

GRAPEVINE VIRUS RESEARCH PROGRAMME

WINETECH DEFINES A RESEARCH PROGRAMME as a number of projects with common goals which either encompass strategic benefits for the industry and/or address industry needs/problems.

INTRODUCTION

The Grapevine Virus Research Programme aims to alleviate the serious virus disease problems in the South African wine industry by thorough characterisation of grapevine viruses, to develop strategies to manage the diseases they cause, to prevent further spread of these diseases, and to use the latest technologies for the establishment of genetic virus resistance in wine grape cultivars in the longer term.

DEFINITION AND STRUCTURE

Grapevine virus research in the South African context is of a multidisciplinary nature and comprises projects addressing the focus areas that make up the three corners of the virus disease triangle. These are the different **Viruses**, the **Vitis** host and the insect **Vector(s)**, which all play equally important roles in the complex nature of virus diseases. Within these focus areas, cross-cutting themes like **identification** and **characterisation, control, detection, epidemiology** and **transformation** are used to encompass existing funded projects, while it makes provision to include future projects on **high-throughput** biology, typical of the “omics” era. The components of the grapevine virus research programme are summarised in Figure 1 below.

Grapevine Virus Research Programme

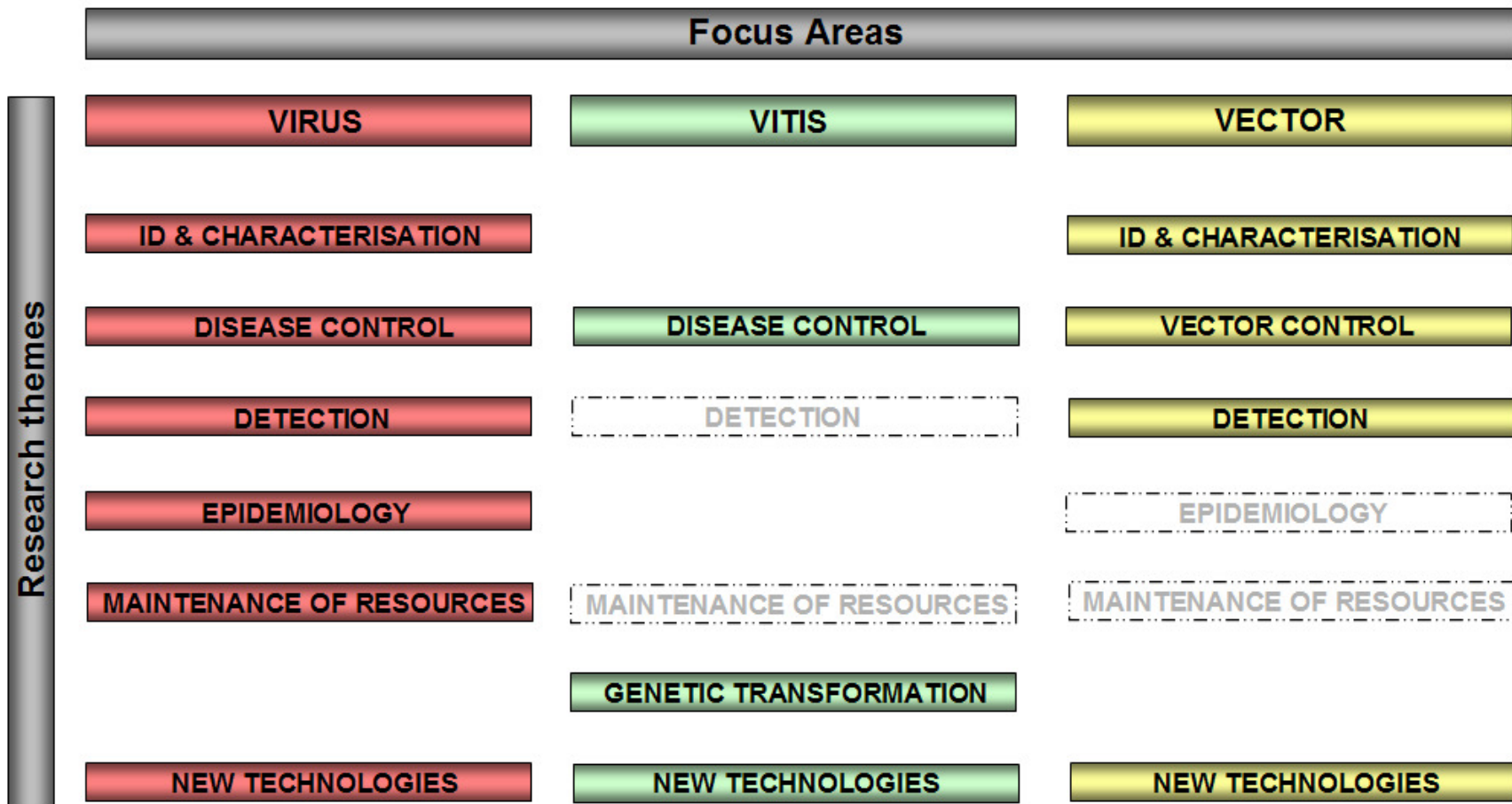


Fig 1. The Grapevine Virus Research Programme. Colour-shaded boxes indicate cross-cutting research themes where current Winetech-funded research projects reside. Non-shaded boxes indicate scope for future expansion of the programme.

OBJECTIVES OF THE VIRUS PROGRAMME

This programme focuses on the improvement of grapevine, specifically to introduce virus resistance, in line with the wine industry's criteria of environmentally friendly production, the optimal use of natural resources and technology transfer. Projects have one of the three key organisms in the disease triangle (Vitis, Virus or Vector) as its focus and each addresses one or more of the following themes:

1. Identification and Characterisation

- Identification and characterisation of new viruses and virus-like organisms (*e.g.* phytoplasmas) in grapevine.
- Identification and characterisation of vectors of established or emerging virus and virus-like diseases in grapevine.
- Biological and molecular characterisation of genetic variants of viruses implicated with diseases.
- Molecular characterisation of the genome structure and functioning of economically important viruses (*e.g.* GLRaV and GVA).
- Etiological studies of established and emerging virus diseases.
- Metagenomic sequencing approaches for entire virus disease complexes.

2. Disease and Vector Control

- Construction of infectious clones for virus-based expression/silencing strategies
- Pathogen-derived resistance approaches
- cDNA library construction of mealy bug
- Vector control strategies based on gene silencing
- Insecticide resistance studies in mealy bug
- Total chemical control of leafroll-infected grapevines

3. Virus, Disease and Vector Detection

- Development of virus diagnostics (semi-commercial)
- Glasshouse and field trials
- Vector transmission studies
- Establishment and maintenance of a grapevine virus reference source

4. Epidemiology

- Epidemiological studies on the current status of grapevine leafroll disease and its spreading patterns
- Epidemiological studies on the status of phytoplasma diseases of grapevine

5. Genetic Transformation

- Development of alternative (somatic) virus-based transformation vectors for grapevine
- Evaluation of transgenic grapevines

6. Maintenance of Resources

- Maintenance of virus isolates and variants
- *In planta* maintenance of disease complexes
- Vector collection and maintenance

7. New Technologies

- Luminex-based high-throughput virus diagnostics
- Grapevine genome analysis using microarray technology
- miRNA discovery in virus-infected grapevine and elucidation of its role in pathogenesis
- Metagenomic high-throughput sequencing of virus disease complexes

8. Other

- Economic assessment of virus elimination programmes in controlled vineyard environments.

PROJECTS IN THIS PROGRAMME

Note: *Projects listed below include all projects funded by Winetech since the inception of the Grapevine Virus Programme in 2000. These projects were approved and funded in the previous Virus Programme structure and have been re-allocated below in the research themes of the new structure.*

1. Identification and Characterisation

- A survey to determine the prevalence of the grapevine stem pitting/stem grooving disease complex.
Project status: Pending
- Bio-assays on natural hosts of mealy bugs (WW 05/12).
Project status: Completed
- Identification of virus vectors (WW 07/03).
Project status: Completed
- Life tables of mealy bugs and their natural enemies (WW 05/13).
Project status: Completed

- Economical thresholds for mealy bug (WW 05/14).
Project status: Completed
- Underground population dynamics of vine mealy bug (230044).
Project status: Completed
- Vectors of leafroll (AD 936).
Project status: Completed
- Establish a correlation between the degree day model, pheromone trap catches and physical vine monitoring of grapevine mealybug (WW 05/16).
Project status: Completed
- Life tables of *Anagyrus pseudococci* (US/E&N/A3).
Project status: Completed
- Quantifying the impact of ants on vine mealybug and its parasitoids (US/E&A/A1).
Project status: Completed
- The development of an action threshold for ants in vineyards (US/E&A/A2).
Project status: Completed
- Molecular study of viruses from LN33 hybrid showing symptoms of corky bark (CB) disease (WW 07/09).
Project status: Completed
- Analysis of grapevine leafroll-associated virus 3 (GLRaV-3) isolates by single-strand conformation polymorphism (SSCP)(WW07/12).
Project status: Completed
- Molecular and biological variability of grapevine leafroll-associated virus-3 (GLRaV-3) in South African vineyards (PPRI-Jooste 1).
Project status: Completed
- Sequencing and sequence analysis of divergent molecular variants of GVA associated with Shiraz disease (PPRI-Gos 1).
Project status: Completed
- Determination of a possible viral etiology of Shiraz disease (WW07/07).
Project status: Completed
- Investigation of the virus status of grapevines cv. Shiraz clone 99 affected with Shiraz decline disorder using molecular methods (PPRI-Gos 2).
Project status: Completed
- Cloning and sequencing of leafroll 3 virus (WW 07/05).
Project status: Completed
- The cloning and molecular characterisation of the coat protein gene of a South African isolate of grapevine leafroll-associated virus 3 (IWB 4/5).
Project status: Completed

- Virome in a vineyard (GenUS 8/1)
Project status: Completed
- Study of association of RSPaV-SY and GVB with Shiraz decline-affected Shiraz clone 99B (PPRI/Gos 3)
Project status: Completed
- Molecular characterisation of the sgRNAs of GLRaV-3 (GenUS 7/3)
Project status: Ongoing
- Study to determine occurrence and impact of Asian ladybird, *Harmonia axyridis* (Pallas), in Western Cape Winelands (230072)
Project status: Ongoing
- Grapevine genome analysis using DNA microarray technology.
Project status: Pending

2. Disease and Vector Control

- Prevention of grapevine leafroll disease spread between successive vineyards on the same site (WW 07/18).
Project status: Ongoing
- The evaluation of different herbicides and herbicide application techniques for total chemical control of leafroll infected grapevines, as part of a strategy to prevent the transfer of the virus to newly planted grapevines (WW 02/19).
Project status: Ongoing
- Determination of the effectiveness of hot water treatment for preventing the spread of vectors for vine leafroll virus (US/E&N/W3).
Project status: Completed
- Ant control (WW 05/08).
Project status: Completed
- Integrated pest management (WW 05/02).
Project status: Completed
- Cover crops for ant management (230041).
Project status: Completed
- Investigation into insecticide resistance in vine mealy bugs (230047).
Project status: Completed
- Assessment of toxic baits for the control of ants in vineyards (230048).
Project status: Completed
- Beskerming van kwekerye en jong stokkies teen rolblaar infeksie deur die toediening van 'n sistemiese middel (WW 07/19).
Project status: Completed

- The use of vine mealybug pheromones for mating disruption in South African vineyards (US/E&N/W1).
Project status: Completed
- Verification of the integrated vine mealybug control programme (US/E&N/W2).
Project status: Completed
- Field effects of pesticides on *Anagyrus pseudococci* (US/E&N/A4).
Project status: Completed
- Effect of mass release *Anagyrus pseudococci* on VMB pest populations (230070).
Project status: Ongoing
- A pathogen-derived resistance strategy for the broad-spectrum control of grapevine leafroll disease (GENUS 1/2).
Project status: Completed
- The construction of an infectious clone of grapevine virus A (GENUS 1/5).
Project status: Completed
- The isolation and characterisation of viral promoters for directed expression of transgenes in grapevine (GENUS 1/3).
Project status: Completed
- Control of grapevine leafroll by means of cross protection (WW07/01).
Project status: Completed
- The introduction of multiple virus resistance in grapevines through virus-induced gene silencing (GENUS 1/4).
Project status: Completed
- Grapevine Transformation with virus resistance constructs and evaluation of resistance (GENUS 6/2)
Project status: Completed
- Identification of vectors of Aster Yellows (AdK1)
Project status: Ongoing
- Chemiese beheer van blaarspringers om Aster Yellows te bekamp (AdK3)
Project status: Ongoing
- Transmission of Aster yellows to grapevine by insect vectors (UP KK1)
Project status: Ongoing

3. Virus, Disease and Vector Detection

- Detection of grapevine viruses in grapevine rootstocks (WW 07/17).
Project status: Completed
- ds-RNA detection of leafroll viruses (WW 07/02).
Project status: Completed

- ELISA detection of grapevine fleck diseases (WW 07/04).
Project status: Completed
- PCR detection of viruses of the grapevine leafroll complex (WW 07/06).
Project status: Completed
- Development of ELISA for simultaneous detection of grapevine virus A (GVA) and grapevine virus B (GVB)(WW 07/08).
Project status: Completed
- An evaluation of commercial grapevine virus diagnostic kits (US-Biochem 03/01).
Project status: Completed
- The development of an ELISA diagnostic kit for Grapevine Fanleaf Nepovirus (GENUS 5/1).
Project status: Completed
- Development of diagnostic assays for the detection of viruses of the rugose wood complex.
Project status: Pending
- Detection of a grapevine viruses in rootstocks using qPCR (GENUS 7/4)
Project status: Completed
- Development of a large-scale detection method for grapevine leafroll associated virus type 3 (PPRI GP2)
Project status: Ongoing

4. Epidemiology

- Analysis of spatial distribution patterns indicative of spread of leafroll disease (WW 07/13).
Project status: Completed
- Epidemiology and control of spread of grapevine leafroll disease (WW 07/16).
Project status: Completed
- Grapevine leafroll disease: Proof and quantification of vector dispersal and disease spread (CRI-GP1)
Project status: Ongoing
- The epidemiology of grapevine yellows disease in South African vineyards (WW 06/40)
Project status: Ongoing
- Epidemiology of the viti-viruses.
Project status: Pending

5. Genetic Transformation

- The establishment of efficient transformation and regeneration systems for grapevines (IWBT 4/1).
Project status: Completed
- The grapevine transformation and regeneration programme (IWBT 4/11).
Project status: Completed
- The molecular characterisation and transformation of the grapevine chloroplast genome (GENUS 2/2).
Project status: Completed
- The establishment of stable and synchronous embryogenic cell lines of grapevine rootstock cultivars for use in transformation systems (IWBT 4/10-2005).
Project status: Completed
- The evaluation of transgenic grapevines (IWBT 5/10).
Project status: Completed
- Developing resistance against phytoplasmas in grapevine (GenUS 8/2)
Project status: Ongoing

6. Maintenance of Resources

- Mass rearing of mealy bugs (WW 05/06).
Project status: Ongoing
- Maintenance of a virus garden (WW07/15).
Project status: Ongoing

7. New Technologies

COMPLETED GRAPEVINE VIRUS RESEARCH PROJECTS

Focus Area ¹	Project Num.	Project Title	Responsibility	Budget	Begin date	End date
Resistance	WW 07/01	Control of grapevine leafroll by means of cross protection.	ARC-Inf/Nietv (Carstens)	0	1992	completed ²
Support technologies	WW 07/02	ds-RNA detection of leafroll viruses	ARC-Inf/Nietv (Carstens)	0	1992	completed ²
Eradication / Support technologies	WW 07/03	Identification of virus vectors.	ARC-Inf/Nietv (Allsopp)	0	1992	completed ²
Support technologies	WW 07/04	ELISA detection of grapevine fleck diseases	ARC-PPRI (Goszczyński)	0	1992	completed ²
Resistance	WW 07/05	Cloning and sequencing of leafroll 3 virus	ARC-Inf/Nietv (Brand)	0	1993	completed ²
Support technologies	IWBT 4/1	The establishment of efficient transformation and regeneration systems for grapevines	US-IWBT (Vivier)	0	1998	completed ²
Eradication	WW 05/08	Ant control	ARC-Inf/Nietv (Addison)	0	1998	completed ²
Eradication	WW 05/12	Bio-assays on natural hosts of mealy bugs	ARC-Inf/Nietv (Walton)	0	1999	completed ²
Eradication	WW 05/13	Life tables of mealy bugs and their natural enemies	ARC-Inf/Nietv (Walton)	0	1999	completed ²
Eradication	WW 07/13	Analysis of spatial distribution patterns indicative of spread of leafroll disease.	ARC-PPRI (Pietersen)	0	2001	completed ²
Support technologies	WW 07/08	Development of ELISA for simultaneous detection of grapevine virus A (GVA) and grapevine virus B (GVB).	ARC-PPRI (Goszczyński)	0	2001	completed ²
Support technologies	IWBT 4/11	The grapevine transformation and regeneration programme	US-IWBT (Vivier)	0	2001	completed ²
Resistance	IWBT 4/5	The cloning and molecular characterisation of the coat protein gene of a South African isolate of grapevine leafroll-associated virus 3	US-GEN (Burger)	0	1998	completed ²
Eradication	WW 05/02	Integrated pest management	ARC-Inf/Nietv (Walton)	0	1993	completed ²
Eradication	WW 05/14	Economical thresholds for mealy bug	ARC-Inf/Nietv (Walton)	0	1999	completed ²
Eradication	230041	Cover crops for ant management	ARC-Inf/Nietv (Addison)	0	2001	completed ²
Eradication	23 0047	Investigation into insecticide resistance in vine mealybugs	ARC-Inf/Nietv (Walton)	0	2002	completed ²
Support technologies	WW 07/06	PCR detection of viruses of the grapevine leafroll complex.	ARC-PPRI (Van der Merwe)	0	1993	completed ²
Etiology	WW 07/12	Analysis of grapevine leafroll-associated virus 3 (GLRaV-3) isolates by single-strand conformation polymorphism (SSCP).	ARC-PPRI (Goszczyński)	0	2001	completed ²
Resistance	GENUS 1/2	A pathogen-derived resistance strategy for the broad-spectrum control of grapevine leafroll disease	US-GEN (Burger)	0	2000	completed ²
Eradication	US/E&N/W3	Sanitation of nursery propagation material	US – Walton	0	2004	completed ²
Eradication	230044	Underground population dynamics of vine mealybug	ARC - Inf/Nietv (Walton)	0	2002	completed ²
Support technologies	GENUS 2/2	The molecular characterisation and transformation of the grapevine chloroplast genome	US-GEN (Burger)	0	2002	completed ²
Etiology	WW 07/07	Determination of a possible viral etiology of Shiraz disease	ARC-PPRI (Goszczyński)	0	1998	completed ²
Etiology	WW 07/09	Molecular study of viruses from LN33 hybrid showing symptoms of corky bark (CB) disease.	ARC-PPRI (Goszczyński)	0	2001	completed ²
Support technologies	WW07/17	Detection of grapevine viruses in grapevine rootstocks	ARC-PPRI (Kasdorf)	0	2003	completed ²
Resistance	GENUS 1/3	The isolation and characterisation of viral promoters for directed expression of transgenes in grapevine	US-GEN (Burger)	0	2001	completed ²
Support technologies	US Biochem 03/01	An evaluation of commercial grapevine virus diagnostic kits	US-GEN (Bellstedt)	0	2004	completed ²
Resistance/Etiology	GENUS 1/5	The construction of an infectious clone of grapevine virus A	US-GEN (Burger)	0	2004	completed ²

Eradication	WW 07/16	Epidemiology and control of spread of grapevine leafroll disease	ARC-PPRI (Pietersen)	0	2002	completed ²
Resistance	GENUS 1/4	The introduction of multiple virus resistance in grapevines through virus-induced gene silencing	US-GEN (Burger)	0	2003	completed ²
Eradication	US/E&N/W1	The use of vine mealybug pheromones for mating disruption in South African vineyards	US – Walton (Walton)	0	2004	completed ²
Eradication	US/E&N/W2	Verification of the integrated vine mealybug control programme	US – Walton (Walton)	0	2004	completed ²
Etiology	PPRI/Gos 1	Sequencing and sequence analysis of divergent molecular variants of GVA associated with Shiraz disease	ARC-PPRI (Goszczyński)	0	2006	completed ²
Etiology	PPRI/Gos 2	Investigation of the virus status of grapevines cv. Shiraz clone 99 affected with Shiraz decline disorder using molecular methods	ARC-PPRI (Goszczyński)	0	2006	completed ²
Support technologies	IWBT 5/10	The evaluation of transgenic grapevines	US-IWBT (Vivier)	0	2003	completed ²
Support technologies	GENUS 5/1	The development of an ELISA diagnostic kit for <i>Grapevine Fanleaf Nepovirus</i>	US-GEN (Burger)	0	2006	completed ²
Eradication	WW 05/16	Establish a correlation between the degree day model, pheromone trap catches and physical vine monitoring of grapevine mealybug	ARC-Inf/Nietv. (Achiano)	0	2004	completed ²
Eradication / Support technologies	AD 936	Vectors of leafroll	UP (Kruiger)	0	2001	completed ²
Support technologies	IWBT 4/10-2005	The establishment of stable and synchronous embryogenic cell lines of grapevine rootstock cultivars for use in transformation systems	US-IWBT (Goussard)	0	2001	completed ²
Eradication	WW 07/19	Beskerming van kwekerie en jong stokkies teen rolblaar infeksie deur die toediening van 'n sistemiese middel	ARC-Inf/Nietv. (Carstens)	0	2004	completed ²
Support technologies	US/E&A/A1	Quantifying the impact of ants on vine mealybug and its parasitoids	US (Addison)	0	2005	completed ²
Support technologies	US/E&A/A2	The development of an action threshold for ants in vineyards	US (Addison)	0	2005	completed ²
Etiology	PPRI/Jooste 1	Molecular and biological characterisation of two divergent molecular variants of GLRaV-3	ARC -PPRI (Jooste)	0	2005	completed ²
Eradication	US/E&N/A3	Life tables on <i>Anagyrus pseudococci</i>	US (Addison)	0	2006	completed ²
Eradication	US/E&N/A4	Field effects of pesticides on <i>Anagyrus pseudococci</i>	US (Addison)	0	2006	completed ²
Eradication	230048	Assessment of toxic baits for the control of ants in vineyards	ARC-Inf/Nietv. (Addison)	0	2003	completed ²
Resistance	GENUS 6/2	Grapevine transformation with virus resistance constructs and evaluation resistance	US-GEN (Burger)	0	2007	completed ²
Support technologies	GENUS 7/4	Optimisation of a quantitative RT-PCR assay for the reproducible detection of grapevine viruses in rootstock material	US-GEN (Burger)	0	2008	completed ²
Etiology	PPRI/Gos3	Study of association of RSPaV-SY and GVB with Shirax decline affected Shiraz clone 99B	ARC-PPRI (Goszczyński)	0	2008	completed ²
Identification and Characterisation	GenUS 8/1	Virome in a vineyard	US-GEN (Burger)	0	2009	completed ²

CURRENT PROGRAMME BUDGET

GRAPEVINE VIRUS RESEARCH PROJECTS 2010

Focus Area ¹	Project Num.	Project Title	Responsibility	Begin date	End date
Maintenance of resources / Eradication	WW 05/06	Mass rearing of mealy bugs	ARC-Inf/Nietv (Achiano)	1993	2004
Maintenance of resources / Support technologies	WW 07/15	Maintenance of a virus garden.	ARC-Inf/Nietv (Carstens)	2001	continuous ³
Disease and Vector control / Eradication	WW07/18	Prevention of grapevine leafroll disease spread between successive vineyards on the same site	ARC-PPRI (Pietersen)	2004	2011
Epidemiology / Eradication	CRI-GP1	Grapevine leafroll disease: proof and quatication of vector dispersal and disease spread	CRI (Pietersen)	2007	2012
Disease and Vector control / Eradication	WW 02/19	The evaluation of different herbicides and herbicide application techniques for total chemical control of leaf-roll infected grapevines, as part of a strategy to prevent the transfer of the virus to newly planted grapevines	ARC-Inf/Nietv. (Fourie)	2006	2009
Identification and Characterisation / Eradication	230070	Effect of mass released <i>Anagyrus pseudococci</i> on VMB pest populations	ARC-Inf/Nietv (Achiano)	2006	2009
Identification and Characterisation	GENUS 7/3	Molecular characterisation of the sgRNAs of GLRaV-3	US-GEN (Burger)	2008	2010
Identification and Characterisation / Resistance	AdK 1	Identifisering van insekvektore van Aster Yellows in die Olifantsrivier-en Breërivier-gebied.	Private	2008	2012
Identification and Characterisation / Resistance	UP KK1	Transmission of Aster yellows to grapevine by insect vectors	UP (Kruger)	2009	2012
Genetic Transformation	GenUS 8/2	Developing resistance against phytoplasmas in grapevine	US-GEN (Burger)	2009	2012
Identification and Characterisation	230072	Study to determine occurrence and impact of Asian ladybird, <i>Harmonia axyridis</i> (Pallas), in Western Cape Winelands	ARC-Inf/Nietv (Achiano)	2009	2012
Epidemiology	WW 06/40	The epidemiology of grapevine yellows disease in South African vineyards	ARC-Inf/Nietv (Carstens)	2009	2012
Disease and Vector Control	AdK 3	Chemiese beheer van blaarspringers om Aster Yellows te bekamp	Private	2010	2012
Virus, Disease and Vector Detection	PPRI GP2	Development of a large-scale detection method for grapevine leafroll associated virus type 3	ARC-PPRI (Pietersen)	2010	2012/2014
Resistance		Grapevine genome analysis using DNA micro-array technology	US-GEN (Burger)		pending ⁴
Support technologies		Development of diagnostic assays for the detection of viruses of the rugose wood complex.	ARC-PPRI (Van der Merwe)		pending ⁴
Eradication		Epidemiology of the viti-viruses	ARC-PPRI		pending ⁴
Eradication		A survey to determine the prevalence of the grapevine stem pitting/stem grooving disease complex	ARC-PPRI		pending ⁴
		Symposium / workshops for researchers			
			Total (R)		

¹ Projects were categorised in four key focus areas that were identified to address the grapevine virus problem

² These projects were completed or terminated, but remain listed here for reference purposes

³ These technologies have largely been optimised, but need to be maintained for use in several of the other listed projects

⁴ These projects have been identified as of major importance for the grapevine virus programme and will be submitted for funding in future

PROGRAMME COMMITTEE

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E Wolf (Chair Plant Biotechnology committee)
E Hoffman (Chair Soil Science committee)
W Botha (Chair Viticulture & Oenology Forum)

GENERAL

The program members meet at least twice a year to discuss research progress and possible problems that have been experienced since the previous reporting occasion, as well as new project applications.

All the research projects in this programme that will involve the use or production of genetically modified organisms (GMOs) will be conducted in facilities that have been registered in terms of Regulation 4 of the Genetically Modified Organisms Act, 1997 (Act No.15 of 1997) with the National Department of Agriculture, Directorate: Genetic Resources.

A workshop is held on a yearly basis in which all the researchers with projects in this programme participate.

PROF JOHAN BURGER

PROGRAMME COORDINATOR

2009