Industry allocated project number

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Indicate (X) client(s) to whom this final report is submitted. Replace any of these with other relevant clients if required.

FINAL REPORT
2014

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
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Project Information

Research Organisation Project number	230073
Project title	Measurement and evaluation of the effects of soil factors on ring nematode (*Criconemoides xenoplax*)

Fruit kind(s)	Stone, Vine		
Start date (mm/yyyy)	01/04/2009	**End date** (mm/yyyy)	31/03/2014

Project keywords	Ring nematode, stone fruit, vines, soil factors

Approved by Research Organisation Programme leader (tick box) ✓

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

Executive Summary

Give an executive summary of the total project.

The objective of this project was three-fold: (1) to determine if soil factors such as soil texture and pH have any effect on ring nematode (*Criconemoides xenoplax*) (RN); (2) determine the vertical distribution of RN in soil for different crops; and (3) determine if RN populations exhibit seasonal fluctuations.

Samples received at ARC Infruitec-Nietvoorbij's nematode diagnostic service and at Nemlab were selected for their RN numbers: either classified as "low" (< 250 RN/250 cm^{-3} soil) or "high" (> 1 000 RN/250 cm^{-3} soil). Seventy-two samples were thus selected, representing all the major fruit and vine areas of the Western Cape, and also the Lower Orange River.

Soil from these samples was analysed for five different soil fractions, namely coarse sand, medium sand, fine sand, loam, and clay.

Our results show that the different soil fractions had no effect on RN numbers and that high RN numbers occurred in all types of soil, both sandy and clayey type soils.

The results also showed that pH values ranging between 4.5 – 7.0 had no effect on RN numbers.

To determine the vertical distribution of RN and also if seasonal fluctuations occur, a vineyard and a nectarine orchard was sampled in January, April, July, and October. Samples were taken at 20 cm intervals down to 1 m deep. Although RN numbers decreased below 60 cm depth, their numbers were still high enough at the 80-100 cm depth to cause serious damage. No seasonal fluctuations were observed.

The outcome of this project was:

1. This study confirms that soil fractions do not affect RN numbers;
2. Although almost 50% of the RN population present in a plant's root zone occurs in the top 40 cm soil, damaging high levels can occur to at least 1 m deep, posing a serious problem for effective nematode control. These high nematode numbers below the zone that can be effectively fumigated provide a source for re-infecting replanted vineyards and orchards;
3. Seasonal fluctuations do not occur, therefore samples for diagnostic analysis can be taken at any time of the year.

Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

During the past 10-15 years both the ARC Infruitec-Nietvoorbij and Nemlab nematode diagnostic laboratories observed an increase in the numbers of ring nematode present in samples from

vineyards and stone fruits. Numbers often exceed 2 000/250 cm^3 soil, whereas in the late 1970's and early 1980's, numbers of over 1 000 were seldom encountered (Personal communication, A.J. Meyer, nematologist, Univ. Stellenbosch, 1980 & P.C. Smith, nematologist, PPRI, 1981) This is regarded as extremely high, since a figure of 500/250 cm^{-3} soil is considered damaging and warranting control. This increase is at least partly due to the increased use of Ramsey and Richter 99 as rootstocks, as both rootstocks are regarded as resistant to root-knot nematode, but very good hosts for RN. The situation is further complicated as the registered nematicides are not very effective in bringing down the numbers of this specific nematode.

According to the manufacturers of the nematicides fenamiphos and cadusafos, both these chemicals do not readily move deeper than 30 cm in soils. We therefore need to know the scope of the vertical distribution of RN in soils and also if different soil factors influence RN numbers, either positively or negatively.

Presently we rely on the study by Seshadri (1964) on the biology of RN in California. Personal observations during ARC Infruitec-Nietvoorbij's nematode diagnostic service and by S. Storey (2008, personal communication) and A. J. Meyer (2008, personal communication) differ from some of Seshadri's findings. Locally, RN has been found in high numbers in soils with a high clay content, low pH, and low soil moisture. This differs from Seshadri's observations in California, where he found that RN prefers sandy soils, a pH of 5-7, and a high soil moisture level. Observations by Hoffmann & Norton (1976) showed that RN can occur in a wide range of soil types, in contradiction to Seshadri's findings.

RN has been found in undisturbed fynbos, indicating that this nematode is indigenous. This means that the South African population has evolved over thousands of years to be completely adapted to South African conditions. This may explain why RN, although occurring worldwide, is such a serious nematode pest in South Africa, but not in some other fruit and vine growing areas in the world. Research by Pinkerton *et al*. (2005) showed that there are differences between RN populations from different areas and those different populations can differ in their pathogenicity.

It is therefore important that the biology of RN under local conditions be researched. This knowledge is essential to develop an effective management strategy.

The objective of this project was to:

- Determine if specific soil factors, such as soil fractions and pH, favour high RN numbers;
- Determine the vertical distribution patterns of RN for vineyards and stone fruit orchards;
- Determine if RN populations exhibit seasonal fluctuations.

HOFFMANN, J.K., & NORTON, D.C. 1976. Distribution patterns of some Criconematidae in different forest associations. J. Nematol. 8, 32-35.

PINKERTON, J.N., VASCONCELOS, M.C., SAMPAIO, T.L., & SHAFFER, R.G. 2005. Reaction of grape rootstocks to ring nematode *Mesocriconema xenoplax*. Am. J. Enol. Vitic. 56, 377-385.

SESHARDI, A.R. 1964. Investigations on the biology and life cycle of *Criconemoides xenoplax* Raski, 1952 (Nematoda: Criconematidae). Nematologica 10, 540-562.

Workplan (materials and methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

A: Effect of soil factors on RN

Seventy-two orchards and vineyards were selected on the basis of the RN numbers present, either high numbers (> 1000/250 cm^{-3} soil) or low numbers (< 250/250 cm^{-3} soil) and from different production areas, including the Lower Orange River, and all the main fruit and vine areas of the Western Cape.

Soil from each of these orchards/vineyards were analysed by Bemlab. A five-fraction soil (coarse sand, medium sand, fine sand, silt, clay) and a pH analysis were done on each sample.

Multivariate Principal Component Analysis (PCA) was performed on the data using XLStat (Version 2012, Addinsoft, New york, USA) to visualise and elucidate the relationships between nematode count classes and soil fraction variables. Pearson correlation analysis was performed to test for linear relationships between nematode numbers and soil fractions. Stepwise regression was performed to determine which soil fractions could contribute significantly to a multiple regression model predicting nematode counts.

B: Vertical distribution and seasonal fluctuation of RN

1. Vine

A Merlot on Ramsey rootstock vineyard with high numbers of RN, was selected in the Franschhoek area.

Sampling commenced in October 2010. Samples were taken at 3-month intervals up to July 2012 to give two years' seasonal data (summer, autumn, winter, spring). Data for the two years was pooled to give an average based on two years' observations.

Soil samples were obtained by digging a hole just over 1 m deep, approximately 1 m from the trunk from the work row side. Samples were taken from the soil profile at 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm depths. To select vines for sampling, a "vakkie" was randomly selected and the third vine in the "vakkie" used for sampling. Thereafter the next four adjacent "vakkies" were used, and the corresponding five "vakkies" in the adjacent row, for a total of 10 "vakkies", equalling 10 replicates per sampling date. The third vine in each "vakkie" was always sampled. Due to the disruptive nature of the sampling process, different "vakkies" were selected on each sampling date.

2. Peach

A nearby nectarine orchard, Alpine on Kakamas, also with high RN numbers, was selected for the peach study. Similar sampling procedures as for the vines were used, except that the first tree to be sampled was randomly selected. Thereafter every second tree in the same row was sampled for a total of five trees, and the corresponding five trees in the adjacent row, to make up a total of 10 trees sampled on each sampling date. Due to the disruptive nature of sampling method, different trees were selected on each sampling date.

RN numbers were determined by using our standard centrifuge-flotation extraction method and counting 2 x 1 ml aliquots from a 20 ml suspension.

Results and discussion

State results obtained and list any industry benefits. If applicable, include a short discussion covering ALL accumulated results from the start of the project. Limit it to essential information only.

A: Effect of soil factors on RN

A Principal Component Analysis (PCA) (Fig 1) of the data showed no definite patterns. This is a very strong indication that high RN numbers are not limited by specific soil fractions.

This contradicts the findings of Seshardi (1964) who found that RN prefers sandy soils, but support Hoffmann & Norton (1976) who showed that RN can occur in a wide range of soil types. Our results from this project also confirm our field observations where on a number of occasions high RN numbers were found in clayey soils.

The implication of this finding is that RN can occur in any soil type, irrespective of the clay content. It also differs from our initial observation based on a small set of samples, which found that high RN numbers do not occur in soils with a clay content of more than 16%.

These findings that RN can occur in high numbers in all soil types dispel the commonly held belief that RN is not a problem in soils with a high clay content. This knowledge will help advisors and producers to take the threat of RN more seriously and to plan accordingly.

Effect of pH on RN

There was no correlation between RN numbers and pH ($r = -0.0647$) (Fig 2). This is further evidence that in general, pH is not a limiting factor for nematodes. It also support the general belief that plant-parasitic nematodes are very hardy animals that are well adapted to live and multiply in a wide range of environmental conditions.

B: Vertical distribution and seasonal fluctuation of RN

1. Vine

The data in Table 1 represents the distribution of RN at 20 cm intervals down to a depth of 1 m.

There were no significant differences ($p = 0.05$) between the different seasons at the shallower depths (0-20 cm; 20-40 cm). This is the depth where almost 50% of the RN populations occur. This confirms our previous findings that sampling for diagnostic purposes can be taken anywhere from just below the surface down to about 25-30 cm at any time of the year. Such samples will give a reliable indication of the RN numbers present.

At the 40-60 cm depth the RN numbers for July (winter) were significantly higher than for the other seasons, but at the deeper levels there were again no differences. We have no explanation for the differences occurring at the 40-60 cm depths.

RN numbers were significantly less below 60 cm depth (p = 0.05). However, this is only of academic interest, as the actual RN numbers are still around 500 RN/250 cm^{-3} soil, which are considered as damaging high levels.

2. Peach

Results from the peach rootstock trial (Table 2) gave similar results as the vine trial. There were no seasonal differences (p = 0.05) at any of the depths, except at the deepest level of 80-100 cm. In this case it can possibly be attributed to differences in the amount of roots available at such a deep depth.

As in the case of the vines, again almost 50% of the RN population occurred in the top 40 cm soil.

There were no statistical differences between these two diverse crops (vines and stone fruit) (p = 0.05) and the results concur with the results obtained with Marianna rootstock (Project 230022; Final Report 2004). We therefore postulate that the presence of roots and not soil depth determines the vertical distribution of RN, and furthermore, that the RN distribution will be similar for all host crops.

The occurrence of damaging high levels of RN occurring at depths below 40 cm, poses a serious challenge for efficient nematode control, as it is extremely difficult, if not impossible, to get any of the present nematicides or pre-plant fumigants to depths deeper than about 30-40 cm depth. As the nematodes at the deeper depths are not controlled, they will migrate to the shallower depths when a suitable food source becomes available and cause damage when a plant is still young and very vulnerable to root damage.

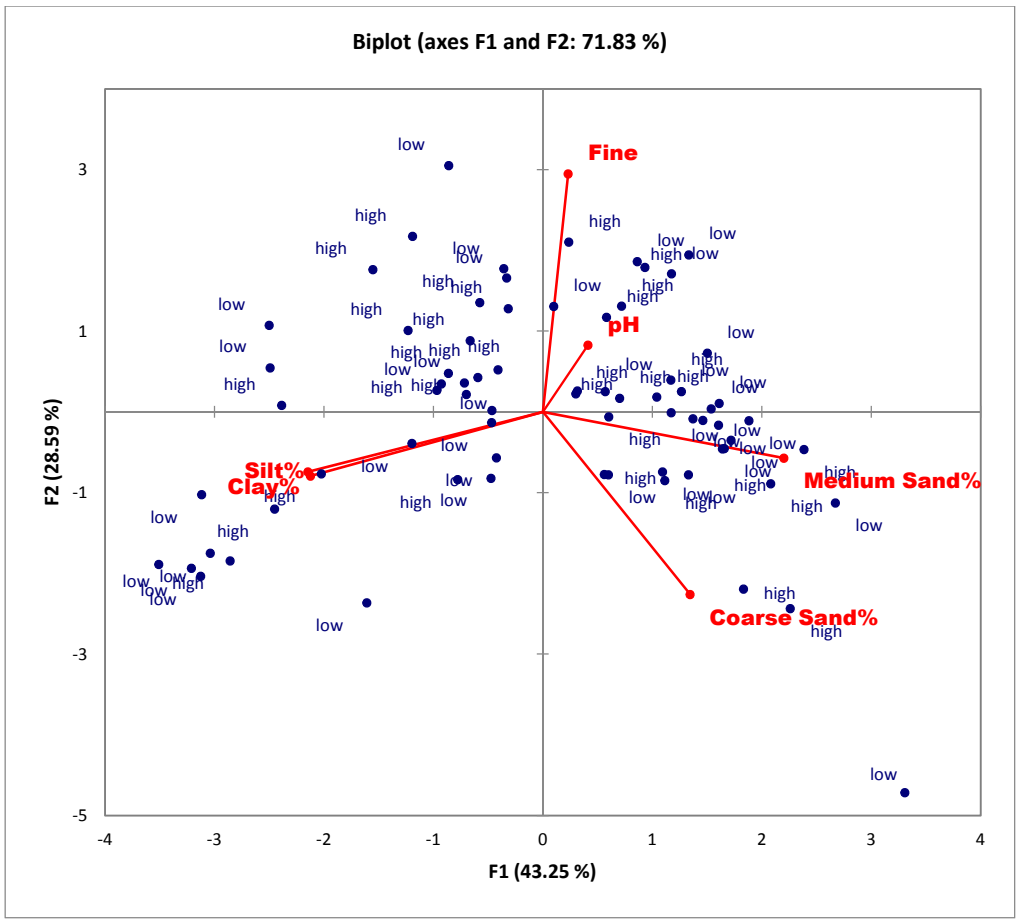

Fig 1. **PCA Biplot of high/low RN numbers vs soil fractions**

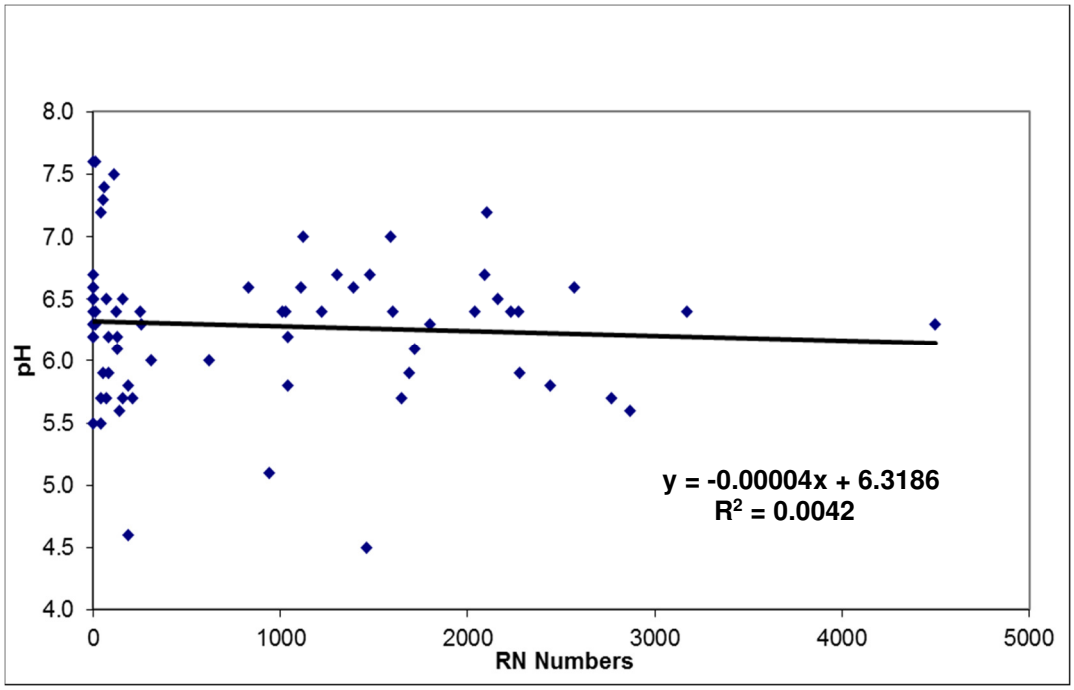

Fig 2. **Effect of pH on RN**

Table 1. Vine: RN numbers at different soil depths (Average of 2 years' data)

	0-20 cm	20-40 cm	40-60 cm	60-80 cm	80-100 cm
January	987 a	1020 a	786 b	810 a	493 a
April	885 a	923 a	586 b	567 a	522 a
July	1042 a	1107 a	1263 a	847 a	593 a
October	842 a	1119 a	731 b	686 a	489 a
Distribution	23,0%	25,6%	20,7%	17,9%	12,9%
	LSD = 332 (p = 0.05)	LSD = 484 (p = 0.05)	LSD = 357 (p = 0.05)	LSD = 390 (p = 0.05)	LSD = 337 p = 0.05)

Table 2. Peach: RN numbers at different soil depths (Average of 2 years' data)

	0-20 cm	20-40 cm	40-60 cm	60-80 cm	80-100 cm
January	981 a	893 a	861 a	691 a	341 b
April	1074 a	806 a	813 a	712 a	519 ab
July	953 a	967 a	712 a	466 a	365 ab
October	903 a	983 a	994 a	753 a	603 a
Distribution	25,4%	23,7%	22,0%	17,0%	11,9%
	LSD = 379 (p = 0.05)	LSD = 369 (p = 0.05)	LSD = 357 (p = 0.05)	LSD = 339 (p = 0.05)	LSD 248 (p= 0.05)

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Literature review: RN on vines	May 2010		May 2010	Achieved
2. Analyse data from 50 orchards/vineyards	March 2011		March 2011	Achieved
3. Complete seasonal fluctuation samples	March 2012		March 2012	Achieved
4. Complete vertical distribution sampling	March 2012		March 2012	Achieved
5. Soil factors: analysis completed	March 2013		March 2013	Achieved
5. Journal publication/s – final milestone	May 2015			Popular article in Wynboer Semi-scientific article in S.A. Fruit Journal

Accumulated outputs

List ALL the outputs from the start of the project. The year of each output must also be indicated.

2010: Submit progress report;
2010: Submit literature review of RN on vines to Winetech;
2011: Submit progress report
2012: Submit progress report;
2014: Submit final report.

Conclusions

Using multiple variable analysis, no association between RN numbers and different soil fractions could be found. Therefore, the percentage sand or clay that is present in a soil is not a limiting factor for high RN numbers.

We also found that soil pH (KCl) between 4.5 – 7.0 had no effect on RN numbers.

RN occurred throughout the soil profile to at least 1 m deep, with almost 50% of the population occurring in the top 40 cm soil. We postulate that soil depth is not a limiting factor for RN, but that RN will follow the roots, wherever the roots occur.

In perennial crops such as vines and deciduous fruit trees, RN occurred in high numbers throughout the year, with no seasonal fluctuations.

Technology development, products and patents

Indicate the commercial potential of this project, eg. Intellectual property rights or commercial product(s)

None

Suggestions for technology transfer

List any suggestions you may have for technology transfer

Talks to wine and fruit producers at Farmers' Days.
Popular article in Wynboer.
Semi-scientific article in S.A. Fruit Journal.

Human resources development/training

Indicate the number and level (eg. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

Student level (BSc, MSc, PhD, Post doc)	Cost to Project
1. None	
2.	

Publications (popular, press releases, semi-scientific, scientific)

None

Presentations/papers delivered

None

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST
YEAR 1	2009/10
YEAR 2	2010/11
YEAR 3	2011/12
YEAR 4	2012/13
TOTAL	

CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
		44969		44970		93606	183546
		48675		49467		102966	201900
		51548		52432		110028	214008
		145192		146869		306600	**599454**