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 MA Vivier	Prof MA Vivier
Present position	Professor	Professor
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Project Information

Research Organisation Project number	IWBT (2008) Grapevine 1
Project title	Understanding and manipulating disease resistance in grapevine

Fruit kind(s)	Grapes		
Start date (mm/yyyy)	1/1/2008	**End date** (mm/yyyy)	31/12/2012

Project keywords	Disease resistance, grapevine, fungal pathogens

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.

Vitis vinifera cultivars are susceptible to a range of economically important pathogens and the practice of vegetative propagation has artificially perpetuated this susceptibility, by excluding cross-breeding and natural adaptation to infections. Significant advances have been made to understand the genomic and genetic basis of resistance and susceptibility towards pathogens in grapevine resources. These advances, supported by the availability of a sequenced genome are also providing momentum to identify and functionally analyse defense and resistance genes and/or control mechanisms to provide functional markers for breeding programmes, or targets for potential genetic manipulation. This project tested two classes of grapevine defense proteins for their effectiveness to improve disease resistance; and also evaluated the functional roles and mechanisms of these proteins. The grapevine defensin gene, VvAMP1 was shown to be effective in orchestrating defense responses against a biotrophic organism (*Erisiphe necator*), but not a necrotrophic pathogen (*Botrytis cinerea*), whereas polygalacturonase-inhibiting proteins from grapevine were shown to effectively "prime" plants in anticipation for attack, but that this strategy did not cause resistance against a nectrotrophic pathogen such as *Botrytis*, but rather hyper-susceptibility. The data also confirmed the importance of the cell wall composition and architecture in plant-pathogen interactions. The functional analysis of the two defense-related genes contributes to future genetic screening of plant material since they can be considered "functional markers" for defense.

Problem identification and objectives

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

Disease management remains one of the key components in viticultural production systems. It also remains one of the major expenses in production and together with unpredictable climatic factors, one of the most important risk factors that could negatively impact grapevine products. Modern agriculture has dealt with this reality by improving natural resistance against pathogens by resistance breeding, as well as comprehensive use of agro-chemicals. Both these strategies have limits, and the use of agrochemicals in addition has extremely negative effects on the environment, as well as poses potential risks to consumers regarding harmful residues and product safety. Most grapevine producers around the world aim to optimise production and product quality; minimise the negative impact on the environment, while maintaining or improving cost-effectiveness and product safety. These broad industry aims provided the motivation of this study where the theme was "Understanding and manipulating disease resistance in grapevine", with the specific objectives to improve our understanding of the biology of grapevine regarding disease resistance.

Workplan (materials and methods)

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

PROJECT 1: ANALYSIS OF TRANSGENIC GRAPEVINE LINES OVEREXPRESSING ANTIFUNGAL PEPTIDE ENCODING GENES

The aim of this project was to evaluate the success of the overexpression of antifungal peptides in grapevine to improve plant resistance to pathogen attack. It also aimed at determining the anti-microbial spectrum of the peptides by testing them against a broad panel of mostly grapevine specific pathogens. Their mode of action as well as their *in planta* roles in pre-formed defense were also evaluated.

- **Milestone 1:** Genetic analysis of putative transgenic populations and functional analysis of the transgene(s) in the grapevine lines.

Putative transgenic Sultana and Red Globe lines overexpressing the VvAMP1 antifungal peptide encoding gene were available from the transformation programme of the IWBT. These lines were analysed for transgene presence and integration patterns (Southern blot), transgene expression (Northern blot or qRT-PCR), presence of antifungal peptides (Western analysis) and confirmation of antifungal activities (quantitative micro-titre plate assays, using *B. cinerea* as pathogen). The confirmed transgenic population producing active antifungal peptides were subsequently multiplied to establish both an *in vitro* and hardened off population of the transgenic lines.

- **Milestone 2:** Establish working cultures of major grapevine fungal pathogens and optimise *in vitro* and *in vivo* quantitative infection assays

The following pathogens have been used in the study

Botrytis cinerea
Uncinula necator (powdery mildew)
Botryosphaeria obtusa
Phaeomoniella chlamydospora
Cylindrocarpon liriodendri
Phomopsis viticola

- **Milestone 3:** Plant infection assays on the transgenic populations to identify resistance phenotypes, as well as determine the effectiveness of the antifungal peptides against *Botrytis* and *Uncinula*

Detached leaf and/or whole plant infection assays have been conducted on representative lines in the populations. These infections have been time-course assays to determine the reduced susceptibility of the transgenic lines against the test pathogens over an extended period.

- **Milestone 4:** In-depth analysis to understand the mode-of-action of the antifungal peptide.

An evaluation of the possible resistance mechanisms being used/induced by the antifungal peptides was conducted.

PROJECT 2: ANALYSIS OF TRANSGENIC GRAPEVINE LINES OVEREXPRESSING POLYGALACTURONASE-INHIBITING PROTEIN (PGIP) ENCODING GENES

- **Milestone 1:** Genetic analysis and PGIP activity assays of putative transgenic populations.

Putative transgenic Sultana, Chardonnay and Merlot lines overexpressing VvPGIP1 were available from the transformation programme of the IWBT. These lines were subjected to comprehensive genotypic and phenotypic screening.

- **Milestone 2:** Plant infection assays on the transgenic populations to identify resistance phenotypes

Whole plant infection assays have been conducted on representative lines in the populations against *Botrytis cinerea*.

- **Milestone 3:** In-depth analysis of the gene-specific resistance phenotypes to understand the mode-of-action and the *in planta* role(s) of PGIPs.

Evaluation of the effective inhibition of the PGIPs against the endopolygalacturonases (endoPGs) of *Botrytis cinerea* have been conducted. *In vitro* plate assays as well as *Agrobacterium* co-infiltration assays according to Joubert et al, 2007 were used.

- **Milestone 4:** Evaluation of cell wall metabolism in PGIP-specific resistance phenotypes to understand PGIP's role in "priming" cells for disease resistance

Recent results in our group have identified a possible role for PGIP in defense priming. From microarray analysis on tobacco overexpressing the grapevine PGIP1 gene, it became clear that the transgenic lines show differential gene expression in genes involved in cell wall metabolism. The differential changes all points to cell wall strengthening. This aspect has been studied further to clarify this "priming" phenotype and included a more comprehensive cell wall analysis

to corroborate the observation of altered cell wall metabolism when multiple copies of PGIP are present.

- **Milestone 5:** Evaluation of PGIP's role in defense signalling following infection

It has been conclusively shown that PGIPs are antifungal proteins that inhibit specific fungal ligands (polygalacturonases). Overexpression in various plant species have confirmed that they do afford the transgenic plants with protection against invading pathogens. What has not been shown is *how* it (PGIP) works in the plant to cause the decreased susceptibility. A transcriptomic approach was used to evaluate the differential response of PGIP overexpressing tobacco lines in comparison with the controls, using a time-course infection assay.

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.

PROJECT 1: ANALYSIS OF TRANSGENIC GRAPEVINE LINES OVEREXPRESSING ANTIFUNGAL PEPTIDE ENCODING GENES

Twenty five putative transgenic lines of *V. vinifera* (cv. Sultana) transformed with the *Vv-AMP1* expression cassette was obtained from the IWBT transformation and regeneration facility and subjected to genetic and phenotypical analysis. Characterisation included PCR screening, Southern and Northern blot analysis. Number of integrations refers to the number of Vv-AMP1 transgene integrated into the genome (Table 1).

Table 1: Summary of molecular characterisation of seven transgenic *V. vinifera* (cv. Sultana) lines overexpressing the Vv-AMP1 plant defensin.

Plant line	Wild type	6	8	9	10	14	18	19
PCR	-	+	+	+	+	+	+	+
Southern Blot	+	+	+	+	+	+	+	+
Northern Blot	-	+	+	+	+	+	+	+
Number of integrations	0	2	2	3	2	3	2	5
Morphological characterisation	+	+	-	-	+	+	+	-

A "+" denotes a positive result and a "-" a negative result. Morphological characterisation refers to the average leaf size and internode lengths (n=10) where a "+" denotes no statistically significant differences to the wild type lines and a "-" denotes significant differences from the wild type for each measurement taken. Wild type refers to the untransformed *V. vinifera* (cv. Sultana) used as a control.

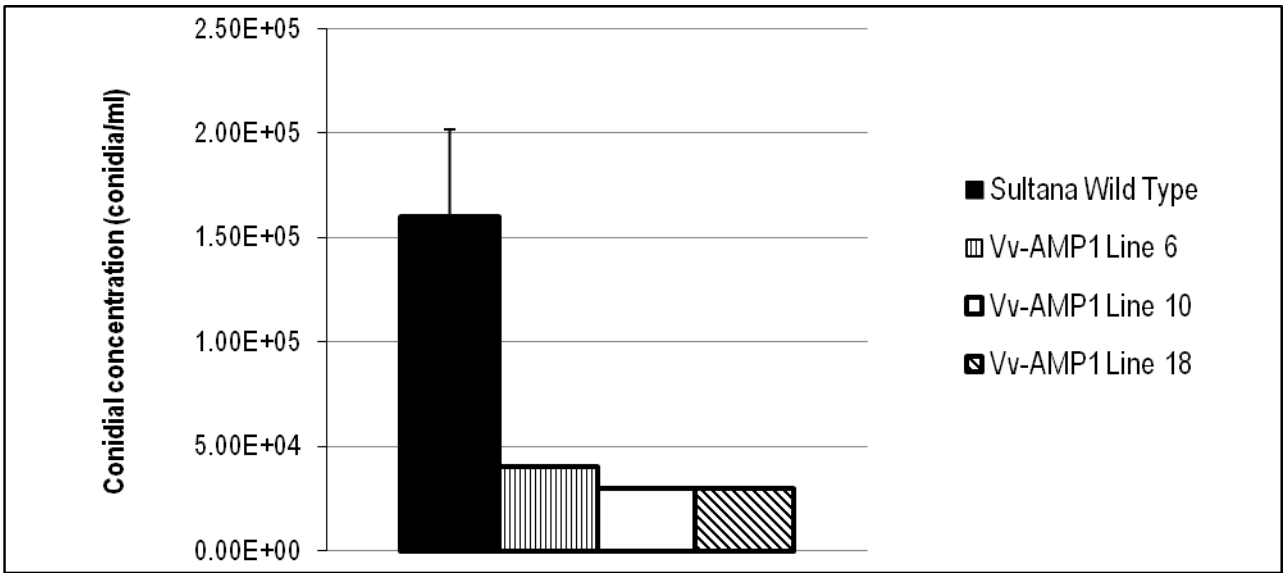
Table 2 lists the pathogens that have been used in the evaluation, as well as how they were prepared and used in assays.

Table 2. Growth and sporulation media for grapevine pathogen cultures

Organism	**Sporulation media**	**Assays**
Fungal isolates:		
Botrytis cinerea	PDA	*In vitro* and *in planta*
Erisiphe necator (powdery mildew)	Infected plant material	*In planta*
Botryosphaeria obtusa	Oatmeal Agar (OA)	*In vitro*
Phaeomoniella chlamydospora	PDA	*In vitro*
Cylindrocarpon liriodendri	PDA	*In vitro*
Phomopsis viticola	PDA/OA	*In vitro*

VvAMP1 had very strong activity against all tested pathogens in *in vitro* assays (De Beer and Vivier, 2008; Tredoux, 2011). Additional defensin peptides were also isolated and shown to be very active against *Botrytis* and *Phomopsis* (De Beer and Vivier, 2011).

Moreover, very strong resistance against powdery mildew (*Erisiphe necator*) have been observed in the transgenic population (Figure 1).



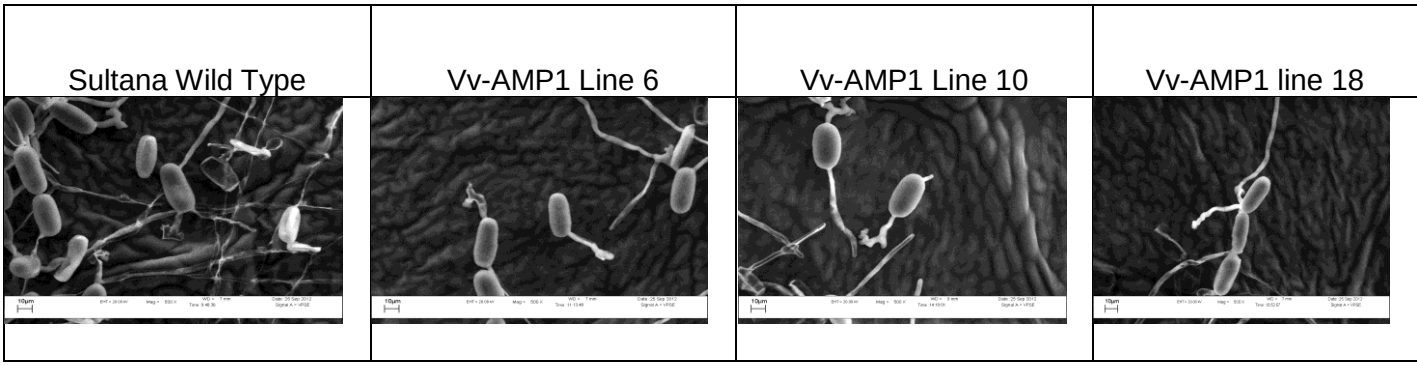


Figure 1. Comparison between three transgenic grapevine lines overexpressing Vv-AMP1 and the untransformed Sultana lines infected with *E. necator* conidia in a detached leaf infection assay. (A): Comparison between the conidial concentration produced at 14 dpi on the surfaces of transgenic Vv-AMP1 lines and wild type Sultana lines. Conidial concentration is the average of five technical repeats. Error bars indicate standard deviation between all detectable haemocytometer readings (n=3). (B): SEM micrographs representative of *E. necator* conidia forming infection structures on three transgenic Vv-AMP1 lines and a wild type Sultana line at 48 hrs post infection. The SEM images confirmed that the fungus could not form normal appressoriaon the transgenic plants.

Characterisation of the resistance mechanism of transgenic Vv-AMP1 lines against *E. necator* infection was performed according to a method described by Feechan et al (2011). Leaf material was harvested at 48 hrs after *E. necator* inoculation in order to determine the infection stage of the successfully germinated conidia through the identification of fungal appressoria and haustorium as well as the prevalence of Programmed Cell Death (PCD). Leaves were stained in trypan blue in order to distinguish fungal structures as well as dead host tissue. Germinated conidia were categorized according to their development of appressorium, haustorium or a combination of haustorium leading to PCD. The frequency of each infection mechanism was calculated in order to scale the level of susceptibility (Figure 2). According to this classification, the two types of *E. necator* resistance can be identified as either penetration resistance or induction of PCD. The wild type Sultana lines showed high susceptibility to *E. necator* infection with 72% of germinated conidia leading to the development of haustoria and secondary hyphae after each infection attempt through appressorium formation. Only 16% of the germinated conidia on the untransformed wild type showed the development of an appressorium without

successful penetration of the epidermal cells, whereas 9% of germinated conidia lead to PCD of the penetrated epidermal cells (Figure 2). At 14 dpi, a dense network of sporulating hyphae was observed for the untransformed control. The transgenic lines tested on the other hand predominantly showed PCD associated resistance. The mechanism of resistance of these transgenic lines could be characterised by rapid host cell death, as well as the presence of abnormal appressorium formation (Figure 1) and epidermal cell penetration of the germinated conidia.

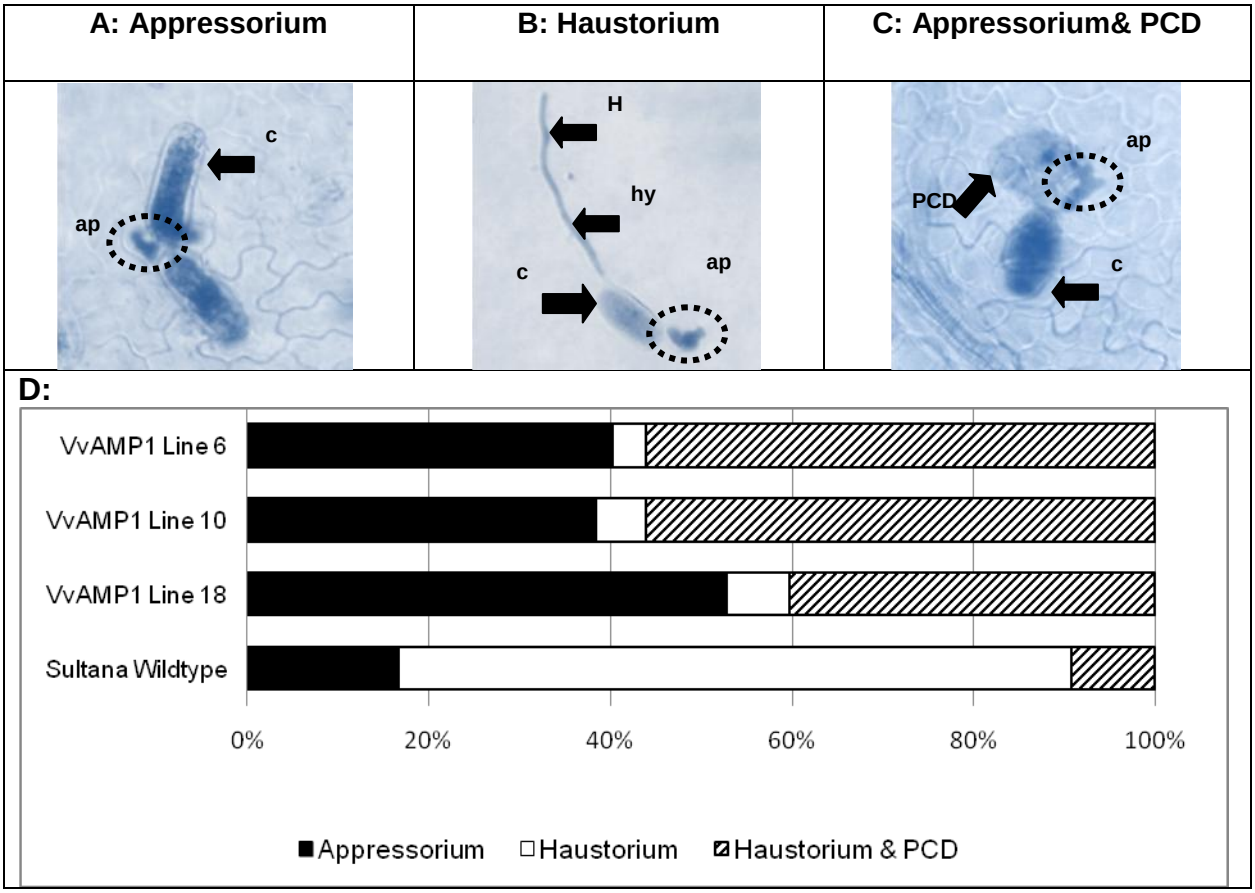


Figure 2. The susceptibility of various grapevine lines overexpressing Vv-AMP1 in comparison to the Sultana wild type in reaction to the infection with dry *E. necator* conidia in a detached leaf infection assay at 48hrs post-inoculation. The evaluation is based on the method described by Feechan *et al*., (2011). The samples had been stained in a trypan blue solution and visualized under a light microscope at 100X magnification. (A): Criteria according to which the infection mechanism of the conidia is classified as "appressorium" due to the visible formation of an appressorium but no successful penetration or subsequent fungal development. (B): Criteria according to which the infection mechanism of the germinating conidia is classified as "haustorium" due to the visible formation of an appressorium and subsequent formation of a haustorium and secondary hyphae. (C): Criteria according to which the infection mechanism of the germinating conidia is classified as "haustorium & PCD" due to the visible formation of an appressorium leading to successful penetration and subsequent programmed cell death. (D): The frequency of *E. necator* conidial penetration events resulting in appressorium formation but no successful penetration, successful penetration, haustorium formation and secondary hyphal development or the development of a haustorium followed by programmed cell death (PCD). Broken black circles indicate the position of an appressorium. ap, appressorium; c, conidium; hy, hypha; H, haustorium. The total number of germinated conidia counted for each line was as follows: Sultana wild type, 55; Vv-AMP1 line 6, 84; Vv-AMP1 line 10, 77; Vv-AMP1 line 18, 74.

The transgenic lines did not show any enhanced resistance against the necrotroph *B. cinerea*. (Figure 3).

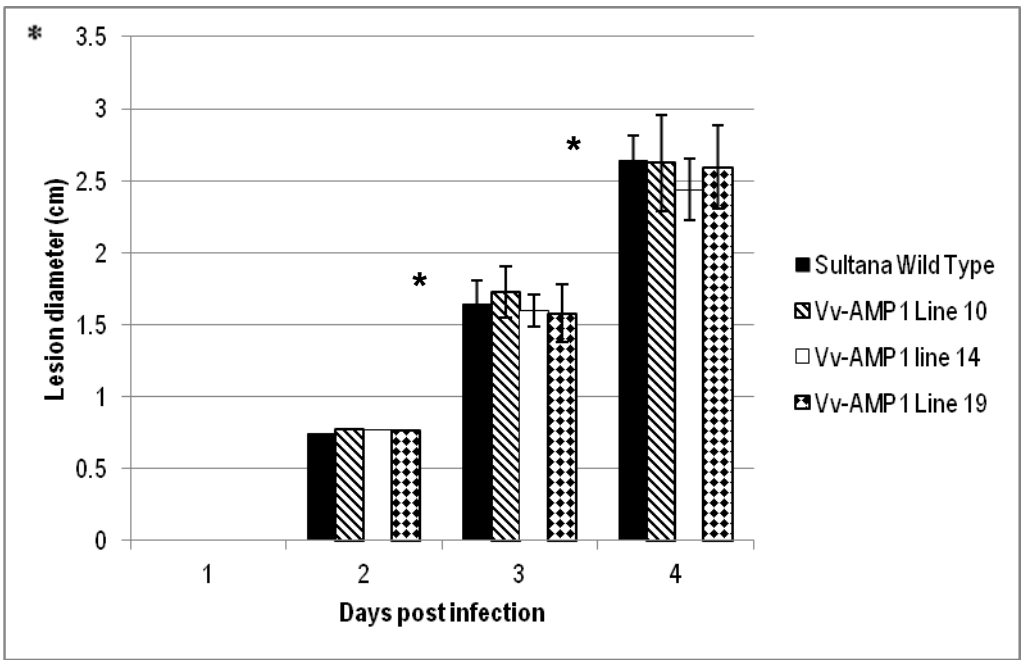


Figure 3. Lesion development of Sultana transgenic lines overexpressing Vv-AMP1 as compared to untransformed wild type Sultana lines in a whole-plant infection assay with *B. cinerea*. Three individuals from each plant line with four leaves per plant infected with three spots per leaf (1000 spores per spot). Error bars indicate standard deviation and asterisks indicate statistical difference from the Sultana wild type ($p<0.05$).

An evaluation into the possible modes-of-action of the antifungal peptides has been completed by Barkhuizen et al., 2012)

Our results show:

- that **cations directly induced conformational changes** in the secondary structure of plant defensins
- Growth inhibition activity of the defensins tested against *B. cinerea* was reduced in the presence of monovalent and divalent cations
- Our results clearly indicate a **definite conformational change in the secondary structure** of Hc-AFP1, 2 (pGlu) and (Gln) in the presence of divalent and monovalent cations.

PROJECT 2: ANALYSIS OF TRANSGENIC GRAPEVINE LINES OVEREXPRESSING POLYGALACTURONASE-INHIBITING PROTEIN (PGIP) ENCODING GENES

A transgenic *V. vinifera* population overexpressing two non-*vinifera* PGIP encoding genes was analysed. Below is a summary of the main findings:

- Transgene presence, expression and integration events were confirmed in the transgenic population (Milestone 1)
- Transgene derived protein activity (crude extracts) were confirmed (Milestone 1)
- The non-vinifera PGIPS inhibited BcPG1 and 6 *in vitro* but no inhibition against BcPG2 was detected *in the plate assays* (similar results as previously obtained for VvPGIP1; Joubert et al, 2007) (Milestone 3)
- The non-vinifera PGIPS however significantly inhibited BcPG2 the *in vivo* assays (*Agrobacterium* co-infiltration studies), confirming again that the pectic substrates in the plant cellular environment are important in the inhibition interaction between the PG-PGIP pairs (similar results as previously obtained for VvPGIP1; Joubert et al, 2007) (Milestone 3)

- **Overexpressing the non-*vinifera* PGIPs in the *V. vinifera* background promoted susceptibility, not resistance, against *B. cinerea* infection in a whole plant infection (Figures 4 and 5).** This is a very surprising result, since the same construct lead to very strong resistance phenotypes in tobacco. Moreover, neither VvPGIP1 nor the non-*vinifera* PGIPs were silenced during the first 48 hours post infection, eliminating induced gene silencing as an underlying cause for this phenotype (Milestone 2).

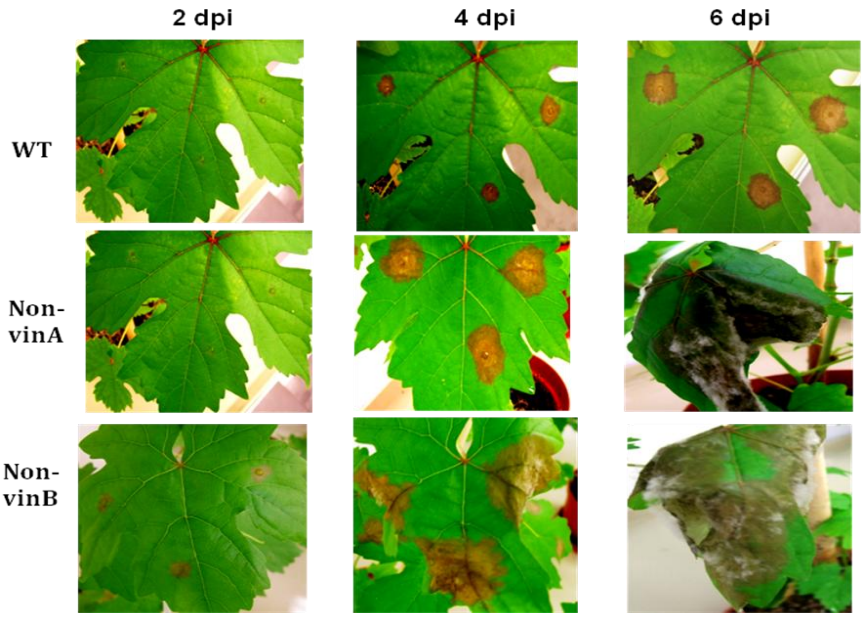


Figure 4. Lesion development of a representative subset of plant lines challenged with *B. cinerea* in a whole plant antifungal assay (1000 spores per spot). Lesions were measured at 24 hour intervals.

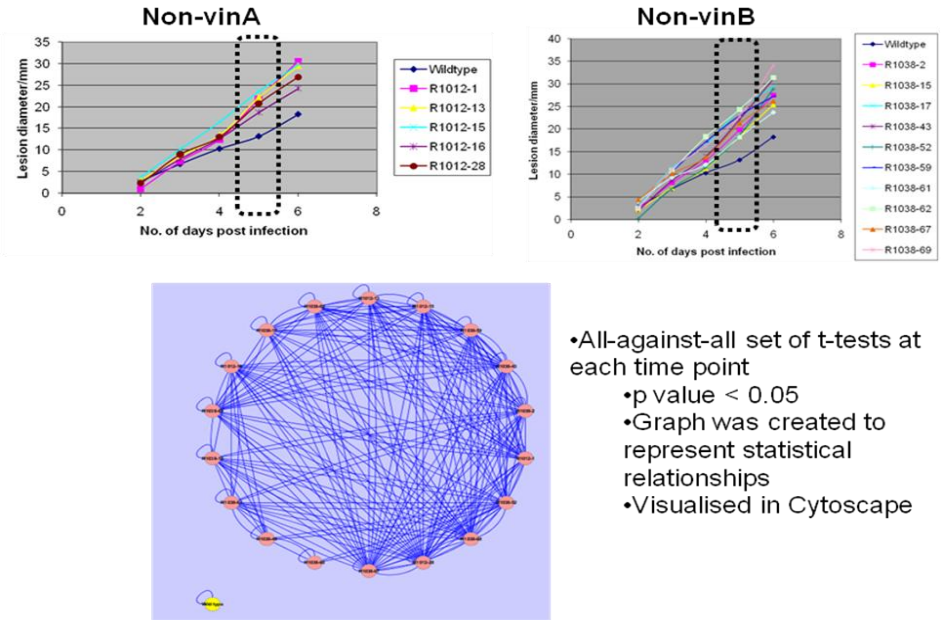


Figure 5. Lesion development on whole plant Redglobe transgenic lines overexpressing (**A**) *PGIP*1012 and (**B**) *PGIP*1038. Untransformed WT lines were used as controls. All plants were challenged with 1000 *B. cinerea* spores per infection spot with four leaves being infected per plant (three infection spots per leaf). Lesions were measured at 24 hour intervals from day two post infection. Cytoscape-generated graph showing the wild type (highlighted in yellow) clearly separated from the transgenic lines (highlighted in orange/dark pink) at five days post infection. The graph illustrates that at day five, the difference in lesion diameter of the wild type compared to all the transgenic lines was statistically significant at $p<0.05$.

The contribution of the grapevine PGIP to activated defense was studied through microarray analysis following a time-course infection of the transgenic population with *B. cinerea*. The results showed that a total of 1270 Gene Ontology terms were enriched across the time-course (Figure 6). (Milestone 5)

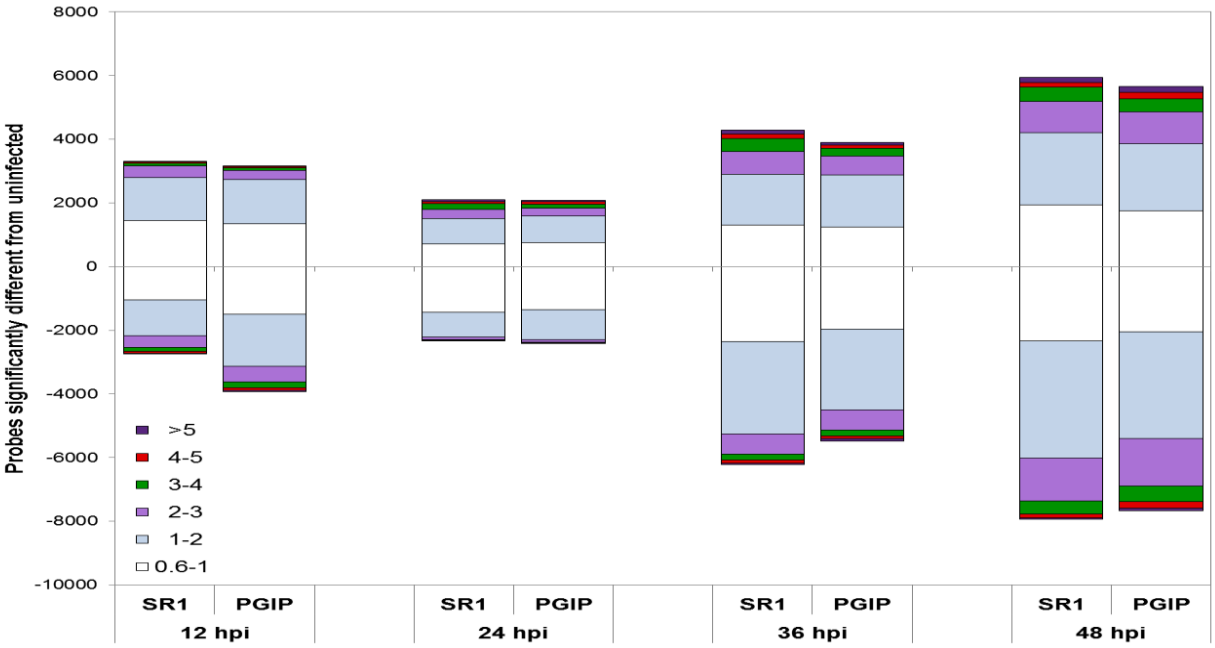


Figure 6. The comparative profile of probes differentially expressed between the transgenic VvPGIP1 and control population following infection with *Botrytis* over a 48 period.

Moreover, the data obtained supported a "priming" role for the PGIP prior to defense:

- Photosynthesis was induced prior to infection and repressed earlier in the transgenic lines
- Jasmonic acid synthesis was induced earlier in the transgenic lines (also corresponded to actual increased levels of this defense signalling hormone (see Figure 7).
- Jasmonic acid-responsive elements were also induced earlier in the transgenic lines.

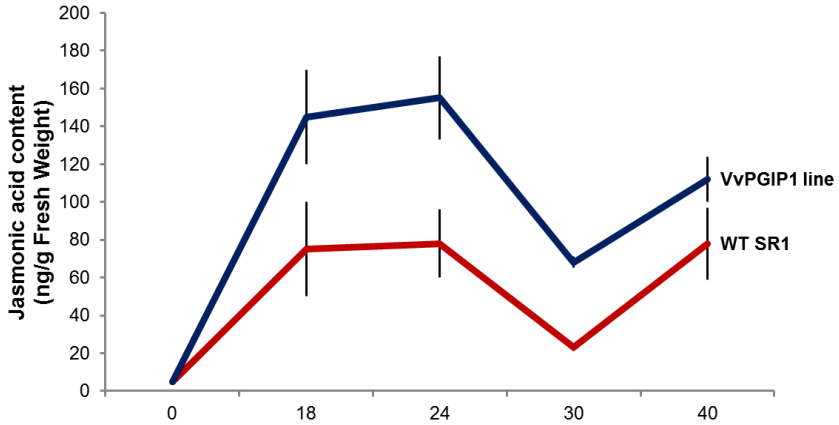


Figure 7. Jasmonic acid levels in VvPGIP1 transgenic lines and the control following *Botrytis* infection over an 48 hour period.

In addition to the results presented and discussed here, the following publications contain data related to the project. Since these publications are available, the results contained in them will not be further discussed here:

- Alexandersson, E.O., J.v.W. Becker, D. Jacobson, E. Nguema-Ona, C. Steyn, K.J. Denby & M.A. Vivier. 2011. Constitutive expression of a grapevine polygalacturonase-inhibiting protein affects gene expression and cell wall properties in uninfected tobacco. *BMC Res Notes* 4:493 ((Milestones 4 and 5)
- Nguema-Ona, E., J.P. Moore, A. Fagerström, W.G.T. Willats, A. Hugo & M.A. Vivier. 2012. Profiling the main cell wall polysaccharides of tobacco leaves using high-throughput techniques. *Carbohydrate Polymers* 88: 939– 949 (Milestone 4)
- Joubert, D.A., G. De Lorenzo & M.A. Vivier. 2013. Regulation of the grapevine polygalacturonase-inhibiting protein encoding gene: expression pattern, induction profile and promoter analysis. *J Plant Res* 126:267–281 (Milestones 3 and 5)
- Nguema-Ona, E. J.P. Moore, A. Fagerström, J.U. Fangel, W.G.T. Willats, A. Hugo & M.A. Vivier. 2013. Overexpression of the grapevine PGIP1 in tobacco results in compositional changes in the leaf arabinoxyloglucan network in the absence of fungal infection. *BMC Plant Biology* 13:46 (Milestones 4 and 5)

Milestones achieved in this study

PROJECT 1: ANALYSIS OF TRANSGENIC GRAPEVINE LINES OVEREXPRESSING ANTIFUNGAL PEPTIDE ENCODING GENES				
Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Genetic analysis of putative transgenic populations and functional analysis of the transgene(s) in the grapevine lines.	2009		2009	Complete characterisation of the transgenic population
2. Establish working cultures of major grapevine fungal pathogens and optimise *in vitro* and *in vivo* quantitative infection assays	2008		2008	Cultures and methods were established for the following pathogens: *B. cinerea; E. necator* (powdery mildew); *B. obtuse; P. chlamydospora; C.liriodendra; P. viticola*
3. Plant infection assays on the transgenic populations to identify resistance phenotypes, as well as determine the effectiveness of the antifungal peptides against *Botrytis* and *Erisiphe*	2011	2012	2012	Disease resistance against *Erisiphe necator* found. The fungal appressoria were abnormal leading to poor penetration on the transgenic plants, as well as a very strong programmed cell death phenotype. The transgenic lines did not show increased resistance against *Botrytis*.
4. In-depth analysis to understand the mode-of-action of the antifungal peptides	2011		2011	Peptides from grapevine and a native South African Brassicaceae were isolated and purified and studied in terms of their ability to disrupt fungal membranes as well as their response to the presence of cations.
5. Journal publication/s – final milestone	2013		In process for 2013	Two papers have been published and two are currently in preparation
PROJECT 2: ANALYSIS OF TRANSGENIC GRAPEVINE LINES OVEREXPRESSING POLYGALACTURONASE-INHIBITING PROTEIN (PGIP) ENCODING GENES				
Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Genetic analysis of putative transgenic populations and functional analysis of the transgene(s) in the grapevine lines.	2009		2009	All populations have been genetically characterised. Functional characterisation of the transgenes confirmed their functional roles in inhibiting endopolygalacturonases from fungi, specifically the six endoPGs studied from *Botrytis.*
2. Plant infection assays on the transgenic populations	2011	2012	2012	A surprising phenotype of increased susceptibility towards *Botrytis* was observed in

to identify resistance phenotypes				overexpressing grapevine lines. This was not due to transgene, or native PGIP gene silencing.
3. In-depth analysis of the gene-specific resistance phenotypes to understand the mode-of-action and the *in planta* role(s) of PGIPs	2010-2011		2010-2011	PGIP transgenics showed that PGIPs also have additional roles unknown until now, specifically with regards to modulating cell wall changes linked to a "priming" phenotype.
4. Evaluation of cell wall metabolism in PGIP-specific resistance phenotypes to understand PGIP's role in "priming" cells for disease resistance	2011		2011	Cell wall profiling of tobacco and grapevine leaf tissues also clarified the priming phenotype further and suggests that the specific characteristics of the cell walls of a species and its organs are important parameters that will influence the success of a pathogen.
5. Evaluation of PGIP's role in defense signalling following infection				A transcriptomic analysis of grapevine transgenics and the controls confirmed that the transgenic lines responded earlier to the infection at the localised lesion. The data also confirms that the transgenic plants are "prepared" for defense since they responded quicker and with higher intensity with regards to subsequent defense signalling and the accumulation of defense-related hormones and hormone signals.
6. Journal publication/s – final milestone	2013		2013	Four papers have already been published and two additional papers are in preparation from this work.

Accumulated outputs

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

Conclusions

The aim of this project was to confirm the functions of two classes of defense proteins of grapevines as possible targets to improved disease resistance. The project was successfully completed and confirmed the functions of both the defensin peptides and the polygalacturonase-inhibiting proteins in plant defense against fungal pathogens. When the grapevine VvAMP1 defensin was overexpressed in its native host, it caused strong resistance against the biotroph *Erisiphe necator*, but did not increase resistance against the necrotropihic *B. cinerea*. Overexpression of PGIPs from non-vinifera grapevine species in *V. vinifera*, resulted in dramatic increase in susceptibility against *B. cinerea*, despite it being shown that those PGIPs protected tobacco against the pathogen through a process of priming for disease and effective inhibition of the endoPGs from *Botrytis*. Cell wall profiling methods optimised for tobacco and grapevine also shed more light on this priming phenotype in tobacco and will be instrumental to understand the hypersusceptible phenotype observed. These transgenic lines are excellent genetic resources to study the interaction with the pathogens more in depth, but it is already clear that PGIPs have the ability to "prime" the host plant against attack.

Technology development, products and patents

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

Transgenic populations with specific traits have been developed and characterised. The results obtained showed that effective resistance against important grapevine pests could be obtained with this strategy. Although GMOs in the grapevine and wine industry are not viable, the information obtained could feed into marker-assisted breeding and selection schemes, since the gene products have been comprehensively characterised, including functional analysis. They

can therefore be considered “functional markers” when analysing defense genes. The optimised methods and techniques, specifically the screening techniques could potentially be developed into commercial services with regards to disease resistance screening of plant material.

Suggestions for technology transfer

List any suggestions you may have for technology transfer

In addition to a popular paper summarising the results, the research will be presented at the next SAJEV conference (November 2013) specifically to put in context with international trends and results obtained in the field of “Improving grapevine Resistance against pathogens”.

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. De Beer, A (PhD; 2008)	R 0
2. Venter, A (MSc; 2009)	R 2 500
3. Moyo, M (MSC; 2010)	R 45 811
4. Tredoux, MM (MSc; 2010)	R 78 097
5. Du Plessis, K (MSc; 2012)	R 34 000
6. Barkhuizen, H (MSc; 2012)	R 74 000
7. Alexandersson, E (Postdoc; 2009-2011)	None

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

1. De Beer, A. & M.A Vivier. 2008. Vv-AMP1, a ripening induced peptide from *Vitis vinifera* shows strong antifungal activity. BMC Plant Biology 8: 75-90.
2. Moore, J.P., B. Divol, P.R. Young, H.H. Nieuwoudt, V. Ramburan, M. du Toit, F.F. Bauer & M.A. Vivier. 2008. Wine biotechnology in South Africa: towards a systems approach to wine science. Biotechnology Journal 3 (11): 1355-1367.
3. Bauer, F.F., T. Naes, K. Esbensen, P.R. Young, M. du Toit & M.A. Vivier. 2008. Functional wine-omics. Blair, R.J.; Williams, P.J., Pretorius, I.S. (eds) Proceedings of the Thirteenth Australian Wine Industry Technical Conference, 29 July to 2 August 2007, Adelaide South Australia. Australian Wine Industry Technical Conference Inc. Adelaide, South Australia. pp 178-183.
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6. Nguema-Ona, E., J.P. Moore, A. Fagerström, W.G.T. Willats, A. Hugo & M.A. Vivier. 2012. Profiling the main cell wall polysaccharides of tobacco leaves using high-throughput techniques. *Carbohydrate Polymers* 88: 939– 949
7. Joubert, D.A., G. De Lorenzo & M.A. Vivier. 2013. Regulation of the grapevine polygalacturonase-inhibiting protein encoding gene: expression pattern, induction profile and promoter analysis. *J Plant Res* 126:267–281
8. Nguema-Ona, E. J.P. Moore, A. Fagerström, J.U. Fangel, W.G.T. Willats, A. Hugo & M.A. Vivier. 2013. Overexpression of the grapevine PGIP1 in tobacco results in compositional changes in the leaf arabinoxyloglucan network in the absence of fungal infection. *BMC Plant Biology* 13:46

Presentations/papers delivered

2008:

(1) Becker, J.v.W., A. Venter, S. Mbewana & M.A. Vivier. 2008. Functional analysis of Polygalacturonase-inhibiting proteins (PGIPs) from *Vitis* species: Identification of an additional role in plant defence. Eighth International Symposium on Grapevine Physiology and Biotechnology, Adelaide, Australia. (23-28 Nov)

(2) Nguema-Ona, E., J.P. Moore & M.A. Vivier. 2008. A method for analysing cell wall reinforcement in transgenic tobacco lines expressing *Vitis vinifera* polygalacturonase-inhibiting proteins (PGIPs). Cape Biotechnology Forum, Lord Charles Hotel, Somerset West. (30 Nov – 2 Dec)

(3) Vivier, M.A. 2008. Understanding and manipulating grapevine stress responses. Cape Biotechnology Forum**,** Lord Charles Hotel, Somerset West. (30 Nov – 2 Dec) (By invitation/Op uitnodiging – Keynote speaker)

(4) Vivier, M.A. 2008. Understanding and manipulating grapevine stress. South African Chemometrics Symposium, Stellenbosch, South Africa. (1-5 Dec)

2009:

(5) Nguema-Ona, E.E., Moyo, M., J. Steyn, J.P. Moore & M.A. Vivier. 2009. Cell wall remodeling in PGIP overexpressing lines of tobacco (*Nicotiana tabacum*) and grapevine (*Vitis vinifera*). Fourth International Viticultural and Oenology Conference, Cape Town International Convention Centre, Cape Town, South Africa. (28 – 30 July)

(6) Nguema-Ona, E., J.P. Moore & M.A. Vivier. 2009. The role of polygalacturonase inhibiting proteins (PGIPs) and the cell wall of grapevine in pathogene defence. South African Association of Botany (SAAB), Stellenbosch University. (19-23 Jan.)

(7) Tredoux M.M., de Beer A., Rautenbach M. and Vivier M.A. 2009. Production and purification of a grapevine antifungal peptide in *Escherichia coli*: An optimised method. SASM 09/BIO-2-BIZ, International Convention Centre, Durban (20 - 23 September).

(8) Venter, A., M.M. Tredoux, A. De Beer, Vivier, M.A**.** 2009. Antifungal genes and proteins from grapevine. SASM 09/BIO-2-BIZ, International Convention Centre, Durban (20 - 23 September).

(9) Moore, J.P., E.E. Nguema-Ona & M.A. Vivier. 2009. Tracking the careers of grape and wine polymers. Fourth International Viticultural and Oenology Conference, Cape Town International Convention Centre, Cape Town, South Africa (28 – 30 July).

(10) Vivier, M.A. 2009. Witroes bestandheid – ontwikkeling van GMO druiwevariëteite. Winetech-VinPro Streeks-inligtingsdag: Witroes Simposium. Augustus/September 2009.

2010:

(11) Nguema-Ona, E., C. Steyn, J.P. Moore, E. Alexandersson, D. Jacobson & M.A. Vivier. 2010. Polygalacturonase-inhibiting proteins (PGIPs), cell wall remodeling and defense: Are there links? *Twelfth Cell Wall Meeting*, Porto, Portugal (25-30 July).

(12) Nguema-Ona, E., J.P. Moore, E. Alexandersson, D. Jacobson & M.A. Vivier. 2010. Analyses of *Botrytis cinerea* defense phenotypes highlight the importance of studying the plant cell wall in plant-pathogen interactions. Fifteenth International *Botrytis* Symposium, Conference Hall: Faculty of Philosophy and Arts, University of Cádiz, Cádiz, Spain (keynote).

(13) Vivier, M.A. 2010. Genetics and genomic approaches to improve grape quality for winemaking. International *Intervitis Interfructa* Congress 2010 [Sixtieth German Grape and Wine Congress], New Stuttgart Trade Fair Centre, Germany. (24 – 28 March)

(14) De Beer, A. & M.A. Vivier. 2010. Intein-mediated expression allows for the production of biologically active plant defensin peptides in *Escherichia coli*. Cape Biotechnology Forum 2010, Lord Charles Hotel, Somerset West. (24-26 March)

(15) Jacobson, D., E. Alexandersson, P.R. Young & M.A. Vivier. 2010. Re-examining microarray probe sets to improve downstream analysis. Cape Biotechnology Forum 2010, Lord Charles Hotel, Somerset West. (24-26 March)

(16) Nguema-Ona, E., J.P. Moore & M.A. Vivier. 2010. Cell walls and plant defense: Using microscopical and analytical techniques to elucidate resistance phenotypes. Cape Biotechnology Forum 2010, Lord Charles Hotel, Somerset West. (24-26 March)

2011 :

(17) Vivier, M.A. & P.R. Young. 2011. GMO grapevines: excellent study models and promising (future) products. Second International Society for Horticulture Science Genetically Modified Organisms in Horticulture (GMO 2011), Nelspruit. (11-15 September) (Keynote)

2012:

(18) Barkhuizen, H., A. de Beer, M. Rautenbach & M.A. Vivier. 2012. Mode of action studies of defensin peptides from native South African *Brassicaceae* species. SASBMB-FASBMB 2012 congress, Champagne Sports Resort, Drakensberg, KwaZulu-Natal. (29 January – 1 February)

Total cost summary of the project

TOTAL COST IN REAL TERMS	YEAR	CFPA	DFTS	DFPT	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1	**2008**	0	0	175 000	0	250 000	201 875	0	626 875
YEAR 2	**2009**	0	0	270 000	0	270 000	270 000	0	810 000
YEAR 3	**2010**	0	0	291 600	0	297 000	294 300	0	882 900
YEAR 4	**2011**	0	0	312 012	0	326 700	319 356	0	958 068
YEAR 5	**2012**	0	0	0	0	0	0	0	0
TOTAL		**R 0**	**R 0**	**R 1 048 612**	**R 0**	**R 1 143 700**	**R 1 085 531**	**R 0**	**R 3 277 843**