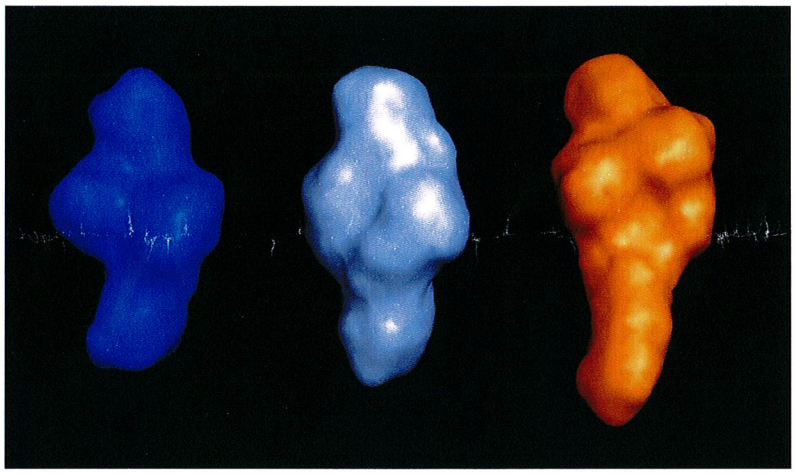
State Government Victoria

Australian Government

Australian Grape and Wine Authority

Building a professional network with tannin researchers in the US and presentation at the 65th ASEV National Conference in Austin, Texas.



FINAL REPORT to
AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **GWT 1323**

Principal Investigator: **Peta Faulkner**

Research Organisation: **Department of Environment and Primary Industries**

Date: **10th July 2014**

Abstract

The award of this travel grant provided Ms Peta Faulkner from the Department of Environment and Primary Industries (DEPI) the opportunity to attend the 65th American Society of Enology and Viticulture National Conference in Austin, Texas (USA) from the 23rd-27th of June 2014. Ms Faulkner presented research outcomes from a GWRDC funded project investigating tannin structure at an Enology – Wine Tannin Chemistry session alongside collaborators Dr Rachel Kilmister (DEPI) and Dr Jim Harbertson (Washington State University). Attendance at the conference facilitated discussions and the cultivation of collaborative networks that will be incorporated into current methodology and research projects on tannin research.

Executive Summary

Peta Faulkner is a Senior Technical Officer working in grape and wine research at the Department of Environment and Primary Industries (DEPI), supervised by Dr Rachel Kilmister. Ms Faulkner attended the 65th Annual American Society of Enology and Viticulture National Conference to present an oral presentation based on a submitted abstract "*Structural Characterisation of Tannin Polymers by Small Angle X-ray Scattering*" (Appendix 1).

The 65th Annual American Society of Enology and Viticulture National Conference was held in Austin, Texas, USA between the 23rd and 27th of June 2014. Over 350 industry and research participants attended with the program consisting of more than 170 presentations including 1 keynote speaker, 80 oral presentations and 90 posters. The award of this travel grant enabled Ms Faulkner to attend and participate in the conference and facilitated the fulfilment of the project objectives to:

- communicate research outputs associated with the completion of GWRDC funded project "Tannin Structure Function Relationships" DPI1001 and Ms Faulkner's completed Honours project to an international audience and wider research community
- and to cultivate and reaffirm collaborative relationships with international research groups, viticultural and winemaking networks

Research presentations

Ms Faulkner's oral presentation was the first of three in the Enology – Wine Tannin Chemistry session moderated by James Kennedy. The three presentations in the Enology – Wine Tannin Chemistry Session were a result of collaborations between researchers from DEPI, Washington State University (US), Constellation Brands (US), University of Sheffield (UK) and the Australian Synchrotron. The second presentation by Dr James Harbertson was titled *"Impact of individual and*

mixed condensed tannin polymers on their ability to precipitate proteins" and the third presentation in the session was by Dr Rachel Kilmister titled *"Effect of condensed tannin size on tannin protein interactions"*. The session was well received by the attendees, with a number of questions asked and several research related discussions taking place following the session.

The research presented by Ms Faulkner outlined methodology for separating and isolating tannin polymers of increasing degree of polymerisation (DP) and further separation and purification of isomeric structures. The 3D structures of isolated tannins characterised by Small Angle X-Ray Scattering (SAXS) were presented. The presentation demonstrated the use of SAXS as a technique that can provide information on gross tannin structure in solution and has the ability to distinguish between discreet structural differences present in isomers. Ongoing experiments are being conducted utilising SAXS to investigate how different components of the wine matrix such as ethanol and sugar effect tannin gross structure. This information will facilitate a better understanding of how tannin structure impacts on its interactions involved in grape tannin extraction, colloid formation and astringency.

The second presentation by Dr Harbertson discussed the interaction of tannins isolated using the methodology presented in Ms Faulkner's presentation with BSA protein. The research presented by Dr Harbertson demonstrated an increase in the effectiveness of tannins to precipitate protein with increasing size (DP). The third and concluding presentation by Dr Kilmister further extended the work presented by Dr Harbertson and discussed the use of Isothermal Titration Calorimetry (ITC) as a technique for measuring the strength of tannin-protein interactions. The work further demonstrated how ITC can be used to measure the effect of different components of the wine matrix such as pH, temperature and ionic strength on the strength of the tannin-protein interactions.

By attending presentations over the duration of the conference, Ms Faulkner was exposed to current wine research with both direct and indirect relevance to current research being undertaken at DEPI.

Presentations focused on challenges faced by the wine industry helped to identify new technologies, with the potential to be adapted to current research. Observations around these agricultural challenges will be communicated to other DEPI researchers working on GWRDC funded projects "Impact of elevated CO_2 and its interaction with elevated temperature on production and physiology of Shiraz" DPI 1202 and "Identifying vineyard and winery management practices that impact on tannin extraction" DPI 1402.

Presentations and discussions of particular interest to continuing work that Ms Faulkner and her group at DEPI are involved in are highlighted below:

- Dr James F. Harbertson (Washington State University, Prosser, USA) *"Impacts of maturity and ethanol concentration on wine tannin extraction"*.
- Larry A. Lerno (Department of Viticulture and Enology, UC Davis, USA) "*Investigating the effect of cold-soak duration on the extraction of phenolics during cabernet sauvignon fermentations*".
- Bruce S. Pan (E&J Gallo Winery, Modesto, USA) "*Development of a grape chemical quality scale for Vitis vinifera L. Cabernet Sauvignon*".
- Matthew R. Revelette (Department of Viticulture and Enology, California State University, Fresno, USA) "*HPLC determination of red wine tannin concentration and prediction of "grippiness" following direct injection"*.
- James M. Myers (Cornell University, Geneva, USA) " *Responses of phenolic compounds to sunlight intensity in cool-climate Cabernet franc.*"
- Karine Pedneault (Quebec Agrifood Development Centre, Quebec, Canada) "*Phenolic and aromatic composition of grapes and wines from five hybrid grape varieties used in northern wine production"*.
- Dr James F. Harbertson (Washington State University, Prosser, USA) *"Impact of individual and mixed condensed tannin polymers on their ability to precipitate proteins"*.
- Julie Mekoue Nguela (National Institute of Agronomic Research, Montpellier, France) "*Sorption of grape proanthocyanidins and wine polyphenols by inactivated yeast fractions"*.
- David E. Block (University of California, Davis, USA) "*Investigation of pump-over frequency on phenolic extraction and fermentation rate during Cabernet Sauvignon production"*.
- Alan Lasko (Cornell University, NY, USA) "*A microtensiometer for continuous monitoring of soil and plant water potentials"*.
- Eugenia Galat (Instituto Nacional de Technologia Agropecuaria, Argentina) "*Heat waves and water deficit: effects on photosynthesis, transpiration and chlorophyll fluorescence of Malbec grapevine"*.

Introductions and Informal Discussions

General discussions provided an opportunity for Ms Faulkner to gain an appreciation of current research directions and issues in the US Wine industry while the opportunity to communicate with other tannin researchers helped to identify strengths and shortcomings of current research and future research directions at DEPI, Mildura.

Discussions took place regarding limitations, solutions and potential improvements to methodology recently developed in the GWRDC funded project "Tannin Structure Function Relationships"

DPI1001 and the opportunity for ongoing collaborations relevant to the GWRDC funded project "Identifying vineyard and winery management practices that impact on tannin extraction" DPI 1402.

Attendance at this conference also provided Ms Faulkner with the opportunity to discuss more general limitations and problems in tannin chemistry, as well as new techniques that can be utilised for tannin research and analysis that can be applied to current and future research projects.

General introductions were made to a number of conference delegates including: Kristen Barnhisel, Winemaker/Enologist (Inglenook, California), L. Federico Casassa, Principal Investigator Enology (Instituto Nacional de Tecnología Agropecuaria, Argentina), Mark Krasnow, Associate Professor (The Culinary Institute of America, New York), Kimmo Siren, Internship at WSU (Hochschule Geisenheim University, Corvallis) and Rob Walker (CSIRO, SA, Australia).

Discussions with delegates of particular interest with relevance to ongoing research at DEPI included:

- Lindsay F. Springer (Food Research Laboratory, Cornell University, Geneva, NY, USA) poster presentation "*Limits of red wine tannin extraction and additions: the role of pathogeniesis related proteins*". Ms Faulkner discussed with Ms Springer the implications of findings of her work into proline rich proteins in red wine and it's possible implication to red wine colloid formation. These discussions will be incorporated into Ms Faulkner's review paper "Wine tannin – solution, colloid or precipitate?" (currently in prep) an outcome of Ms Faulkner's Honours thesis and GWRDC funded project "Tannin Structure Function Relationships" DPI1001. Implications to the current GWRDC funded project "Identifying vineyard and winery management practices that impact on tannin extraction" DPI 1402 will also be considered.
- Richard C. Larsen, Research Winemaker (Washington State University, Prosser, USA). Discussions around differences in methodology for tannin extract preparation at Washington State University and DEPI were discussed and possible limitations of particular instrumentation were identified. This knowledge will help to further optimise current methodology and ensure maximum product yields during sample preparation.
- Carolyn Merrell, Masters Student (Washington State University, Prosser, USA). Ms Merrell was able to provide insight into new methodology developed by Dr Harbertson's research group to isolate the anthocyanin, malvidin-3-glucoside, by column chromatography. The new methodology significantly improves the yield for isolation of pure anthocyanin. Further collaboration and exchange with Ms Merrell will allow for the adoption of this methodology by researchers at DEPI to facilitate future research into tannin-anthocyanin interactions using ITC and SAXS technologies.

- Dr James F. Harbertson (Washington State University, Prosser, USA). Continuing talks with Dr Harbertson over the four days of the conference allowed for discussions regarding Ms Faulkner's review paper "Wine tannin – solution, colloid or precipitate?" and a second paper "*Isolation and structural characterisation of condensed tannins by SAXS and NMR*" which are both outcomes of Ms Faulkner's Honours thesis and GWRDC funded project "Tannin Structure Function Relationships" DPI1001. Comments and suggestions from Dr Harbertson will be incorporated into the final draft papers prior to submission.

Conclusions

Attendance by Ms Faulkner at the 65th ASEV conference allowed for the communication of current research outputs associated with Ms Faulkner's completed Honours project and GWRDC funded project "Tannin Structure Function Relationships" DPI1001 to an international audience and wider research community. The poster sessions, lunches and networking events enabled opportunities to cultivate and reaffirm collaborative relationships with international research organisations, viticultural and winemaking networks. They also facilitated discussions which will be useful for guiding improvements to current and future research.

Australian Government
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END OF PROJECT

FINANCIAL STATEMENT

Statement of Receipts and Expenditure

At the conclusion of each Project, AGWA requires a Statement of Receipts and Expenditure of AGWA funds received in relation to the Project.

Project Title:	Building a professional network with tannin researchers in the US and presentation at the 65th ASEV National Conference in Austin, Texas.

AGWA/GWRDC Project Number	Project Start Date	Project End Date
GWT 1323	20th June 2014	29th June 2014

Financial Year (e.g. 2010-11)	(a) *Funds brought forward from previous year	(b) #Approved GWRDC Budget (full year)	(c) Total budget available (a)+(b)	(d) Actual expenditure ($)	(e) Difference ($) (c)-(d)
Year 1: 2014/06	n/a	1,950.00	1,950.00	1,950.00	0.00
TOTAL		1,950.00		1,950.00	**0.00**

* "Funds brought forward" refers to any unspent AGWA funds from the previous year and approved by AGWA/GWRDC to be spent the following year (i.e. column *(e)* from previous year).

"Approved GWRDC Budget" (now administered by AGWA) is the funding amount specified in the Project Agreement, including any approved Variations after Project commencement.

Was funding expended as approved in the Project Agreement? Yes ☒ No ☐

*If **NO**, please provide reasons:*

Has the cash and/or in-kind funding from Contributing Agencies been received and applied to the Project as approved in the Project Agreement? Yes ☐ No ☒

*If **NO**, please provide reasons:*

See budgetary notes in Appendix 2.

I hereby certify that this statement is true and accurate.



Signature of duly authorised representative

Name: Peta Faulkner *Title:* Senior Technical Officer *Date:* *11/07/14*

Appendix 1.

Abstract submitted for an oral presentation at the 65th American Society for Enology and Viticulture National Conference, June 23-27, 2014, Austin, Texas USA

Investigating the gross structure of condensed tannins by small angle x-ray scattering analysis

Peta Faulkner[1], Nigel Kirby[2], Rachel L. Kilmister[1]

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2. SAXS/WAXS Beamline, Australian Synchrotron, Clayton, Victoria, 3168, Australia

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The gross structure of condensed tannins found in grapes and extracted into wine influences the astringent properties of wine. For example, tannin interactions with salivary proteins involved in astringency is governed by the availability of binding sites on the tannin polymer, which is influenced by polymer tertiary structure. The tertiary structure of tannins is in turn determined by interflavan linkage, subunit composition and sequence, and branching within a polymer. It is also influenced by components of the wine matrix such as ethanol that determine tannin solubility. The aim of this study was to isolate tannins with different structural characteristics and investigate their gross structure using small angle x-ray scattering analysis (SAXS). Tannin polymers found in winegrapes that increase in size from monomer to octamer were isolated from cacao beans using preparative hydrophilic interaction chromatography (HILIC), while tetrameric polymers were further separated using semi-preparative C18 liquid chromatography to isolate three structurally different tetrameric isomers. Three dimensional models of each tannin polymer in aqueous solution were generated following analysis by SAXS. Analysis of the three tetramer isomers demonstrated the ability of SAXS to distinguish tannin isomers likely differing in interflavan bond position. Further investigation will determine the effect of different components of the wine matrix such as ethanol, sugar and anthocyanin on tannin structure.

Appendix 2.

The initial budget and itinerary submitted for approval to GWRDC was $8000 supported by 50:50 expenditure by both GWRDC and DEPI. As the agreed contribution by GWRDC in the signed Project Agreement was $1950 the number of days travel was reduced to minimise additional expenditure that would be incurred by DEPI.

	Initial Budget GWRDC (GST exc)	Initial Agency Contribution (DEPI)	**Actual Travel Costs GWRDC** (GST exc)	**Actual Travel Costs DEPI** (GST exc)	**Reason for variation**
Domestic airfares	$0	$300.00	$0	$0	Airfares were all purchased on an international airfare due to reduced itinerary.
International fares	$3200.00	$0	$1950.00	$1403.78	DEPI contributed additional airfare expenses not covered by agreed GWRDC contribution
Hire car/taxi expenses	$0	$700.00	$0	$360.00	Reduced travel itinerary
Accommodation	$	$1500.00	$0	$1230.00	
Living allowance	$	$1500.00	$0	$387.42	Reduced travel itinerary
Conference Fees	$800.00	$0	$0	$510.00	Discount received as a presenter
Total :	$4000.00	$4000.00	$1950.00	$3891.20	Agreed GWRDC contribution less than that requested