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Participation in the Wartburg Flavour Chemistry and Biology symposium in Germany and the Macrowine 2016 symposium in Switzerland - The biochemical response of grapevines to smoke exposure





FINAL REPORT to **AUSTRALIAN GRAPE AND WINE AUTHORITY**

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Principal Investigator: **Lieke van der Hulst**

Research Organisation: **The University of Adelaide**

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Abstract

This travel grant provided University of Adelaide PhD student, Lieke van der Hulst, the opportunity to attend and present at (i) the 11th Wartburg Symposium on Flavor Chemistry and Biology in Eisenach, Germany (June 21–24) and (ii) the Macrowine 2016 Symposium in Nyon, Switzerland (June 27–30). The focus of these symposia are flavour chemistry and biology, and grape and wine macromolecules and secondary metabolites, respectively. Lieke van der Hulst presented research findings from her PhD at each conference, outlining the impact of smoke exposure by grapevines on the chemical composition of fruit, in particular grapes of different cultivars. Feedback from conference delegates was favourable and, together with networking opportunities and the knowledge gained from other presentation, which spanned flavour chemistry, analytical chemistry, biochemistry, sensory science and wine science fields, was immensely valuable.

Executive summary

In June 2016, Lieke van der Hulst attended two conferences in Germany and Switzerland, during which she presented aspects of her research on the occurrence of smoke taint in fruit. The Wartburg Symposium on Flavor Chemistry and Biology conference convenes every three years at the UNESCO heritage-listed Wartburg Castle in Eisenach, Germany. This symposium covers a broad range of topics over four days, and included sessions on chemosensory reception and perception, functional flavour genomics, and flavour generation and precursors. The latter of these three sessions included an oral presentation by Lieke titled '*Impact of smoke exposure on the composition of different fruit'* (Appendix 5).

Macrowine is a bi-annual symposium held at different locations, organised by the international wine science community. In 2016, Macrowine was held in Changins-Nyon in Switzerland, home of Chasselas wines. Macrowine is a multi-disciplinary conference, but with a focus on viticulture, wine production, and wine aroma and flavour perception. At this symposium, Lieke presented research findings from a field trial involving the application of kaolin to grapevines, as a potential method of ameliorating smoke taint in the vineyard. Her oral presentation was titled '*Impact of smoke exposure on the chemical composition of grapes'* (Appendix 5) and was scheduled in the session on '*Modulators of aroma and taste'*.

Both presentations initiated interesting discussions with conference delegates, which not only validated project scope and methodology, but provided new ideas for future experiments based in the vineyard or laboratory.

Project aims were successfully accomplished and included the following targets:

- For Wartburg: dissemination of research findings via a conference presentation concerning the accumulation of glycoconjugate precursors of smoke derived volatile phenols in grapes and other agricultural crops following smoke exposure;
- For Macrowine: dissemination of research findings via a conference presentation concerning the effect of wax and cuticle development on the uptake of smoke derived volatile compounds by grape berries following grapevine exposure to smoke;
- Expansion of academic network and promotion of the Australian wine industry and wine industry stakeholders, such as Wine Australia.

Targets that have changed following travel include:

- For Wartburg: dissemination of research findings via an article to be published as a refereed book chapter in the conference proceedings.

  Based on feedback obtained during the Wartburg symposium, additional experimental work will be undertaken prior to publication of existing data sets. This will enable

preparation of a manuscript for submission to a refereed journal, such as the *Journal of Agricultural and Food Chemistry*, rather than inclusion in the Wartburg conference proceedings. However, a manuscript will be prepared for submission to a Special Issue of the refereed *Food Research International* journal, based on work presented at Macrowine; the deadline for this manuscript will be 30th of October 2016.

- Research visit to Wageningen University (in the Netherlands): The duration of time spend in the Netherlands did not permit a visit to the Wageningen University (and therefore a presentation to be given on smoke taint research being conducted within the ARC TC-IWP). However, a meeting with Dr. Hosseini from Leiden University was arranged and enabled a valuable discussion on microscopy techniques available for studying plant material. Furthermore, the conferences enabled contacts to be established with: the Wageningen Food Science Hub (Catrienus de Jong); the NIZO Food Innovators; Yannick Weesepoel, Wageningen University and Research Centre; and Stellenbosch University (Dr. Astrid Buica).

The Wartburg symposium started every session with a key note speaker, which included amongst others Dr. Ross Atkinson from the New Zealand Institute for Plant and Food Research, Dr. Markus Herderich from the Australian Wine Research Institute and Dr. Bettina Malnic from the University of São Paulo in Brazil. Especially the presentation by Dr. Ross Atkinson resonated with me, as his presentation was titled '*Unravelling the genetics of flavour/aroma compound production in fruit*', and introduced a full overview of challenges and opportunities in introducing genetics in flavour development. This work and the presented work on smoke taint and flavour development in several types of fruit invited both Dr. Atkinson and Lieke to discuss this topic later during the conference.

Macrowine 2016 showcased many interesting topics and presentations, and highlights during this conference included some of the key note speakers, such as Dr. Liz Waters from Wine Australia and her presentation on white wine haze. Although not linked directly to the research performed on smoke taint, her work presented an all-encompassing overview of research in wine science and connecting this work to the wine industry. Over the course of the two conferences it was also a pleasure to be able to hear Prof. Vicente Ferreira speak on the origin of reduction and oxidation aromas.

In summary, the opportunity to attend these conferences was of considerable value, in terms of both professional development (and networking), and ongoing research efforts.

Background

11th Wartburg Symposium on Flavor Chemistry and Biology

Since its inception in 1978, the Wartburg symposium has convened every three years. The symposium covers a broad range of topics in flavour chemistry and biology, including emerging challenges at the intersection of odour/taste chemistry and sensory science, with biology, neurophysiology, nutrition, post-genomic (genetics, biotechnology) and advanced imaging technologies. The symposium was divided into sessions with themes such as '*chemosensory reception, processing, and perception'*, '*food-born bio-actives and chemosensory health prevention'*, *'flavour generation and precursors'* and '*functional flavour genomics and biotechnology'*. The conference aimed to present cutting edge research in multi-disciplinary fields, and brought together delegates from industry and academia, via 50 oral presentations and 64 poster presentations. The conference provided a fitting platform to give an oral presentation in the session of '*Flavor generation and precursors'* titled '*Impact of smoke exposure on the composition of different fruit'*, in which the chemical profiles of smoke exposed fruit were discussed (Abstract provided in Appendix 5). The presentation was well received and generated considerable discussion during the conference.

The location of the conference was the Wartburg hotel, next to the UNESCO world heritage Wartburg Castle, in the beautiful Thuringia region of Germany. Apart from the striking environment of the conference venue, there was also an opportunity to taste local wines. Saale-Unstrut is the local wine region in Thuringia, which mainly produces white wines from local German grape varieties such as Silvaner, Riesling and Müller-Thurgau.

Macrowine 2016

Macrowine is a bi-annual conference spanning multiple grape and wine-related disciplines, and provides a platform for disseminating high-quality research and technical innovations concerning grape and wine macromolecules and secondary metabolites. The conference also promotes scientific debate that helps to shape the future of wine science, viticulture and wine making. A total of 43 oral and 151 poster presentations were given by delegates from academia and industry, covering the challenges faced, and opportunities to be seized, by grape growers and wine makers around the world. During the conference session '*Modulators of aroma and taste'*, Lieke presented her work on the '*Impact of smoke exposure on the chemical composition of grapes'*. The presentation outlined a field trial conducted in 2016 evaluating the potential for kaolin to be used as a protective spray to prevent the uptake

of smoke derived volatile phenols by several varieties of grapevines. The work was well received and again led to interesting discussions with fellow researchers.

During the conference special attention was paid to the location of Nyon-Changins, as it is in the wine region of Lavaux which produces the unique Swiss Chasselas wine. During a visit of the terraced vineyards of Lavaux, the specific challenges of wine makers in the area were discussed, followed by a wine tasting that introduced the varietal expression of this interesting grape variety.

Project aims and performance targets

The overarching aims of this project comprised disseminating ongoing research at the Wartburg and Macrowine symposia. As such, the performance targets involving dissemination of research findings and promotion of the Australian wine industry and wine industry stakeholders were successfully accomplished, i.e.:

- For Wartburg: dissemination of research findings via a conference presentation concerning the accumulation of glycoconjugate precursors of smoke derived volatile phenols in grapes and other agricultural crops following smoke exposure;
- For Macrowine: dissemination of research findings via a conference presentation concerning the effect of wax and cuticle development on the uptake of smoke derived volatile compounds by grape berries following grapevine exposure to smoke;
- Expansion of academic network and promotion of the Australian wine industry and wine industry stakeholders, such as Wine Australia.

Targets that have changed following travel include:

- For Wartburg: dissemination of research findings via an article to be published as a refereed book chapter in the conference proceedings.

  Based on feedback obtained during the Wartburg symposium, additional experimental work will be undertaken prior to publication of existing data sets. This will enable preparation of a manuscript for submission to a refereed journal, such as the *Journal of Agricultural and Food Chemistry*, rather than inclusion in the Wartburg conference proceedings. However, a manuscript will be prepared for submission to a Special Issue of the refereed *Food Research International* journal, based on work presented at Macrowine; the deadline for this manuscript will be 30th of October 2016.

- Research visit to Wageningen University (in the Netherlands): The duration of time spend in the Netherlands did not permit a visit to the Wageningen University (and therefore a presentation to be given on smoke taint research being conducted within the ARC TC-IWP) However, a meeting with Dr. Hosseini from Leiden University was arranged and enabled a valuable discussion on microscopy techniques available for studying plant material. Furthermore, the conferences enabled contacts to be established with: the Wageningen Food Science Hub (Catrienus de Jong); the NIZO Food Innovators; Yannick Weesepoel, Wageningen University and Research Centre; and Stellenbosch University (Dr. Astrid Buica).

The introduction to Dr. Astrid Buica from Stellenbosch University will be of particular value given researchers at Stellenbosch have recently initiated research into smoke taint, following significant bushfires in early 2016. Overall, networking opportunities were very motivational, in terms of establishing contacts with other early-career researchers, as well as discussing research ideas with prominent researchers in the flavour chemistry and wine science fields.

Conference and travel outcomes

As discussed under ‘project aims and performance targets’ the overall outcome for this travel has been validation of project scope and methodology, by both the scientific community and the wine industry. Publications based on research findings presented at both conferences are anticipated, albeit not necessarily in the conference proceedings, as proposed. Attendance at the conferences has resulted in other opportunities for publication being identified, together with opportunities for collaborations, for example with Stellenbosch University in South Africa or the Wageningen University in the Netherlands.

Recommendations

Both conference presentations were met with interest from other delegates, which resulted in valuable opportunities for further discussing research findings and future work. As such, the main outcome from this travel will relate to interpretation of existing data sets and/or new ideas being incorporated into future field trials and laboratory-based experiments; in particular, in combining chemical analysis with genetic screening following grapevine exposure to smoke exposure. Compositional and molecular analyses features in several other presentation given during the Wartburg symposium. Following the presentation at Macrowine, it became clear that mostly industry would be interested in seeing more work

done towards a ‘quick and easy’ method for preventing smoke taint in the vineyard. The relevance of Lieke’s research to industry was therefore validated.

Appendices

Appendix 1: Communication

A manuscripts based on the research findings presented at Macrowine will be prepared for submission to a Special Issue of *Food Research International*.

Appendix 2: Intellectual Property

Not applicable - This project has no intellectual property attached to it.

Appendix 3: References

Not applicable – refer to conference abstracts provided as Appendix 5.

Appendix 4: Staff

Principal Investigator: Lieke van der Hulst

Principal Supervisor: Associate Professor Kerry Wilkinson

Appendix 5: Abstracts

11th Wartburg Flavour Chemistry and Biology Symposium

Impact of smoke exposure on the composition of different fruit

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Vineyards exposed to smoke can lead to tainted grapes and wine, characterised by objectionable *smoky*, *ashy* aromas and flavours, commonly referred to as 'smoke taint' [1, 2]. In recent years, the occurrence of significant bushfires in close proximity to wine regions in Australia, Canada, South Africa and the USA has increased, due to the warmer, drier conditions associated with climate change. As a consequence, considerable research has been undertaken to understand the chemical, sensory and physiological consequences of grapevine exposure to smoke.

The *smoky*, *ashy* attributes typically associated with smoke tainted wine are thought to result from the presence of several smoke-derived volatile phenols, including guaiacol, 4-methylguaiacol, syringol and cresols, in wine made from smoke-affected grapes [2]. These phenols are known to accumulate in glycoconjugate precursor forms, i.e. with one or more sugar molecules attached [3, 4]. During fermentation and ageing, glycoconjugates can be hydrolysed to release the volatile phenols and thus, the intensity of smoke-related sensory attributes increases [4].

To date, research has focussed on the impact of bushfire smoke on grapes and wine, but bushfires often occur in regions in which other agricultural crops are grown. This study therefore aimed to investigate the potential for other fruits, i.e. apples, strawberries and tomatoes, to be tainted as a consequence of smoke exposure. Apple trees, strawberry and tomato plants, and grapevines, were exposed to smoke under experimental conditions during fruit development. Fruit samples were harvested at commercial ripeness and analysed by GC-

MS and UHPLC-MS/MS, to quantify smoke-derived volatile phenols and their glycoconjugates, in order to determine the extent to which fruit was affected by smoke. Research findings will demonstrate the impact of smoke exposure on crops other than grapevines and provide insight into the broader risk of smoke taint to primary production.

Literature

1. Kennison, KR, Gibberd, MR, Pollnitz, AP and Wilkinson, KL (2008) Smoke-derived taint in wine: The release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. *Journal of Agricultural and Food Chemistry, 56*, 7379-7383.
2. Parker, M, Osidacz, P, Baldock, G, Hayasaka, Y, Black, C, Pardon, K, Jeffery, D, Geue, J, Herderich, M and Francis, I (2012) Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine. *Journal of Agricultural and Food Chemistry, 60*, 2629-2637.
3. Hayasaka, Y, Dungey, KA, Baldock, GA, Kennison, KR and Wilkinson, KL (2010) Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke. *Analytica Chimica Acta, 660*, 143-148.
4. Ristic, R, Osidacz, P, Pinchbeck, KA, Hayasaka, Y, Fudge, AL and Wilkinson, KL (2011) The effect of winemaking techniques on the intensity of smoke taint in wine. *Australian Journal of Grape and Wine Research, 17*, S29-40.

Impact of smoke exposure on the chemical composition of grapes.

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Theme: Life in the vineyard: influence of vineyard biological activity on the composition of grapes and wine

Vineyard exposure to smoke can lead to grapes and wine which exhibit objectionable *smoky* and *ashy* aromas and flavours, more commonly known as 'smoke taint' [1, 2]. In the last decade, significant bushfires have occurred around the world, including near wine regions in Australia, Canada, South Africa and the USA, as a consequence of the warmer, drier conditions associated with climate change. Considerable research has subsequently been undertaken to determine the chemical, sensory and physiological consequences of grapevine exposure to smoke.

The sensory attributes associated with smoke-tainted wine have been linked to the presence of several smoke-derived volatile phenols, such as guaiacols, syringols and cresols [2]. These volatile phenols have been shown to accumulate in grapes in glycoconjugate forms, following grapevine exposure to smoke [3, 4]. However, their mode of entry, and therefore the factors influencing their uptake from smoke by grapevine leaves and fruit, have not as yet, been adequately investigated.

This study aimed to investigate the extent to which berry physiology, in particular, the development of the berry cuticle and epicuticular wax, influences the uptake of volatile compounds from smoke. Potted Chardonnay and Shiraz grapevines were exposed to smoke for 60 minutes, at approximately one week prior to maturity. Fruit samples were collected immediately after smoke treatment, and again at maturity (i.e. one week later), for imaging

(using an environmental scanning electron microscope) to identify any differences in the physiology of control and smoke-affected berries. Fruit sampled at maturity was also analysed by gas chromatography-mass spectrometry and ultrahigh performance liquid chromatography-tandem mass spectrometry to determine concentrations of volatile phenols and their glycoconjugates, respectively. The potential for a commercial particle film, i.e. kaolin, to act as a protective barrier against smoke, was also investigated, by comparing the volatile phenol and glycoconjugate concentrations of fruit harvested from grapevines treated with kaolin and/or smoke.

Literature:

1. Kennison, K.R., et al., *Smoke-derived taint in wine: The release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke.* Journal of Agricultural and Food Chemistry, 2008, **56**(16): 7379-7383.
2. Parker, M., et al., *Contribution of several volatile phenols and their glycoconjugates to smoke-related sensory properties of red wine.* Journal of Agricultural and Food Chemistry, 2012, **60**(10): 2629-2637.
3. Hayasaka, Y., et al., *Identification of a beta-D-glucopyranoside precursor to guaiacol in grape juice following grapevine exposure to smoke.* Analytica Chimica Acta, 2010, **660**(1-2): 143-148.
4. Ristic, R., et al., *The effect of winemaking techniques on the intensity of smoke taint in wine.* Australian Journal of Grape and Wine Research, 2011, **17**(2): S29-S40.

Appendix 6: Budget reconciliation

Provided online via CIMS