GFLV strain F13 particles and from total RNAs of 20 aviruliferous *X. index* cultured on fig plants, respectively.

5. Bitterlin, M. W., and Gonsalves, D. 1987. Spatial distribution of *Xiphinema rivesi* and persistence of *Tomato ringspot virus* and its vector in soil. *Plant Dis.* 71:408-411.
6. Brown, D. J. F., and Weischer, B. 1998. Specificity, exclusivity and complementarity in the transmission of plant viruses by plant parasitic nematodes: An annotated terminology. *Fundam. Appl. Nematol.* 21:1-11.
7. Cohn, E., and Mordechai, M. 1969. Investigations on the life cycle and host preferences of some species of *Xiphinema* and *Longidorus* under controlled conditions. *Nematologica* 15:295-302.
8. Dalmaso, A. 1970. Influence directe de quelques facteurs écologiques sur l'activité biologique et la distribution des espèces françaises de la famille des *Longidoridae* (*Dorylaimida*). *Ann. Zool. Ecol. Anim.* 2:163-200.
9. Dalmaso, A., and Younes, T. 1969. Ovogénèse et embryogénèse chez *Xiphinema index* (Nematoda: *Dorylaimida*). *Ann. Zool. Ecol. Anim.* 1:265-272.
10. Das, S., and Raski, D. J. 1969. Effect of grapevine fanleaf virus on the reproduction and the survival of its nematode vector, *Xiphinema index* Thorne & Allen. *J. Nematol.* 1:107-110.
11. Demangeat, G., Komar, V., Cornuet, P., Esmenjaud, D., and Fuchs, M. 2004. Sensitive and reliable detection of *Grapevine fanleaf virus* in a single *Xiphinema index* nematode vector. *J. Virol. Methods* 122:79-86.
12. Descotes, A., and Moncomble, D. 1995. Lutte contre le court-noué. *Vignerons Champenois* 9:20-24.
13. Esmenjaud, D., Walter, B., Minot, J. C., Voisin, R., and Cornuet, P. 1993. Biotin-avidin ELISA detection of *Grapevine fanleaf virus* in the vector nematode *Xiphinema index*. *J. Nematol.* 25:401-405.
14. Esmenjaud, D., Walter, B., Valentin, G., and Cluzeau, D. 1992. Vertical distribution and infectious potential of *Xiphinema index* (Thorne et Allen, 1950) (Nematoda: *Longidoridae*) in fields affected by grapevine fanleaf virus in vineyards in the Champagne region of France. *Agronomie* 12:395-399.
15. Feil, H., Westerdahl, B. B., Smith, R. J., and Verdegaal, P. 1997. Effects of seasonal and site factors on *Xiphinema index* in two California vineyards. *J. Nematol.* 29:491-500.
16. Harris, A. R. 1979. Seasonal populations of *Xiphinema index* in vineyard soils of Northern-Eastern Victoria, Australia. *Nematologica* 25:336-347.
17. Hooper, D. J. 1986. Extraction of free-living stages from soil. Pages 5-30 in: *Laboratory Methods for Work with Plant and Soil Nematodes*. J. F. Southey, ed. Ministry of Agriculture, Fisheries and Food, London.
18. Lamberti, F. 1981. Combatting nematode vectors of plants viruses. *Plant Dis.* 65:113-117.
19. Lamberti, F., and Carone, M. 1991. A dichotomous key for the identification of species of *Xiphinema* (Nematoda: *Dorylaimida*) within the *X. americanum* group. *Nematol. Mediterr.* 19:341-348.
20. Loof, P. A. A., and Luc, M. 1989. A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb, 1913 (Nematoda: *Longidoridae*) with exclusion of the *X. americanum* group. *Syst. Parasitol.* 16:35-66.
21. Martelli, G. P., and Savino, V. 1991. Fanleaf degeneration. Pages 48-49 in: *Compendium of Grape Diseases*. R. C. Pearson and A. Goheen, eds. The American Phytopathological Society, St. Paul, MN.
22. Mc Farlane, S. A. 2003. Molecular determinants of the transmission of plant viruses by nematodes. *Mol. Plant Pathol.* 4:211-215.
23. Mc Guire, J. M. 1973. Retention of *Tobacco ringspot virus* by *Xiphinema americanum*. *Phytopathology* 63:324-326.
24. Mc Namara, D. G. 1980. The survival of *Xiphinema diversicaudatum* in plant-free soil. *Nematologica* 26:170-181.
25. Pinck, L., Fuchs, M., Pinck, M., Ravelonandro, M., and Walter, B. 1988. A satellite RNA in grapevine fanleaf virus strain F13. *J. Gen. Virol.* 69:233-239.
26. Raski, D. J., Goheen, A. C., Lider, L. A., and Meredith, C. P. 1983. Strategies against *Grapevine fanleaf virus* and its nematode vector. *Plant Dis.* 67:335-339.
27. Raski, D. J., Hewitt, W. B., Goheen, A. C., Taylor, C. E., and Taylor, R. H. 1965. Survival of *Xiphinema index* and reservoirs of fanleaf virus in fallowed vineyard soil. *Nematologica* 11:349-352.
28. Serghini, M. A., Fuchs, M., Pinck, M., Reinbolt, J., Walter, B., and Pinck, L. 1990. RNA2 of grapevine fanleaf virus: Sequence analysis and coat protein cistron location. *J. Gen. Virol.* 71:1433-1441.
29. Taylor, C. E., and Brown D. J. F. 1997. *Nematode Vectors of Plant Viruses*. CAB International, Wallingford, U.K.
30. Taylor, C. E., and Raski, D. J. 1964. On the transmission of grapevine fanleaf by *Xiphinema index*. *Nematologica* 10:489-495.
31. Taylor, C. E., and Thomas P. R. 1968. The association of *Xiphinema diversicaudatum* (Micoletzky) with strawberry latent ringspot and arabis mosaic viruses in a raspberry plantation. *Ann. Appl. Biol.* 62:147-157.
32. Vovlas, N., and Larizza, A. 1994. Life stages identification and embryology of the virus-vector nematode *Xiphinema index*. *Nematol. Mediterr.* 22:109-113.
33. Vuittenéz, A., and Legin, R. 1964. Recherches sur les vecteurs naturels de la dégénérescence infectieuse. *C. R. Acad. Agric. Fr.* 12 février 1964:286-306.
34. Vuittenéz, A., Legin, R., and Kuszala, J. 1969. Les viroses de la vigne. *Journées françaises d'études et d'information*, Paris 5-7:557-578.
35. Vuittenéz, A., Munck, M. C., and Kuszala, J. 1964. Souches de virus à hautes agressivité isolées de vignes atteintes de dégénérescence infectieuse. *Etud. Virol. Appl.* 5:69-78.
36. Walter, B., and Etienne, L. 1987. Detection of the grapevine fanleaf viruses away from the period of vegetation. *J. Phytopathol.* 120:355-364.
37. Wang, S., Gergerich, R. C., Wickizer, S. L., and Kim, K. S. 2002. Localization of transmissible and nontransmissible viruses in the vector nematode *Xiphinema americanum*. *Phytopathology* 92:646-653.
38. Wang, X., Bosselut, N., Castagnone, C., Voisin, R., Abad, P., and Esmenjaud, D. 2003. Multiplex polymerase chain reaction identification of single individuals of the Longidorid nematodes *Xiphinema index*, *X. diversicaudatum*, *X. vuittenézi*, and *X. italiae* using specific primers from ribosomal genes. *Phytopathology* 93:160-166.
39. Weisher, B. 1975. Ecology of *Xiphinema* and *Longidorus*. Pages 291-307 in: *Nematode Vectors of Plant Viruses*. F. Lamberti, C. E. Taylor, and J. W. Seinhorst, eds. Plenum Press, New York.
40. Wyss, U. 2000. *Xiphinema index*, maintenance, and feeding in monoxenic cultures. Pages 251-281 in: *Maintenance of Human, Animal and Plant Pathogen Vectors*. K. Maramorosch and F. Mahmood, eds. Science Publishers Inc., Enfield, NH.