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
2013

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
Title, initials, surname	Prof JT Burger	Dr HJ Maree
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Project Information

Research Organisation Project number	GenUS 11/2
Project title	Determination of the incidence and distribution of GVE in vineyards of the Stellenbosch region

Fruit kind(s)	Wine grapes		
Start date (mm/yyyy)	01/08/2010	**End date** (mm/yyyy)	31/12/2012

Project keywords	GVE

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.

This study was aimed at determining the incidence and distribution of the newly identified virus, Grapevine virus E (GVE) in South African vineyards.

A survey was performed to determine the incidence of GVE in the vineyard (*Vitis vinifera* cv Merlot) where GVE was first detected in Stellenbosch. The amount of samples needed for the survey was determined for a 95% confidence. Out of the approximate 1320 plants, 139 plants were randomly selected for testing. A RT-PCR diagnostic assay was developed and used to screen the samples determining the incidence of GVE. The incidence in this vineyard was low, with only 3% of the surveyed vineyard positive for GVE infection. Symptoms of GVE infected plants were documented to determine if there is any disease association with GVE. All GVE positive vines were also infected with grapevine leafroll-associated virus 3 (GLRaV-3) displayed typical **grapevine leafroll disease** (GLD) symptoms, this includes down rolling of leaf margins and the interveinal leaf areas turning red. Because of the low incidence and presence of GLRaV-3 no disease association could be made with GVE infection and no clear distribution pattern could be described.

The relative virus titre of GVE was calculated over the growing season of 2010/2011, using a newly developed RT-qPCR assay. Grapevine virus E infected grapevine plants were randomly sampled fortnightly from the 15 November 2010 – 30 May 2011. The results obtained from the relative GVE titre calculation did not indicate significant titre fluctuations throughout the season.

Two additional surveys were added to the project.

In the first survey GLD'ed vines were screened. The incidence of co-infection of GVE with GLRaV-3 was ~20%.

The second survey was performed on nuclear material from Vititec. As expected no GVE was detected in these samples.

Problem identification and objectives

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

In 2010, a new grapevine-infecting virus, *Grapevine virus E* (GVE), a member of the genus Vitivirus, was discovered in South African vineyards by our laboratory. Its involvement and impact in grapevine diseases has not been resolved anywhere in the world. Since its recent discovery, no survey to establish the incidence and distribution of the virus has been undertaken. This project aimed to determine the prevalence of GVE, its involvement in disease complexes and to develop a standard detection protocol for GVE.

Workplan (materials and methods)

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

- Determine with statistical analysis the number of samples that need to be tested for a 95% confidence value in the percentage occurrence of GVE
- Document the disease symptoms of plant material that will be used in the survey
- Collect plant material and perform RNA extraction via the CTAB method (White et al, 2008)
- Perform and compare end-point and real-time RT-PCR to determine the presence of GVE
- Use RT-PCR to screen for other known viruses that cause disease
- Determine if GVE is associated with a disease complex

- Determine the virus titer of GVE in infected vines over the growing season using qRT-PCR

Additional work

- Two surveys were added to this project.
    1. Geographical distribution: GVE is only found in co-infections with GLRaV-3. A survey will be conducted to determine the incidence on GVE in GLRaV-3 infected vines sampled across the Western Cape.
    2. Nuclear material: Nucleus material (Vititec) will be screen for GVE infection.

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.

To detect GVE, a RT-PCR diagnostic assay was developed. The assay was then used to screen samples from the vineyard where GVE was first discovered to determine the incidence. The amount of samples needed for the survey was statistically determined for a confidence interval of 95%. Out of the approximate 1320 plants it was determined that 139 plants should be randomly selected for testing. The incidence in this vineyard was low, with only 3% of the surveyed vineyard testing positive for GVE infection.

Symptoms of GVE infected plants were documented to determine if there is any disease association with GVE. All GVE positive vines displayed typical GLD symptoms, that included down rolling of leaf margins and the interveinal leaf areas turning red. The GVE positive samples were also screened for other grapevine viruses and determined that all plants were co-infected with GLRaV-3. Because of the low incidence and presence of GLRaV-3 no disease association could be made with GVE infection and no clear distribution pattern could be described.

The relative virus titre of GVE was calculated over the growing season of 2010/2011, using RT-qPCR. Grapevine virus E infected grapevine plants were randomly sampled every second week from the 15 November 2010 – 30 May 2011. The results obtained from the relative GVE titre calculation did not indicate significant titre fluctuations throughout the season.

Two additional surveys were added to the project. In the first survey GLD'ed vines were screened. Samples were collected across the Western Cape. Different cultivars, all affected by GLD, were screened for GVE. The incidence of co-infection of GVE with GLRaV-3 was ~20%. See table for results.

The second survey was performed on nuclear material from Vititec. Hundred and seventy-eight samples were screened and as expected, tested negative for GVE.

Table: GLD positive samples that also tested positive for GVE.

Region	GVE Positive	Total GLD Samples
Rawsonville	6	36
Somerset	5	16
Hermanus	5	9
Worcester	3	8
Rawsonville	6	32
Paarl	2	9
Wolseley	1	17
Porterville	0	12
Darling	0	16
Malmesbury	4	8
Total	**26**	**127**

Complete the following table

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Determine the incidence				3% of surveyed samples infected with GVE
2. Determine the disease association				No disease symptoms has been associated with GVE infection
3. Determine the relative virus titre				No fluctuation during 2010/2011growing season
4. Two surveys: 1) Geographical distribution Western Cape 2) Nucleus material Vititec (179 samples)				1) ~20% of GLD affected vines are co-infected with GVE. 2) No GVE detected in nuclear block material.
5. Journal publication/s – final milestone				

Accumulated outputs

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

Extended abstract ICVG Conference p86:
http://ucanr.edu/sites/ICVG/files/156711.pdf

MSc thesis, WC de Koker:
http://scholar.sun.ac.za/bitstream/handle/10019.1/71709/de%20koker_molecular_2012.pdf?sequence=2

Conclusions

GVE was only detected in co-infections with GLRaV-3 and therefor no association with any specific disease symptom could be determined. The role of GVE in disease symptom expression and severity is unknown. The possibility that GVE requires GLRaV-3 for systemic infection seems likely but remains to be proven.
Recommendation: Until more research has been conducted to proof otherwise the recommendation is that GVE be listed as one of the viruses (along with the other vitiviruses) to be excluded from all plant material the Improvement scheme. The current observations that the vitiviruses requires additional viruses to establish systemic infection means that the inclusion of GVE will not have a significant impact on the current scheme since the "helper" viruses are already on the list.

Technology development, products and patents

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

GVE diagnostic assays: conventional RT-PCR and RT-qPCR

Suggestions for technology transfer

List any suggestions you may have for technology transfer

GVE diagnostic tests are available

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.MSc (B Coetzee)	0
2.MSc (WC de Koker)	0
3.Postdoc (HJ Maree)	0
4.BSc (Hons) (R Bester)	0
5.	

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

Presentations/papers delivered

Oral presentations:

De Koker, W.C., Coetzee, B., Maree, H.J., Bester, R., Nel, Y., Stephan, D., Freeborough, M-J. and Burger, J.T. 2010. Investigation into the incidence of Grapevine virus E in South African vineyards. 32nd Conference of the South African Society for Enology and Viticulture, Somerset West, South Africa. 18-19 November 2010. p.15

De Koker, W.C., Bester, R., Jooste, A.E.C., Maree, H.J., Coetzee, B., Stephan, D., Freeborough, M-J. and Burger, J.T. 2012. Determining the incidence of grapevine virus E in South African vineyards. (Paper) 34th Conference of the South African Society for Enology and Viticulture, Franschhoek, South Africa. 14-16 November 2012.

Poster Presentation:
De Koker, W.C., Coetzee, B., Bester, R., Jooste, A.E.C., Maree, H.J., Stephan, D., Freeborough, M.-J., and Burger, J.T. 2012. Sequencing and prevalence of grapevine virus E in South African vineyards. p86. (Poster 14) 17th meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG), Davis, USA. 8-14 October 2012.

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST
YEAR 1	
YEAR 2	
YEAR 3	
YEAR 4	
YEAR 5	
TOTAL	

CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
				0	0		0
				0	0		0
				60000	30000		90000
				60000	30000		**90000**