Industry allocated project number	PHI allocated project number

AGRICULTURAL RESEARCH COUNCIL
PLANT PROTECTION RESEARCH INSTITUTE
P/Bag X134, Queenswood, Pretoria 0121

FINAL REPORT

Ref: PPRI 11/18

Study of the insect transmission efficiency of known Grapevine leafroll-associated virus 3 (GLRaV-3) genetic variants

Prepared by: AEC Jooste & K. Krüger
Tel: (012) 8088197 Fax: (012) 8088299
Email: JoosteE@arc.agric.za

PREPARED FOR:
Client: Winetech
Contact person: Ms Anel Andrag
Tel: (021) 276 0499 Fax: 0866117817
Email: andraga@winetech.co.za

July 2015

SATI	CFPA	SAAPPA/SASPA	DFTS	Winetech
tarryn@satgi.co.za Tel: 021 863-0366	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: 021 276 0499
				X

FINAL REPORT
2015

1. PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	Research Team Manager	Project leader
Title, initials, surname	Dr. IH Rong	Dr. SPN Mativandlela	Dr. AEC Jooste
Present position	Acting Senior Manager-Plant Health	Research Team Manager	Researcher
Address	ARC-PPR Private bag X134 Queenswood, 0121	ARC-PPR Private bag X134 Queenswood, 0121	ARC-PPR Private bag X134 Queenswood, 0121
Tel. / Cell no.	012 8088138	012 8088108	012 8088197
Fax	012 8088299	012 8088299	012 8088299
E-mail	RongI@arc.agric.za	MativandlelaS@arc.agric.za	JoosteE@arc.agri.za
	Co-worker	**Student**	
Title, initials, surname	Prof K. Krüger		
Present position	Associate Professor		
Address	Department of Zoology and Entomology, University of Pretoria		
Tel. / Cell no.	012 420 4619		
Fax			
E-mail	kkrüger@zoology.up.ac.za		

2. PROJECT INFORMATION

Research Organisation Project number	PPRI11/18
Project title	Study of the insect transmission efficiency of known Grapevine leafroll-associated virus 3 (GLRaV-3) genetic variants
Short title	Insect transmission of GLRaV-3 variants

Fruit kind(s)	Grapevine		
Start date (mm/yyyy)	01/04/2012	**End date** (mm/yyyy)	31/03/2014

Key words	GLRaV-3 variants, transmission efficiency, *Planococcus ficus*

Approved by Research Organisation Programme leader (tick box) [X]

3. EXECUTIVE SUMMARY

The rapid spread of Grapevine leafroll disease (GLD) in South African vineyards is of major concern to the wine industry. No biological data are currently available on the transmission efficiency of GLRaV-3 variants in vineyards. The importance of the interaction between the mealybug vector and specific GLRaV-3 variants warrants further investigation and was the main objective of this study.

The incidence of GLRaV-3 variants in local vineyards, their molecular characterisation and transmission efficiency by mealybug vectors play a key role in understanding the biological aspects of LRD spread. In a recent study the relative abundance of five GLRaV-3 variants in vineyards of the Western Cape was determined (Jooste et al., 2015). The overall analysis showed that infections with variant groups II and VI were the most abundant among the samples. The question arose if specific GLRaV-3 variants are transmitted more frequently by the mealybug vector in vineyards or is a combination of aspects influencing the prevalence of the different variants.

The objective of the project was to study the transmission efficiency of four known GLRaV-3 variants by the vine mealybug (*Planococcus ficus* (Hemiptera: Pseudococcidae)), the most common vector in South African vineyards. The transmission efficiency of four characterised GLRaV-3 variants, i.e. groups I (represented by isolate 621), group II (represented by isolate 623), group III (represented by PL-20) and group VI (represented by isolate GH11), is unknown at present. In three consecutive years, GLRaV-3 variants from singly-infected plants were transmitted to *Vitis vinifera* cv Cabernet Franc plants by mealybugs under controlled conditions. A total of 368 single mealybug transmissions were carried out over a three-year period (2011-2013), with 50 to 64 replicates for each GLRaV-3 variant-infected source plant (621, 623, PL-20 and GH11). Results of the study demonstrated that the four GLRaV-3 variants in single infected vines, or when occurring in combination with GVA, are transmitted equally well under controlled conditions. The results suggest that factors other than the mealybug vector play a role in the prevalence of group II and group VI variants in vineyards.

The results from this study created knowledge on virus-vector interactions and will impact future management strategies of leafroll disease spread.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

Planococcus ficus is considered the most important vector of GLRaV-3 in South African vineyards, and it was shown that a single mealybug can transmit GLRaV-3 to a healthy grapevine plant (Douglas and Krüger, 2008). No biological data are currently available on the transmission efficiency of GLRaV-3 variants in South African vineyards.

To date, eight genetic variant groups of GLRaV-3 have been proposed to occur world-wide (Maree et al., 2015). The relative abundance of five of these GLRaV-3 variants occurring in South African vineyards was determined recently (Jooste et al., 2015). In this study, GLRaV-3 variant groups II and VI were the most prevalent as single infections and in combination with each other and with other variants (Jooste et al., 2015). However, the importance of the interaction between the mealybug vector and specific GLRaV-3 variants warranted further investigation and thus was the main objective of this study. The transmission efficiency of specific GLRaV-3 variants with *P. ficus* as vector was determined using source plants infected with four characterised GLRaV-3 variants, *i.e.* group I (represented by isolate 621), group II (represented by isolate 623), group III (represented by PL-20), and group VI (represented by isolate GH11).

5. WORKPLAN (MATERIALS AND METHODS)

Singly-infected reference plants of four known GLRaV-3 variants (groups I, II, III and VI) were established in a greenhouse. Two additional combinations included *Grapevine virus A* (GVA) with GLRaV-3 group I and group II variants, respectively. A non-viruliferous culture of *P. ficus* was maintained on butternut (*Cucurbita moschata,* Cucurbitaceae). Virus-free grapevines, *Vitis vinifera* (Vitaceae) cv. Cabernet franc, were propagated at Vititec (Paarl)) and served as recipient vines for the experiment. The virus-free status of the plants was confirmed in a GLRaV-3 specific nested RT-PCR (Ling et al., 2001).

To determine the transmission efficiency of the four GLRaV-3 variants as well as the combinations that included GLRaV-3/GVA, single nymphs were carefully transferred with a fine paint brush from infected leaf material after acquisition access periods (AAP) of 48 hours to healthy recipient plants and given an inoculation access period (IAP) of 48 hours. After transmissions the plants were treated with a systemic insecticide. Plants were maintained at 25 ^{o}C, 16:8 h L:D and natural humidity. Total RNA was extracted from petioles 8 months after transmissions and tested in a RT-PCR for GLRaV-3 (LR3.HRM4F+R) (Bester et al., 2012) using GoScript and GoTaq (Promega) and nested RT-PCR for detecting GVA (Dovas and Katis, 2003).

Chi-square tests were used to determine transmission differences between GLRaV-3 variants and the combinations with GVA. The significance level was set at 5 %.

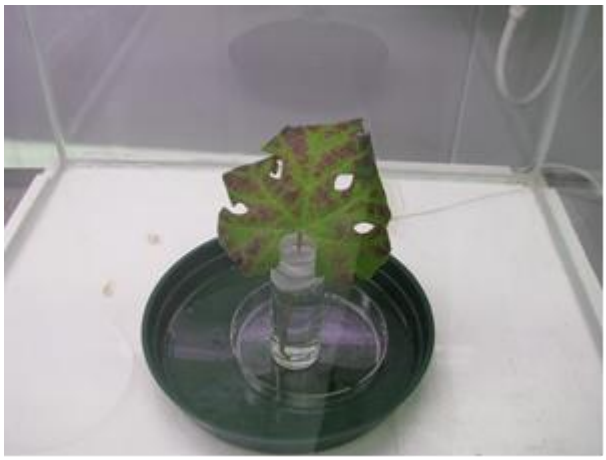

Figure 1. Mealybugs were allowed to feed on infected source plants for 48 hours

Figure 2. Single mealybug transmissions were carried out and clip-on leaf cages placed around the feeding area.

Figure 3. Plants in the transmission experiments were kept in a temperature controlled tunnel and tested after extracting RNA from petioles.

6. RESULTS AND DISCUSSION

A total of 368 single mealybug transmissions were carried out over a three-year period, with 50 to 64 replicates for each GLRaV-3 variant infected source plant (621, 623, PL-20 and GH11). Between 9 and 18% of plants became infected (Figure 1). The number of plants infected did not differ significantly between the GLRaV-3 variant groups (X^2 = 2.14, df = 5, P = 0.828), demonstrating that the four GLRaV-3 variants in single infected vines, or when occurring in combination with GVA, are transmitted equally well under controlled conditions. The results suggest that factors other than the mealybug vector play a role in the prevalence of group II and group VI variants in the field.

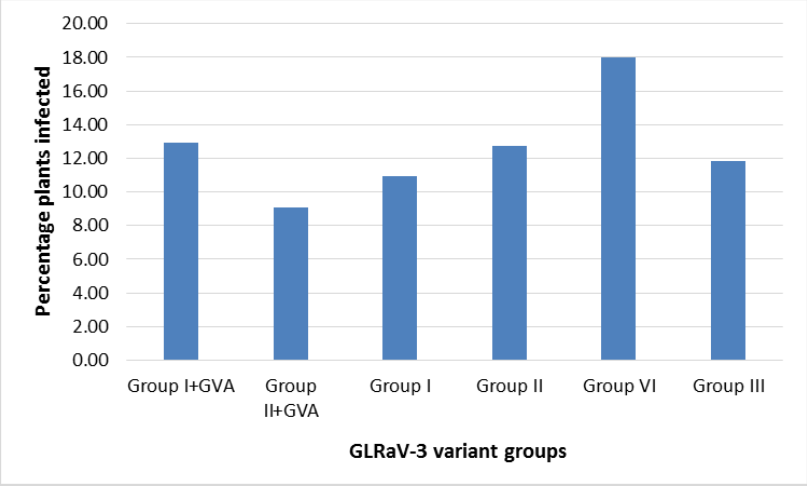

Figure 1. Percentage transmissions of different GLRaV-3 variant groups as well as GLRaV-3/GVA combinations.

Table 1. Number of plants infected from source plants with mixed infections of GLRaV-3 and GVA.

Source plants	GLRaV-3	GVA	GLRaV-3+GVA	Not infected	Total
GLRaV-3 group I/GVA	2 (6%)	16 (47%)	6 (18%)	10 (29%)	34
GLRaV-3 group II/GVA	2 (7%)	18 (60%)	3 (10%)	7 (23%)	30

Mealybugs can transmit GVA from plants with mixed infections without transmitting GLRaV-3 (Table 1). GVA was transmitted more frequently than GLRaV-3. The GLRaV-3 variant had no influence on the number of plants infected with GVA (X^2 = 0.20, df = 1, P = 0.652). With GLRaV-3 group I/GVA and GLRaV-3 group II/GVA as source plants, 65% of and 70% of recipient plants, respectively, became infected with GVA either singly or in combination with GLRaV-3. Blaisdell et al. (2012) also observed that GVA was transmitted more frequently than GLRaV-3 from mixed infected plants and that this can be a major concern for managing GVA spread in vineyards.

References:

Bester, R., Jooste, A. E. C., Maree, H. J., and Burger, J. T. (2012). Real- time RT-PCR high-resolution melting curve analysis and multiplex RT-PCR to detect and differentiate grapevine leafroll-associated virus 3 variant groups I, II, III and VI. Virology Journal, 9: 219.

Douglas, N., and Krüger, K. (2008). Transmission efficiency of Grapevine leafroll-associated virus 3 (GLRaV-3) by the mealybugs Planococcus ficus and Pseudococcus longispinus (Hemiptera: Pseudococcidae). European Journal of Plant Pathology, 122: 207–212.

Dovas, C. I., and Katis, N. I. (2003). A spot multiplex nested RT-PCR for the simultaneous and generic detection of viruses involved in the aetiology of grapevine leafroll and rugose wood of grapevine. Journal of Virological Methods, 109: 217–226.

Jooste, A.E.C., Molenaar, N. Maree, H.J., Bester, R, Morey, L., de Koker, W.C.and Burger, J.B. Identification and distribution of multiple virus infections in grapevine leafroll diseased vineyards. European Journal of Plant Pathology, 142(2): 363-375

Ling, K.-S., Zhu, H. –Y., Petrovic, N., & Gonsalves, D. (2001). Comparative effectiveness of ELISA and RT-PCR for detecting Grapevine leafroll-associated virus 3 in field samples. American Journal of Enology and Viticulture, 52: 21-27.

COMPLETE THE FOLLOWING TABLE

Objectives	Milestones	Target Date	Extension Date	Date completed	Achievement
1. Transmit different GLRaV-3 variants from singly-infected grapevines to healthy vines (cv Cabernet franc) with mealybugs. A reliable transmission protocol was developed in the UP laboratory and will be used.	1. Ordered healthy C franc plants from Vititec yearly 2. Tested recipient plants to ensure virus-free 3. Did single mealybug transmissions from plants infected with different GLRaV-3 variants (GH11, PL-20, 621, 623,623+GVA, 621+GVA)	May 2011 May 2012 May 2013	N/A	May 2011 May 2012 May 2013	368 single mealybug transmissions were carried out over a three-year period, with 50 to 64 replicates for each GLRaV-3 variant infected source plant (621, 623, PL-20 and GH11)
2. Determine the transmission efficiency of GLRaV-3 variants according to established and tested protocols.	1. Collected petioles from recipient plants of 2011, 2012 and 2013 transmission experiment after 8 months and stored petioles at -80 ^{o}C 2. Extracted RNA and tested plants collected after 8 months of 2011, 2012 and 2013 transmission experiment 3. Analysed results statistically	May 2011 May 2012 May 2013	2015	May 2015	RNA extracted from petioles was tested under the same RT-PCR conditions and results were concluded
3. Finalise project	Do combined statistical analyses		2015		Final results were obtained
4. Journal publication(s) – final milestone	In progress				In progress. Results will be presented at ICVG, Turkey and popular and scientific publications will follow.

7. CONCLUSIONS

In this study, the transmission efficiency of plants with single GLRaV-3 variant infections were tested. The objective was to study if a certain GLRaV-3 variant can be transmitted more readily from infected to healthy plants by the mealybug vector, *P. ficus.* Investigations on the relative abundance of GLRaV-3 variants in vineyards showed that GLRaV-3 variants from groups II and VI were found more often in South African vineyards.

After results were analysed statistically we did not observe any differences in transmission efficiency between the four GLRaV-3 variants studied here. The results suggest that factors

other than the mealybug vector play a role in the prevalence of group II and group VI variants in the field. GLRaV-3 variants from groups II and VI represent genetically distinct variants, and although the percentage plants infected with group VI variants were slightly higher, statistically there were no differences. We found no evidence of vector-variant specificity.

In the follow-up study, PPRI 13-16, plants infected with multiple GLRaV-3 variants and viruses were used in a transmission experiment to determine how GLRaV-3 variants are transmitted with competition. Results will show if virus-virus interactions during establishment in a new host plant may be more important than interactions within the vector. These results will be available in the next reporting year.

Two of the source plant combinations in this study included GVA with GLRaV-3 variant group I- and II, respectively. Mealybugs can transmit GVA from infected to susceptible *V. vinifera* plants without simultaneous transmission of GLRaV-3. Results showed that GVA was transmitted more frequently than GLRaV-3, and this can be a major concern for managing GVA spread in vineyards.

Although we did not find evidence of vector-variant specificity here, further studies are needed to explore why certain GLRaV-3 variants occur predominantly in vineyards. Understanding molecular interactions between the host plant, vector and specific variant/virus is crucial for prediction of pathogen spread.

8. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

None

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Popular publication in Winelands Magazine

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

No students trained in this study

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

To be published

e) PRESENTATIONS/PAPERS DELIVERED

E. Jooste & K. Krüger (2012). Initial results of the transmission efficiency of GLRaV-3 variants. Grapevine Virus Workshop XI, Olive Grove, Infruitec, 29 May 2012

A.E.C. Jooste & K. Krüger (2015). Mealybug transmission efficiency of four Grapevine leafroll-associated virus 3 (GLRaV-3) genetic variants. Proceedings of the 18th Congress of ICVG, Ankara, Turkey, September 2015 (abstract submitted and will participate with an oral presentation)

9. BUDGET

a) TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
1					60 000		80 000	140 000
2					65 000		104 574	169 574
					125 000		184 574	**309574**

b) FINAL BUDGET/FINANCIALS OF PROJECT

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
TOTAL INCOME	309 574		None	
Industry Funding	125 000	125 000	None	
PHI Funding				
Other Funding	184 574	184 574	None	Additional funding was from ARC-PPRI towards salary and UP towards healthy plant material, testing of samples prior to experiment to confirm virus-free and use of facilities during transmission experiments
TOTAL EXPENDITURE	309 574			
Running Expenses				
General operating costs (printing, communication, etc.)	110 000		None	Winetech contribution
Local Travel	15 000		None	Winetech contribution
Publication costs				

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
Lab Analysis				
Lab Consumables				
Other (facilities)	50 000		None	UP contribution
Running expenses SUB-TOTAL	175 000			
HR Administration and Project Management				
HR Technical				
HR Research	134 574		None	ARC-PPR contribution towards salary component
Student Bursaries				
HR SUB-TOTAL	134 574			
OTHER EXPENSES				
SURPLUS / DEFICIT				Income and expenditure were equal, no surplus or deficit

EVALUATION BY INDUSTRY

This section is for office use only

Project number	
Project name	
Name of Sub-Committee*	

Comments on project

Committee's recommendation	
• Accepted.	☐
• Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this final report by_____	☐
• Unacceptable. Must resubmit final report.	☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech
Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit
Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer
Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology