Plant parasitic nematode quantification and management strategies in viticulture

FINAL REPORT to GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Project Number: **CRV 99/4**

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Research Organisation:
Cooperative Research Centre for Viticulture

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Final report to Grape and Wine Research & Development Corporation On
Plant Parasitic Nematode Quantification and Management Strategies in Viticulture

Authors:
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The cooperation and assistance of the owners of the vineyards on whose sites sampling was undertaken is acknowledged. Dr Greg Walker, SARDI, provided valuable co-supervision of Dr Motiul Quader during his Ph.D. studies. Dr Quader received support and assistance in this work from colleagues, family and friends, for which he is most grateful. The collaboration of Dr Alan McKay of the Diagnostics Group, SARDI Field Crops Pathology, Waite Campus and his staff was essential for the inclusion of the DNA assay evaluated in the study.

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CRV 99/4 Plant parasitic nematode quantification and management strategies in viticulture

1. Abstract

Parasitic nematodes of grapevines in South Australia were surveyed and prevalence and distribution determined. Root-knot nematode (RKN) was found to be the most prevalent while distribution varied depending on nematode type. A standardised sampling method was developed and its genetic diversity studied. Low densities of RKN at vine establishment were shown to be damaging. A novel assay of DNA from soil proved a reliable method for RKN quantification and estimation of potential for damage. A pilot evaluation, involving industry collaborators, showed the DNA test to be robust and acceptable. This technology could assist researchers and vineyard managers to monitor RKN, leading to a greater understanding of its importance and need for management.

2. Executive Summary

The occurrence of plant parasitic nematodes associated with grapevines in South Australia was surveyed. Root knot nematode (*Meloidogyne* spp.) was the most prevalent. Root lesion (*Pratylenchus*), pin (*Paratylenchus*), ring (Criconematids) and dagger (*Xiphinema*) nematodes (in decreasing order of prevalence) were the other plant parasitic nematodes found.

The distribution of root knot nematode relative to the vine in the row and inter-row was determined and a standard sampling protocol developed Meloidogyne spp. and Dagger nematodes occurred mainly along the vine rows whereas root lesion nematodes had similar densities in rows and inter-rows. Genetic (specific and subspecific) diversity of root knot nematodes were determined by various techniques including sequencing of ribosomal DNA.

Available DNA probe and testing protocols were evaluated for ability to quantify root knot nematode population density with comparison to extraction of live nematodes and bioassay using a susceptible host plant. The DNA assay proved to be a useful tool for population estimation and was more reliable than other approaches.

The effect of initial population density on grapevine growth at establishment was assessed. The damage threshold at this stage is low. The population rose rapidly prohibiting the determination of critical densities for later growth or quality. Damage risk categories were developed for the DNA test based on published literature

A pilot evaluation of DNA detection and quantification of root knot nematode was conducted. The data confirmed the high incidence of root knot nematode (41% of sites) at potentially damaging densities (88% of infested sites medium to high densities). Collaborating growers provided feedback on the acceptably of the test, the usefulness of the results and the likelihood of adopting this technology, if made available.

It is recommended that the provision of a commercial test for root knot nematode be encouraged and that additional work necessary be undertaken in partnership with potential service providers. Synergies with other horticulture industries needing to test for root knot nematode, and potential opportunities to include the test with survey work for phylloxera should be explored.

Given the prevalence of root knot nematode in grapevines in South Australia and that root feeding nematodes impair the uptake of water and nutrients, further work on the impact of these nematodes should be undertaken, especially in the context of efforts to improve the efficiency of water use in viticulture.

This project was supported by the Commonwealth Cooperative Research Centres Program and conducted by the CRC for Viticulture (CRCV). Australian grape growers and winemakers are key stakeholders in the CRCV, contributing levies matched by the Commonwealth Government and invested by the Grape and Wine Research and Development Corporation in the Centre.

The collaboration of Dr Alan McKay of the Diagnostics Group, SARDI Field Crops Pathology, Waite Campus and his staff was essential for the inclusion of the DNA assay evaluated in the study.

3. Background

Industry Context

Plant parasitic nematodes are insidious pests of agriculture and are particularly problematic in perennial species such as grapevine. There are a number of nematode species that are either well-recognised or potential pests in grapevine. Root knot nematode (*Meloidogyne* spp.) is able to cause yield losses of up to 60% in grapevine and citrus nematode (*Tylenchulus semipenetrans*) can cause losses of 20 to 30%. Damage caused by other parasitic species has not been quantified. In addition to direct yield losses, high nematode populations at planting may result in establishment problems, delayed development and uneven vine performance.

Unreliable quantification of nematodes has limited the definition of economic thresholds and the assessment of management options. Recent developments in nematology have seen the provision of a commercial service for the quantification of nematodes in soil used for field crops based on DNA technologies. The approach offers promise for viticulture to better define pest levels and to assess the applicability of various control strategies. However, despite DNA probes being available considerable further development is needed before a commercial service can be offered to industry.

Once the techniques for extracting and quantifying parasitic nematodes from vineyard soils are developed, the challenge is to determine economic thresholds (or probably, more realistically, impact risk categories) and to assess management options based on the levels detected. This may lead to recommendations based on current options or highlight the economic imperative for identifying novel approaches to nematode control in grapevine.

Relevance and priority assessment

In a competitive international environment identifying and minimising constraints to production is critical to maintaining advantage. Insidious pests such as plant parasitic nematodes have non-specific affects on grapevine growth and production and if the cause is unrecognised amelioration strategies will be misdirected, ineffective and wasteful. It is important for industry to be provided with reliable tools for nematode detection, quantification, impact prediction and management. This project addressed each of these priority issues.

History of R & D in the area

The development of DNA-based diagnostic tests for nematode pests of agriculture is relatively recent. A testing service of cereal nematodes based on probes developed by CSIRO Entomology is being offered commercially in Australia by SARDI. CSIRO has developed specific probes for other nematodes including many of those problematic in grape production. These researchers are world leaders in this area and Australian technology is likely to be applied in many countries.

With respect to nematodes the focus has been on identification and use of resistant rootstocks. However, choice of rootstock for commercial planting and rootstock evaluation programs are constrained by the ability to reliably identify and quantify pest nematodes. Research on other forms of control in Australia, including nematicides, biological control, intercropping, organic

amendments and the like is limited. This is in part due to difficulties in use of available methods for characterising nematode populations and their impact.

Success and limitations to development and adoption
Nematode resistant rootstocks are used widely in viticulture and provide a level of control. However, limitations to the adoption of this approach are evident. Reliable diagnostic tools and damage risk assessments are needed to ensure best practice is adopted and to underpin the development of alternative methods.

4. Project Aims and Performance Targets

4.1 Objectives

- Develop sample handling and DNA extraction methods for the detection and quantification of nematode parasites of grapevine in conjunction with the SARDI root disease testing service.

- Using DNA-based quantification to determine economic thresholds and/or impact risk categories for the most prevalent and important nematode species affecting grapevines.

- Assess available and potential management options for responding to damaging nematode populations.

- Facilitate the establishment of a commercial testing service for viticulture through SARDI root disease testing service.

4.2 Project Milestones and Outputs

These are summarised in the following table: -

Milestone	Output
1. Research scientist trained in viticultural nematology	Student completed research, submitted thesis and awarded degree
2. Sample handling and DNA extraction methods for the detection and quantification of nematode parasites of grapevine	Optimal methods and target nematodes for detection recommended
3. Risk categories for the most prevalent and important nematode species affecting grapevines	Threshold categories developed for key nematode species and reported
4. Management options for reducing nematode populations evaluated	Effect of management options evaluated and reported

5. Method

The project involved designed experiments in the field, glasshouse and laboratory and included considerable field sampling.

Determination of nematode incidence, spatial and temporal distribution was done by sampling commercial grapevine plantings in various districts of South Australia. Nematode quantification was achieved by live extraction, bioassay and direct DNA assay of soil. The DNA assay was verified and calibrated by direct addition of known numbers of nematodes and by parallel assessment of populations by standard methods.

Identification of nematodes was achieved by morphology, differential hosts and various DNA methods, including published methods and those developed by the project. Genetic study of nematode populations was done by sequence analysis.

Assessment of impact of root knot nematode was undertaken using grapevines grown in microplots (small field plots surrounded by a barrier to prevent nematode movement and filled with nematode-free soil). Microplots of single vines were established in the vineyard at the Waite campus.

Assays of soil DNA were conducted by the SARDI diagnostic group at the Waite Campus on a fee for service basis.

Pilot evaluation of a DNA test for root knot nematode for viticulture was conducted by recruiting industry collaborators and providing directions on sampling protocol. Samples provided by collaborators were assayed by SARDI

Appropriate statistical analysis was performed using GENSTAT (Release 6, Lawes Agricultural Trust, Rothamsted Experimental Station, UK)

6. Results and Discussion

Development of sampling methods

The distribution pattern of RKN was studied in five vineyards in three locations in South Australia. Nematodes were extracted from soil samples at two depths (0-300 and 300-600 mm) for each of five positions; three along the vine row, one under cover crops and one next to cover crops in the inter-row. *Meloidogyne* spp. were found to be aggregated along the vine rows. The highest RKN population was found in samples taken close to vines, especially those at about 100 mm from the base of vine, and the second highest was in the row between two vines. No significant difference was found between RKN population levels at the two soil depths. RKN populations under cover crops were significantly lower than in the vine rows. It was concluded that core samples for quantification of RKN population in vineyards should only be collected along the rows. To standardise the collection procedure for RKN, it is recommended that soil samples should be collected about 100 mm from the vine to a depth of 300 mm. The effect of cover crops on RKN populations in vineyards is considered to be less important than suggested by others.

Detailed methods, results and discussion for this component of the study can be found in the attached papers.

Quader, M., I. T. Riley and G. E. Walker (2001). Distribution pattern of root-knot nematodes (*Meloidogyne* spp.) in South Australian vineyards. *Australasian Plant Pathology* **30**, 357-360.

Application of sampling method to other grapevine nematodes.

The sampling method developed for RKN was tested for its suitability for ectoparasitic *Xiphinema* spp. and migratory endoparasitic *Pratylenchus* spp., nematodes that also affect grapevines. Using the same methodology, the horizontal and vertical distribution of dagger (*Xiphinema* spp.) and root lesion (*Pratylenchus* spp.) in vineyards was determined. The seasonal distribution pattern of these nematodes was also determined. Dagger nematodes occurred mainly along the rows and their highest density was at 300-600 mm, whereas root lesion nematodes had similar densities in rows and inter-rows and were at highest density at 0-300 mm. The population densities of both the nematodes were greater in October and November (spring) and lowest in February (late summer). The implications of these spatial and temporal patterns on routine sampling for quantification purposes were discussed in the more detailed reports.

Detailed methods, results and discussion for this component of the study can be found in the attached papers.

Quader, M., I. T. Riley and G. E. Walker (2003) Spatial and temporal distribution patterns of dagger (*Xiphinema* spp.) and root-lesion (*Pratylenchus* spp.) nematodes in a South Australian vineyard. *Australasian Plant Pathology* **32**, 81-86.

Quader, M., I. T. Riley and G. Walker (2002c) Nematode parasites in South Australian vineyards. *The Australian & New Zealand Grapegrower and Winemaker* September, 62-64.

Identification of root knot nematode

Identification methods based on the North Carolina differential host test and DNA methods were assessed for their ability to distinguish a collection of South Australian populations of RKN from vineyards. The NC differential host test differentiated *M. incognita* but not *M. arenaria* race 2 from *M. javanica*. A combination of the NC host test and PCR amplification of mtDNA could differentiate between *M. arenaria, M. incognita* and *M. javanica.* A mtDNA based method was successfully used to differentiate *M. arenaria* from *M. incognita* and *M. javanica* by PCR amplification. However, subsequent RFLP analysis of PCR-mtDNA product did not differentiate between *M. incognita* and *M. javanica.* The PCR amplifications of D3 expansion region of 28S rRNA gene and intergenic sequences of ribosomal DNA (IGS-rDNA) were also made to distinguish *M. arenaria, M. incognita* and *M. javanica.* The identification of these species with D3 expansion region of 28S rRNA gene was not possible. The sequences of this region are highly conserved among the species, limiting the possibility of their identification based on this D3 expansion region alone. PCR amplification of IGS-rDNA of genomic DNA from a single female of each species produced distinct banding patterns that can differentiate the species from each other. These species-specific banding patterns were reproducible across a range of individual nematodes of each species collected from different geographical locations of Australia. The method may also be applied to the examination of intraspecific variation of *Meloidogyne.*

DNA assay for quantification of root knot nematode

A DNA based quantification method was evaluated under controlled conditions on species of RKN from grapevines. A clear relationship was found when the DNA assay was applied to soil samples with addition of known numbers of RKN juveniles. A strong relationship was also found between the DNA assay and addition of nematodes for both *M. incognita* and *M. javanica.* The relationship between the DNA assay and number of nematodes added remained robust in both sand and clay soil types. In these experiments, the DNA assay could detect levels as low as 40 juveniles per 400 g soil. The DNA assay appears not only to be adequately sensitive but is consistent for the accurate estimation of both important species (*M. incognita* and *M. javanica*) in both clay and sandy soils, so it is likely that the method could be successfully applied to a range of soils occurring in Australian vineyards.

An overview of aspects of this component of the study can be found in the attached report.

Quader, M., I. T. Riley, Herdina, K. Ophel-Keller and G. Walker (2002b) Root-knot nematode quantification for management options in grapevines. *The Australian & New Zealand Grapegrower and Winemaker* March, 13-16.

Application to the diversity of root knot nematode in South Australia

The sampling and identification methods developed were used to validate the DNA quantification method under vineyard conditions and to survey vineyards of South Australia (SA). A comparative study, based on extraction, bioassay and DNA methods, was performed for the quantification of *Meloidogyne* spp. in vineyards of SA. DNA based species identity and differences in the sequences of internal transcribed spacers (ITS-1 and ITS-2) of rRNA genes in individuals of *Meloidogyne* were also determined. The DNA method was consistently better than commonly used methods for quantification of RKN in various vineyard soils. Four species, *M. javanica, M. incognita, M. arenaria* and *M. hapla*, were recorded in vineyards of SA. The former three species were predominantly found in warmer and *M. hapla* in cooler regions. The DNA sequences in ITS regions of rRNA genes were highly conserved (<2% divergence) among the individuals of the

main species in SA vineyards. Variability in rRNA genes and its relation to the DNA based method for quantification of RKN is discussed.

Damage threshold

A microplot experiment was conducted over two years (2000-2002) to determine the effect of *Meloidogyne incognita* population densities on the growth of grapevine (*Vitis vinifera*) during establishment. Four RKN population densities, 25, 154, 960 and 2400 per 1000 ml soil, were assessed on a susceptible cultivar Colombard and a moderately resistant cultivar Sultana. At the first assessment, there was a direct relationship between inoculum density and root gall number in Colombard, but no galls were found in Sultana roots. In the first growing season, RKN did not reduce the growth of either cultivar. However, in the second season, RKN population densities greater than 25 per 1000 ml soil significantly reduced the pruned weight of Colombard but increased pruned weight in Sultana. Therefore, the damage threshold of RKN for grapevines will vary between cultivars. However, for an apparently intolerant cultivar, such as Colombard, the damage threshold for RKN would be about 1 to 25 per 1000 ml soil at establishment. The damage threshold density was found to be 1.5 *M. incognita* per 1000 ml soil by the Seinhorst crop-loss model. This damage threshold for *M. incognita* on grapevines and its implication to the decision making process for the establishment of a vineyard is discussed.

Detailed methods, results and discussion for this component of the study can be found in the attached paper.

Quader, M., I. T. Riley and G. E. Walker (2002a). Damage threshold of *Meloidogyne incognita* for the establishment of grapevines. *International Journal of Nematology* **12**, 125-130.

Pilot industry evaluation of DNA

Of the 123 sites assessed, RKN was detected in 41% with 88% of those having densities considered to pose a medium to high risk of yield loss. The samples came from six different regions in South Australia, McLaren Vale (45 sites), Willunga (26), Langhorne Creek (31), Clare (5), Barossa (3) and Adelaide Hills (13), with the proportion of sites infested varying between regions. Excluding Clare and Barossa, for which too few sites were sampled, Langhorne Creek had the highest proportion of infested samples (65%). Only one infested sample came from Willunga and Adelaide Hills. Since, this pattern will reflect the composition of collaborators participating in the study, a systematic investigation of the incidence of RKN is needed for confirmation.

Like previous investigations, this study found that RKN is significantly ($P<0.001$) more common in light soil (71%) than in heavier clay soils (17%). With the grapevine cultivars grouped as Shiraz, Cabernet Sauvignon and other (61, 22 and 39 samples respectively), there was no statistically significant difference in the frequency of RKN infestation. The age of grapevines was found to have a significant ($P=0.003$) effect, with older vines more commonly infested (65% vs 27% for more than and less than 10 years old). The frequency of infestations was greater ($P=0.02$) when a cover crop was grown (44% n=96 vs. 8% n=25 for cover crop vs. no cover crop). This was an unexpected finding and the mechanism is not clear. Earlier work indicated that the inter-row population did not effect the population in the grapevine row. Further investigation of the relationship between cover crops and RKN infestation is clearly warranted.

The relationships between RKN infestations and other factors found in this work should only be considered descriptive. The data set was not large enough to examine interactions between factors and causality cannot be assumed.

The sampling protocol used in this study was found to be acceptable to collaborators, being easy to understand and perform. The DNA assay appears to be a robust means of detecting and quantifying RKN infestations. The feedback from the collaborators was positive and largely identified an interest in access to a reliable test for RKN as provided by the DNA assay.

In addition to obtaining industry feedback on the use of the DNA assay, the study reconfirmed the high incidence and potentially damaging levels of RKN in SA vineyards. The data can only be interpreted within the constraints of the study, but it does indicate that further work on the impact and management of RKN in grapevines is necessary.

7. Outcome/Conclusions

Root knot nematode species were found to be the most common plant parasitic nematodes in grapevines in South Australia. The four most agriculturally important species were found and most detected populations where at levels likely to be causing damage. The DNA test as developed by SARDI was confirmed to be a robust and acceptable means of quantifying nematode populations including populations of root knot nematode in grapevines. The study has highlighted the potential importance of root knot nematode to viticulture in Australia and has provided support for the application of DNA testing for quantification of population densities. This technology overcomes a serious constraint to determining the likely impact of the nematode and the effect of various management activities on its population dynamics.

The project has met its aims in that a research scientist has been trained in viticultural nematology and is currently available for employment within Australia. Sample handling and DNA extraction methods for the detection and quantification of nematode parasites of grapevine have been developed and where appropriate published. Studies on the impact of initial population densities of root knot nematode on vine establishment has permitted the establishment of risk categories for root knot nematode, the most prevalent and important nematode species affecting grapevines. Experiments evaluating management options for reducing nematode populations were established but proved inconclusive and could not be pursued further with the time and resources available.

8. Recommendations

It is recommended that the provision of a commercial test for root knot nematode be encouraged and that additional work necessary be undertaking in partnership with potential service providers. Synergies with other horticulture industries needing to test for root knot nematode, and potentially with survey work for phylloxera, should be explored.

Given the prevalence of root knot nematode in grapevines in South Australia and that root feeding nematodes impair the uptake of water and nutrients, further work on the impact of these nematodes should be undertaken, especially in the context of efforts to improve the efficiency of water use in viticulture.

Appendix 1: Communication

The following publications have arisen from the project

Thesis

Quader, M. (2003). *DNA based sampling, detection, quantification and damage threshold of Meloidogyne spp. in grapevine.* PhD thesis (Department of Applied and Molecular Ecology, University of Adelaide) [Copy supplied to GWRDC]

Scientific Journals

Quader, M., I. T. Riley and G. E. Walker (2001). Distribution pattern of root-knot nematodes (*Meloidogyne* spp.) in South Australian vineyards. *Australasian Plant Pathology* **30**, 357-360. [Attached]

Quader, M., I. T. Riley and G. E. Walker (2002a). Damage threshold of *Meloidogyne incognita* for the establishment of grapevines. *International Journal of Nematology* **12**, 125-130. [Attached]

Quader, M., I. T. Riley and G. E. Walker (2003) Spatial and temporal distribution patterns of dagger (*Xiphinema* spp.) and root-lesion (*Pratylenchus* spp.) nematodes in a South Australian vineyard. *Australasian Plant Pathology* **32**, 81-86. [Attached]

Walker, G. E, M. Quader and I. T. Riley (2004) Root-knot nematode species from South Australian vineyards. Australian Journal of Grape and Wine Research [Submitted 3.10.2003]

Extension articles

Quader, M., I. T. Riley, Herdina, K. Ophel-Keller and G. Walker (2002b) Root-knot nematode quantification for management options in grapevines. *The Australian & New Zealand Grapegrower and Winemaker* March, 13-16. [Attached]

Quader, M., I. T. Riley and G. Walker (2002c) Nematode parasites in South Australian vineyards. *The Australian & New Zealand Grapegrower and Winemaker* September, 62-64. [Attached]

Conference presentations

Quader M. and I. Riley. (2003) Critical level of root-knot nematode for the establishment of grapevines. 8th International Congress of Plant Pathology, 2-7 February 2003, Christchurch, New Zealand.

Quader M. and I. Riley. (2003) The host test and DNA methods to distinguish root-knot nematodes from grapevine. 8th International Congress of Plant Pathology, 2-7 February 2003, Christchurch, New Zealand.

Collins C. and I. T. Riley (2004) Populations of root-knot nematode (*Meloidogyne* spp.) in SA vineyards determined by DNA assay of soil. 3rd Australasian Soilborne Disease Symposium, 8-11 February, 2004, Rowland Flat, South Australia. [Summary submitted]

Appendix 2: Intellectual Property

Substantial data under pining the application of the DNA test for root knot nematode in vineyard soil has been provided by this project. Methods for the extraction of soil DNA and the quantitative PCR for estimation of *Meloidogyne* in soil are the intellectual property of SARDI and have not been provided to this project. All use of this technology has been on a fee for service base.

The project has clearly shown that the DNA assay is a robust and acceptable means of estimating root knot nematode numbers associated with grapevines. Provision of a testing service to the viticultural industry will be dependant on the commercial parameters, the interest of a commercial provider and the involvement of SARDI. Commercial development is more likely if a root knot nematode assay could be conducted in parallel to other tests, for instance a test for phylloxera.

Appendix 3: References

Relevent publications/references:

Brown D. J. F., Dalmasso A. and Trudgill D. L. (1993). Nematode pests of soft fruits and vines. *In:* Plant Parasitic Nematodes in Temperature Agriculture (eds K. Evans, D. L. Trudgill and J. M. Webster). CAB International, Wallingford UK. pp. 427-462.

Esnard J. and Zuckerman B. M. (1998). Small Fruits. *In:* Nematode Interactions (eds K. R. Barker, G. A. Pederson and G. L. Windam). American Society of Agronomy, Madison, Wisconsin. pp. 685-725

New Cereal Root Disease Testing Service. Background, South Australian Research and Development Institute, 4 pp.

May P. (1994) Using grapevine rootstocks: the Australian perspective. Winetitles, Cowandilla, SA. pp. 62

Walker G. E. (1994) Growth of grapevine rootlings in soil from a field nursery naturally infested with *Meloidogyne incognita* and *Rhizoctonia solani*. *South African Journal for Enology and Viticulture* 15: 26-32

Walker G. E. (1994) Evaluation of Nemacur for control of citrus nematode in grapevine rootlings, 1992. *Fungicide and Nematicide Tests* 49, 180.

Walker G. E. (1997) Effects of *Meloidogyne* spp. and *Rhizoctonia solani on* the growth of grapevine rootlings. *Journal of Nematology* 29, 190-198.

Appendix 4: Staff

Dr Motiul Quader, University of Adelaide, PhD student (provide scholarship from project)

Ms Cassandra Collins, University of Adelaide, causal research officer

Dr Ian Riley, University of Adelaide, PhD student supervisor

Dr Greg Walker, SARDI, PhD student supervisor

Dr K Ophel-Keller, SARDI, collaborator

Ms Herdina, SARDI, collaborator

Dr A. McKay, SARDI, collaborator

Appendix 5: Other relevant Material

1. A CD with copies of each of the published papers has been provided as an addendum to this report.

2. Evaluating the need for a DNA-based assay service for RKN in grapevine (see below for details)

«FirstNames» «Surname»
«ContractTitle»
«Address1»
«Address2» «State» «PC»

Dear «FirstNames»

Thank you for your participation in a field evaluation of DNA testing for root-knot nematodes (RKN) in South Australian vineyards. All soil samples have been analysed for DNA to estimate the population density of RKN in the soil. A summary of the data is enclosed.

RKN was detected in about 40% of the samples, with about 90% of these at medium to high population densities, densities that are likely to be damaging to the grapevines. The frequency and level of these detections was greater than we expected.

Details of the sample you provide are listed below and copies of your submission sheet enclosed for reference.

[place results here – Sample number, RKN estimate and ranking.]

Your participation will help us evaluate the need for a DNA-based assay service for RKN in grapevine. To further assist with this, please complete the enclosed response sheet and return it in the reply paid envelope provided. Also, we may need to contact some participants in this study by telephone for further views on the usefulness of such a service.

Yours sincerely

DR IAN RILEY
Lecturer in Nematology

DNA testing for root-knot nematode in grapevines

Summary of results[§]

Cassandra Collins and Ian Riley, CRC for Viticulture

School of Agriculture and Wine, Waite Campus

Please keep these results confidential. They are preliminary only and we ask that no one distribute them beyond the growers who cooperated in the trial. Any enquiries regarding their use should be directed to Dr Ian Riley ph 8303 7259.

Samples and overall results

- 123 samples from 39 vineyards were assayed for root-knot nematode DNA
- 50 samples (41%) tested positive for root knot nematode (RKN)

Conclusion; RKN is common in grapevines in South Australia

Population densities

Number*	Ranking	Samples
<50	Low	6
50-100	Medium	3
>100	High	41

*Estimated RKN number per 400 g of soil. Range 0 to 1885. Median = 1022.

Conclusion; RKN densities reach potentially damaging levels in most of these infestations.

Region

Region	Samples	RKN infested*	Proportion (%)
McLaren Vale	45	18	40
Langhorne Creek	31	20	65
Willunga	26	1	4
Adelaide Hills	13	0	0
Clare	5	2	40
Barossa	3	3	100
Total	**123**	**44**	**36**

*Medium and high population densities only

Conclusion; RKN infestation levels varied between regions.

Langhorne Creek was the highest. Adelaide Hills and Willunga were the lowest. Too few samples collected in Clare and Barossa to give a reliable comparison.

Soil type

[§] Note - Raw data presented without statistical analysis. Relationship may not indicate cause and effect. Further analysis will be needed to give strong conclusions.

Soil type	Samples	RKN infested	Proportion (%)
Light	42	30	71
Medium	48	9	19
Heavy	30	5	17
Total	**120**	**44**	**37**

*Medium and high population densities only

Conclusion; As expected, light soils favour RKN. Damaging densities can occur in all soil types.

Vineyard age

Age	Samples	RKN infested	Proportion (%)
1-10 yrs	90	24	27
>10 yrs	31	20	65
Total	**121**	**44**	**36**

*Medium and high population densities only

Conclusion; Older vineyards were more commonly infested.

Variety (cultivar)

Variety	Samples	RKN infested	Proportion (%)
Shiraz	61	18	30
Cabernet Sauvignon	22	9	41
Other	39	17	44
Total	**122**	**44**	**36**

*Medium and high population densities only

Conclusion; Own-rooted plants of common varieties are all susceptible.

Appendix 6: Budget Reconciliation