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Nematodes and Viticulture

Poor vineyard performance? Consider nematodes as a likely participant.

James Stamp

Dr. **James A. Stamp** is a Sebastopol, California, scientist specializing in critical evaluation of vineyard performance issues and grapevine nursery plant material quality and propagation. He has more than 25 years of experience in West Coast viticulture and established Stamp Associates after founding Novavine grapevine nursery, working in the plant biotech industry and completing a post-doctoral scholarship at UC Davis. Stamp Associates advises growers and winemakers in the U.S. and overseas in the establishment and management of high quality, pathogen-tested vineyards. He is also founder of winecountrycoops.com. Please contact him at *james@jamesstamp.net* or 707-828-8405.

What are Nematodes?

Nematodes are a natural component of the rhizosphere, the ecosystem comprising the space around and within a plant's root system. These often microscopic animals are one of the world's most diverse groups of organisms, occupying habitats ranging from the tropics to deep sea trenches to the polar ice caps. Nematodes are the most numerous multicellular animals on earth. A handful of soil may contain thousands of these so-called roundworms, many of them parasites of insects, plants or animals. There are nearly 20,000 described species classified in the phylum Nemata (University of Nebraska-Lincoln, 2010).

Nematodes are structurally simple organisms. Adult nematodes are typically comprised of up to a few thousand cells, with a few hundred cells associated with the reproductive system. Nematodes have been characterized as a tube within a tube, in reference to the alimentary canal, which extends the length of the organism, creating a fluid-filled cavity between the outer body wall and the digestive tube. Nematodes possess digestive, nervous, excretory and reproductive systems but lack a discrete circulatory or respiratory system. They range from 0.3 mm to more than 8 meters in length (University of Nebraska-Lincoln, 2010).

The nematode species that feed in and around grapevine plants are generally too small to see with the naked eye. They are, however, ubiquitous in the vineyard, and it is most unusual to find a soil sample that does not contain at least some nematode species.

In addition to vectoring important virus diseases (Grapevine Fanleaf Virus, for example), nematodes are one of many factors that must be considered when evaluating the cause of poor vineyard performance. Like other grapevine pests and pathogens, nematodes can be considered opportunistic organisms, with harmful populations becoming established around vines that are under stress. This stress may be the result of management practices and/or other biological pressure. Any event that weakens the plant renders the vine more susceptible to the establishment of harmful nematode populations.

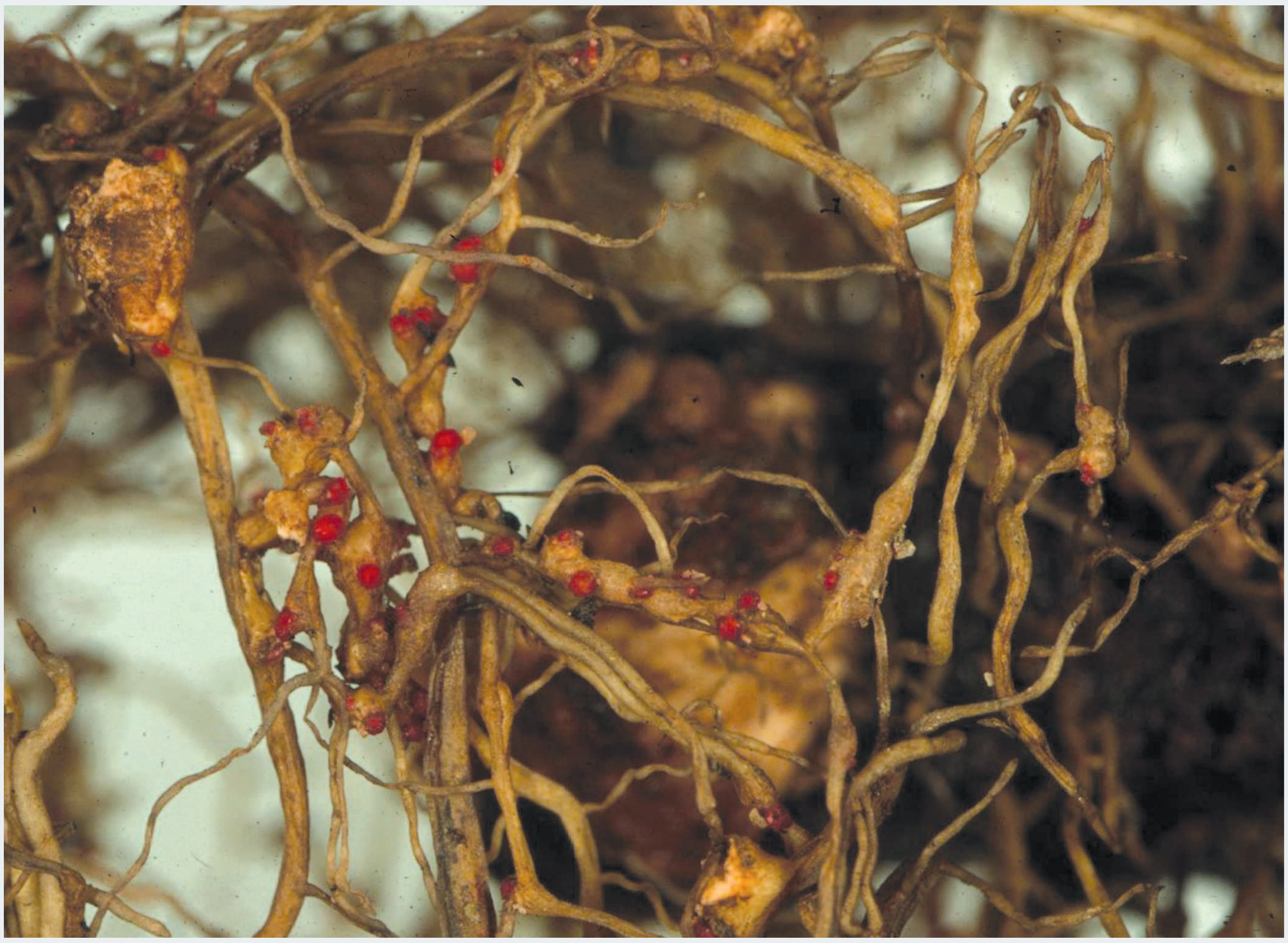

PHOTO 1: In this photo of grapevine roots infected with root-knot nematodes, note the swelling and galling of the roots. Egg masses are stained red. COURTESY OF PETER COUSINS, USDA-ARS, GRAPE GENETICS RESEARCH UNIT, GENEVA, NY

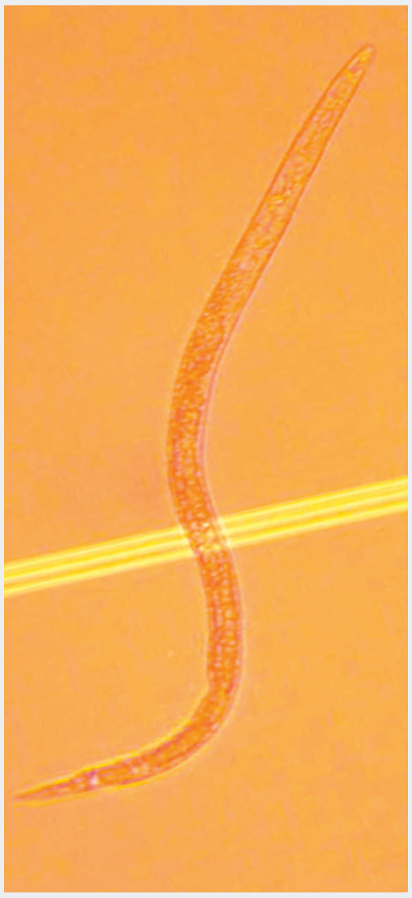

PHOTO 2: Juvenile *Meloidogyne arenaria*

COURTESY OF LANCE CADLE-DAVIDSON AND DEBRA JOHNSTON. USDA-ARS, GRAPE GENETICS RESEARCH UNIT, GENEVA, NY

These events include insufficient irrigation, incorrect planting practice (J-rooting, for example), presence of stress-inducing viral or fungal pathogens (leaf roll viruses, Petri disease pathogens, for example), improperly healed graft unions, over-cropping and premature cropping, to name but a few. In short, in situations where vine performance is substandard, nematodes must be considered a possible and likely participant.

Parasitic nematodes impact grapevines by reducing root efficiency and acting as a sink for photosynthates. Not only do nematodes reduce root efficiency and vector harmful viruses, but their activities render otherwise healthy vines susceptible to root-colonizing pathogenic fungi, such as Pythium, Fusarium, Rhizoctonia and Phytophthora.

Nematode Parasites of Grapevines

The most important nematode parasites of grapevines are root-knot (*Meloidogyne spp.*), dagger (*Xiphinema index*), lesion (*Pratylenchus vulnus*) and ring (*Criconemella xenoplax*). There are several other species that are mildly parasitic, including pin and dagger (*Xiphinema americanum*), but these are not considered to cause significant root damage and yield reduction.

Root-knot and lesion nematodes are endoparasites: they invade the roots and live within the root tissues, thus disrupting the water and nutrient-conducting function of the roots. Ring and dagger nematodes have similar life cycles except they feed only at the root surface (ectoparasites) via an epidermal-penetrating stylet and cause only minor disruption to the internal structure of the roots.

Reports suggest that root-knot and lesion nematodes cause the greatest physical damage to grapevine roots, resulting in up to 20 percent yield reductions in experimental plantings in California. In comparison, minimum yield reductions of 5 to 15 percent were recently estimated for nematode-infested vineyards in the Murray-Darling basin of Australia (Walker and Stirling, 2008). Dagger, ring and lesion nematodes are most prevalent in North and Central Coast vineyards and in the San Joaquin Valley. Root-knot nematodes occur most commonly in the San Joaquin Valley and southern California and prefer sandy soils.

Root-knot (Meloidogyne spp.) and Lesion (Pratylenchus spp.) Nematodes

Meloidogyne species, including M. incognita, M. hapla and M. javanica, are responsible for significant yield reduction in grapevines. Juvenile nematodes penetrate the vine just below the root tip where they feed in the xylem and phloem (water and nutrient-conducting) vessels of the root. Under warm conditions, females will mature into egg-laying adults in a matter of a few weeks (*Grape Pest Management*, 1992). The plant responds to this invasion by the production of a typical gall or "knot" in and around the conductive tissues, thus disrupting fluid movement through affected roots (SEE PHOTOS 1 AND 2).

Egg laying continues through the summer with greatest populations found in late summer or early fall when soils are driest. Eggs hatch and release second-stage juvenile nematodes to the rhizosphere, which are then available for the colonization of fresh roots. Under optimal climatic conditions, the life cycle may be completed in only four to five weeks, thereby allowing the production of several parasitic generations per season. Juvenile nematodes can move quite rapidly, traveling between vines in a matter of days. Root-knot nematode galls can remain viable for several years, which provide an ongoing source of new nematode contamination.

Dagger Nematodes (*Xiphinema americanum* and *X. index*)

Both species parasitize grapevines, but *X. index* is by far the more serious of the two as it is the vector for Grapevine Fanleaf Virus (GFLV). GFLV is one of the most debilitating grapevine virus pathogens, causing severe reductions in yield and vigor in affected plants. Once a field is contaminated with GFLV, it can take up to 10 years before it can be assumed with a reasonable level of confidence that old vine root fragments are no longer infective. Furthermore, it has been demonstrated that GFLV can remain viable in X. index nematodes for four years without the presence of grapevine roots. As a practical matter, the use of *X. index*-resistant rootstocks is the only way to farm infected land. *X. index* are most readily detected in the surface 18 inches beneath the vine row where winter populations are approximately double those in the summer (*Grape Pest Management*, 1992). Frequent tillage will effectively reduce nematode populations. Perhaps the most efficient strategy for replanting in *X. index*/GFLV-infected vineyard sites is to kill diseased vines and roots with systemic herbicides, such as glyphosate, before their removal. The success of the technique is not guaranteed, however, and depends on the vine age and soil depth. *X. index* is most prevalent in the North Coast of California.

Ring (*Criconemella xenoplax*)

Ring nematodes may cause significant damage to vines and are most frequently found in sandy or fine clay soils. Populations are often greatest in soils subject to temporary or periodic water logging. These species are easiest to detect after rain or irrigation and are responsible for significant root damage and related vigor and yield reduction.

Detection of Nematodes in the Vineyard

It is advisable to test for nematodes before planting and replanting and when a vineyard shows decline for non-obvious reasons. There are no recently published procedures on best methods to monitor nematode populations, but the guidelines quoted most frequently are found in UC Davis' *Grape Pest Management, Second Edition*. That which follows is a brief summary of the main points in combination with practical field notes.

1. Collect soil (it is okay to include root tissues) from beneath the irrigation emitter from several vines in the affected area. Samples should be collected from a depth of 10 to 25 inches but should be from a zone that includes actively growing root tips. Mix soils together and submit one sample from a defined area in a half-gallon plastic bag.
2. Avoid collecting samples from the most severely affected vines as it is likely that nematode populations are declining here.
3. Remember to include control samples from adjacent vines in areas that are seemingly not affected by decline.
4. The best time for sampling is after rain or irrigation. However, in practice, vines can be sampled year-round—as required—and it is far easier to collect samples from wet ground. When taking samples on an annual basis, endeavor to collect them at the same time each year.
5. Submit samples for analysis to a recommended laboratory. The laboratory will provide a report of the counts of nematode species per unit volume or weight of soil and should note the extraction procedure used to isolate them. Four reliable laboratories are noted in SIDEBAR 1.

SIDEBAR 1. **Commercial Nematode Diagnostic Laboratories**

Laboratory	Contact	Telephone	Email
A&L Western	Mike Buttress	209-529-4080	*reports@al-lab-west.com*
Dellavalle	Danyal Kasapligil	800-228-9896	*danyal@dellavallelab.com*
EverGreen	Nancy Hammack	425-417-8407	*GreenNema@qwestoffice.net*
Nematodes, Inc.	Douglas Anderson	559-891-9073	*nema@msn.com*

6. Because of the wide array of variables that impact nematode populations and their effect on grapevine productivity, it is difficult to interpret the results of laboratory analysis. However, guidelines are established in *Grape Pest Management, Second Edition*. SIDEBAR 2 presents a rough guideline to damaging populations of nematodes extracted from several sources.

SIDEBAR 2. **Nematode Populations Damaging to Grapevine**

Nematode	Nematodes/Kg soil*
Root-knot	500+
X. americanum	300+
X. index	200+
Lesion	100+
Ring	500+

*Adjusted to 100% nematode extraction efficiency

7. A lack of damaging population thresholds for the various grapevine nematode species is noticeable throughout contemporary literature although this is understandable because of the variety of factors that can influence vine interaction with the nematodes, nematode/nematode interactions and interactions with other pests and pathogens, and the difficulty in assessing damage in the field (Peter Cousins, personal communication).

Dealing with Nematodes: Grapevine Nursery Stock

The vast majority of commercially available grapevine nursery stock in California is produced under the guidelines of the **California Department of Agriculture** nursery certification program (*www.cdfa.ca.gov/phpps/PE/Nursery/pdfs/nipm_5_regs_grades_stds.pdf*). Materials released from this program are expected to be nematode-free. Program staff inspect vines and sample soils around the vines growing at high density (17,000 vines per acre) in the nursery field row. If economically important nematodes are detected, then vines must be subjected to hot water treatment at the nursery before shipping. The hot water treatment is designed to kill nematode endoparasites, such as root-knot, which may be present within the vine. This same hot water treatment is also effective against Vine Mealybug (vector of Grapevine Leafroll-associated virus 3), and so it is unusual these days to find nursery stock that has not been subject to hot water treatment. Occasionally, vines may be delivered with obvious root-knot nematode galls (PHOTO 3); it is strongly advised that these plants be rejected or damaged roots be removed before planting. When ordering plants, it is advisable to inquire as to the nursery's policy on hot water treatment.

Dealing with Nematodes: Contaminated Vineyards

When confronted with a nematode-contaminated vineyard, the options available are to treat with various chemicals and/or replant—especially if dealing with GFLV-contaminated *X. index*. Unfortunately, the available chemical remedies are either extremely environmentally-unfriendly or only moderately effective. As with most other disease conditions that affect grapevines, however, it is important to devise a sustainable and environmentally-sound approach to nematode management and to consider that the overall health and individual components of the rhizosphere have a direct impact on pathogen populations. As UC Davis professor of nematology **Howard Ferris** recently noted, soil pathogen control would, ideally, be achieved through transitioning from conventional pesticide-based management practices (which often inadvertently harm many of the beneficial species components of the rhizosphere, taken as a whole—the soil food web) to a situation where sustainable stewardship of the soil food web encompasses the development of eco-friendly approaches that include regulation of pest species, soil fertility and nutrition. While much of this work is ongoing for annual crops, few, if any, studies have been undertaken with grapevines or other perennials.

Several chemical control treatments for nematodes are recommended by the University of California IPM guidelines for grapevine management (*www.ipm.ucdavis.edu/PMG/r302200111.html*). These include the following preplant treatments:

1. DiTERA. This is one of the most environmentally-sound approaches to nematode control in pre-plant or replant vineyard situations. DiTERA is a biologic, which acts most effectively against ectoparasite nematodes (that feed at the root surface but do not enter the root)—for example, ring species. DiTERA is a killed mixture of parasitic fungal tissues that naturally control some nematode species. This material is moderately effective, and its activity is influenced by soil type and conditions.

2. Methyl bromide. This is extremely toxic and damaging to the environment.

3. Metam Sodium. This is extremely toxic and damaging to the environment.

4. Sodium tetrathiocarbonate (ENZONE). This compound is also toxic and kills many beneficial soil microorganisms. Enzone can work quite well in sandy soils on ectoparasitic nematodes, but the same nematodes in a soil with higher clay content are not controlled.

5. 1,3-Dichloropropene (TELONE II). This is extremely toxic and damaging to the environment.

PHOTO 3: Pictured are root-knot nematode galls on recently-harvested 3309C rootings.

IPM-recommended treatments for nematodes in existing vineyards include the following:

1. Fenamiphos (NEMACUR 3)
2. Sodium tetrathiocarbonate (ENZONE)
3. DiTERA

Cover Crops

Cover crops are being studied as potential tools in combating nematodes that parasitize grapevines. However, this is a relatively new field of research, and there are no clear guidelines as to which plant species have the greatest beneficial impact on damaging populations of nematodes (Westerdahl, 1998). It is understood, however, that Sudan grass is a poor host for lesion and root-knot nematodes but a good host for *X. americanum*.

Nematode-resistant Rootstocks

Selection of nematode-resistant rootstocks is the only reliable strategy for dealing with parasitic nematodes prevalent in replant or virgin vineyard sites. When planting in soils infested with *X. index*, the only rootstock recommended until very recently was VR039-16. This rootstock imparts substantial vigor to the scion and is considered difficult to field-graft, but it does allow vines to thrive in *X. index*-contaminated soils. Recent work has shown, however, that at least under prevailing springtime conditions in Napa and Sonoma, VR039-16 field grafting can be successful. SIDEBAR 3 summarizes the relative resistances and tolerances of standard and newly released rootstocks that should be considered when dealing with nematode-contaminated soils.

SIDEBAR 3 **Relative resistance/susceptibility of rootstock to nemotodes**

Rootstock	Root Knot *Meloidogyne*	Dagger *X. index*	Dagger *X. americanum*	Ring *C. xenoplax*	Lesion *P. vulnus*
Freedom	R	R	MR	HS	SS
1613C	R	MR	HS	HS	SS
Harmony	MR-R	MR	HS	MS	SS
5C	SS-MR	MR	HS	HS	S
SO4	SS-MR	MR	S	HS	S
Schwarzmann	S-MR	MR	SS	MS	SS
VR039-16	S	R	MR	na	S
3309C	HS-S	SS	S	HS	SS
101-14 MG	MR	HS	na	HS	S
5BB	S	HS	na	HS	S
110R	R	HS	na	HS	S
RS-2	SS	HS	na	HS	R
RS-3	R	MR	MR	MS	na
RS-9	R	MR	MR	HS	na
GRN-1	R	R	R	R	R
GRN-2	R	R	R	S	R
GRN-3	R	R	R	S	R
GRN-4	MR	R	R	MS	R
GRN-5*	R	R	R	MS	R

*Does support low populations of phylloxera on roots but this has not been shown to damage vines.

Adapted from Grape Pest Management, 1992; McKenry, 2000; Covert, 2008

R resistant **SS** slightly susceptible **HS** high susceptible
MR moderately resistant **S** susceptible **na** not available

In 2008, the UC Davis-patented GRN-1 through GRN-5 series of rootstocks, developed by Dr. **Andy Walker** in collaboration with Dr. Howard Ferris (both at UC Davis), were released to California nurseries, and they are now available in small quantities (Covert, 2008) (PHOTO 4). These rootstocks, especially GRN-1, are reported to have excellent resistance to root-knot, lesion, dagger and ring nematodes as well as resistance to Phylloxera and Pierce's Disease. GRN-1 may possess the ability to tolerate Fanleaf Virus infection in a manner similar to VR039-16. This tolerance is critical since resistance to *X. index* feeding does not prevent the vectoring of, and infection by, GFLV. Work is underway to determine whether GRN-1 is capable of preventing fanleaf disease (Covert, 2008). The viticultural characteristics of these vines will be largely discovered by those who plant them.

PHOTO 4: UCD GRN-4 rootings are seen under cultivation in summer 2010.

Breeding Improved Rootstocks: Ongoing Research

Because nematodes can persist for long periods in isolated grapevine roots and because of the difficulty in penetrating soils with environmentally-unsound nematicides, breeding nematode-resistant rootstocks is the best approach to dealing with these pest species.

Current research underway at UC Davis in the laboratories of Drs. Walker and Ferris is focused on identifying naturally-occurring resistance in wild and weedy relatives of commercial grapevines (Ferris and Walker, 2010). They are working to develop sources of resistance to ring nematode and a broader genetic base for resistance to root-knot and dagger nematode (*X. index*).

The goal of their studies is to provide a range of rootstock choices for the nematode pressures experienced in four general grape-growing regions: North Coast X. index/GFLV areas; Northern San Joaquin Valley areas where pressures are experienced from root-knot nematodes, *X. index* and GFLV and other nematode pests, including ring, pin and root-lesion nematodes; Central Coast regions with *X. index*, root-knot and ring nematodes; and the central and southern San Joaquin Valley and the Coachella Valley, with nematode pressures from the root-knot nematode complexes, ring nematode, lesion nematode, citrus nematode and others.

In 2009, the Walker/Ferris group tested the susceptibility and resistance of new and existing rootstocks to Meloidogyne arenaria strain Harmony A, M. incognita strain Harmony C, alone and in combination, *X. index* and ring nematode. They report that six rootstocks exhibited sufficiently few root-tip gall indicators of nematode feeding to be considered resistant to *X. index*: Harmony, Freedom, Schwarzmann, Boerner, O39-16 and Riparia Gloire (Ferris and Walker, 2010). Of these, Boerner and Riparia Gloire also appeared to have resistance to root-knot nematodes. Schwarzmann and O39-16 appeared to have resistance to ring nematode while all the others were susceptible.

Conclusion

Although nematode species undeniably cause significant damage and yield loss in all commercial grapevine production areas in California, there are really no complete or up-to-date sources of information on their biology, control and impact. This is in large part due to the myriad of soil, climate and biotic factors that affect grapevines and the difficulty in performing controlled, replicated experiments in important viticultural regions. It is clear, however, that for the foreseeable future, traditional and newly released nematode-resistant rootstocks will remain the keystone of our strategies to combat these parasites. It is also apparent that sustainable and integrated approaches must be devised to allow the harnessing of the natural cleansing properties of healthy soils to combat the pests that accompany intensive agriculture. **WBM**

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- Dr. Andy Walker, Department of Viticulture and Enology, UC Davis, CA.

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