CFPA	SAAPPA / SASPA / SAT	DFTS	Winetech
Canning Fruit Producers' Assoc. Submit to: Wiehahn Victor Tel: +27 (0)21 872 1501 inmaak@mweb.co.za	Fruitgro Science Submit to: Louise Liebenberg Tel: +27 (0)21 882 8470/1 louise@fruitgro.co.za	Dried Fruit Technical Services Submit to: Dappie Smit Tel: +27 (0)21 870 2900 dappies@dtd.co.za	Submit to: Jan Booysen Tel: +27 (0)21 807 3324 booysenj@winetech.co.za
	X		X

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

FINAL REPORT
FOR 2012

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Professor MA Vivier	Dr JP Moore
Present position	Professor	Researcher
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PROJECT INFORMATION

Project number	IWBT-P 09/01
Project title	Evaluating cell wall properties of wine associated organisms
Project Keywords	Cell walls, wine, grapes, yeast, technical methods

Industry programme	**CFPA**	
	Deciduous	
	DFTS	
	Winetech	X
	Other	SATI

Fruit kind(s)	Wine and Table Grapes
Start date (dd/mm/yyyy)	01/11/2008
End date (dd/mm/yyyy)	01/11//2011

FINAL REPORT

(Completion of points 1-5 is compulsory)

1. Executive summary

Give an executive summary of the *total* project in no more than 250 words

The cell wall of biological organisms (such as plants, yeast or bacteria) constitutes the interface between the external environment and the interior cellular apparatus. All of the major wine-associated organisms (*Vitis vinifera*, *Saccharomyces cerevisiae*, and the various wine bacteria) possess cell walls, although, the molecular composition and arrangement differ widely between the specific organisms. A number of reference methods on cell wall composition (monosaccharide composition and associated linkage analysis using GC-MS), lignin profiling methods (through collaboration), amino acid analysis and enzymatic (e.g. XET) analysis, have been successfully implemented. ATR-FT-MIR and NIR methods have been applied to plant and microbial (yeast) samples (both dry powders and wet samples) during normal (and fermentative growth in the case of yeast) growth and development. NMR methods are being performed through collaboration with research groups in Sweden and the USA. MS-based screening methods (using commercial enzyme preparations) have been performed on plant and microbial samples. Carbohydrate Microarray Polymer Profiling (CoMPP) analysis has been performed on tobacco, grapevine and yeast samples through collaboration with Prof. Willats in Copenhagen, Denmark. All of these accurate, reference methods for cell wall samples are now being implemented in plant and microbial projects with research groups at the Institute for Wine Biotechnology (Grapevine and Yeast Groups). A number of publications are emanating from this work in international agricultural, microbial and plant science journals.

2. Problem identification and objectives

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

This project should be classified as a technology-development project in support of projects studying the biology of plants and microorganisms (that have cell walls) in the wine and table grape industries.

The cell wall of biological organisms (such as plants, yeast or bacteria) constitutes the interface between the external environment and the interior cellular apparatus. All of the major wine-associated organisms (*Vitis vinifera*, *Saccharomyces cerevisiae*, and the various wine bacteria) possess cell walls, although, the molecular composition and arrangement differ widely between the specific organisms. The cell wall is of fundamental importance in the vineyard, since grapevine plants undergo changes in wall composition in roots, berries and leaves due to developmental and environmental (e.g. *climate and disease*) influences. Cell walls play a direct role in textural and colour properties of grape berries during ripening in both the wine- and table grape industries. Wall properties and processes (such as phenolic oxidation commonly referred to as *browning reactions*) are also known to strongly influence storage potential of berries and post-harvest quality of many fruits (including grapes). Similarly, during the winemaking process numerous wall-derived polymers (commonly *polysaccharides, proteins and phenolic polymers*) are supplied to the fermenting must from the grape berries as well as yeast and bacteria present. These polymers interact with numerous components present in the must affecting filtration efficiency, *aroma compound retention*, *solution clarity*, *organoleptic properties (e.g. mouth-feel)* and the potential *health-improving* properties of the wine produced. Clearly, wall derived polymers and processes impart significant desirable and undesirable characteristics, during grape-growing (e.g. *berry ripening, browning potential, pathogen susceptibility*), grape storage (e.g. *texture modification, browning reactions*), fermentation (such as *yeast and bacterial growth phenotypes*) and wine properties (e.g. *aroma, mouth-feel, turbidity, health properties*).

The problem: Despite the obvious importance associated with cell wall properties in the grape and wine industries, very little is known regarding the fundamental scientific processes that relate to these quality parameters (aroma, storage (browning), texture). Accurate, but high-throughput analytical and profiling methods for cell walls are lacking. What is greatly needed is a set of methodologies to study these processes by ***'Tracking the careers of grape and wine polymers'*** from the vineyard, through the storage and processing steps, during winemaking, to the shelf and/or glass. Developing relatively rapid, but also highly accurate techniques to dissect and monitor wall processes under defined conditions *(model vineyards, standardised samples (e.g. genetic negative and positive controls)) and 'controlled' field and cellar conditions* will be of tremendous value. Understanding the underlying scientifically defined processes (*causes*) which govern the various quality parameters (*effects*) discussed previously is an absolute requirement for rational and effective experimentation in the table grape and wine industries.

The aims of the project are:

The ultimate aim of this project is to develop **'cell wall platform analytical technologies and capacity'** to support the various research themes under study in the table grape- and wine research programmes. The main project is divided into three sub-projects which integrate with each other through the use of *Chemometrics and Statistical analysis*. Sub-project 1 is to develop a set of reproducible and highly accurate reference methods for the various wall components to be analysed. Sub-project 1 includes the use of sophisticated analytical techniques such as Spectrophotometry, Gas Chromatography (**GC**), High Performance Liquid Chromatography (**HPLC**) and Mass Spectrometry (**MS**). Sub-project 2 is to develop rapid non-invasive 'screening' methods using Fourier Transform (**FT**) Mid or Near Infrared (**MIR and NIR**) and Nuclear Magnetic Resonance (**NMR**) spectroscopy. This will allow the application of *Chemometric* techniques to develop test models with the aid of the reference data generated in Sub-project 1. Sub-project 3 will involve the use of non-spectroscopic, semi-rapid but highly specific analytical techniques to analyses wall components. These techniques principally involve the development of '***glycan arrays*** *using commercial cell wall antibodies*' and '***mass profiling*** *using mass spectrometry*' to analyse wall sub-structures. Each sub-project integrates through the use of metric techniques to provide complementary information on the particular cell wall system under study.

Although the main thrust of this project is to develop and evaluate the technologies for cell wall profiling and analysis, it will be integrated with current research projects in grapevine biotechnology. This will provide this project with fully characterised materials to work with and would lead to immediately useful datasets from the analysis of the prototype grapevines.

THE IMPORTANCE OF A CELL WALL PLATFORM FOR THE GRAPEVINE BIOTECHNOLOGY PROGRAMME OF THE TABLE AND WINE GRAPE INDUSTRIES

The specific aims of this portfolio of projects (Programme leader: Prof MA Vivier, IWBT, Stellenbosch University) are in support of the broad goals of the South African grapevine industries towards sustainable, environmentally-friendly production practices and excellent product quality and safety. The projects use molecular biology tools and related technologies to enhance viticultural research. The projects are designed to generate basic scientific data that would further increase our knowledge of processes that are either linked to production and/or quality aspects of grapes, but would also lead to new and/or improved biotechnology strategies and products. The questions being asking are: (i) How does grapevine defend itself against pathogens and how can it be improved; (ii) How does grapevine deal with environmental stresses and how can stress-related pathways be used to improve environmental stress resistance and other quality impact factors? These research projects are based on analyses within grapevine, building on the results and advances made within this programme that previously focused mostly on model plants. This research are supported

by the availability of a sequenced grapevine genome and presents the opportunity to implement the technologies in grapevine research where the complex genome-environmental interactions are studied on a systems level by using –omics (transcriptomic, proteomic and metabolomic) technologies.

THE IMPORTANCE OF A CELL WALL PLATFORM FOR THE YEAST AND BACTERIAL BIOTECHNOLOGY PROGRAMME OF THE WINE GRAPE INDUSTRY

Fermentation processes during the production of wine requires the maceration of grape berries (releasing diverse viscous mostly wall derived polysaccharides) and supply of microbial cultures (yeast and bacteria produce wall glycoproteins and polysaccharides) (Dallies *et al.*, 1998; Lipke and Ovalle, 1998). These polysaccharides are partially degraded *releasing new wine compounds*, can be viscous leading to *blocked filters* and can *encapsulate aroma compounds*. Yeast and bacterial cells can participate in diverse interactions (such as *adhesion, flocculation and biofilm* (velum) formation) which are of direct relevance to clarification and sedimentation pocesses during winemaking. These interactions are directly related to wall surface properties which are similarly connected with polysaccharide and protein composition of the microbes responsible. Certain types of polysaccharides supplied by the plant, yeast and bacteria are able to survive the winemaking process ending up in the bottled wine. These glycoproteins and polysaccharides can *participate in or prevent the production of haze* resulting in the formation of cloudy wines. These cloudy wines are negatively perceived by the market in general and thus research is necessary to understand these processes. Furthermore, these polysaccharides and proteins contribute to the *mouthfeel properties* of the wine and may also possess *health benefits*.

3. Workplan (materials & methods)

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

Materials:

- Wild type and transgenic plants overexpressing grapevine antifungal peptide genes, PGIP genes, and various carotenoid genes (for plant-pathogen studies, environmental stress studies, as well as grape-derived flavour/aroma studies)
- Grapevine and tobacco suspension cultured material
- Grape berries collected at different stages of ripening from a ‘model vineyard’ being developed under stressed and unstressed conditions
- Laboratory yeast strains (and deletion library strains)
- Industrial yeast strains (BM45 and VIN13)
- Lactic acid bacteria strains
- Molecular and biochemical laboratory equipment (solvents, reagents and benchware)
- Commercially available standards, enzymes and antibodies.

Instruments:

- Fourier transform mid infrared (FT-MIR) spectrometer – solid KBr and ATR
- Fourier transform mid infrared (FT-MIR) spectrometer– liquid
- Fourier transform near infrared (FT-NIR) spectrometer – liquid and solid
- Varian Solid state nuclear magnetic resonance spectrometer - solid
- Varian nuclear magnetic resonance spectrometer – liquid
- Gas Chromatograph with FID detection
- Dionex HPLC anion exchange chromatography
- MALDI TOF mass spectrometer
- Gas Chromatograph coupled Mass Spectrometer

- High Performance Liquid Chromatograph
- Micro-array Instrument (CPGR, University of Cape Town)

All instruments are available at Stellenbosch University (at the IWBT chemical-analytical facility and university Central Analytical Facility) except for the specific work needing to be conducted with the Centre for Proteomic and Genomic Research (CPGR) at the University of Cape Town.

Data analysis:

Spectral profiles and quantitative data will be analysed by means of chemometric methods. Data sets will be subjected to principal component analysis (PCA) and partial least square (PLS) discrimination in order to differentiate between samples and to test predictive power of the respective data sets.

Sub-Project 1:

Milestone 1: Development of a method to accurately determine monosaccharide composition.

The following steps will be included:

- Develop a Gas Chromatography method to analyse cell wall monosaccharides using TMS glycosides (Doco et al., 2001).
- Develop a Gas Chromatography method to analyse cell wall monosaccharides using alditol acetates (Blakeney et al., 1983).
- Development of a method to accurately determine monosaccharide linkages.
- Develop a Gas Chromatography method to analyse cell wall monosaccharides using alditol acetates and then to perform methylation analysis (Fry, 2000).

Milestone 2: Development of a method to accurately measure lignin levels.

- Utilise spectrophotometric reference methods such as thioglycolic acid and/or acetyl bromide (Fry, 2000).
- Develop HPLC method using thioacidolysis (Lapierre et al., 1985)

Milestone 3:

- Development of a method to accurately measure phenolic polymers (wall bound and free) using spectrophotometric reference methods and HPLC following saponification (Fry, 2000).
- Determine the utility of 2D HPLC as a method to investigate complexity and insoluble complexes.
- Determine amino acid composition after hydrolysis using liquid chromatography coupled mass spectrometry (Fry, 2000).

Milestone 4:

- Development of spectrophotometric assays for cell wall enzymatic assays and simple 1D (SDS) PAGE analysis of wall protein complexity (Jamet et al., 2006).
- Utilise zymograms to assay potentially important enzymes (e.g. wall cross-linkers)

Sub-Project 2:

Milestones 1-3:

Most spectroscopic methods require minimal sample pre-treatment. Therefore we will have to optimise methods for cell walls FTMIR using dry powders (KBr pellets) and wet suspensions (ATR).

- Alcohol insoluble residue preparation (plant, yeast and bacteria system) or liquid paste following centrifugation. Identical treatments for NIR and solid state NMR spectroscopy. (Brinkman et al., 2002; Chatjigakis et al., 1998; Galichet et al., 2001).
- Solution and solid state NMR requires sample dissolution/preparation therefore various sample pre-treatments required (Ha et al., 2005, Yelle et al., 2008).
- Commercially available wall polymers will be purchased and tested to determine the utility of creating model polymer blends.

Sub-Project 3:

Milestones 1-3:

- Alcohol insoluble residues will be prepared and dot blotted onto nitrocellulose membranes which will be probed with commercial cell wall antibodies.
- Development of micro (glyco-) array slides loaded with cell wall fractions and probed with fluorescent antibodies (Moller et al., 2007). This would need to be conducted with the CPGR (University of Cape Town) or other similar facility.
- For the mass spectrometric screening will require the use of a range of potentially suitable enzymes commercially available (Lerouxel et al., 2002).
- Suitable standards will have to be generated for mass spectrometry and high performance anion exchange chromatography (Lerouxel et al., 2002).

Supporting References

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Weber B, Hoesch L, Rast DM. 1995. Protocatechualdehyde and other phenols as cell wall components of grapevine leaves. *Phytochemistry* 40: 433-437.

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4. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results.
This final discussion must cover ALL accumulated results from the start of the project, but please limit it to *essential* information.

Sub-project 1: Establishing a set of highly accurate reference methods for cell walls

Milestone	Achievement
1. Development and application of a method to accurately determine monosaccharide composition and application of a method to accurately determine monosaccharide linkages	A derivatisation-based gas chromatography (GC-FID) method (TMS-glycosides) was optimised for cell wall monosaccharides present in tobacco and grapevine leaf tissue. Linkage analysis was optimised on isolated polysaccharides and enriched fractions from tobacco material using GC-EI-MS analysis

	after methylation using a published protocol. (see Nguema-Ona et al. 2012 Carb. Pol.)
2. Development and application of a method to accurately measure lignin levels spectrophotometrically and application of a method to determine lignin composition.	Lignin had been measured spectrophotometrically using the acetyl-bromide method (Alexadersson et al. 2011), but more detailed information regarding the lignin content and composition is required. Due to a lack of expertise in lignin analysis available locally, overseas expertise has been sought. Collaborations to conduct lignin compositional analysis using pyrolysis GC-MS at Umea Plant Science (Sweden) was established. This established method (routine on *Arabidopsis* and Poplar) will be evaluated on samples of tobacco and grapevine we are providing.
3. Determination of cell wall bound phenolic quantity and cell wall amino acid composition	Due to a change in the focus of the project to assess pectin and xyloglucan (i.e. cell wall carbohydrate polymers), phenolic quantification was not pursued. Amino acid analysis is available through collaboration with the University of Cape Town for our samples.
4. Analysis of cell wall enzymatic activities and basic protein composition	Enzymatic analysis (specifically XET/XTH enzymatic fluorescent quantification method) and protein quantitation were performed on tobacco leaf samples successfully (Alexandersson et al. 2011).

Sub-project 2: Establishing a set of non-invasive spectroscopic and chemometric tools

Milestone	**Achievement**
1. Optimise methods for cell wall ATR-FT-MIR using dry powders and also	ATR-FT-IR analysis using a Thermo Instrument (Chemistry) was successfully

methods for plant and microbial (yeast) wet suspensions.	performed on plant (see Nguema-Ona et al. 2012 Carb. Pol.) and microbial samples (see Moore et al. 2012 J. Agric. Food Chem. (in preparation)). Large datasets have been collected for analysis and publication.
2. Develop and apply methods for cell wall NIR analysis using dry powders and also methods for plant and microbial (yeast) wet suspensions.	NIR analysis using a Bruker instrument was successfully performed on plant and microbial samples (see Moore et al. 2012 J. Agric. Food Chem. (in preparation)). Large datasets have been collected for analysis and publication.
3. Develop and apply methods for cell wall NMR analysis for plant and microbial samples for solid-state and solution-state analysis.	Due to a lack of expertise and necessary equipment for the application of NMR methods to cell wall samples locally, international support/collaboration has been sought. Solid-state NMR proved to be insufficient for our needs and a sophisticated solution-state NMR technique (which we lack the expertise to perform) is required. Collaborative support is available through Umea Chemistry Biology Centre (Sweden) and University Wisconsin-Madison (USA), Professor Johan Ralph. We have recently received financial support to purchase the necessary preparation equipment for preparing samples to be sent to Sweden and/or USA. We hope in the next year to be in a position to perform some of these NMR methods locally.

Sub-project 3: Establishing rapid, analytical tools for analysing wall sub-structures.

Milestone	**Achievement**
1. Develop and apply a MALDI-TOF mass spectrometry screening method and nitrocellulose dot blot assays for plant	MALDI-TOF- MS and an ESI-MS oligosaccharide fingerprinting method was applied to plant samples following enzymatic

cell walls polymers.	digestion (see Nguema-Ona et al. 2012 Carb. Pol.) Dot blot assays proved insufficient for our needs and were abandoned.
2. Develop and apply a MALDI-TOF mass spectrometry screening method for microbial cell walls (yeast).	Screening of yeast samples via 'ordinary' MALDI-TOF MS proved unreliable, and with the recent advent of a specialized Bruker MALDI Biotyper a 'new' attempt will be made to screen yeast and bacterial samples.
3. Develop and apply a glycan array screening method using printed microarray slides and plant cell wall antibodies	Carbohydrate Microarray Polymer Profiling analysis (CoMPP) has successfully been performed on tobacco, grapevine and yeast samples. This analysis is performed in collaboration the Prof. William Willats at the University of Copenhagen, Denmark, where the technique originated. These data are performing a valuable basis for a number of publications (see Nguema-Ona et al. 2012 Carb. Pol.)

5. Accumulated outputs

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

Technology development, products and patents

Indicate the commercial potential of this project (intellectual property rights or a commercial product(s)).

1. Technical methods for evaluating cell wall composition and content of tobacco, grapevine material (i.e. leaves, berries) and yeast cell walls are now available.
2. Spectroscopic (NIR and MIR) methods, coupled to multivariate methods, protocols are now established (with an associated spectral database of plant and microbial samples) which could be utilised by industry.

Human resources development/training

Indicate the number and level (e.g. 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 (R)
1.	Dr Eric Nguema-Ona (Post-doctoral fellow)	113,000
2.	Mr Cobus Steyn (MSc student)	23,500
3.	Mr Jason Zhang (BTech/research assistant)	41,440

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

(1) Erik Alexandersson, John W. Becker, Dan Jacobson, Eric Nguema-Ona, Cobus Steyn, Katherine Denby and Melané A Vivier. **2011.** Constitutive expression of a grapevine polygalacturonase-inhibiting protein affects gene expression and cell wall properties in uninfected tobacco. *BMC Research Notes* 4:493.

(2) Eric Nguema-Ona, **John P. Moore,** Alexandra Fagerstrom, Jonatan U. Fangel, William G.T. Willats, Annatjie Hugo and Melané A Vivier. **2012.** Profiling the main cell wall polysaccharides of tobacco leaves using high-throughput and fractionation techniques. *Carbohydrate Polymers* 88(3): 939-949.

(3) Eric E. Nguema-Ona, **John P. Moore**, Alexandra Fagerstrom, William Willats, Annatjie Hugo and Melané A Vivier. **2012.** Overexpression of the grapevine PGIP1 in tobacco leaves results in an altered arabinoxyloglucan composition in the absence of fungal infection. *Journal of Experimental Botany* (in preparation).

(4) **John P. Moore,** Eric Nguema-Ona, Alexandra Fagerstrom, Jonatan U. Fangel, William G.T. Willats, Annatjie Hugo and Melané A Vivier. **2012.** Profiling the main cell wall polysaccharides of grapevine leaves using high-throughput and fractionation techniques. *Carbohydrate Polymers* (in preparation)

(5) **John P. Moore,** Song-lei Zhang, Helene Nieuwoudt, Benoit Divol, and Florian F. Bauer. **2012.** Monitoring mannoprotein and β-glucan content in a variety of laboratory and industrial wine yeast strains under fermentative conditions using ATR-FT-MIR spectroscopy and multivariate data analysis. *Journal of Agricultural and Food Chemistry* (in preparation)

(6) **John P. Moore,** Song-lei Zhang, Helene Nieuwoudt, Benoit Divol, and Florian F. Bauer. **2012.** Using NIR spectroscopy and PLS calibration to model the mannoprotein and β-glucan content in a variety of laboratory and industrial wine yeast strains under fermentative conditions. *Journal of Agricultural and Food Chemistry* (in preparation)

(7) **John P Moore,** Eric E. Nguema-Ona, Florian F Bauer and Melané A Vivier. **2012**. Cell wall-omics technology platform – applications to yeast and grapevine biotechnology. *South African Journal of Viticulture and Enology* (in preparation)

Presentations/papers delivered

(1) Eric E Nguema-Ona, **John P Moore** and Melané A Vivier. **2008.** Cell wall reinforcement in transgenic tobacco lines expressing *Vitis vinifera* polygalacturonase inhibiting proteins (PGIPs) in *Cape Biotechnology Forum Abstracts*, 30 November - 2 December 2008, Somerset West, South Africa.

(2) Mukani Moyo, Jacobus Steyn, E. Eric Nguema-Ona, **John P. Moore**, Melané A. Vivier. **2009.** Cell wall remodeling in VvPGIP1 overexpressed in tobacco (*Nicotiana tabacum*) and grapevine (*Vitis vinifera*) plants in *International SASEV Conference on Enology and Viticulture*, p. 63, 28-30th July 2009, Cape Town, South Africa. **(POSTER)**

(3) **John P. Moore,** E. Eric Nguema-Ona, Melané A. Vivier. **2009.** Tracking the careers of grape and wine polymers in *International SASEV Conference on Enology and Viticulture*, p. 61, 28-30th July 2009, Cape Town, South Africa. **(POSTER)**

(4) Eric E Nguema-Ona, **John P Moore** and Melané A Vivier. **2009.** The role of polygalacturonase inhibiting proteins (PGIPs) and the cell wall of grapevine in pathogen defence in *South African Journal of Botany,* South African Association of Botanists Conference, 19-22 January 2009, Stellenbosch, South Africa.

(5) **John P. Moore**, Eric E. Nguema-Ona, Jill M. Farrant and Melané A. Vivier. **2010.** Towards a systems based approach to plant drought and desiccation tolerance – a role for cell wall processes and –omics studies. COST Action F0605 conference on '*plant abiotic stress from signaling to crop improvement'*, 26-27 May 2010, Valencia, Spain. **(POSTER)**

(6) Eric E. Nguema-Ona, **John P. Moore**, Eric Alexandersson, Daniel Jacobson and Melané A. Vivier. **2010.** Analyses of *Botrytis cinerea* defense phenotypes highlight the importance of studying the plant cell wall in plant-pathogen interactions. *Botrytis Symposium*, 30-31 May 2010, Cadiz, Spain.

(7) **John P. Moore**, Eric E. Nguema-Ona, Thulile Ndlovu, Benoit Divol, Hélène Nieuwoudt, Florian F. Bauer, Melané A. Vivier. **2010.** Methods to track grape and yeast derived cell wall polymers in wine – applications for wine biotechnology. *12th International Cell Wall Meeting* 25-30 July 2010, Porto, Portugal. **(POSTER)**

(8) Eric E. Nguema-Ona, Cobus Steyn, **John P. Moore,** Eric Alexandersson, Daniel Jacobson and Melané A. Vivier. **2010.** Polygalacturonase-inhibiting proteins (PGIPs), cell wall remodeling and defense: Are there links? *12th International Cell Wall Meeting* 25-30 July 2010, Porto, Portugal.

(9) **John P. Moore**, Eric E. Nguema-Ona and Melané A Vivier. **2010.** Cell Wall-Omics: Tracking the careers of grape and wine polymers using biotechnology and systems biology. *Cape Biotechnology Forum 2010*, (24-26 March) Somerset West, South Africa

(10) Eric E Nguema-Ona, **John P. Moore** and Melané A Vivier. **2010.** Cell walls and plant defense: Using microscopical and analytical techniques to elucidate resistance phenotypes. *Cape Biotechnology Forum 2010*, (24-26 March) Somerset West, South Africa.

(11) **John P. Moore**, Eric E. Nguema-Ona, Thulile Ndlovu, Benoit Divol, Hélène Nieuwoudt, Florian F. Bauer and Melané A Vivier. **2010.** Developing cell wall modified and mannoprotein releasing wine yeast *(Saccharomyces cerevisiae)* strains for improved wine protectant properties (e.g. haze protection). *SASEV Conference on Enology and Viticulture*, November 2010, Somerset West, South Africa.

(12) Song-lei Zhang, **John P. Moore**, Helene Nieuwoudt, Benoit Divol and Florian Bauer. **2011.** Evaluating cell wall properties and mannoprotein content in laboratory and industrial wine yeast strains using mid- and near-infrared spectroscopy. *International conference on near-infrared spectroscopy.* 13-20 May, Cape Town, South Africa. **(POSTER)**

4. Total cost summary of project

	Year
Total cost in real terms for year 1	2008
Total cost in real terms for year 2	2009
Total cost in real terms for year 3	2010
Total cost in real terms for year 4	2011
Total cost in real terms for year 5	2012
TOTAL	

CFPA	Deciduous	DFTS	Winetech	THRIP	Other	TOTAL
	R 0		R 0	R 0		**R 0**
	R 150,000		R 150,000	R 150,000		**R 450,000**
	R 163,500		R 165,000	R 164,250		**R 492,750**
	R 150,000		R 150,000	R 150,000		**R 450,000**
	R 0		R 0	R 0		**R 0**
	R 463,500		**R 465,000**	**R 464,250**		**R 1,392,750**