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Strategies Against Grapevine Fanleaf Virus and Its Nematode Vector

Soil fumigation for nematode control was first developed to protect high-value annual and biennial crops, such as beans, carrots, cotton, and pineapple. Fumigants were placed at a depth of 15–30 cm by chisels set 30 cm apart. The treatments worked well for short-lived crops, but the shallow placement did not give adequate control for disease of long-term perennials, such as grapes.

A new strategy, in which fumigants were placed at a depth of 75–90 cm at 90-cm intervals, was developed against the degeneration of grapes caused by grapevine fanleaf virus (GFV) and *Xiphinema index*. This disease complex is devastating to new vines planted in soils from which affected vines have been removed. Deep placement and high dosage rates have given economically successful control of this complex (8) and have also been used extensively against root-knot nematode and other associated nematode species.

Grapevine Fanleaf Virus and *Xiphinema index* Complex

Infectious degeneration. The disease caused by GFV is of paramount importance as a threat to the production of grapes, and infectious degeneration was the first generally recognized name applied to it. The causal agent is a nepovirus (*ne*matode-transmitted, *po*lyhedral-shaped) related to arabis mosaic virus. In Europe, it has been recognized as a soilborne disease for approximately 100 years, the first report being that of Rathay in 1883 (14). Various names have been applied to the disease, including court noué, arriciamento, urticado, and Reisigkrankheit. In California it is known as fanleaf, yellow mosaic, and veinbanding, names derived from symptom patterns in the leaves of affected vines.

Infectious degeneration was first reported in California in 1950 by Hewitt, who also determined it was soilborne (5). The vector was discovered to be the dagger nematode, *Xiphinema index* (6), and GFV was isolated and purified shortly after that (4). The virus along with its vector undoubtedly was introduced to this country through infected cuttings or rootings. There is no evidence to indicate the virus is native to California or the United States, but both virus and vector are found in old vineyards in the eastern Mediterranean area.

Symptoms and pathology. Infectious degeneration produces a variety of symptoms expressed in the foliage. The name *fanleaf* is derived from the sharply toothed leaf margin, mottling, closeness of primary veins (as in a partly closed fan), and open petiolar sinus (Fig. 1). Other symptom types include *yellow mosaic*, with leaves partially or completely a deep chrome-yellow (Fig. 2), and *veinbanding*, with light-green to chrome-yellow chlorotic bands along the veins (Fig. 3). Often, the only leaf symptoms are obscure speckles or small yellow spots (Fig. 3). The malformations on canes include short internodes, double nodes, fasciations, and zigzag growth between nodes (Fig. 4). Perhaps the most striking symptoms are in fruit—poor set, loose clusters, and excessive "shot berries" (small seedless berries that may not mature) (Fig. 5). The effect on fruiting lowers yields and can result in total loss of production.

The Nematode Vector

X. index was first collected and described in California in 1950 from fig trees showing leaf drop and poor growth in Madera County. First reported as a pathogen of grapevines in 1954, it was

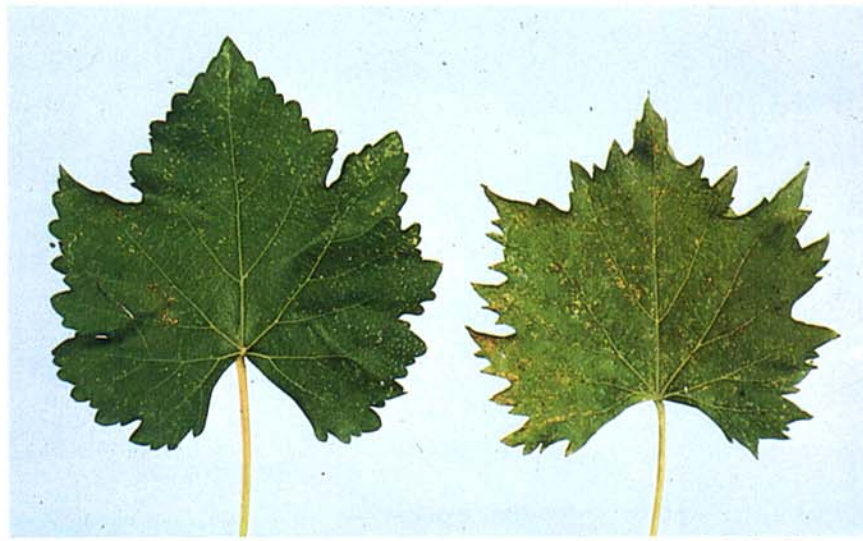

Fig. 1. Healthy leaf of grape cultivar French Columbard (left) compared with leaf infected by grapevine fanleaf virus (right).

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soon after (6) proved the vector of fanleaf. Just as with GFV, *X. index* almost certainly was introduced into California, because no evidence exists to suggest it is native there.

Life history. *X. index* has four larval stages. Males are rare, and females reproduce parthenogenetically. The first stage develops to an elongate form, then emerges from the egg and almost immediately sheds its cuticle, a molting process leading to the second stage. This happens three more times, resulting in the third and fourth stages, then the adult female. At every molt the entire cuticular covering is cast off, including the lining of the esophagus. This is important because infectivity is also lost at molting. The virus particles are located in the lumen of the esophagus and are shed along with the cuticular lining. To become infective again, the nematode must feed on roots of infected grape.

Life cycle from egg to egg is quite short, as little as 15 days, so the reproductive rate is high. Although susceptible to drying or excessive heat, the nematodes are protected in the soil and survive for months as infective vectors in the absence of host roots. They are obligate plant parasites, however, and have few or no suitable hosts other than grape in vineyard plantings.

Symptoms and pathology. *X. index* feeds entirely externally on the tips of grape roots, causing curvature or bending with swelling (slightly reminiscent of phylloxera damage) and often accompanied by necrosis appearing as irregular dark-brown to black patches (Fig. 6). Root tips may be totally blighted and produce no further growth. Excess production of lateral feeders, which in turn are killed, may result in a matted effect. General dearth of feeder roots leads ultimately to poor vine vigor and productivity.

Epidemiology. Infectious degeneration may be spread through the use of infected planting stock, whether bench-grafted rootstocks, field-budded rootstocks, or own-rooted cuttings. If such plantings are free of the nematode vector, no further spread will occur and disease can be controlled simply by replanting with healthy plant materials.

Contaminated soil on rooted plants is by far the most efficient means of spreading the nematode vector. Nematode infection alone is serious because *X. index* is a dangerous pathogen capable of reducing vines to weakened, unproductive plants. Infection with both GFV and the vector is particularly distressing because of the inexorable, slow spread from infection foci in every direction. Absolute control is at present unattainable because total eradication of either the vector or the virus-infected root fragments is not possible. Reinfestations by both pathogens occur regardless of measures taken. Simple rotation is not effective either,

because infective nematodes and roots are known to survive in the soil for 5 years or longer after removal of infected vines.

Control Measures

Side-dressing treatment. Vineyards infested with *X. index* alone responded remarkably in the past to treatments with 1,2-dibromo-3-chloropropane (DBCP) (13), an especially effective chemical against ectoparasitic nematodes such as *Xiphinema* species. DBCP was also widely used in vineyards against root-knot nematode and other species. DBCP was withdrawn in 1976 and no alternative was available until 1981, when two chemicals became available on a limited basis (12). Fenamiphos (Nemacur) and carbofuran (Furadan) are nonfumigant, systemic-type treatments that show promise as side-dressing treatments of dagger and other nematodes in vineyards. Both are sold and applied in California under Emergency Exemption Permits issued under Environmental Protection Agency Section 18.

Vineyards with infectious degeneration, with or without the nematode vector, will not respond to chemical treatments. The only control measure to consider is replanting.

Replanting. If GFV is present alone, replanting with healthy stocks is a totally effective means of permanently eradicating the disease. Presence of both vector and GFV requires replanting after preplant soil fumigation. High-dosage, deep-placement fumigation is recommended after at least 1 year of fallow rotation, preferably more. Soil preparation also must include deep-ripping in at least two directions perpendicular to each other. This is normal soil preparation for all new plantings but is even more essential for fumigation because the equipment cannot deliver precision applications in unbroken soil. Removal of old root systems as completely as possible also is important, especially where root-knot nematode is the principal problem.

Two chemicals are currently in use for preplant soil treatments: methyl bromide (MBr) and 1,3-dichloropropene (1,3-D). Deep-placement testing started about 1968–1969 and was a significant departure from the conventional fumigation practices in general use. Deep-placement necessitated wider spacing and higher dosages to achieve control through a greater total soil profile in order to protect perennial plants longer.

The 1,3-D dosage is 2,336 L/ha for D-D and 1,400 L/ha for Telone II; both should be applied 75–90 cm deep with 90-cm spacing (Fig. 7A). Roller-packing with a ring roller should follow as soon as possible, and planting should be delayed 5–6 months after treatment.

MBr is a highly volatile fumigant for which a continuous cover of 1-mil polyethylene sheeting is required (Fig. 7B). The dosage is 448 kg/ha applied by chisels 50–75 cm deep with 1.65-m spacing. For control of root-knot nematodes, 560 kg/ha without a cover is now possible, and experimental tests are under way to determine the feasibility and efficacy of the same treatment for *X. index*/GFV control. Fumigation without a cover should be followed immediately with roller-packing with a ring roller to seal the chisel marks.

It has been established that 2.5–2.7 ppm of 1,3-D for 3 days (1) or 500–650 μl of MBr per liter air for 3 days (2) is required for 100% kill of *X. index* and *Meloidogyne incognita* (root-knot). The distribution of MBr from the point of field injection was followed by gas chromatographic analyses. The material first surged upward through the broken soil to the surface, then stabilized; by the third day after treatment, the gas followed gravity to depths of 2.5 m or more, even into the unbroken lower soil layers. Dosages well in excess of the requirements were found at all levels for MBr, but dosages of 1,3-D were less than 2.5–2.7 ppm at levels below 61 cm. Nevertheless, the excellent control attained by 1,3-D suggests that longer exposures (up to 21 days) at lesser concentrations are sufficient to kill the nematodes.

Cost of treatment varies according to

acreage treated, ie, the greater the acreage, the lower the cost. Estimates for treating 40 acres, including tax and cost of application, are: 1) D-D at 2,336 L/ha or Telone II at 1,400 L/ha = $3,519/ha ($1,425/acre); 2) MBr at 448 kg/ha with plastic cover = $2,150/ha ($870/acre), plus $50–62/ha ($20–25/acre) for removing the cover; and 3) MBr at 560 kg/ha without plastic cover = $1,395/ha ($565/acre).

When conditions are optimum, control assessment by normal soil sampling procedures shows no recurrence of nematodes for 4–5 years. Then, isolated foci of nematodes and scattered symptoms of infectious degeneration begin to appear. Surveys of vineyards in the Napa Valley–Gilroy areas replanted in *X. index*/GFV-infested soils after commercial treatments with 1,3-D or MBr have shown a gradual buildup of nematodes and affected vines (8). Because only 3–5% of the vines show disease symptoms after 10 years' growth, however, the treatment is considered economically successful. A replacement or alternative to DBCP is urgently needed to deter buildup of nematodes after preplant fumigation and to mitigate damage resulting from nematode feeding.

Fig. 2. Yellow mosaic symptoms of infectious degeneration on leaves of grape cultivar Thompson Seedless.

Neither 1,3-D nor MBr will disperse through highly organic soils or through clay layers in soil. Moisture also limits penetration of the fumigants when it reaches the saturation point or becomes standing water.

Another precaution concerns use of MBr in soils low in zinc or phosphorus. Fumigation has resulted in severe stunting of new plants in such soils. Experimental results (J. A. Menge, D. J. Raski, L. A. Lider et al, *unpublished*) suggest that elimination of mycorrhizal fungi may be an important factor in this deleterious effect. Growers planning soil fumigation should check target soils for mineral analyses and avoid MBr in areas of low zinc or phosphorus until exact causes are determined and means of avoiding this stunting are definitely established. Commercially available mycorrhizal fungi for inoculating nursery rootings intended for such soils may be a valuable help, but knowledge in this area needs development.

Tolerant rootstocks. Rootstocks selected from American *Vitis* species were introduced in France over 100 years ago to combat ravages of phylloxera, a root aphid. These were successful, and *V. vinifera* vineyards of France and the rest of the world were saved. Early rootstock testing was carried out empirically over long evaluation periods with little regard for soil problems other than phylloxera. The pure American rootstocks, such as Rupestris St. George, are very sensitive to GFV. Recent work shows that some rootstocks may be useful for controlling nematodes (9), specifically *X. index* (7).

The value of rootstocks for use against the *X. index*/GFV complex is beginning to receive attention. Certain rootstocks and selections appear tolerant to GFV (F. Jimenez, *unpublished*) and to feeding by the nematode vector (3). Tolerance to GFV appears to come from *V. vinifera* selections of western and central Asia where the disease probably originated. Tolerance to a disease resembling infectious degeneration was reported from *V. vinifera* grapes (Malvasia bianca, Somarello, and Pagadebito) in Italy over 40 years ago (11). Tolerance to GFV transmission by *X. index* appears to be present in muscadine grapes and actually may be tolerance to the nematode vector itself (3).

The prospect for combining germ plasm of different grape species to achieve a genetic solution to the *X. index*/GFV problem is promising. A rootstock with tolerance to *X. index* alone will not protect the vine from GFV because only a very brief feeding period is sufficient to transmit the virus. Tolerance to GFV alone is insufficient because the nematode can greatly weaken the vine even in the absence of the virus. Because most *X. index*/GFV sites are also plagued with phylloxera, the virus and nematode tolerance ideally should be supplemented with a high level of tolerance to the insect. By combining, in a carefully conceived breeding program, it should be possible to produce a superior rootstock with horizontal, multigenic resistance for use in affected areas. It is on the basis of such a premise that a breeding program is currently under way at Davis.

Cultural practices. Special care is needed for new vines growing in fumigated soils. Usually the plants grow

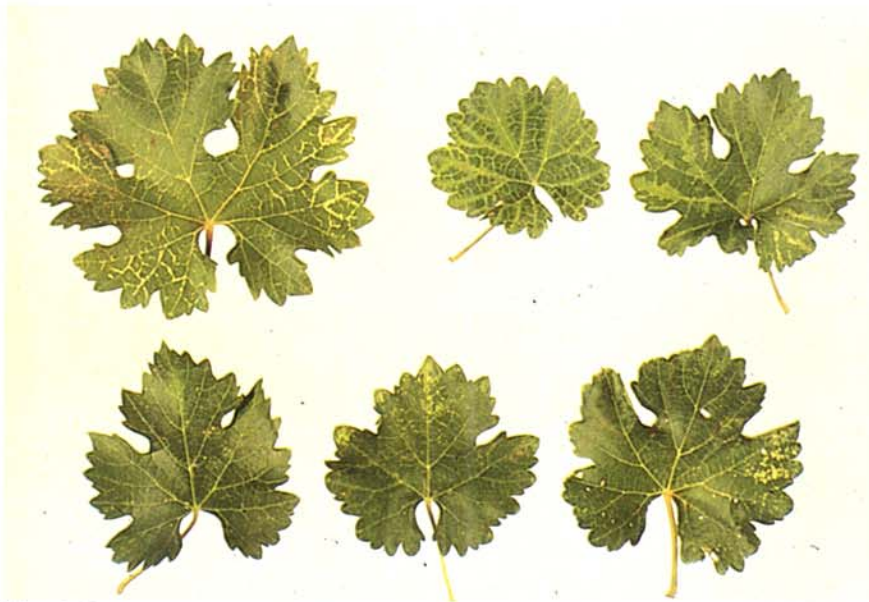

Fig. 3. Symptoms of infectious degeneration on leaves of grape cultivar Cabernet Sauvignon include veinbanding (top row) and obscure speckles or small yellow spots (bottom row).

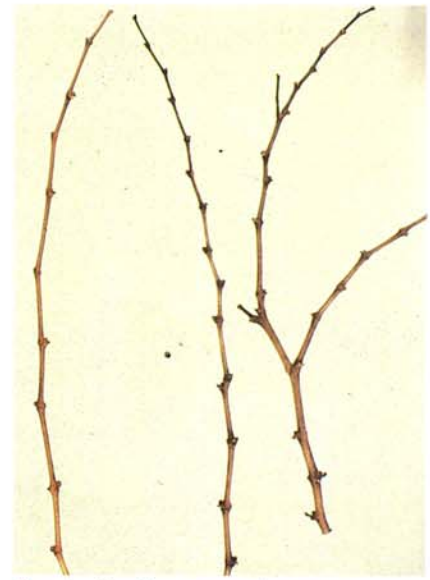

Fig. 4. Healthy cane of grape cultivar Cabernet Sauvignon (left) compared with canes with infectious degeneration symptoms (center and right).

with remarkable vigor compared to untreated checks (Fig. 8). These vigorous plants must be managed to avoid overcropping, especially when the vines are young. Excessive fruit production is a common stress factor on young vines. Adequate water is critical, especially in the Central Valley and on light, sandy soils. Timely insect and fungus disease control is important on young plants to help establish the strongest vine structure possible before reinfestation with nematodes occurs.

Summary

Grapevine fanleaf virus and its nematode vector can be expected to pose an increasing threat to the grape industry. The known distribution of infectious degeneration is increasing, with new records of infection being added every year. Replanted vines in untreated infested soils cannot grow to maturity or sustain productivity. Soil fumigation is the only effective control measure now available. Many vineyards have succeeded after being replanted in soils infested with *X. index* and fanleaf virus that have been treated with nematicidal fumigants, but this method is very costly and not without risk.

A few reports have been made of new plantings that show infectious degeneration symptoms after only 2–3 years' growth and accompanied by high populations of nematodes. Soil preparation, moisture content, and details of the actual conditions of application are not always known. But it is quite clear that the requirements for fumigation must be followed as carefully and exactly as possible to achieve maximum control.

With careful management, strong, thrifty vines can be produced during the nematode-free years with a structure and productivity that can be sustained even after the nematodes begin to build up again. The replanting process can be scheduled every 15–20 years and still be successful economically.

The development of a hybrid rootstock with horizontal, multigenic resistance to GFV, *X. index*, and phylloxera is an exciting possibility and one that is being explored in the rootstock breeding program at Davis (10). Such a rootstock would be of inestimable value for controlling infectious degeneration and its nematode vector, a condition inadequately controlled by soil fumigation at the present time.

Fig. 5. Healthy fruit of grape cultivar Cabernet Sauvignon (left) compared with fruit with infectious degeneration symptoms (right).

Fig. 6. Grape root damaged by *Xiphinema index* (left) compared with healthy root (right).

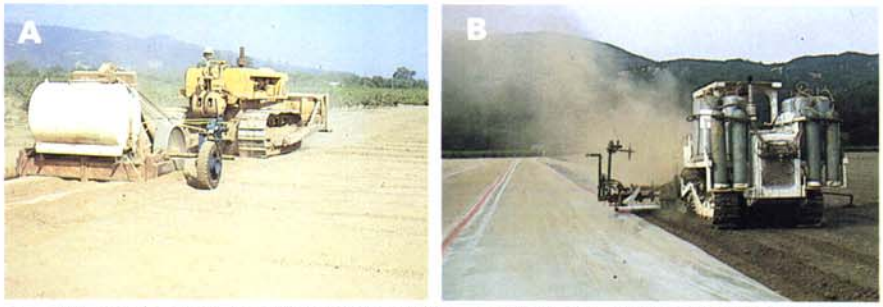

Fig. 7. Deep placement of (A) 1,3-D and (B) methyl bromide under polyethylene cover.

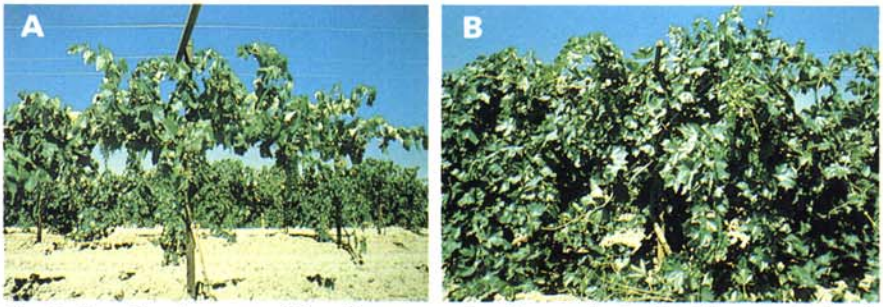

Fig. 8. Growth of grape cultivar Thompson Seedless replanted in (A) untreated soil infested with root-knot nematode and (B) nematode-infested soil treated with 1,3-D.

Acknowledgments

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