Nematodes in WA vineyards: A study by Professor Franco Lamberti, visiting scientist

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **RT 02/29-3**

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Research Organisation: **The Wine Industry Association of WA**

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Executive summary

Nematologist Professor Franco Lamberti (Institute of Agricultural Nematology, Bari, Italy) spent one month of 2003 in Western Australia conducting a survey of vineyards to identify nematode genera and species. One hundred and thirty four sites were sampled, covering all the major vine-growing areas of the State.

Root Knot Nematode (RKN, *Meloidogyne* spp.) was detected in 31% of the vineyards sampled. Fourteen of the vineyards showed obvious symptoms of decline ,and these were all infested with RKN. On adjacent areas where vine decline was not apparent, RKN was not detected or was present at only low levels. RKN was more likely to occur on sandy soils, but economic damage was also observed on gravelly or loamy soils. RKN was present on land that had been used previously for horticultural production. The Root Knot Nematode was found on properties in all of the regions investigated.

Xiphinema (Dagger Nematode) was detected in 20% of the vineyards sampled. *Xiphinema index*, which is a vector for the devastating Grapevine Fan Leaf Virus (GFLV), was not detected in any of the samples. Other *Xiphinema* species were observed in samples from all regions, though the presence of these species was not obviously associated with a decline in vine performance. The economic importance of these species to vines in Western Australia is uncertain.

Pratylenchus (Root Lesion Nematode, RLN) was observed in extracts from some of the soil samples although levels were considered too low to cause significant economic damage. However, several species of Root Lesion Nematode have been associated with vine damage in Australia, and these nematodes would need to be considered further if levels were to increase.

Background

The aim of this study was to sample as many vineyards as possible, across all the major Western Australian vine-growing regions, to investigate nematode species and genera associated with vines. Vineyards with possible decline due to nematode attack, as well as vineyards with no history of nematode problems, were sampled. In February-March 2003, Professor Franco Lamberti (in conjunction with Neil Lantzke of the DAWA Wine Grape Project, Vivien Vanstone of DAWA Nematology, and numerous regional Horticulture staff) sampled 70 vineyards plus 64 fruit and other trees.

Several nematode genera are potentially harmful to grape production in Australia (Nicol *et al.* 1999):

Meloidogyne: Four Root Knot Nematode species have been found in major vine-growing areas of Australia: *M. javanica, M. hapla, M. arenaria, M. incognita*. It is not uncommon for plants to be infected by more than one of these species.

Xiphinema: One or more species of Dagger Nematode are present in grape-growing areas worldwide, and at least 28 species have been reported on vines. These nematodes are common in Australian vineyard soils, but no detailed study of the species or the damage they may cause has been made.

X. index is of particular concern, since it transmits the devastating nepovirus Grapevine Fan Leaf (GFLV). Fortunately, the only record of *X. index* from Australia is from a limited area around Rutherglen, Victoria, where it has been contained. Other species of *Xiphinema* are often associated with vines, but their economic impact is largely unknown. Species other than *X. index* can transmit economically important viruses to a wide range of crops.

There is substantial morphometric and morphological variation between *Xiphinema* populations, and *X. americanum* alone has been defined by Lamberti *et al.* (2000, 2002) as a complex ("the *X. americanum*-group") comprising 51 species. This has been arrived at by principal component and hierarchical cluster analysis of up to 17 morphometric characters, with identifications confirmed by isozyme and DNA analyses. Identifications are further confounded by deviations from the type species populations due to environmental and/or geographic influences, possibly leading to incorrect identifications if based solely on phenotypic morphometric data. Professor Lamberti has accumulated morphometric and molecular data for *Xiphinema* from over 50 countries. The specimens from Western Australia will represent the first inclusion of Australian data to these comprehensive studies.

Pratylenchus: Root Lesion Nematode usually occurs in low populations on vines, but is widespread and potentially more damaging than RKN if high population levels develop. Several species are possibly associated with vines in Australia: *P. vulnus, P. scribneri, P. brachyurus, P. neglectus, P. pratensis, P. thornei, P. zeae*.

Objectives

The objectives were to:

- Gain a better understanding of the effect of nematodes on wine grape performance in Western Australian vineyards via an extensive vineyard survey.
- Identify each nematode genus and species present.
- Hold seminars at three locations in Western Australia to present the results of the survey and to discuss management options (chemicals, biofumigation, use of resistant rootstocks).
- Train Department of Agriculture staff in extraction and identification of nematodes from vineyard soils.

Method

Rhizosphere soil (~ 5kg per sample) was collected from 70 vineyards and from 64 fruit and other trees during February-March, 2003.

Soil was wet sieved (710μm followed by 63μm) in the laboratory, and the resulting slurry placed in water overnight at room temperature in 90μm sieves in Petri dishes to obtain "clean" nematode extracts. All extractions were qualitative rather than quantitative.

Professor Lamberti investigated each extract microscopically, and picked out individual *Xiphinema* specimens into 1M NaCl for transport to Italy. These specimens will be subjected to isozyme and DNA studies.

Remaining extract was fixed in 4% formalin to obtain *Xiphinema* for detailed morphometric and morphological study. Nicola Vovlas in Bari examined *Pratylenchus* from these extracts for species identification.

Galled vine roots (i.e. showing symptoms of RKN infection) contained in the soil samples were removed prior to sieving. Molecular studies and identification of the RKN species from these samples will be done in conjunction with Professor Lamberti's collaborators in Portugal.

Results/Discussion

A combination of factors predispose Western Australian vineyards to damage from nematodes: sandy soils; warm climate; cultivation of “own rooted” vines which are susceptible to nematodes; and use of land previously planted to horticultural crops such as potato and fruit trees. Significant levels of RKN are likely to have developed on horticultural land. Since the 1960’s and 1970’s the majority of vines in the Swan Valley, the oldest viticultural region in the State, have been planted on resistant rootstocks to overcome nematodes. However, the newer viticultural areas in the State are almost exclusively planted on “own rooted” vines, which are susceptible to nematodes.

Increasing numbers of grape growers in Western Australia are submitting samples to *AGWEST* Plant Laboratories for identification and enumeration of nematodes. Large areas of vines have, in recent times, been planted on sandier soils (Jindong, Swan Coastal Plain and Gingin) and on old horticultural land where significant levels of damaging nematodes may occur. Many gravelly soils in the Margaret River region have loamy sand topsoils. The nematode population levels in this region, and their likely impact on plant growth, is unknown but is of concern.

Representative samples were collected from all major vine-growing areas of the State. Table 1 shows the locations of the properties surveyed. At the time of producing this report, nematode species identification by the European experts had not been completed. This information will follow in a scientific paper that is in preparation.

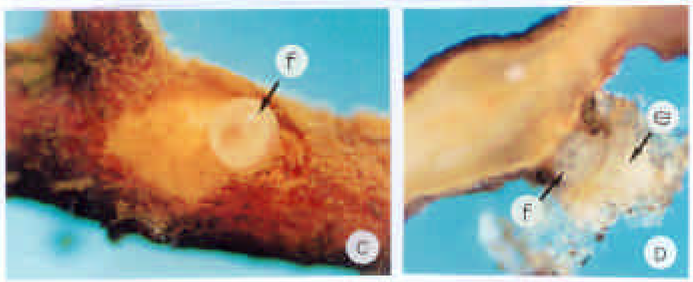
Root knot nematode

Root Knot Nematode was detected in 31% of the vineyards surveyed. Fourteen vineyards with obvious symptoms of decline were sampled, and all were infested with RKN. Roots displayed moderate to severe galling, indicative of RKN infection. On adjacent vines that did not show obvious symptoms of decline, RKN was not detected or was present at low levels (Figure 1). Both *M. javanica* and *M. arenaria* were identified, with *M. javanica* being more common. In many vineyards where RKN was detected it is likely that levels were not high enough to cause economic damage. Once present, however, these nematodes can not only increase in density with time, but also spread within a vineyard.

Figure 1. Growth of vines of the same age and cultivar in Swan Region in areas infested (A) or not infested (B) with Root Knot Nematode (*Meloidogyne javanica*).

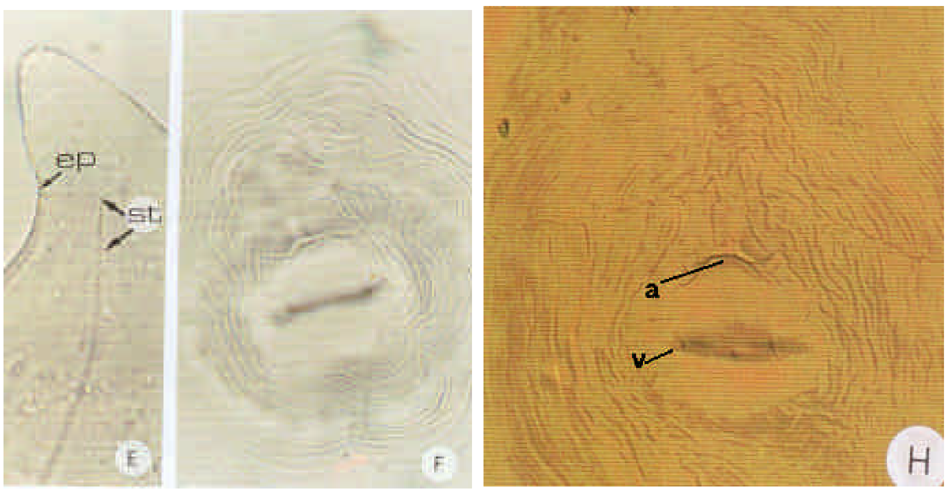
RKN was the predominant nematode pest detected in this survey of grapevines in Western Australia. Growth in infested vineyards was patchy, with vines growing in these patches showing stunting, shorter internodes, reduced canopy and early senescence. Root galls typical of RKN infection were observed along the roots, combined with abnormal proliferation of lateral roots. Distorted and swollen roots showed small, dark galls from which mature female nematodes and their associated egg masses protruded (Figure 2). Within the roots, necrotic areas associated with the nematodes were evident, along with the production of multinucleate giant cells induced by the feeding activities of RKN.

Figure 2. Root galls showing protruding females (f) and egg masses (e) of *Meloidogyne arenaria* (C) and *M. javanica* from vine root samples collected from Swan Valley and Bindoon, respectively.



In Italy, the RKN species were identified as either *M. javanica* or *M. arenaria* on the basis of the diagnostic morphology of female nematode perineal patterns (Figure 3F, 3H), excretory pore position (Figure 3E), and also with esterase isozyme phenotyping. *M. javanica* occurred more often than *M. arenaria,* although both species can be equally destructive. It is important to identify the nematode species present, as host plant (or rootstock) resistance can vary with species: resistance to one nematode species will not necessarily confer resistance to another. Although presence of RKN can be determined by investigation of root symptoms, this is not indicative of the individual species that can occur.

Figure 3. Anterior of female *Meloidogyne arenaria* (E) showing position of excretory pore (ep) and stylet (st); diagnostic morphology of female perineal patterns for *M. arenaria* (F) and *M. javanica* (H). Diagnostic features of the perineal pattern are based on the number and patterns of cuticular ridges between and around the anus (a) and vulva (v) of the mature female Root Knot Nematode.



Soil type

RKN infestation was more likely to occur on the sandy soils investigated, and nematode reproduction is greater under these growing conditions. In addition, the lower water and nutrient holding capabilities of sands exacerbate the water and nutrient stresses experienced by nematode infected vines. However, not all vineyards located on sands showed signs of RKN infection.

Economic damage from RKN was not commonly observed for vines planted on gravelly duplex or loamy soils in the south west of Western Australia. Where damage did occur, this was associated with older vines (>20 years) or where the vines were planted on old horticultural land. Vigorously growing vines on loamy soils are better able to tolerate nematode infestation without loss of performance. These soils have higher water and nutrient holding capacities, so can more readily supply the vine if root function has been impaired by nematode attack.

Table 1. Location of vineyards, soil type, property history and presence of Root Knot Nematode (RKN, *Meloidogyne* spp.) species in Western Australian vineyards, February-March 2003.

Sample number	Location	Soil type	Old horticulture land	Symptoms of decline	Nematode identification
1	West Gingin	Sand	No	Yes	*M. javanica*
3	West Gingin	Sand	No	No	absent
5	Gingin	Sand	No	No	absent
6	Gingin	Sand	No	No	*M. javanica*
12	Gingin	Sand	No	No	absent
13	Gingin	Sand	No	No	absent
14	Bindoon	Loam	No/possibly	Yes	*M. javanica*
32	Capel	Sand	No	Yes	*Meloidogyne* spp.
33	Capel	Sand	No	Yes	*Meloidogyne* spp.
37	Jindong	Sand	Possibly	Yes	*M. arenaria*
38	Jindong	Loamy sand	Possibly	No	absent
41	Jindong	Loamy sand	Yes	No	absent
42	Jindong	Loamy sand	Yes	No	absent
45	Yallingup	Gravelly loam	No	No	absent
47	Yallingup	Gravelly loam	No	No	absent
49	Margaret River	Gravelly loam	No	No	absent
50	Margaret River	Gravelly loam	No	No	absent
55	Margaret River	Gravelly loam	No	No	*M. javanica*
56	Margaret River	Gravelly loam	No	No	absent
57	Margaret River	Gravelly loam	No	No	*M. javanica*
58	Margaret River	Gravelly loam	No	No	absent
59	Margaret River	Sand	No	Yes	*Meloidogyne* spp.
61	Margaret River	Gravelly loam	No	No	absent
64	Margaret River	Gravelly loam	No	No	absent
66	Margaret River	Gravelly loam	No	No	absent
68	Margaret River	Sand	No	Yes	*M. javanica*
69	Margaret River	Sand	No	No	absent
71	Margaret River	Sand	No	Yes	*M. javanica*
72	Pemberton	Gravelly loam	No	No	absent
75	Pemberton	Gravelly loam	No	No	absent
76	Pemberton	Gravelly loam	Yes	No	absent
77	Pemberton	Gravelly loam	Yes	No	absent
78	Pemberton	Gravelly loam	Yes	No	absent
80	Pemberton	Gravelly loam	Yes	No	absent
84	Manjimup	Gravelly loam	Yes	No	absent
88	Manjimup	Gravelly loam	Yes	No	absent
91	Manjimup	Gravelly loam	No	No	absent
93	Manjimup	Gravelly loam	No	No	absent

94	Manjimup	Gravelly loam	Unknown	No	absent
95	Manjimup	Gravelly loam	Unknown	No	absent
96	Manjimup	Gravelly loam	No	No	absent
97	Manjimup	Gravelly loam	No	No	absent
98	Manjimup	Gravelly loam	Possibly	No	*Meloidogyne* spp.
99	Frankland	Gravelly loam	No	No	absent
100	Frankland	Gravelly loam	No	Yes	*Meloidogyne* spp.
101	Frankland	Gravelly loam	No	No	absent
102	Frankland	Gravelly loam	No	Yes	*Meloidogyne* spp.
107	Mt Barker	Gravelly loam	Possibly	Yes	*Meloidogyne* spp.
108	Mt Barker	Gravelly loam	Yes	Yes	*Meloidogyne* spp.
109	Mt Barker	Gravelly loam	No	No	absent
110	Mt Barker	Gravelly loam	No	No	absent
111	Mt Barker	Gravelly loam	No	No	absent
112	Mt Barker	Gravelly loam	No	No	absent
113	Albany	Loam	Yes	No	absent
115	Albany	Loam	Yes	No	absent
116	Porongurup	Gravelly loam	No	No	absent
117	Porongurup	Gravel	No	No	absent
121	Swan	Sand over clay	Yes	No	*M. arenaria*
122	Swan	Loam	Yes	Yes	*M. arenaria*
123*	Swan	Loam over clay	Yes	No	absent
125**	Wanneroo	Sand	No	No	absent
126	Baldivis	Sand	No	Yes	*M. arenaria*
127**	Baldivis	Sand	No	No	absent

* Planted on 34EM rootstock
** Planted on Schwarzmann rootstock
All other vines sampled were planted on "own roots"

Previous land use history

RKN was common on land that had previously been used for horticulture. However, this was not always the case, with some vines planted on old horticultural sites having no nematodes detected in the root zone. In the Swan Valley, where vines have been replanted on old vineyard land, severe infestations of nematodes often occur.

Pasture species and weeds such as Deadly Nightshade (*Solanum nigra*) can act as nematode hosts and allow build-up of RKN populations. If vineyard cover crops susceptible to RKN and/or RLN are grown, it is also possible that levels of these nematodes could increase and threaten vine production.

Age of vines

Older vines in the Great Southern and Swan regions showed signs of decline, and this was often associated with the presence of RKN. Over time, nematode numbers in the soil increase until they reach a level where economic damage begins to occur. In the Manjimup/Pemberton area, the vines sampled were generally on higher vigour sites that would, to some extent, alleviate nematode damage. The vines sampled in the Margaret River area were generally younger.

Planting material

The use of planting material that is free of nematodes is essential. On one of the properties, rootlings that were infected with RKN were planted into a sandy soil. These vines yielded very poorly, and nematodes spread to an adjacent block causing these vines to also decline. The rate of spread was about one vine row every one to two years.

Type of rootstock

Soil samples from a rootstock trial in the Swan Valley showed differences in levels of nematode infection (Table 2). Ruggeri 140 was a better host (i.e. was more susceptible) to RKN than Paulsen 1103, Schwarzmann or Ramsey. Trial results (Table 3) from table grapes at Carnarvon also indicate differences between rootstocks for RKN susceptibility (Ian Cameron & Vivien Vanstone, pers. com.). RKN risk categories reported by Stirling *et al.* (1999) are: low risk with < 40 juveniles / 200g soil; moderate risk with 40-400 / 200g soil; and high risk with >400 / 200g soil. Own rooted vines assessed from both the Swan Valley and Carnarvon are therefore subject to at least a moderate risk of damage from RKN.

Table 2. Density of Root Knot Nematode (RKN, *Meloidogyne* spp.) juveniles associated with vine root stocks, Swan Valley, Western Australia, February-March 2003.

Root stock	**RKN juveniles / 200 g soil**
Ruggeri 140	312
Own roots	136
Paulsen 1103	85
Ramsey	40
Schwarzmann	20

Table 3. Density of Root Knot Nematode (RKN, *Meloidogyne* spp.) juveniles associated with vine root stocks, Gascoyne Research Station, Carnarvon, Western Australia, September 2003.

Root stock	**RKN juveniles / 200 g soil**
Own roots	424
K51-40	330
Kober 5BB	158
99R	156
Ramsey	104

Xiphinema spp

Xiphinema spp were identified in 20% of the vineyards sampled. However, growth and performance of these vines did not appear adversely affected. Professor Lamberti was surprised at the lack of species diversity in the *Xiphinema*, compared to his observations from other countries. It seemed, from his initial investigations, that there were only two species (both of the *X. americanum*-group), and most were probably *X. rivesi*. However, identifications remain to be confirmed with the more detailed work being conducted in Italy.

X. rivesi was originally described from France, and has since been found in Bulgaria, Germany, Spain, Portugal, North and Central America, South America and Pakistan. In North America, *X. rivesi* is the most widespread species of the *X. americanum*-group. This species transmits at least three nepoviruses. It is parthenogenetic, and among one of the five *X. americanum*-group that has only three juvenile stages (most nematodes have four juvenile stages, so this feature can be partially diagnostic of *X. rivesi*).

The distinctive *X. index*, which is a vector for the devastating Grapevine Fan Leaf Virus, was not detected in any of the Western Australian vineyard samples.

Pratylenchus

Low levels of *Pratylenchus* were observed in extracts from most vineyard soils, and these nematodes did not seem to be associated with any economic decline. Too few RLN were present in extracts from soil samples to allow any detailed identification of the species present, and these nematodes were not observed in any of the roots returned to Italy. However, time of sampling is critical for detection of RLN in vineyards (Walker and Morey 2001). Levels in the soil can appear low over most of the season, but increase to coincide with flushes of root growth in spring. This is also the time when vine roots are most vulnerable to infection and damage from these nematodes. To make any further conclusions on the role of RLN in Western Australian vineyards it would be necessary to conduct more detailed sampling of both roots and soil throughout the season.

Outcome

- Individual reports outlining the identity of nematode species present at each sampling site will be sent to each property, and indications given of the possible implications of this to current and future vine productivity.

- As a result of this study, a detailed Farmnote titled 'Nematodes in Western Australian Vineyards' is being produced.

- At a DAWA seminar (attended also by researchers from UWA and Murdoch University), Professor Lamberti summarised some of his 40 years of nematology experience. He also addressed a grower meeting, and a press release was prepared to publicise his activities in Western Australia. A summary of this work appeared in the July 2003 *Newsletter of the Australasian Association of Nematologists*, and in the DAWA *Wine Industry Newsletter* (No. 70 December 2002; No. 72 October 2003).

- Professor Lamberti demonstrated to staff and students from DAWA, Murdoch University and UWA a comparison of the nematode specimens extracted, and demonstrated the features of *Xiphinema* species.

- A scientific paper on root knot nematodes in Western Australian vineyards is in preparation.

- Professor Lamberti examined and re-confirmed as *X. index* fixed nematode specimens from Rutherglen, Victoria, that had been collected in 1963.

Recommendations

1. All grapevines planted on sands should be planted on nematode resistant rootstocks.
2. All grapevines planted on old horticultural land should be planted on nematode resistant rootstocks.
3. Where old viticultural land is to be replanted, nematode resistant rootstocks should be used.
4. Confirmation should be made that planting material is not contaminated with nematodes.
5. Conduct trials to determine the best nematode resistant rootstocks for conditions in the South West of Western Australia.
6. Continued education of growers, DAWA personnel and consultants on the risks of nematode infestation to current and future vine production.
7. Further targeted sampling of infested vineyards identified in this study to enumerate levels of RKN, and estimations of the economic impact of these nematodes on vine productivity.
8. Monitoring of vineyards where RKN was detected to determine changes in levels of nematode infestation, spread, and vine decline with time.
9. Vigilance on RLN levels and species present in vineyards, and additional sampling of roots and soil at times more appropriate for successful detection and population estimates of these nematodes.
10. Encourage growers to employ soil testing to determine nematode levels, particularly prior to planting or re-planting vines.

References

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Stirling G, J Nicol & F Reay 1999 – Advisory services for nematode pests: operational guidelines. RIRDC Publication 99/41.

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Budget Reconciliation

Budget	Funding required from GWRDC	Actual expenditure
Accommodation and meals		
(30 days x $180)	5,400.00	5,455.30
Car hire		
(20 days x $85)	1,700.00	1,000.00
Laboratory assessment (Technician)		
(30 days @ $200 /day)	6,000.00	7,954.70
Total	13,100.00	14,410.00
Add 10 % GST	1,310.00	
Total funds requested from GWRDC	14,410.00	