Transmission Competency of Single-Female *Xiphinema index* Lines for Grapevine fanleaf virus

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ABSTRACT

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Grapevine fanleaf virus (GFLV) is vectored specifically from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index*. Limited information is available on the vector competency of *X. index* populations from diverse geographical origins. We determined the transmissibility of two GFLV strains showing 4.6% amino acid divergence within their coat protein (e.g., strains F13 and GHu) by seven clonal lines of *X. index* developed from seven distinct populations from the Mediterranean basin (Cyprus, southern France, Israel, Italy, and Spain), northern France, and California. *X. index* lines derived from

single adult females were produced on fig (*Ficus carica*) plants to obtain genetically homogenous aviruliferous clones. A comparative reproductive rate analysis on *Vitis rupestris* du Lot and *V. vinifera* cv. Cabernet Sauvignon showed significant differences among clones, with the single-female Cyprus line showing the highest rate (30-fold the initial population) and the Spain and California lines showing the lowest rate (10-fold increase), regardless of the grapevine genotype. However, there was no differential vector competency among the seven *X. index* lines for GFLV strains F13 and GHu. The implications of our findings for the dynamic of GFLV transmission in vineyards and screening of *Vitis* spp. for resistance to GFLV are discussed.

Additional keywords: longidorid.

Grapevine fanleaf virus (GFLV) is responsible for fanleaf degeneration, the most severe viral disease of grapevines worldwide. This virus reduces vigor, crop yield (up to 80%), and fruit quality (1). It also reduces the lifespan of vineyards and can cause plant mortality (1). GFLV belongs to the genus *Nepovirus* in the family *Secoviridae*, subfamily *Comovirinae*. It is specifically transmitted from grapevine to grapevine by the soilborne longidorid ectoparasitic nematode *Xiphinema index*. GFLV has a bipartite genome consisting of two single-stranded RNA molecules, each encoding for a polyprotein, which are cleaved by the viral protease into mature proteins (Fig. 1). RNA1 encodes proteins involved in viral replication and polyprotein maturation whereas RNA2 encodes a protein involved in replication (2A^{HP}), the movement protein (2B^{MP}), and the coat protein (2C^{CP}). The coat protein of GFLV is responsible for the specificity of transmission by *X. index* (2,4). GFLV isolates differing by 0.2 to 6.9% at the amino acid level have been identified (42).

GFLV and *X. index* have an almost worldwide distribution (1,39). It is hypothesized that *X. index* originated from the Middle East, where it is found in natural woodlands associated with grapevines (30). From this area, *X. index* and GFLV probably spread to most grapevine-growing regions of the world via the Mediterranean basin through rooted propagation material. Results of a recent phylogeographical study based on genetic diversity of

the *X. index* cytochrome B gene (*cytB*) were consistent with this hypothesis (44). Indeed, analyses of *cytB* nucleotide substitution pattern of *X. index* from diverse geographical origins revealed that all the haplotypes identified were present in populations from Cyprus, Israel, Palestine, and Iran whereas populations from America (Chile and the United States) and Europe (France, Greece, Italy, and Spain) had only one of the haplotypes identified. These results suggested that *cytB* haplotype distribution paralleled the history of grapevine cultivation (44).

X. index has a narrow host range, with grapevine as the major natural host (1,39). The reproduction rate of *X. index* varies with the grapevine genotypes (15,28). For example, a high rate was achieved on *Vitis riparia* Gloire de Montpellier and SO4 (*V. berlandieri* × *V. riparia*) but a low rate on Freedom (*V. longii* × *Othello*) × Dogridge) and 161-49 Couderc (*V. riparia* × *V. berlandieri*) (28). Rootstocks with *Muscadinia rotundifolia* parentage are resistant to *X. index* feeding (5,20,47,48). However, the nematode can probe and transmit GFLV to *X. index*-resistant rootstocks. Therefore, virus translocation occurs from *X. index*-resistant rootstocks into scions, although the debilitating effects of fanleaf degeneration are reduced (47,48). For most susceptible grapevine genotypes, the feeding process of *X. index* on actively growing roots causes malformation, swelling, galling, or necrosis of grapevine root tips. Fig (*Ficus carica*) is another host for *X. index* but fig is not a host for GFLV (32,40).

X. index reproduces parthenogenetically, although a sexual reproduction may occur occasionally in spite of rare males (16). A single female is able to reproduce and generate a clonal lineage (15,32). Eggs are laid in the spring and develop into adults through four juvenile stages. The life cycle of *X. index* lasts from

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one to several months depending on biotic and abiotic factors (3,39). *X. index*, including viruliferous specimens, can survive for at least 4 years when stored in vineyard soil in the absence of host plants (18) and for 4.5 years in a fallowed vineyard (33).

Nematodes from distinct geographical origins can differ in their efficiency of virus transmission. For example, *X. diversicaudatum* populations from France, Italy, and Spain transmitted *Arabidopsis mosaic virus* (ArMV) poorly, whereas populations from Bulgaria, England, New Zealand, Norway, Scotland, Switzerland, and the United States were efficient vectors of ArMV (6,7). A similar situation applies to *Longidorus arthensis*, the specific vector of *Cherry rosette virus* (CRV), for which populations from four regions in Switzerland (Arth, Rossberg, Steinberg, and Talmatte) transmitted CRV efficiently whereas populations from two other regions (Baselland and Maiberg) did not vector CRV (9). Limited information is available for *X. index* and GFLV. Studying the vector competency of geographically diverse *X. index* populations would provide useful information into virus–vector relationships and fanleaf epidemiology. The transmission of GFLV by different *X. index* populations was reported earlier (12–14). However, the low number of *X. index* populations studied and their restricted geographical diversity precluded any conclusions on the effect of the origin of *X. index* on GFLV transmission competency. Also, the transmission procedure was not optimal for comparative transmissibility assays (12,14).

We evaluated the transmission competency of seven *X. index* lines of different geographical origins by using two genetically distinct GFLV strains. Single-female lines were produced for each population and their multiplication rate was analyzed on two *Vitis* spp. Here, we report a similar competency of acquisition and transmission of the two GFLV strains by the seven single-female-derived *X. index* lines despite different multiplication rates. The implications of these findings for the GFLV spread in vineyards and screening of *Vitis* spp. for GFLV resistance are discussed.

MATERIALS AND METHODS

Virus strains. GFLV strains F13 (46) and GHu (25) were isolated from naturally infected *V. vinifera* cvs. Muscat de Frontignan and Glorie de Hungariae, respectively. Sequences of GFLV-F13 and GFLV-GHu RNA2 were determined previously (34,35,43) and obtained from GenBank accession numbers X16907, D00915, and EF426852. GFLV-F13 and -GHu were transferred by mechanical inoculation from infected grapevine leaf tissue to *Chenopodium quinoa* and propagated in this systemic herbaceous host. GFLV-F13 and -GHu were back trans-

mitted onto the certified, virus-tested rootstock Kober 5BB (*V. berlandieri* × *V. riparia*) clone 259 by in vitro heterologous grafting, as described previously (4).

Plant material. Fig (*F. carica*) plants were used to produce single-female-derived *X. index* lines and establish rearings of aviruliferous *X. index*. Fig plants were produced either by in vitro multiplication (26) or propagation of green cuttings on rockwool (Grodan, Roermond, The Netherlands) in a growth chamber under controlled temperature (20 to 26°C) and light (16-h photoperiod and 60 μEinstein/m²/s) conditions.

V. rupestris du Lot and *V. vinifera* cv. Cabernet Sauvignon were used to determine the reproduction rate of single-female *X. index* lines. Plants of the two grapevine genotypes were micropropagated in vitro and acclimatized in a greenhouse (41).

Healthy and GFLV-F13- or -GHu-infected grapevine rootstock Kober 5BB were used in nematode transmission experiments as virus recipient and virus donor plants, respectively. Healthy and virus-infected rooted Kober 5BB plants were produced from green cuttings (17).

Production and multiplication of single-female-derived *X. index* lines. Seven *X. index* populations from naturally GFLV-infected vineyards were collected and maintained on grapevines in a greenhouse at the Institut National de la Recherche Agronomique (INRA) in Sophia-Antipolis, France (Table 1). For each population, single-female lines were obtained by transferring a single adult female into a 200-ml container planted with one rooted fig plant produced in vitro. After 1 year, nematode specimens were isolated using an Oostenbrink elutriator and a Baermann funnel (24). Females and the four juvenile stages were identified following morphometric *X. index* characteristics (27). Specimens of each *X. index* line were transferred into 10-liter containers planted with fig plants and cultured in a greenhouse to increase population density. Increased single-female *X. index* clonal lines progeny were used in this study.

Determining the multiplication rate of *X. index* lines. Rooted cuttings of *V. rupestris* and *V. vinifera* were micropropagated in vitro and planted in sandy soil in 2-liter containers in a greenhouse. Vines showing homogenous growth were selected and their roots were exposed to 250 aviruliferous single-female-derived *X. index* that were extracted from soil samples recovered from containers planted with fig using an Oostenbrink elutriator and a Baermann funnel (24). Five to eight replicates were used per grapevine cultivar and single-female *X. index* line combinations. After 5 months of cultivation, the soil of each 2-liter container was recovered in 10-liter buckets and plant roots were carefully washed using tap water and dried. Roots were collected and weighted, and *X. index* were extracted from the water suspension based on an adapted Oostenbrink method (24). Nematodes were counted with a stereomicroscope to determine their total number per plant. The ratio of the total number of *X. index* recovered (final population [fp]) over the initial number of *X. index* (initial population [ip]) was calculated to determine the multiplication rate (fp/ip) for each nematode line.

GFLV transmission by single-female-derived *X. index* lines. The vector transmissibility of GFLV-F13 and -GHu was determined using aviruliferous single-female-derived *X. index* lines

Fig. 1. Genome organization and expression of *Grapevine fanleaf virus* (GFLV). Open boxes represent open reading frames encoded by the two genomic RNAs. The 5' and 3' untranslated regions are denoted by a black line, the viral protein genomic (VPg) is represented by a black circle and the cleavage sites are indicated by arrows below the polyproteins. Processed proteins encoded by the two genomic RNAs are indicated within the polyproteins: 1A, putative proteinase cofactor; 1B^{Hel}, putative helicase; 1C^{VPg}, VPg; 1D^{Pro}, proteinase; 1E^{Pol}, putative RNA-dependant RNA polymerase; 2A^{HP}, homing protein; 2B^{MP}, movement protein; and 2C^{CP}, coat protein. The position of primers CPS, EV1, and EV3 used to detect GFLV by immunocapture (IC) reverse-transcription polymerase chain reaction (RT-PCR) or RT-PCR assays is indicated.

TABLE 1. Origin of the *Xiphinema index* populations collected from vineyards naturally infected by *Grapevine fanleaf virus* (GFLV) and used to produce single-female lines

Line ID	Country	Region
Cyprus	Cyprus	Unknown
Israel	Israel	Unknown
Italy	Italy	Apulia
France-1	France	Champagne
France-2	France	Provence
Spain	Spain	South Mancha
California	United States	Napa Valley

reared on fig, as described previously (4). Transmission experiments used a two-step procedure with infected rooted Kober 5BB grapevines as donors and rooted healthy Kober 5BB grapevines as recipients. Two repetitions with 10 to 14 individual transmission assays were run per GFLV strain and single-female *X. index* line. The transmissibility of GFLV isolates was assessed by double-antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) using root samples of recipient grapevines (4,6). The systemic GFLV infection of recipient grapevines was analyzed by DAS-ELISA in newly emerging leaves after a cold treatment.

GFLV detection in *X. index* by reverse transcription-polymerase chain reaction. The presence of GFLV was assessed in groups of 20 *X. index* by reverse-transcription polymerase chain reaction (RT-PCR) using primers specific to GFLV-F13 RNA2 (17). The RT reaction used 50 pmol of reverse primer EV1 (5' ³⁵⁷⁴GACTATCTAGACACATATATACACTTGGGTCTTTAA³⁶⁰¹ 3') (Fig. 1) with an additional *Xba*I site underlined. The PCR reaction was performed in a final volume of 50 µl containing 10 µl of the RT reaction and 50 pmol of primers EV1 and CPS (5' ³⁰⁴⁵TTGTGCGCCAGATCTCTCTTTACCA³⁰⁷⁰ 3') (Fig. 1) in a Master thermocycler (Eppendorf, Hamburg, Germany). RT-PCR products were analyzed under UV illumination following electrophoresis on 1% agarose gels and ethidium bromide staining.

GFLV detection in grapevines by DAS-ELISA and immunocapture RT-PCR restriction fragment length polymorphism. GFLV was detected in leaf and root samples of individual vines by DAS-ELISA using specific antibodies as described (4,6). The antibodies used in DAS-ELISA recognized equally GFLV strains F13 and GHu. The full-length 2C^{CP} gene of GFLV was further characterized by immunocapture (IC)-RT-PCR using root samples of recipient grapevines that reacted positively in DAS-ELISA and primers EV1 and EV3 (42,43). IC-RT-PCR-amplified DNA products were purified from agarose gels using the QIAquick extraction kit following the supplier's instructions (Qiagen, Hilden, Germany) and characterized by restriction fragment length polymorphism (RFLP) using *Eco*RI and *Sty*I digestion to discriminate the two GFLV strains (42). IC-RT-PCR and RFLP products were resolved by electrophoresis on 1% agarose and 7% polyacrylamide gels, respectively, and analyzed after ethidium bromide staining using a UV light transilluminator.

Statistical analyses. Data collected on *X. index* multiplication rates on two grapevine genotypes and GFLV transmission assays were processed for statistical analysis. Multiplication rates were subjected to analysis of covariance, for which nematode lines and grapevine genotype were factors studied. Root weight was used as covariate. Comparisons of means were done using a "Dunnett" test with the single-female *X. index* line France-2 as reference, because it is used routinely in transmission experiments at INRA in Colmar, France. The transmission rates were subjected to analysis of variance using line and genotype as fixed factors. In

order to satisfy hypotheses of normality of the residuals and homoscedasticity of the variances, multiplication and transmission rates were log and square-root transformed, respectively, and hypotheses were tested using a Shapiro-Wilk normality test and a studentized Breusch-Pagan test, respectively. Statistical analyses were run using R package (The R Project for Statistical Computing, version 2.8.1, available at <http://www.r-project.org/>).

RESULTS

Individual adult females were isolated from seven *X. index* populations collected from various vineyards in the Mediterranean basin (Cyprus, southern France, Israel, Italy, and Spain), northern France, and California (Table 1). Individual *X. index* females were deposited on roots of young fig plants and nematodes derived from a single adult female were further increased on rooted fig plants to obtain genetically homogenous aviruliferous clonal lines for comparative reproduction assays and GFLV transmission experiments.

The reproduction rate of groups of 250 *X. index* individuals from single-female lines reared on fig was determined on *V. rupestris* and *V. vinifera*. Multiplication ratios were positive for all *X. index* lines after 5 months of culture in a greenhouse (Table 2), indicating that the seven single-female lines multiplied on the two grapevine genotypes. However, the multiplication rate varied among the seven lines, regardless of the grapevine genotype. Analysis of covariance (type III) showed that the multiplication rate was strongly linked to the *X. index* line ($F = 13.88$; $Df = 6$; $P < 0.0001$). The effect of grapevine genotype ($F = 0.0171$; $Df = 1$; $P = 0.75$), grapevine root weight ($F = 0.0004$; $Df = 1$; $P = 0.98$) and line-genotype interaction ($F = 1.11$; $Df = 6$; $P = 0.37$) were not significant (Table 2). Considering only single-female *X. index* lines as factor, hypotheses of normality of the residuals (Shapiro-Wilk test: $W = 0.99$, $P = 0.82$) and homoscedasticity of the variances (Breusch-Pagan test: $BP = 7.526$; $Df = 6$; $P = 0.275$) were satisfied. The multiplication rate of the Cyprus single-female line was approximately three times higher compared with the Spain and California single-female lines, while other lines showed intermediary multiplication ratios (Table 2). A comparison of means (Dunnett Test) indicated that the Cyprus, Spain, and California lines were significantly different from France-2 whereas the three others lines (France-1, Italy, and Israel) were not significantly different from France-2 (Table 2).

The transmissibility of GFLV strains F13 and GHu by aviruliferous nematodes of the seven *X. index* single-female lines was determined. GFLV strains F13 and GHu were selected for comparative transmission assays because their coat proteins, which are involved in transmission specificity (4), have 4.6% amino acid divergence. Prior to initiating the nematode transmission tests, the presence of GFLV was confirmed in roots of source grapevines by

TABLE 2. Multiplication of single-female *Xiphinema index* lines on *Vitis rupestris* cv. du Lot and *V. vinifera* cv. Cabernet Sauvignon, and root weight of grapevine hosts

<i>X. index</i> line	<i>V. vinifera</i> cv. Cabernet			<i>V. rupestris</i> du Lot			Log (fp/ip) ^a	
	fp ^b	fp/ip ^c	Root (g/vine) ^d	fp ^b	fp/ip ^c	Root (g/vine) ^d	Means	Standard error
Cyprus	7,573	30.3	43.4	7,525	30.1	42	3.332***	0.115
Israel	3,225	12.9	49.8	4,150	16.6	43	2.606	0.111
Italy	4,965	19.9	39	3,850	15.4	38	2.786	0.111
France-1	3,896	15.6	50	5,005	20	37	2.876	0.111
France-2	4,409	17.6	38	3,846	15.4	37	2.666	0.107
Spain	1,982	7.9	47	2,303	9.2	40	2.163**	0.103
California	2,441	9.8	39	2,207	8.8	40	2.160**	0.107

^a Data represent the means of the multiplication rates log transformed for each line; fp = final population and ip = initial population. Statistically different means at $P \leq 0.05$, 0.01, and 0.001 indicated by *, **, and ***, respectively, using Dunnett's test are indicated.

^b Data represent the total number of *X. index* recovered after 5 months of culture (fp). Five to eight plants were used for each combination of *X. index* line and genotype.

^c Ratio of the total number of *X. index* recovered after 5 months of culture over the initial number of *X. index* exposed to grapevine roots (ip = 250).

^d Data represent root weight mean after 5 months of contact with nematodes.

DAS-ELISA (data not shown). For transmission tests, 300 aviruliferous nematodes were exposed to roots of GFLV-infected grapevine plants for 6 weeks. After completion of the virus acquisition step, virus source plants were uprooted and replaced by healthy grapevines. Nematodes were then allowed to feed for six additional weeks. Results from two independent experiments indicated that the seven single-female lines transmitted both GFLV strains with a mean rate of 93% (321 infected out of 344 grapevines tested) (Table 3). These data were consistent with the notion that GFLV transmission is not influenced by the geographical origin of *X. index*. Analysis of variance (ANOVA type III) showed that the effect of *X. index* lines ($F = 0.59$; $Df = 6$; $P = 0.74$), GFLV strains ($F = 0.18$; $Df = 1$; $P = 0.67$), and line–GFLV strain interaction ($F = 0.39$; $Df = 6$; $P = 0.87$) on transmission rate were not significant. A model, including lines and GFLV strains as factors, satisfied the hypothesis of the normality of the residuals (Shapiro-Wilk test: $W = 0.97$; $P = 0.51$) and the homoscedasticity of the variance (Breusch-Pagan test: $BP = 9.96$; $Df = 7$; $P = 0.191$).

To validate transmission results, three criteria of the nematode transmission process described by Brown and Weischer (11) were verified. First, grapevine roots were examined for malformation as indicative of nematode feeding. Roots of the majority of source (89%, 336 of 378) and recipient (93%, 320 of 344) grapevines showed galls or swelling, indicating that the seven *X. index* lines were actively feeding. Second, the viruliferous status of nematodes was determined after the acquisition step. GFLV-F13 and -GHu were detected in *X. index* by RT-PCR using total nematode RNA extracted from randomly selected samples of 20 adult nematodes (Fig. 2). A specific DNA fragment of the expected size (566 bp) was obtained for all the *X. index* tested (Fig. 2A and B). These results showed that some specimens of the seven single-

Fig. 2. Reverse transcription-polymerase chain reaction (RT-PCR) detection of **A**, *Grapevine fanleaf virus* (GFLV)-F13 and **B**, GFLV-GHu in *Xiphinema index* after the acquisition step. Total RNA were extracted from samples of 20 nematodes isolated from single-female lines from Cyprus (lane 1), Israel (lane 2), Italy (lane 3), France-1 (lane 4), France-2 (lane 5), Spain (lane 6), and the United States (lane 7). Lanes 8 and 9 correspond to RT-PCR products of 20 nematodes reared on GFLV-F13-infected and healthy grapevines, respectively. RT-PCR products were analyzed by electrophoresis on 1% agarose gels and staining with ethidium bromide.

TABLE 3. Transmission rate of *Grapevine fanleaf virus* (GFLV) strains F13 and GHu by seven single-female *Xiphinema index* lines from diverse geographical origins

<i>X. index</i> line	GFLV-F13		GFLV-GHu		Mock	
	Ratio	%	Ratio	%	Ratio	%
Cyprus	22/24	92	23/24	96	0/9	0
Israel	24/26	92	22/24	92	0/10	0
Italy	25/26	96	23/24	96	0/10	0
France-1	24/22	92	21/24	88	0/10	0
France-2	23/26	88	23/24	96	0/8	0
Spain	22/24	92	23/24	96	0/9	0
United States	25/26	96	23/24	96	0/10	0
Total	163/176	93	158/168	94	0/66	0

^a Data represent the total number of bait grapevines infected by GFLV, as shown by double-antibody sandwich enzyme-linked immunosorbent assay, following *X. index*-mediated transmission over the total number of bait grapevines used in transmission assays. Data from two independent transmission experiments with 10 to 14 plants each were pooled.

female lines acquired GFLV. In addition, none of the bait grapevines was infected by GFLV after exposure to *X. index* from fig plants (Table 3), indicating that the seven single-female lines were aviruliferous when reared on fig. Third, the genetic integrity of the GFLV strains was analyzed after transmission by characterizing the coding region of the 2C^{CP} gene in leaves of infected recipient grapevines by IC-RT-PCR using primers EV1 and EV3 (Fig. 1). The 2C^{CP} amplicons obtained had the expected DNA size fragment (1,573 bp) (Fig. 3A and D, lanes 1 to 7) and co-migrated with DNA fragments amplified from infected source grapevines (Fig. 3A and D, lane 8). RFLP analysis of purified 2C^{CP} amplicons showed the expected banding patterns for GFLV-F13 with 1,075-, 330-, and 168-bp *Eco*RI fragments (Fig. 3B) and 690-, 350-, 276-, and 110-bp *Sty*I fragments (Fig. 3C) for all the recipient grapevines tested as well as one source grapevine. Similarly, the expected RFLP patterns for the GFLV-GHu 2C^{CP} gene were obtained from recipient grapevines with undigested *Eco*RI products (Fig. 3E) and 510-, 394-, 350-, and 276-bp *Sty*I fragments (Fig. 3F) from all recipient and source grapevines tested. These results confirmed that the seven *X. index* lines acquired and transmitted GFLV strains F13 and GHu.

DISCUSSION

The transmission competency of seven *X. index* lines from diverse geographical origins was determined with two genetically divergent GFLV strains. Although the seven nematode lines showed differences in their reproductive rate on two *Vitis* spp., they vectored the two GFLV strains with a 93% mean transmission rate regardless of their country of origin. These results clearly indicated no geographical competency of *X. index* for GFLV transmission.

Fig. 3. Characterization of the coat protein (2C^{CP}) gene of *Grapevine fanleaf virus* (GFLV) strains after *Xiphinema index* transmission. The 2C^{CP} gene was amplified by immunocapture reverse-transcription polymerase chain reaction from infected bait grapevines exposed to single- female *X. index* lines from Cyprus (lane 1), Israel (lane 2), Italy (lane 3), France-1 (lane 4), France-2 (lane 5), Spain (lane 6), and the United States (lane 7). **A and D**, Amplicons of the 2C^{CP} gene were digested by **B and E**, *Eco*RI and **C and F**, *Sty*I, respectively. Restriction fragment length polymorphism patterns obtained from bait plants were compared with those from source plants infected with either **A to C**, GFLV-F13 (lane 8) or **D to F**, GFLV-GHu (lane 8). DNA fragments less than 100 bp are not visible.

Previous studies reported an efficient transmission of GFLV isolates by different populations of *X. index* (12–14). However, the impact of the geographical origin could not be investigated rigorously because the number of *X. index* populations and their geographical diversity were limited. Also, the transmission procedure was not designed for comparative evaluations because it did not rely on a standardized protocol. In our study, *X. index* origins were chosen to represent a broad geographical diversity, with populations from Europe (mainly from Mediterranean countries), the Middle East, and North America. Also, a standardized procedure was used to assess the transmissibility of GFLV strains with a single *Vitis* genotype (e.g., Kober 5BB) as source and bait plants. Furthermore, the susceptibility of Kober 5BB to *X. index* feeding and GFLV transmission is similar to that of *V. rupestris*, one of the grape genotypes used in our study to assess multiplication rates (15,36). This similarity enabled us to make inferences between the multiplication rate and vector competency of the seven *X. index* lines. Moreover, *X. index* lines representing genetically homogenous material were used for optimal comparative transmission assays. Altogether, our experimental conditions enabled us to compare the competency of different *X. index* lines for GFLV transmission. To our knowledge, this is the first in-depth evaluation on a possible association between the geographical origin of *X. index* and its GFLV vectoring capacity. It would have been interesting to investigate the transmissibility of the two GFLV strains used in this study with lower numbers of *X. index*, as reported previously for ArMV and *X. diversicaudatum* (7), *Grapevine leafroll-associated virus 3* and mealybugs (19), and *Zucchini yellow mosaic virus* and *Myzus persicae* (38), among other examples. Unfortunately, reliable GFLV transmission assays require at least 300 *X. index* individuals, as shown by several investigators (2,4,14,36,37), although a single viruliferous individual can be vector competent (D. Esmenjaud, unpublished data).

The seven *X. index* lines used in our study transmitted the two divergent GFLV isolates at similar rates. This situation contrasts with the lack of transmission of strains of ArMV and *Strawberry latent ringspot virus* (SLRV) by some *X. diversicaudatum* populations (6–8,10). A difference in vector capacity and efficiency between *X. diversicaudatum* and *X. index*, two closely related dagger nematode species, is likely associated with their host range. *X. diversicaudatum* has a broad host range whereas *X. index* has a narrow host range (39). Both *Xiphinema* spp. vary also in their reproduction mode. The amphimictic reproduction of *X. diversicaudatum* underlies a higher genetic variability that may explain differences in vector capacity. To this extent, a loss in transmission efficiency was reported in progenies from two *X. diversicaudatum* populations (8). Because *X. index* mainly reproduces by meiotic parthenogenesis, a loss of GFLV vector capacity among progenies is less likely to occur.

The reproduction rate of the seven single-female *X. index* lines characterized in this study varied considerably. Variations were not linked to grapevine root vigor or grapevine genotype but to *X. index* lines. However, differences in reproduction rate were not associated with differences in the ability to transmit the two GFLV strains. Probing behavior or genetic factors of *X. index* lines could account for differential reproduction rates. More work is needed to verify these hypotheses. A difference between reproduction and virus vectoring capacity was described for *X. diversicaudatum* populations of different geographical origin (10).

Nematode transmission of viruses is characterized by the vector's ability to acquire and retain virus particles at specific sites within the feeding apparatus and, subsequently, release them into a recipient plant (11). *X. diversicaudatum* populations that are poor vectors or fail to transmit viruses do not efficiently retain ArMV or SLRV particles (10). Our experimental data indicated that the two GFLV isolates were equally acquired and retained by the seven single-female lines, because GFLV 2C^{CP} amplicons were clearly detected in all the nematodes randomly recovered

and tested after the acquisition step. Similarly, GFLV 2C^{CP} amplifiable DNA products were obtained in the root zone of bait grapevine plants at the end of the transmission experiments.

X. index is considered to be the only natural vector of GFLV (1,12,28,39). A strong and specific interaction characterizes the association between *X. index* and GFLV. Indeed, *X. index* can retain GFLV for at least 4 years (18). Based on our findings with seven *X. index* lines from diverse geographical locations, it is reasonable to anticipate that most, if not all, *X. index* populations are able to transmit natural GFLV isolates. This hypothesis is also supported by the fact that no natural nontransmissible GFLV isolate has been recovered from naturally infected vineyards. This is in contrast with SLRV isolates not transmitted by several *X. diversicaudatum* populations; *Tomato ringspot virus* strains differentially transmitted by populations of *X. americanum*, *X. bricolensis*, *X. californicum*, and *X. rivesi* (39); and *Tobacco rattle virus* isolates differentially transmitted by *Paratrichodorus pachydermus* nematodes (31). Our observations on GFLV confirm the strong genetic stability of its coat protein (42) and support a strong selection pressure on the corresponding gene to conserve the potential to be transmitted by *X. index*.

GFLV is exclusively transmitted by *X. index* whereas ArMV is specifically transmitted by *X. diversicaudatum* (11,29). The transmissibility of chimeric viruses for which the 2A^{HP}, 2B^{MP}, or 2C^{CP} genes of GFLV were replaced by their ArMV counterpart sequences have shown that protein 2C^{CP} is the sole viral determinant responsible for the specific transmission of GFLV (2,4). Recently, GFLV-GHu was shown to result from three interspecies recombination events between GFLV and ArMV with gene 2B^{MP} of ArMV origin and gene 2C^{CP} of GFLV origin (43). Interestingly, the seven *X. index* lines used in this study efficiently transmitted this natural chimeric virus, confirming that gene 2B^{MP} is not involved in the specificity of transmission of GFLV by *X. index*.

The dynamic of GFLV spread by *X. index* in vineyards may differ from site to site (D. Esmenjaud, unpublished). These differences may be correlated to *X. index*–grapevine cultivar interactions, pedoclimatic characteristics acting on the dynamic of *X. index* populations (39), and differential GFLV transmission efficiency (12). Based on our findings, it seems that differences in GFLV spread in vineyards are more likely due to the dynamic of *X. index* populations in relation to crop management practices (45) and soil texture and moisture (18,23), as well as host susceptibility and plant vigor (23).

Rouging is recommended to control GFLV in infected vineyards in combination with soil disinfection with nematicides and extended fallow (1). Agrochemicals are usually not efficient to control viruliferous nematode populations and constitute a hazard to the environment (1). Their use was restricted or banned recently in Europe (21,22). Extended periods of fallow are difficult to implement for economic reasons, especially in premium vineyards. *X. index*-tolerant rootstocks have been developed. They reduce *X. index* numbers but do not prevent GFLV transmission (5,20,47,48). Therefore, breeding programs are currently working to develop plant material resistant to *X. index* and GFLV (20). Our findings suggest that any *X. index* population should be suitable to evaluate plant reaction to virus infection following nematode transmission of GFLV. This should facilitate the screening of *Vitis* spp. for resistance to GFLV.

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