Industry allocated project number

SATI	CFPA	SAAPPA/SASPA	DFTS	Winetech
tarryn@satgi.co.za Tel: 021 872-1438	inmaak@mweb.co.za Tel: 021 872-1501	theresa@hortgro.co.za Tel: 021 882-8470	dappies@dtd.co.za Tel: 021 870 2900	andraga@winetech.co.za Tel: 021 807 3387
				x

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

FINAL REPORT
2013

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
Title, initials, surname	Ms R Carstens	Dr. Francois Halleen
Present position	Acting Research Leader: Plant Protection	Specialist Researcher
Address	ACR Infruitec-Nietvoorbij Private Bag X5026 Stellenbosch 7599	ARC Infruitec-Nietvoorbij Private Bag X5026 Stellenbosch 7599
Tel. / Cell no.	(021) 809 3023	(021) 809 3040
Fax	(021) 809 3002	(021) 809 3002
E-mail	CarstensR@arc.agric.za	HalleenF@arc.agric.za

Project Information

Research Organisation Project number	WW06/39
Project title	The role of insects in the dispersal of trunk disease pathogens.

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	01/04/2009	**End date** (mm/yyyy)	31/03/2013

Project keywords	Arthropods, Petri disease, esca, Botryosphaeria canker and dieback, Eutypa dieback, *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., Botryosphaeriaceae, Diatrypaceae, Diaporthales, *Vitis vinifera*, pruning wounds

Approved by Research Organisation Programme leader (tick box) **X**

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

Executive Summary

Give an executive summary of the total project.

Petri disease and esca are devastating grapevine trunk diseases and compromise the sustainability of viticulture world-wide. Despite being extensively studied, knowledge of inoculum sources and mechanisms of spread of the causal pathogens is limited. Arthropods have been suspected to play a role in the spread of Petri disease and esca pathogens. However, little information is known about the extent to which arthropods are associated with these pathogens. This study aimed to determine whether arthropods occurring within or on declining grapevines, are associated with trunk disease pathogens and to identify arthropods associated with pruning wounds. The potential of selected arthropods to act as vectors of trunk disease pathogens was also investigated.

Two vineyards exhibiting grapevine trunk disease infections were sampled weekly for two years for collection of arthropods. Arthropods were collected using pruning wound traps, visual searches as well as trunk and cordon traps. Fungal spores from surfaces of arthropods were collected in water. Samples were subjected to nested PCR using primers Pm1/Pm2 and Pch1/Pch2 to verify the presence of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora*, respectively. Water samples were also cultured and grapevine trunk disease pathogens obtained were identified by sequencing the internal transcribed spacers 1 and 2 and the 5.8S rRNA gene or the partial beta-tubulin gene. A total of 10 875 arthropod individuals, belonging to more than 31 families, were collected from declining grapevines. The most abundant arthropods included millipedes, ants, spiders and beetles. Portuguese millipedes and cocktail ants were associated with fresh grapevine pruning wounds. Thirty-three percent of the 5677 water samples analysed, contained propagules of pathogens associated with Petri disease and esca. Of these, 37 % were recovered from millipedes, 22 % from cocktail ants, 15 % from spiders and 10 % from beetles. All the major groups of grapevine trunk diseases were detected on the arthropods. *Phaeoacremonium* species were detected in 1242 samples while *Phaeomoniella chlamydospora* was identified from 855 samples. Other fungi isolated included members of the Botryosphaeriaceae, Diatrypaceae and Diaporthales.

The potential of grapevine sap as a food source for Portuguese millipedes and cocktail ants was investigated, *in vitro*. Millipede individuals were offered a choice between water and grapevine sap while ants in nests were presented with grapevine sap, tuna and water and monitored for ingestion of sap. Both taxa preferred grapevine sap over the other food items, indicating close association with pruning wounds. Subsequently, the ability of both taxa to transmit a DsRed-transformed *Phaeomoniella chlamydospora* isolate to fresh pruning wounds of canes in polystyrene strips, floating in water, and potted vines was tested. Arthropods were exposed to the fungus for 24 hours and transferred to the base of the plants and canes and were removed after three days. Isolations after a month revealed that millipedes and ants were capable of transmitting the fungus onto wounds and cause infection. Millipede faecal pellets were also evaluated as potential sources of inoculum. Millipedes were fed on *Phaeomoniella chlamydospora* for 24 hours, surface sterilised and allowed to defaecate in sterile Petri dishes overnight. Faecal material was collected, macerated in water and plated onto potato dextrose agar. Propagules of *Phaeomoniella chlamydospora* survived passage through the gut of millipedes and were passed out in a viable state to form colonies of *Phaeomoniella chlamydospora*.

This study concludes that a wide variety of arthropods can be a source of inoculum of trunk diseases in vineyards. The results of the dissemination trial provides evidence that millipedes and ants are able to disseminate and infect vines with *Phaeomoniella chlamydospora.* It is therefore, highly likely that other grapevine trunk disease pathogens are transmitted in the same manner.

This knowledge highlights the need for control of certain arthropods to be taken into consideration when managing grapevine trunk disease pathogens.

Problem identification and objectives

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

Several pathogens are associated with trunk diseases of grapevines. These pathogens cause the decline and dieback of grapevines, which leads to severe economic losses due to the reduction in quantity and quality of grapes. The control of trunk disease pathogens is difficult since no fungicides are available or registered. Consequently, the management of inoculum sources must form part of an integrated disease management strategy. Infected planting material is a known source of inoculum; however, the spread from infected vines to healthy vines remains unclear. Insects could be role players in the spread of trunk disease pathogens, but very little research has been done in this area.

The objectives of this study will be to:

(1) determine if any particular insects are associated with declining grapevines and
(2) determine if specific trunk disease pathogens occur on these insects.

Workplan (materials and methods)

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

See attached MSc Thesis "The role of arthropods in the dispersal of trunk disease pathogens associated with petri disease and esca" by P. Moyo.

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.

See attached MSc Thesis "The role of arthropods in the dispersal of trunk disease pathogens associated with petri disease and esca" by P. Moyo.

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Monitoring and collection of arthropods from diseased plants.	continuous		Nov 2011	Trunk and cordon traps, manual searches, and pruning wound traps were all effective in collecting 10 875 "insects" from diseased vines (over two seasons).
2. Screening of arthropods for fungal spores.	continuous		Nov 2011	5 677 water samples from arthropod washings were obtained.
3. Identification of pathogens collected on arthropods.	continuous		July 2012	*Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, Botryosphaeriaceae, Diatrypaceae and Diaporthales were identified.
4. Identification of arthropods that tested positive for trunk disease pathogens.	continuous		July 2012	Several arthropod species tested positive, including millipedes, ants, spiders, beetles, cockroaches, earwigs, springtails, etc. 52 spider species were associated with declining vines, 39 of which tested positive for a trunk pathogen. This is the 1st report of the spider *Euryopes episiniodes* in Africa. It occurred frequently and tested positive for *Phaeoacremonium*, *Phaeomoniella* and Botryosphaeriaceae.
5. Arthropods as possible vectors of grapevine trunk disease pathogens	Nov 2012		Aug 2012	Spiders, ants, beetles and springtails were directly associated with infected pruning wounds.
6. Attraction to pruning wounds (Food choice studies).	Nov 2012		Aug 2012	Millipedes and ants were attracted to and feed on grapevine sap indicating close association with pruning wounds.
7. Transmission of fungal propagules from arthropods to pruning wounds	Nov 2012		Aug 2012	Controlled experiments revealed that the ant *Crematogaster peringueyi* and millipede *Ommattoiulus moreleti* could effectively vector trunk disease pathogens.
8. Millipede faecal pellets as sources of inoculum on grapevine pruning wounds	Nov 2012		Aug 2012	The excreta of millipedes had viable spores of *Phaeomoniella chlamydospora* and may serve as inoculum source.
5. Journal publication/s – final milestone			Dec 2012 July 2013	1 MSc Thesis (Cum Laude), P. Moyo 1 Scientific Publication submitted(Journal of Applied Ecology), Impact Factor 4.74

Accumulated outputs

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

Conclusions

Numerous arthropods, including beneficial predators, are potential vectors of grapevine trunk disease pathogens. Our results highlight the need for an integrated approach, including targeted management of pests such as ants and millipedes at the time of pruning, to effectively control the spread of grapevine trunk diseases.

Technology development, products and patents

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

Identification of arthropods associated with grapevine trunk diseases.

Suggestions for technology transfer

List any suggestions you may have for technology transfer

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. BSc Hons (M vd Merwe)	R0
2. BSc Hons (P. Moyo)	R0
3. MSc (P. Moyo)	R0

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

Scientific Publication:
Providence Moyo, Elleunorah Allsopp, Francois Roets, Lizel Mostert and Francois Halleen (2013) Arthropods as vectors of grapevine trunk disease pathogens. Journal of Applied Ecology (Submitted).

Presentations/papers delivered

Papers at International Conferences:
Arthropods disseminate Petri and several other grapevine trunk disease pathogens. 8th International Workshop on Grapevine Trunk Diseases (18-21 June 2012, Valencia, **Spain**). P. Moyo, F. Halleen, F. Roets, E. Allsopp & L. Mostert

Papers at National Conferences:
Dissemination of Petri disease and esca pathogens by arthropods. 34th SASEV Congress (14-16 November 2012, Simondium), P. Moyo, F. Halleen, F. Roets & L. Mostert

Detection of Petri Disease and esca pathogens on arthropods found in vineyards. 48th Congress of the Southern African Society for Plant Pathology (20-24 Jan 2013, Bela Bela). P. Moyo, F. Halleen, E. Allsopp, F. Roets & L. Mostert

Posters at International Conferences:
Ants and millipedes as vectors of *Phaeomoniella chlamydospora* to grapevine pruning wounds. 10th International Congress of Plant Pathology (25-30 August 2013, Beijing, China). P. Moyo, F. Roets, L. Mostert & F. Halleen.

Posters at National Conferences:
Grapevine pruning wound sap as food source for millipedes and cocktail ants, two of the main carriers of Petri disease pathogens. 48th Congress of the Southern African Society for Plant Pathology (20-24 Jan 2013, Bela Bela). P. Moyo, F. Halleen, E. Allsopp, F. Roets & L. Mostert

Presentations at local Information Days, University lectures, etc.:
University of Stellenbosch (Dep. Plant Pathology, Friday Forum, 13 November 2009)
The role of arthropods in the dispersal of Petri and esca diseases of grapevine.
M. van der Merwe, F. Halleen, L. Mostert, F. Roets & E. Allsopp

University of Stellenbosch (Dep. Plant Pathology, 12 November 2010)
The role of arthropods in the dispersal of grapevine trunk disease pathogens associated with Petri disease and esca.
P. Moyo, L. Mostert & F. Halleen

University of Stellenbosch (Dep. Plant Pathology, 15 April 2011)
Detection of Petri disease and esca fungi on arthropods associated with grapevines.
P. Moyo, L. Mostert F. Halleen, F. Roets & E. Allsopp

University of Stellenbosch (Dep. Plant Pathology, 12 August 2011)
The role of arthropods in the dispersal of fungi associated with Petri disease and esca of grapevine.
P. Moyo, L. Mostert, F. Halleen, F. Roets & E. Allsopp

ARC Infruitec-Nietvoorbij Information Day (Stellenbosch, 26 July 2012)
Grapevine trunk disease research.
F. Halleen

THRIP Audit (ARC Infruitec-Nietvoorbij, Stellenbosch, 28 August 2012)
Epidemiology and etiology of esca on grapevine in South Africa.
F. Halleen

University of Stellenbosch (Dep. Plant Pathology, 18 October 2012)
Grapevine sap as food source for millipedes and ants, the main carriers of Petri disease pathogens.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

VinPro Information Day (Bien Donne, 18 April 2013)
The role of arthropods in the dispersal of grapevine trunk diseases.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

VinPro Regional Information Day (Robertson, 6 Aug 2013).
Oordra van patogene deur duisendpote op snoeiwonde.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

VinPro Regional Information Day (Worcester, 7 Aug 2013).
Oordra van patogene deur duisendpote op snoeiwonde.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

VinPro Regional Information Day (Paarl, 8 Aug 2013).
Oordra van patogene deur duisendpote op snoeiwonde.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

VinPro Regional Information Day (Vredendal, 13 Aug 2013).
Oordra van patogene deur duisendpote op snoeiwonde.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

VinPro Regional Information Day (Stellenbosch, 4 Sept 2013).
Oordra van patogene deur duisendpote op snoeiwonde.
P. Moyo, L. Mostert, F. Roets, E. Allsopp & F. Halleen

Total cost summary of the project

TOTAL COST IN REAL TERMS	Years	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1	2009/10					155912		146664	287576
YEAR 2	2010/11					171502		178502	350004
YEAR 3	2011/12					181791		189212	371003
YEAR 4	2012/13					181791		189212	371003
TOTAL						690996		703590	**1379586**