

Xiphinema index and its Relationship to Grapevines: A review

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The dagger nematode, *Xiphinema index*, is considered a major pest in grape growing countries. *Xiphinema index* is especially important because of its ability to transmit Grapevine fanleaf virus when feeding on grapevine roots. This paper provides a comprehensive and updated review of the classification, genetics and biology of *Xiphinema index*, and its relationship with grapevine fanleaf virus. Current control measures, as well as past and present efforts to breed resistant grapevine rootstocks, are presented.

Grapevines are cultivated in temperate and Mediterranean climates around the world. Grapevines have been moved between countries and continents, following human migration and settlement, and have been imported and cultivated in numerous countries. These events have increased the incidence and spread of injurious pests and diseases (Esmenjaud & Bouquet, 2009). Three major pests are known to attack the root system of grapevines: grape phylloxera (*Daktulosphaira vitifoliae* Fitch); ground pearls (*Margarodes* spp.); and a wide range of root-feeding nematodes. These pests damage roots, leading to their decay, the prevention of new root development and may result in vine decline and eventual death. The initial impact of these pests may not be severe, but the cumulative impact over years intensifies and causes significant losses (De Klerk & Loubser, 1988). Nematodes associated with vine damage are root-knot nematodes (*Meloidogyne* spp.), citrus nematodes (*Tylenchulus semipenetrans*), root-lesion nematodes (*Pratylenchus vulnus*) and dagger nematodes (*Xiphinema* spp.) (Nicholas *et al.*, 2007).

All of the nematodes that have an economic impact on grapevines are present in South Africa (Smith, 1977). Dagger nematodes are considered to be major pests (Brown *et al.*, 1995), because they are often associated with woody plants and are generally associated with specific viruses, which they carry from plant to plant through feeding. More than 170 species of *Xiphinema* have been identified worldwide on a wide range of hosts. Sixty-nine *Xiphinema* species have been reported in South Africa, although only four were implicated in plant virus transmission: *X. americanum* Cobb, *X. diversicaudatum* Thorne, *X. index* Thorne and Allen, and

X. italiae Meyl (Loubser & Meyer, 1987a); the first three are common in South African vineyards (Malan, 1995). They are found in a variety of soils and are migratory ectoparasites (Shurtleff & Averre III, 2000). This review focus on *X. index*, its interaction with grapevines and its role as vector for grapevine fanleaf virus (GFLV).

Classification, description and identification of *Xiphinema index*

Xiphinema index is in the order Dorylaimida, suborder Dorylaimina, and superfamily Dorylaimoidea, family Longidoridae, subfamily Xiphineminae and genus *Xiphinema* (Taylor & Brown, 1997). The genus *Xiphinema* was first described by Thorne (1939) and *X. index* was first identified and described by Thorne & Allen (1950).

The body of an adult female *X. index* is about 3 mm long. The lip region is hemispherical and almost continuous along the body. The odontostyle is approximately 126 µm long, the odontophore 70 µm and has large flanges. There is a guide ring at approximately 108 µm from the anterior end (Decraemer & Geraert, 2006). The female body is elongate-cylindrical, forming an open spiral with a greater curvature in the posterior half. The cuticle is thick with fine, superficial striations. Eight or nine lateral body pores are present in the oesophageal region, 13 or 14 between the oesophagus and vulva and 21 or 22 between the vulva and anus (Siddiqi, 1974). The female has one or two ovaries, which are usually paired and reflexed, one reduced and extending anteriorly, the other posteriorly (Shurtleff & Averre III, 2000). Reproduction is parthenogenetic and males are extremely rare. Their body shape is the same as that of the female (Siddiqi, 1974). Males

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have two opposed, outstretched testes and the spicules are strong with lateral guiding pieces (Siddiqi, 1979). Both males and females have short, dorsally rounded tails. The tail has a terminal peg situated ventrally and is 8 to 12 μm long. This peg is a distinct characteristic of the species (Figure 1) (Luc & Cohn, 1982).

Descriptions of this nematode are varied, for example; the listed length of females ranges from 2.8 to 3.4 mm and the odontostyle length vary from 120 to 144 μm (Thorne & Allen, 1950; Lamberti *et al.*, 1985; Barsi, 1989; Coiro *et al.*, 1992). However, the soil environment might play a role in this variation since this factor is often ignored during collection (Prins, 1997). In 1977, Garau & Prota described the four juvenile stages of *X. index* using three different measurements: body length, functional odontostyle length and replacement odontostyle length. The data varied considerably within each of these measurements, particularly across juvenile stages. Separation of the first and second stage juveniles was particularly difficult, but with any single measurement used, the third and fourth stages were readily identified with a high degree of accuracy (Garau & Prota, 1977).

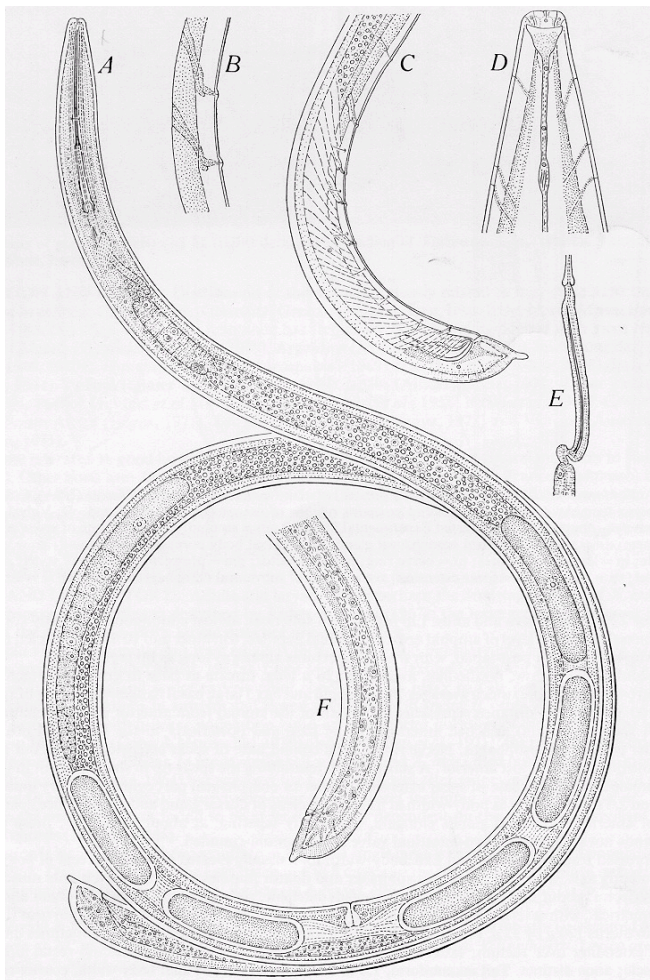


FIGURE 1

X. index as described by Thorne & Allen, 1950. A: Female. B: Detail of supplements. C: Male posterior. D: Head end showing amphid. E: Replacement spear in anterior portion of oesophagus of larvae. F: Female posterior (Siddiqi, 1974).

It is important to be able to distinguish different species of *Xiphinema* from each other. *Xiphinema index*, *X. diversicaudatum*, *X. vuittenezi* and *X. italiae* are closely related taxonomically and therefore difficult to distinguish regarding morphological and morphometrical characteristics. This has led to efforts using PCR (Polymerase Chain Reaction) techniques and species-specific primers (Esmenjaud & Bouquet, 2009) to distinguish between the species on molecular level. Specific ribosomal regions of the genome were sequenced in one population of each species and species-specific primers were developed from the sequencing data and allowed to identify single individuals at different developmental stages (Wang *et al.*, 2002). The species-specificity of those primers for *X. index*, *X. diversicaudatum* and *X. vuittenezi* detection was then confirmed (Hübschen *et al.*, 2004a) and have become a tool that facilitates taxonomic identification for non-specialists. Similarly, Hübschen *et al.* (2004b) developed species-specific ribosomal primers for seven Longidorid species of which some are also known as virus vectors. These Longidorid primers were tested for sensitivity and selectivity on closely related species and proven to be highly specific in detecting all developmental stages within a species, and also in distinguishing a single target nematode from a community (Hübschen *et al.*, 2004b).

Range, habitat, biology and culturing of *Xiphinema index*

Dagger nematodes are found in all soil types. In South Africa, 16 species of *Xiphinema* were found in soil samples analysed from five viticulture regions in the Cape Province and *X. index* was present in three of these regions (Malan & Meyer, 1994). The population of *X. index* decreases with soil depth. More than 92% of all nematodes are found in the 0 to 300 mm zone where most vine roots occur (De Klerk & Loubser, 1988). Earlier research done in California, showed that *X. index* could be found as deep as 360 cm (Raski *et al.*, 1965a) and are likely to be found wherever roots are. In France, the highest number of individuals occurred at 40-110 cm depth, corresponding to the layers where the highest densities of fine roots were observed in both Champagne (Esmenjaud *et al.*, 1992) and Bordeaux (Villate *et al.*, 2009) vineyards. Light to medium textured soils with a pH between 6.5 and 7.5 seem to be preferred (Siddiqi, 1974). Based on a study done in a Barossa Valley vineyard in Australia, the best time to determine *X. index* densities was in the late spring (Quader *et al.*, 2003).

Temperature is an important modulating factor on the reproduction and life cycle of *X. index*, which is typically associated with grapevines in warm climates. The *X. index* population increased more rapidly as the soil temperature increased from 16 to 28°C. In Italy, it was found that *X. index* numbers are lower in winter (Coiro *et al.*, 1987; Coiro *et al.*, 1991), but a study in California found that the populations peaked in the winter (Feil *et al.*, 1997) possibly due to more accurate sampling in moist soils. A study done in England under experimental conditions showed that *X. index* egg-laying peaked during summer months, with maximum populations in autumn and lowest populations in spring (Siddiqi, 1974).

Xiphinema index has been shown to survive in a wide range of soil temperatures ranging from -11°C to 35°C,

but constant temperatures for 10 days of 45°C or -22°C killed the nematodes. Fluctuations in diurnal temperatures also lowered *X. index* survival rates (Cotten *et al.*, 1971). Females typically produce an egg every 24 to 26 days when the temperature is above a minimum daily threshold of 10°C. Eggs are laid singly in the soil close to the feeding site (Weischer & Wyss, 1976) and the life cycle takes 3 to 5 months to complete at 28°C, but slows down to 7 to 9 months at lower temperatures (Nicholas *et al.*, 2007). Reproduction rate has been shown to be highest at 29.4°C (Siddiqi, 1974). As mentioned, reproduction is by parthenogenesis (Dalmaso, 1975) and a single larva is capable of generating a population. Eggs hatch in 6 to 8 days and the first molt takes place outside the egg 24 to 48 hours after hatching. Dagger nematodes have four juvenile stages; the 2nd, 3rd and 4th molts occur at six-day intervals (Siddiqi, 1974). The nematode reproduces by meiotic parthenogenesis (Dalmaso, 1975) but sexual reproduction has been observed from a recent field population genetics study (Villate *et al.*, 2009) even though it appears to be a rare event. Thus, increasing genetic diversity through sexual recombination in *X. index*, involving the rare male has been observed, does exist and explains the occasional detection of females having spermatozoa in the uterus (Luc & Cohn, 1982). Initial studies found that the *X. index* genome consisted of 20 chromosomes and suggested that it might be a tetraploid (Dalmaso & Younes, 1969), but it was later reported that the genome consisted of 10 chromosomes (Dalmaso, 1975).

Earlier studies showed substantial variations in reproduction rates and life cycle stages under greenhouse conditions (Cohn & Mordechai, 1970; Coiro *et al.*, 1990); *X. index* reproduced faster in non-clay soils under these conditions (Coiro *et al.*, 1987). Fine sand and sandy loam soils with a soil moisture content of 10 to 15% induced higher reproduction results than coarse sand (Sultan & Ferris, 1991). In a Californian greenhouse study the cycle from egg to female has been reported to be 22 to 27 days (Pearson & Goheen, 1988), whereas others report a 60-day life cycle (McKenry, 2000). Individuals can live for many years (Nicholas *et al.*, 2007) as claimed by a French report of survival in stored soil for four years (Demangeat, *et al.*, 2005). In a study done by Brown & Coiro (1985), it was shown that the longevity of *X. index* on *Ficus carica* was 60 to 64 weeks, with a total reproductive capacity of 140 to 160 progeny. Longevities and reproductive capacities for female *X. index* from Italy and the U.S.A. were similar when raised on *F. carica* (Brown & Coiro, 1985).

Effect of Xiphinema index feeding on grapevines

Xiphinema index feeding initially causes a swollen club-like gall on root tips, which varies in size with the size and vigor of the root. The feeding wound then becomes reddish brown to black and forms slightly sunken lesions on the roots (Wyss, 1978). Infested root systems are stunted and have a witch's broom appearance after successive rounds of new roots branching and being damaged from behind the original damaged root tip (Pearson & Goheen, 1988). Extensive root damage eventually results in reduced shoot growth and yield. Common symptoms of *X. index* feeding are plant stunting, chlorosis, root swellings or galls and root necrosis (Figure 2)

(Wyss *et al.*, 1980). The number of galls formed has been correlated with the size of a nematode population and with size of the root system in potted plants (Xu *et al.*, 2008). The clubbed galls suggest that the nematodes discharge some substance into the roots to induce swelling (O'Bannon & Inserra, 1990) and this galling has been shown to occur as early as 24 hours after feeding (Fisher & Raski, 1967).



FIGURE 2

Feeding damage (galling) caused by *X. index* on roots of St. George, a highly susceptible variety.

Nematode feeding damage induces water and nutrient stress, which in turn reduces vine vigor and yield. Penetration of roots by nematode stylet also makes them more susceptible to root-rotting fungi (Nicholas *et al.*, 2007), which contributes to vine death.

The foliage symptoms caused by root damage from *X. index* feeding are similar to those caused by root rots, drought and other root-feeding pests. Soil conditions can also restrict root growth and consequently, damage done by *X. index* can be made worse. These conditions include drought, compact soils, shallow water tables, saline soils and highly acidic or alkaline soils. In addition, it is common to have more than one type of nematode attacking the roots, which often intensifies the damage (Nicholas *et al.*, 2007).

The combined effect of *X. index* feeding and its association with GFLV may kill grapevines (Nicholas *et al.*, 2007). Cultural practices, such as girdling, which put grapevines under stress, can further intensify the deleterious effects of nematode feeding (Raski, 1955). If soil and cultural conditions are favorable, infested grapevines are able to better tolerate the presence of nematodes (Anwar *et al.*, 2003).

All stages, including adult females, move through the soil to find and feed on roots (Nicholas *et al.*, 2007). *Xiphinema index* prefers to feed near the root tips (Weischer & Wyss, 1976) by inserting their mouth parts (stylets) into the root tissue (Figure 3) (De Klerk & Loubser, 1988). This nematode perforates 5 to 7 cells deep with a twisting action of the odontostyle, followed by rhythmical contractions of the oesophageal bulb and feeding actions (Weischer & Wyss, 1976; Taylor & Brown, 1997).

The time that *X. index* stay at one feeding site can vary from several minutes to several days. Root areas already fed on, attract more nematodes and can result in crowding (Weischer & Wyss, 1976).

Non-grape hosts

Xiphinema index has been reported to attack figs, roses and citrus (Nicholas *et al.*, 2007). In Italy, *X. index* was also found on the roots of mulberry trees (Siddiqi, 1974). *Xiphinema* species in general are associated with root damage on ornamental shrubs, corn, lawn grasses, oats, roses, pines, peanuts (Garrett *et al.*, 1966), as well as pistachio (Weiner & Raski, 1966). Coiro & Serino (1991) reported that *X. index* reproduction could occur on petunia and tomato, which render them hosts. A lesser extent of reproduction was found on *Chenopodium amaranticolor* and tobacco plants, showing that some herbaceous plants may be suitable as bait plants, but differences in host status are likely between different *X. index* populations (Coiro & Serino, 1991). Brown & Coiro (1985) reported that *F. carica* can be a more suitable host for *X. index* than *V. vinifera* under controlled greenhouse conditions. They found that *Olea europaea*, *Citrus aurantium* and four tomato cultivars were poor hosts (Brown & Coiro, 1985).

In vitro culture

No quick method exists to screen grapevines for *X. index* resistance. *In vitro* dual culture on grape roots might overcome this problem. For *in vitro* culture to be successful,

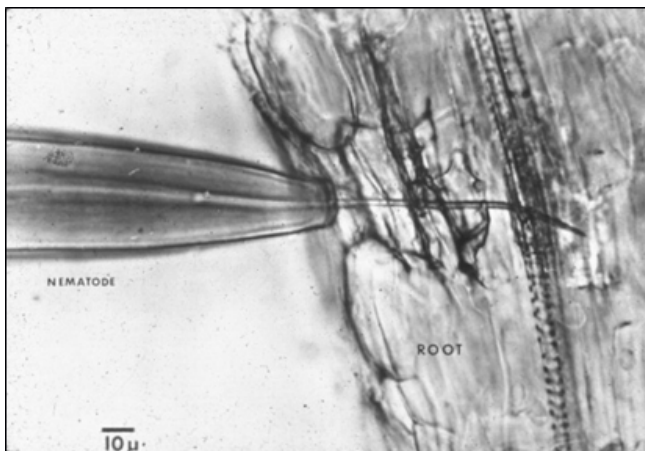


FIGURE 3

Nematode feeding on grapevine roots (Wylie *et al.*, 2004).

nematodes have to be surface sterilised. In 1978, Wyss successfully surface sterilised *X. index* using a 0.03% NaN_3 solution. The nematodes were transferred to a 0.6% agar media where they were left to feed on fig roots. In 1983, Bleve-Zacheo & Zacheo did a similar study, but they used a 2% agar media. In both these studies, *X. index* were alive and feeding on fig roots within a few days. They observed reproduction and growth of juveniles *in vitro*. However, a study done by Bavaresco & Walker (1994) on different sterilisation methods showed that no nematodes survived the NaN_3 treatment. The only surface sterilisation treatment *X. index* survived was a Sigma A-7292 antibiotic antimycotic compound. After this treatment root tip swelling and egg production were observed after 50 days and first stage larvae were observed after 60 days (Bavaresco & Walker, 1994).

Extraction methods

Nematodes can be extracted from plants and soil in several ways. Soil samples are usually taken near the vine up to a depth of 600 mm (Quader *et al.*, 2003) and the method of extraction is usually dependent on the nematode species (Brown & Boag, 1988) and soil type (Viglierchio & Schmitt, 1983). Brown and Boag (1988) showed that care should be taken when handling soil samples containing virus vector nematodes. It was shown that *X. index* were more susceptible to rough handling than some *Longidorus* species, and that dropping soil samples can kill nematodes (Brown & Boag, 1988). Four different methods for nematode extraction are summarised in Table 1.

Xiphinema index as a vector of grapevine fanleaf virus

Grapevine fanleaf virus is a member of the nepovirus (nematode vectored polyhedral particle shape) group (Pearson & Goheen, 1988). This group contains 37 viral species that have isometric particles of about 28 nm in diameter. One-third of the viruses in this group are known to be transmitted by nematodes (Taylor & Brown, 1997).

Genetics

Nepoviruses are positive-sense single-stranded RNA viruses, and have two genomic RNA's. The larger one is referred to as RNA1 and the smaller as RNA2. The large RNA1 molecule carries the genetic determinants for host-range, seed transmissibility and some types of symptom expression, while the small RNA2 molecule contains genes for the coat protein, nematode transmissibility and some symptom expression (Taylor & Brown, 1997). Full-length cDNA

TABLE 1

Nematode extraction methods (Viglierchio & Schmitt, 1983; Evans *et al.*, 1993; Shurtleff & Averre III, 2000).

Method	Advantage	Disadvantage
Cobb's sieving and gravity method	- Rapid method - Larger soil samples used	- Samples not always clean - Egg and juveniles not retained
Baermann funnel method	- Active adult and juveniles extracted - Used in combination with first method - Limits soil and root debris	- Time consuming: - hours to days - depending on sample size temperature and species - Anaerobic conditions in funnels
Mist extraction method	- No anaerobic conditions	- Most time consuming method
Centrifugal flotation method	- Active and sedentary nematodes recovered - Good for large samples	- High mortality rate for <i>X. index</i>

clones of GFLV RNA1 and RNA2 have been constructed for the synthesis of infectious transcripts (Viry *et al.*, 1993).

The determinants responsible for the specific spread of GFLV by *X. index* are located within the 513 C-terminal residues of the polyprotein encoded by RNA2. Findings suggest that the coat protein provides the basic determinants for the specificity of GFLV transmission by *X. index* (Belin *et al.*, 2001). In 2004 it was confirmed that the viral coat protein was the key determinant for GFLV transmission of GFLV (Andret-Link *et al.*, 2004a). Genetic variability exists within the RNA2 molecule of GFLV (Pompe-Novak *et al.*, 2007). Multiple interspecies recombination events were identified within the RNA2 molecule of strains from GFLV and the arabis mosaic virus (Vigne *et al.*, 2008).

GFLV can be inoculated by grafting so that the impact of virus resistance can be studied without the impact of nematode feeding or transmission. Approach grafting techniques were used to study GFLV resistance in *V. vinifera* (Walker & Meredith, 1989). Bouquet (1981) also used graft transmission to examine resistance in *Vitis* species. Valat *et al.* (2000) developed a biolistic method to inoculate *Vitis* species with GFLV to enable the examination of GFLV genetics and resistance on a molecular level. However, consistent detection of the virus in grapevine tissue after bombardment was not successful. The transmission and infectivity of GFLV might also vary among virus strains (Valat *et al.*, 2003). Fattouch *et al.* (2005) detected and characterised two different strains of GFLV in Tunisia. Different grapevine samples were subjected to ELISA (enzyme-linked immunosorbent assay) techniques and then amplified by using RT-PCR (Reverse Transcription Polymerase Chain Reaction). The PCR products were used for RFLP (Restriction Fragment Length Polymorphism) analysis and data showed a clear distinction between two GFLV strains. This study was the first report to show molecular variability of GFLV (Fattouch *et al.*, 2005).

Symptoms

Grapevine fanleaf virus is one of the oldest viruses of *V. vinifera* (Pearson & Goheen, 1988) and is still one of the most economically impacting pathogens (Vigne *et al.*, 2005). Records of this disease date back 200 years and it is believed that GFLV may have existed in the Mediterranean Basin and the Near East since the earliest cultivation of grapes (Pearson & Goheen, 1988).

Vines infected with GFLV are generally seen in patches within a vineyard (Andret-Link *et al.*, 2004b; Nicholas *et al.*, 2007) and are normally smaller than healthy vines (Golino *et al.*, 1992). In 1954 Hewitt documented the symptoms and the use of indicator plants for GFLV. The impact of GFLV varies with the tolerance of the cultivar and more tolerant cultivars can still produce good crops (Pearson & Goheen, 1988).

The disease is characterised by four distinct symptoms:

- Infected leaves exhibit widely open petiolar sinuses and abnormally gathered primary veins causing a fan-like shape (Figure 4A). This leaf deformity gave origin to the name of the virus (Pearson & Goheen, 1988). Leaf and shoot deformities develop early in the season but fade later (Hewitt, 1954). Vine shoots can also be malformed, showing abnormal branching, double nodes, short internodes and zigzag growth (Raski *et al.*, 1983).
- Yellow mosaic develops on leaves of affected vines in early spring. Specks vary from a few scattered spots to total yellowing. In summer, the vegetation resumes its normal colour (Pearson & Goheen, 1988).
- Bunches are fewer and smaller than usual with shot berries and irregular ripening (Figure 4B) (Pearson & Goheen, 1988). The GFLV can cause up to 80% reduction in fruit set. Symptoms can be confused with herbicide damage and mite injury (Nicholas *et al.*, 2007).
- Affected vines show yellow vein banding along the main veins of mature leaves. These symptoms are seen in mid to late summer (Figure 4C). Discoloured leaves show little malformation (Pearson & Goheen, 1988). This symptom has been shown to be the result of cross infection with yellow speckle viroid (Szychowski *et al.*, 1995).

Diagnosis and detection

Grapevine fanleaf virus is one of a number of viruses for which woody indexing is used to verify virus-free status. The rootstock variety St. George is the standard indicator for the presence of GFLV, but symptoms are common on most *V. vinifera* varieties. Woody indexing involves grafting

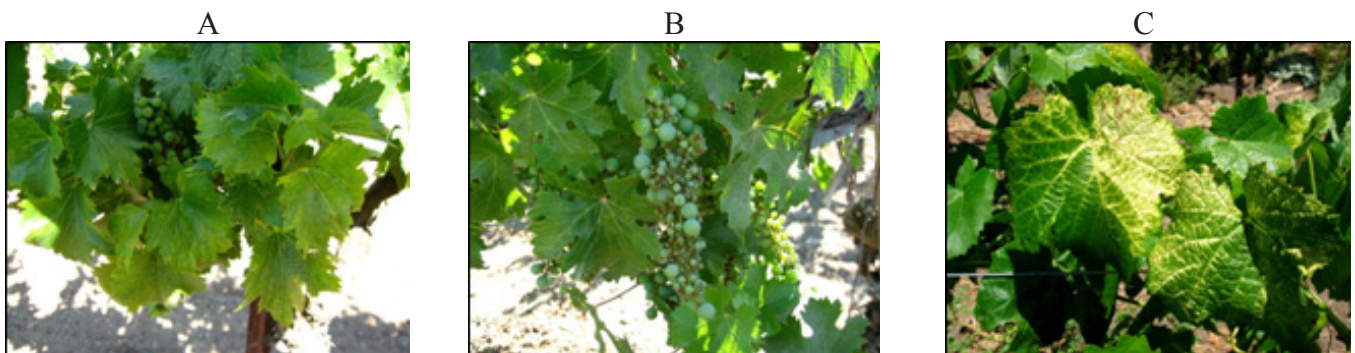


FIGURE 4

- A. Grapevine leaves showing the fan-like symptoms of the GFLV.
 B. Vines infected with GFLV show smaller, fewer bunches per vine with a high number of shot berries.
 C. Late-summer yellow vein-banding symptoms of vines infected with GFLV.

a candidate plant bud onto the highly reactive indicator variety. This index requires at least 18 months for reliable assays with grapevine viruses (Alley, 1955).

To accelerate the time required for detection of GFLV infection, serological techniques such as ELISA were developed (Rowhani, 1992). However, immunoassays are much less sensitive than techniques based on nucleic acid hybridisations (Fuchs *et al.*, 1991) and PCR. Both RT-PCR (Fattouch *et al.*, 2001) and immunocapture (IC)-RT-PCR (Acheche *et al.*, 1999) have been shown to be successful as very sensitive GFLV detection methods.

In 2001, Fattouch *et al.* developed a RNA oligoprobe capture technique to detect GFLV in grapevine tissue. This procedure was compared to an IC technique using commercial antibodies. Grapevine fanleaf virus isolates from vineyards in northern Tunisia showed negative results with IC-RT-PCR, but were detected by the RNA oligoprobe capture technique (Fattouch *et al.*, 2001). Detection of GFLV in the vector nematode firstly relied on ELISA (Bouquet, 1981; Esmenjaud *et al.*, 1992; 1993) and then on RT-PCR, a method that allowed GFLV detection from a single viruliferous nematode as soon as 1994 (Esmenjaud *et al.*, 1994). The RT-PCR was then refined (Demangeat *et al.*, 2004) using a bead mill to disrupt the nematode and amplifying 555-bp fragment of the coat protein gene with two primers designed from conserved sequences. *Styl* RFLP analysis on the coat protein amplicon is used in addition to RT-PCR to enable the GFLV isolate carried by a single nematode to be characterised (Demangeat *et al.*, 2004).

Significant progress has been made on the elucidation of the functions of most GFLV proteins, specifically those involved in the virus multiplication cycle, RNA replication, cell-to-cell movement and transmission by *X. index*. New insights into the genomic variability among isolates from naturally infected vineyards have also been made (Andret-Link *et al.*, 2004b).

Acquisition and transmission

In 1958, Hewitt *et al.* showed that *X. index* is the natural vector of the GFLV and that GFLV is soil-borne and not air-borne. This study was also the first to prove that nematodes are able to vector soil-borne viruses and that spread is typically slow and in a concentric pattern (Hewitt *et al.*, 1958).

Laboratory methods for assessing the transmission of nepoviruses were established by Trudgill *et al.* (1983). Nematode vectors that feed on plant roots can transmit viruses in all development stages, but GFLV is lost with each molt and needs to be reacquired (Taylor & Raski, 1964). However, GFLV is not passed through nematode eggs (Taylor & Raski, 1964; McFarlane *et al.*, 2002). *Xiphinema index* has the ability to ingest GFLV particles from an infected grapevine, retain the virions at specific retention sites within its feeding apparatus and subsequently infect a recipient vine when feeding (Andret-Link *et al.*, 2004b). The virus also occurs in grapevine pollen (Cory & Hewitt, 1968), but not in seeds (Shurtleff & Averre III, 2000).

The virus is acquired by *X. index*, feeding first on the roots of an infected vine and then transferring the virus by feeding on healthy vines (Leavitt, 2000). A single brief feeding on an infected vine root can make nematodes viruliferous.

The nematode can retain the virus for up to eight months in the absence of host plants or up to three months when feeding on resistant host plants (Taylor & Raski, 1964). When nematodes were stored in soil (and thus starved) under controlled conditions, the virus was detected for up to four years in nematode samples of 20 adult individuals (Demangeat *et al.*, 2005). The minimum GFLV acquisition threshold for transmission from *X. index* to the grapevine was established by Alfaro & Goheen (1974), and proved to be five minutes. The virus has no measurable effect on the rate of reproduction of its vector, but improved its survival rate during starvation (Das & Raski, 1969).

In laboratory and greenhouse studies, temperature, soil moisture, the host plant, the population and developmental stages of the nematode and even the size of the pot affected the rate of virus transmission. In general, increasing the acquisition and transmission access periods from hours to several weeks increased the frequency of transmission (Shurtleff & Averre III, 2000). The virus is acquired and transmitted with an access time of 5 to 15 minutes in a soil temperature of 13 to 24°C (Siddiqi, 1974). Even when *X. index* does not carry the virus, roots are still damaged (McKenry, 1992). The nematodes retain the ability to transmit the virus for 4 to 8 weeks when feeding on non-viruliferous plants (Taylor & Raski, 1964) and for up to nine months under starvation conditions (Raski & Hewitt, 1960). Successful virus transmission requires that infective virus particles be inoculated into plant cells that are healthy and undamaged (O'Bannon & Inserra, 1990).

Vector method, spread and specificity

According to Pearson & Goheen (1988), GFLV's natural host range is limited to *Vitis* species. Recent studies showed that Bermuda grass in Iran is infected with GFLV. The virus was detected by RT-PCR using two different pairs of GFLV specific primers and ELISA. However, the Bermuda grass expressed few or no symptoms of GFLV infection (Izadpanah *et al.*, 2003). In addition to *X. index*, *X. italiae* has been reported to spread GFLV (Cohn *et al.*, 1970), but these results were not corroborated (Esmenjaud & Bouquet, 2009). Long-range spread of the GFLV is limited to the spread of infected plant material. Short-range spread depends on nematodes (Pearson & Goheen, 1988).

The transmission process is characterised by a high degree of specificity between GFLV and *X. index*. Viruses are attached to the cuticular lining and the lumen of the odontophore and the pharynx. They are shed with the cuticle when the nematode molts (Taylor & Robertson, 1970). During feeding, virus particles dissociate from the cuticular lining at the retention site and are carried by the saliva of the nematode to the grapevine plant cells. Dissociation of the virus particles occurs when saliva passes through the lumen of the oesophagus and absorbs the virus at the retention site. Virus particles are released into the grapevine cells during the initial feeding phases (O'Bannon & Inserra, 1990). Limited information is available on the mechanisms of the transmission process of GFLV (Belin *et al.*, 2001).

Management strategies for *Xiphinema index* and grapevine fanleaf virus

Each disease and pest requires a different control strategy. For example, foliar diseases of grapes need specific weather patterns. Some diseases and pests spread quickly, others slowly and viruses live within the vine. Nematodes are primarily spread by the movement of contaminated soil or infested plant material (Nicolas *et al.*, 2007). Preventative measures for controlling *X. index* and GFLV are usually the best (Hewitt, 1954), but not always practical given high grower demand for specific clones of varieties that might also be infected with fanleaf. It is advisable to plant only certified planting stock (Golino, 1993), but studies have shown that even healthy grapevines can become infected with GFLV transmitted by *X. index* from the soil within three years after planting (Hewitt *et al.*, 1962).

Grapevine rootstocks

The use of resistant rootstocks upon which fruiting cultivars are grafted is often the best way to overcome nematode problems in perennial crops. Rootstocks for use against the *X. index* / GFLV disease complex must resist both the nematode and virus. However, resistance to both does not exist within commonly used commercial rootstocks (Meredith *et al.*, 1982; Harris, 1983). The 110R rootstock, which is often used in South Africa for its phylloxera resistance and good vigor, is susceptible to *X. index* feeding. However, Harmony, Freedom, 3309C and Schwarzmann had some degree of resistance (Harris, 1983; Malan & Meyer, 1993). More rootstock examples are named and described in Table 2 in terms of their resistance or susceptibility towards *X. index* feeding.

Hot water treatment, heat therapy and somatic embryogenesis

A common means of spreading *X. index* is by the distribution of infested dormant rootings or bench grafts from nurseries or from vineyards where rootstocks are planted between rows in infested areas and then later moved to other areas. A hot water (52°C) agitated soak for five minutes is recommended for treatment of infested materials (Nicholas *et al.*, 2007). However, to avoid damaging roots or buds,

accurate temperature control is essential and low numbers of nematodes may survive (Raski *et al.*, 1965b).

Grape viruses are widely spread and controlling the distribution of infected plant materials was the genesis of clean stock/certification programs in the world's grape growing regions. Infected plants can be freed of viruses by heat therapy and/or meristem culture (Torres-Viñals *et al.*, 2004). Meristem culture is effective in eliminating phloem-limited viruses, while heat therapy is normally required for viruses that readily invade plant meristems such as nepoviruses (Gambino *et al.*, 2009). Buds from a candidate vine of unknown virus status can be grafted onto a nurse plant and heat-treated in a growth chamber at 37°C for two to three months. After this treatment, the buds are forced to grow and the resulting shoots are checked for the presence of virus by indexing or PCR-based testing. Heat therapy works because RNA based viruses degrade at high temperature and are eliminated before plant cells can be damaged. The process is not highly efficient, but was widely used in the past (Gifford & Hewitt, 1961). Alternatively, a small segment, less than one mm, of the shoot tip can be excised and grown in sterile culture. In many cases this small piece of tissue has escaped virus infection and can be grown into a new plant (Barlass & Skene 1978) which can be verified free of virus by indexing and PCR testing. In some cases these two techniques can be combined but in most cases meristem culture is effective (Gambino *et al.*, 2009).

Somatic embryogenesis has also been used to efficiently eliminate several phloem-limited viruses from grapevine material (Goussard *et al.*, 1991). By using this technique, GFLV was eliminated from grapevine tissue in combination with heat therapy of the explants (Goussard & Wiid, 1992). In a study done by Gambino *et al.* (2009), it was possible to eliminate GFLV from plantlets by using somatic embryogenesis without using heat therapy with a success rate close to 100%. The virus was however detected in all tested anthers and ovaries by using RT-PCR techniques, but not in the regenerated plantlets two years after transfer to greenhouse conditions (Gambino *et al.*, 2009).

Crop rotation and fallow periods

Before vineyards are replanted with grapevines, the land

TABLE 2

Description of rootstock characteristics in terms of *X. index* resistance with S = susceptible, R = resistant and MR = moderately resistant.

Rootstock	Genetic origin	Resistance	Reference
110R	<i>V. berlandieri</i> x <i>V. rupestris</i>	S	Malan & Meyer (1993)
Harmony	(<i>V. longii</i> x Othello) x Dog Ridge	R	Harris (1983)
Freedom	(<i>V. longii</i> x Othello) x Dog Ridge	R	Harris (1983)
3309C	<i>V. rupestris</i> x <i>V. riparia</i>	S	McKenry <i>et al.</i> (2004)
Schwarzmann	<i>V. riparia</i>	MR	Harris (1983)
O39-16	<i>V. vinifera</i> x <i>M. rotundifolia</i>	R	McKenry <i>et al.</i> (2004)
Ramsey	<i>V. champinii</i>	S	Ambrosi <i>et al.</i> (1966)
Dog Ridge	<i>V. rupestris</i> x <i>V. candicans</i>	S	Ambrosi <i>et al.</i> (1966)
Fairy	Not known	MR	Ambrosi <i>et al.</i> (1966)
Jacquez	<i>V. aestivalis</i> x <i>V. cinerea</i> x <i>V. vinifera</i>	S	Ambrosi <i>et al.</i> (1966)
775 Paulsen	<i>V. berlandieri</i> x <i>V. rupestris</i>	S	Ambrosi <i>et al.</i> (1966)

can be cropped with cereals or grains to suppress grapevine-attacking nematodes. Some crops can increase nematode populations, as is the case with growing pumpkins or tomatoes before replanting grapevines (Nicholas *et al.*, 2007). An early study done by Raski (1955) suggested that three years is an adequate period for crop rotation. However, more recent studies suggest that *X. index* infested sites should be left fallow or rotated to crops other than grapes or figs for at least 10 years (McKenry, 2000). In moist sterile soil without food, *X. index* died after 9 to 10 months, but survived for 4 to 5 years in soil where grapevines were removed, but roots remained (Raski *et al.*, 1965a). Since vine roots decay very slowly and act as a reservoir for *X. index*, it is beneficial (but not necessarily economically viable) to wait at least six to ten years before replanting (Golino *et al.*, 1992). It must also be kept in mind that GFLV can still be detected in nematodes kept in soil without roots for four years (Demangeat *et al.*, 2005).

Nematicides

Soil may be fumigated before planting, although such treatments rarely penetrate to depths greater than one meter and thus do not eradicate nematodes on deep perennial root systems (Lear *et al.*, 1981). This is especially true for California where the soils are often deep and fine-structured (Raski *et al.*, 1983). Broad-spectrum fumigants are expensive, but they also kill soil insects, fungi and weeds as well as beneficial organisms. Before nematicides and fumigants can be applied, soil must be ripped and cleared of as many old roots as possible and dried to as great a depth as possible (Nicholas *et al.*, 2007).

Non-fumigant nematicides can be applied to established vineyards by using soil drenches or applied through the drip irrigation system. These nematicides must be applied with care, as they are toxic to humans and may leave residues in or on fruit (Nicholas *et al.*, 2007). Due to the high toxicity levels of nematicides and because they are unsafe for the environment and human health, their use is being highly restricted in the vineyards of the world (Bouquet *et al.*, 2000).

Breeding Xiphinema index and grapevine fanleaf virus resistant vines

Breeding fanleaf degeneration resistant grape rootstocks would be a logical step in the process of controlling this disease, however as with all perennial crops, the process can be slow and difficult (Esmenjaud & Bouquet, 2009). Resistance to GFLV has been identified in *Muscadinia rotundifolia* (Boubals & Pistre 1978; Walker & Jin, 2000) and in some Middle Eastern *V. vinifera* cultivars (Walker *et al.*, 1985), although the latter sources have not been studied further.

Resistance to *X. index* has been found in a number of *Vitis* species, notably *V. arizonica*, *V. candicans*, *V. rufotomentosa*, *V. solonis* (Kunde *et al.*, 1968) and *M. rotundifolia* (Bouquet *et al.*, 2000). During a breeding program at the University of California, Davis found that two *V. vinifera* x *M. rotundifolia* (VR) hybrids, O39-16 and O43-43, were highly resistant to *X. index* and prevented fanleaf degeneration. These two rootstocks were patented and released (Walker *et al.*, 1991),

although the recommendation for O43-43 was subsequently withdrawn due to insufficient phylloxera resistance (Walker *et al.*, 1994). Once these rootstocks were used in field situations it became clear that although they had strong resistance to *X. index* feeding, they did not prevent the vectoring of GFLV as *X. index* probed for feeding sites. However, although scions grafted on these rootstocks became infected with GFLV, disease was not expressed (Walker *et al.*, 1994; Walker & Wolpert, 1994). Unfortunately these hybrids cannot be used as parents in future crosses due to sterility from the incomplete pairing of chromosomes resulting from a *Vitis* (2n=38) x *Muscadinia* (2n=40) cross (Walker *et al.*, 1994). In France a nematode-resistant accession, obtained from a *Vitis* x *Muscadinia* F1 hybrid (VRH8771) backcrossed to a *Vitis* rootstock, was patented in 2011 and is being released as the new rootstock 'Nemadex AB'. In the vineyard, it highly delays GFLV infection (Esmenjaud & Bouquet, 2009), but the durability of its resistance to *X. index* over years will be challenged under controlled conditions (Esmenjaud *et al.*, 2010).

The need for a broader range of rootstocks with strong resistance to fanleaf degeneration continues and efforts to discover strong sources of *X. index* resistance have built on the work of Kunde *et al.* (1968) mentioned above. Coiro *et al.* (1985) found that *V. riparia* and hybrids containing *V. riparia* also had degrees of resistance. *Muscadinia* species have also been studied to determine their *X. index* resistance and consequently their resistance to the vectoring of GFLV. *Xiphinema index* was found to attack *Muscadinia* roots very reluctantly and the few feeding sites that developed rapidly became necrotic indicating a hypersensitivity. Apparently this reaction prevented viruses from being transmitted, suggesting that these species are resistant to *X. index* feeding and the transmission of GFLV (Staudt & Weischer, 1992). The basis for GFLV resistance in grapevines is not yet fully understood and need further investigation.

Inheritance and mapping of DNA markers for resistance to Xiphinema index

The highly heterozygous nature of grapevine limits the study of its genetics, but has been an advantage for breeding, which has led to a long history of breeding and domestication compared to other perennial crops (Myles *et al.*, 2010). The publication of the first grapevine genome sequences (Jaillon, *et al.*, 2007; Velasco *et al.*, 2007) provided a new generation of molecular tools for grapevine breeding efforts. Doors were opened to identify genes responsible for agronomic traits and disease resistance as well as the assignment of biological functions to annotated sequences (Martínez-Zapater *et al.*, 2009). Technologies such as QTL- and linkage disequilibrium-based mapping are implemented to better understand the genetic structure of grapevines. Only a small portion of the genetic diversity of grapes has been explored. The grape genome sequence, in addition to rapidly developing technologies, will provide easier ways to improve existing grape cultivars while incorporating specific traits and disease resistance (Martínez-Zapater *et al.*, 2009; Myles *et al.*, 2010).

Since the 1970s at the University of California, Davis has been developing rootstocks to resist fanleaf degeneration. As

part of this effort, *V. rupestris* x *M. rotundifolia* hybrids were produced and 60 of 200 seedlings tested highly resistant to *X. index* feeding, several of which, including R8913-02 and R8913-21, were also resistant to the root-knot nematode and phylloxera. Genetic mapping efforts found that the RAPD (Random Amplified Polymorphic DNA) marker OPA-12 (Operon) was tightly linked to *X. index* resistance (Walker & Jin, 1998). These *V. rupestris* x *M. rotundifolia* seedling populations were later found to be largely mistaken outcrosses of *V. rupestris* by forms of *V. arizonica* (Riaz *et al.*, 2007). Two half siblings, R8909-15 x R8909-17 were used to create a mapping population 9621, in which resistance to *X. index* segregated as a single dominant resistance gene. Initial mapping efforts used AFLP (Amplified Fragment Length Polymorphism) technology to identify over 500 segregating markers on 19 linkage groups (Walker & Jin, 2000). This map was later used to position resistance to the bacterial causal agent of Pierce's disease, *Xylella fastidiosa* (Douceff *et al.*, 2004).

Previous work found that *V. arizonica* was resistant to *X. index* (Kunde *et al.*, 1968) and suggested that resistance was inherited as a single heterozygous gene (Meredith *et al.*, 1982). More recently, the 9621 population has been mapped with highly informative and co-dominant simple sequence repeat (SSR) markers further positioning resistance to *X. fastidiosa* and placing a major quantitative trait locus (QTL) for *X. index* resistance (*XiR1*) on chromosome 19 (Xu *et al.*, 2008).

These studies as well as the agronomical importance of nematodes and the viruses they vector, prompt interest in determining the extent of *X. index* resistance in *V. arizonica* and whether other accessions had the same degree and genetics of resistance to this nematode pest.

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