WARMING EFFECTS ON GRAPE ORGANIC ACID METABOLISM





FINAL REPORT to GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Research Organisation: **UNIVERSITY OF ADELAIDE**

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1. Abstract

This travel had three major objectives. The first was to participate at an international grapevine conference in La Serena Chile, where I gave a seminar outlining our current GWRDC-supported research progress. The second was to carry out a small research project in the lab of a collaborator in Dundee, Scotland, in order to advance an aspect of our current GWRDC-supported research programme through the use of a chromatography technique. The third was to interact with grapevine researchers at their host institutions in Chile, Spain and France, where I gave seminars and learned a new technique for grapevine leaf transformation.

2. Executive Summary

The major objectives of this travel were three-fold. Firstly, to attend the 9th International Symposium on Grapevine Physiology and Biotechnology (ISGPB) in La Serena, Chile, to communicate results from our GWRDC-supported research. This objective was met. I gave a seminar at the symposium on Friday 26th April, dealing with the effect of elevated temperature on organic acid metabolism in grape berries. I also gained information from the research of prominent international grape and wine scientists.
The second objective was to conduct a research project with a collaborator, Rob Hancock, at The James Hutton Institute in Dundee, Scotland. This objective was also met. In Dundee I used a chromatography technique to facilitate three aspects of our research: (i) confirm L-idonic acid as the reaction product of an enzyme of our interest and therefore provide evidence for the enzyme's role in tartaric acid biosynthesis in grapes; (ii) measure metabolite levels in fruit lacking the tartaric acid biosynthesis gene, L-idonate dehydrogenase, and (iii) measure metabolite levels in grape berry samples that had been exposed to elevated temperature treatments and exhibited lower levels of malic acid, and in grape berries of various varieties differing in malic acid levels.
Finally, the travel was also used as an opportunity to visit various institutes and universities throughout South America and Western Europe, to meet with leading researchers in the grape and wine field. Institutes that were visited included INIA and The University of Chile (both in Santiago, Chile), The University of Rioja (Logroño, Spain), as well as INRA institutes in Bordeaux and Colmar (France). Meetings with scientists in the field of grapevine genetics and molecular biology, as well as grapevine breeding, physiology, microbiology and grapevine bioinformatics, opened some opportunities for idea-swapping, networking and potential future collaborations.

3. Details of the travel

3.1 INIA, Santiago, Chile

In Chile, I visited, along with PhD student David Contreras-Pezoa (also from our laboratory), the INIA institute in Santiago, Chile. We arrived on Monday 15th April, and met with Dr Patricio Hinrichsen. Dr Hinrichsen told us about the INIA grape-breeding programme, where they are interested in obtaining table grapes with optimal characteristics such as high crop load, loose bunch structure, particular physical properties of the berries (e.g. high crunchiness), and of course pleasant taste and aroma characteristics. Dr Hinrichsen showed me the glasshouses and vineyards containing hundreds of progeny

from various crosses that had been conducted on-site, and introduced me to the researchers of his laboratory.
I had intended to also meet with Professor Manuel Pinto, however he was called away during my visit, to work on the organisation of the 9th ISGPB conference in La Serena. I gave a seminar in the afternoon, entitled "Warming effects on grape organic acid and primary metabolism" that lasted approximately 40 mins. Many of the questions from the researchers were to do with the physiology of the fruit that was used in the experiments I had discussed.



Photo1: Myself and Dr Patricio Hinrichsen at the experimental vineyard, INIA, Santiago.

3.2 Universidad de Chile, Santiago, Chile

The next day, Tuesday 16th April, I visited the Universidad de Chile, Santiago. Here I was hosted by Dr Claudio Pastenes of the Faculty of Agronomical Sciences, a fascinating grapevine physiologist/biochemist with many interests, but mostly involved in photosynthesis, photoinhibition and carbon partitioning research. I met the students and researchers of his laboratory, and he introduced me to many other academics in his, and other departments, including Dr Herman Silva Ascencio, who is a fruit bioinformaticist interested in functional genomics, and has recently been involved in annotating the strawberry genome, and Dr Alvaro Peña, an oenologist with an interest in the effects of terroir. After I gave my seminar, this time titled "Warming effects on wine-grape organic acid metabolism" Dr Peña gave us a short seminar on the different wine regions of Chile, which are extremely diverse due to the range of geographical conditions throughout the country.
Dr Peña also arranged a wine-tasting session. He chose Chilean wines that relied on natural acidity and no additions of tartaric acid, due to my interest in the organic acids of grapes and wine. This was interesting, as many of these wines had a bitter, tart acidity that can be attributed to the high levels of malic acid, only found in cool-climate wines. The varieties that we tasted included Sauvignon Blanc, Chardonnay, Cabernet Sauvignon and Carignan. I also learned that the Carignan variety can retain high levels of acidity even in hot climates. This may be an interesting variety to subject to elevated temperature trials alongside Shiraz in the future. There are three Carignan plants in the Coombe Vineyard at The Adelaide of University, Waite campus.



Photo 2: Giving my presentation at the Universidad de Chile, Santiago.



Photo 3: Wine tasting session at the Universidad de Chile, arranged by Dr Alvaro Peña (standing behind students, on the right) and Dr Claudio Pastenes (standing at the back, in front of the window)

3.3 IX ISGPB Conference, La Serena, Chile

The International Symposium on Grapevine Physiology and Biotechnology is held every 3 years, and enables grapevine researchers to present data and exchange ideas over a 5 day conference. This year the ninth symposium was held at the Enjoy Hotel Convention Centre in La Serena, Chile, and featured prominent grapevine researchers from all over the world, including Dr Anne Francoise Adam-Blondon (France), Prof. Grant Cramer (U.S.A.), Dr Chris Davies (Australia), Dr Nick Dokoozlian (U.S.A.), Dr Eric Duchene (France), Prof. Eric Gomes (France), Dr Patricio Hinrichsen (Chile), Dr Claudio Moser (Italy), Dr Nathalie Ollat (France), Dr Etti Or (Israel), Prof. Mario Pezotti (Italy), Prof. Manuel Pinto (Chile), Dr Laurent Torregrosa (France), Prof. Steve Tyerman (Australia), Prof. Larry Williams (USA) and Prof. Melanie Vivier (South Africa). There were also several student and early-career researcher participants, including myself.
The conference was convened by Professor Manuel Pinto (INIA, Chile). It began on Sunday 21st April with an opening lecture from Dr Chris Davies (CSIRO, Adelaide), and came to a close on Friday 26th April. My seminar, entitled “Warming effects on organic acid metabolism in berries of field-grown vines”, was presented on the morning of the final

day, in a 15-minute slot of the “Grapevine and Global Change” session, to an estimated 100 conference attendees.

Highlights from the conference included the seminar by Eric Gomes (INRA, Bordeaux), whom I later visited at INRA. His presentation gave results from a transcriptomic and metabolomic analysis of berries from heat-stressed Cabernet Sauvignon fruiting cuttings. He has identified a transcription factor that is involved in the heat-stress response, and could be involved in regulating galactinol levels, which increased in response to heat stress. I was interested in the fruit cutting system, and the methods that his laboratory used for their elevated temperature experiments. The data from his publication will be useful to compare with our own elevated temperature treatments that we have carried out with potted vines and field vines.

I also enjoyed the seminar of Dr Etti Or (Israel), although she presented such a large volume of data that it was difficult to take it all in. She spoke of the use of heat shock and application of hydroxycinamide in breaking bud dormancy. These treatments led to some changes in hormone levels, oxidative stress and signalling pathways that could be interesting to look into with respect to our own heat treatments during berry development.

Dr Eric Duchene also gave an interesting talk, which made me think more about different ways to express data during berry development. For example, to compare different varieties at a particular stage of development, it may be better to collect the samples at a particular “thermal time” (i.e. 233°C after veraison), rather than collecting them on the same calendar date, or at the same °Brix.

Assistant Prof. Laurent Deluc presented some interesting and controversial data about synchronisation of berry development during berry ripening. That is, at véraison on a single bunch there may be berries that are green and hard (GH), green and soft (GS), pink and soft (PS), and red and soft (RS). In this situation, berries that are GH will develop more quickly than berries that are more advanced, in order to “catch up”. At least, this is his hypothesis. I have noticed myself many times in the past, that early during ripening there will be a high variation in °Brix between individual berries on a bunch, but as the berries ripen this variation decreases, until the individual berries seem to reach a point where they are all more-or-less at a similar °Brix.

Dr Paul Haynes presented data from a spectral counting technique that can be used to quantify protein profiles in grapevine fruit tips. I would like to find out whether this technique could be applicable to fruit tissues, as it would be interesting to gain protein level data in addition to gene transcript and metabolite level data from some of our experiments.

Professor Anne Francoise Adam-Blondon presented information on a database for up-to-date grape genome sequence data, including annotation information.

There were also several posters that were interesting and relevant to our work. Two separate groups are working on identifying QTLs associated with grape berry acidity. One from a group at Geisenheim University, Germany, presented by Mr Oliver Bitz, and another from Miss Maribel Viviana of INIA, Santiago, Chile. I spoke with both of these authors and we plan to share information about genes that could be involved, and whether these are located in areas that show some potential as QTLs.

The conference also included a day trip to two wineries and a pisco distillery in the Elqui Valley, as well as a visit to the INIA grapevine breeding station at Vicuña. At the latter, we participated in a survey of some new grape varieties that have been bred on-site, at least one of which is soon to be commercially released. We were given three new varieties and asked to score them on various taste and texture parameters.



Photo 4: Organic vineyard in one of the highest regions of the Elqui valley, demonstrating over-head trellising to improve picking efficiency and reduced incidence of disease.

3.4 The James Hutton Institute, Dundee, Scotland

After the conference, the next stop was the James Hutton Institute (JHI) in Dundee, Scotland. I arrived in Dundee on Thursday 2nd May, and went to the institute on Friday 3rd May to sort out paperwork and inductions with Rob Hancock, who kindly welcomed me into his laboratory for the four weeks that followed. The purpose of this visit was to conduct some analyses using Gas Chromatography – Mass Spectrometry (GC-MS), using a protocol commonly used within the Cellular & Molecular Biology group at the JHI with potato tubers and berry tissues (including raspberries and currants). There were several objectives to the analyses that I carried out at the JHI, summarised below as two separate projects.

3.4.1 Project 1: Enzyme characterisation

One of the main interests of our lab is in characterising the biosynthetic pathway of tartaric acid in grape berries. A previous PhD student identified and confirmed the involvement of one enzyme of the pathway, L-idonate dehydrogenase (DeBolt et al. 2006). However, there are at least another three enzymes that must be involved in this pathway, and are as yet unidentified. A candidate gene for one of these enzymes was identified by another PhD student in the lab (Burbidge, 2011). However, while a recombinant enzyme was successfully expressed in *E. coli* from this grapevine gene, and was shown by a spectrophotometric assay to catalyse a reaction involving the expected substrate, 2-keto-L-gulonic acid, the product for the reaction was thus far unidentified. We expect that the product of this reaction is L-idonic acid, if this enzyme can indeed catalyse a step of the tartaric acid biosynthesis pathway. Dr Rob Hancock indicated to us that a GC-MS method at his institute could be used to identify L-idonic acid.
The first part of my work at the JHI was to use a standard of L-idonic acid to check that it could be identified using GC-MS, and to confirm the mass:charge fragmentation profile. The compound consistently eluted in two peaks; one small peak at 6.04 mins and another larger peak at 6.42 mins. According to the fragmentation patterns, the peak at 6.04 mins correlated with a lactone form of L-idonic acid from the GC-MS NIST database, while the peak at 6.43 mins correlated with a galactonic acid compound (Figure 1). Upon further

conversation with biochemists and organic chemists, we have concluded that the L-idonic acid fractionation profile in the NIST database was not a true representative of L-idonic acid as it appears in solution, but rather the less common lactone ring form. It is also likely that the peak at 6.42 mins is not galactonic acid, but rather the open-chain form of L-idonic acid, which is only correlating with galactonic acid due to the absence of the standard profile for L-idonic acid in the NIST database. Online literature and database searches are yet to yield a profile for the open-chain form of L-idonic acid, which is the form that we expect to occur in solution, and therefore in our enzyme assay product. It may be that we are the first to characterise the mass:charge fractionation profile of L-idonic acid.

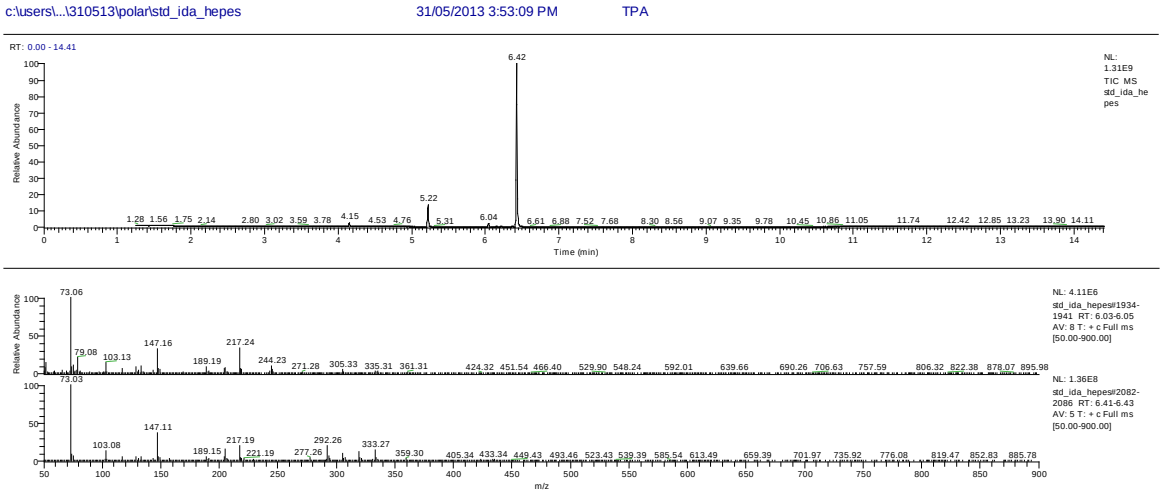


Figure 1: Characterisation of L-idonic acid standard using GC-MS. The top plot shows the Total Ion Count, which essentially gives an elution profile throughout the 15 minute run. Four peaks can be seen. The first at 4.15 mins, which is consistent with silane, used in the extraction process. The second peak elutes at 5.22 mins, and is an internal standard, ribitol. The third peak is at 6.04 mins and is the first peak of the L-idonic acid standard, thought to be the lactone ring form. The final and largest peak is the second L-idonic acid peak, eluting at 6.42 mins, thought to be the open-chain form of L-idonic acid. Below the elution profile are the fragmentation patterns of the two L-idonic acid peaks, the first from the 6.04 min peak and the second from the 6.42 min peak. When searching through the NIST chemical database, the first profile is consistent with the lacone ring form of L-idonic acid. The second fits closest with galactonic acid, however we note that there is no database entry for the open-chain form of L-idonic acid. Therefore we propose that this profile is that of the linear form of L-idonic acid, which is the predominant form of L-idonic acid when in solution.

Having characterised the mass-spec profile of the expected enzyme assay product, L-idonic acid, I then used plasmid from Burbidge (2011) to express the candidate gene of the tartaric acid biosynthesis pathway in *E.coli* (DH5α) cells, then purified the protein and ran assays with the substrate 2-keto-L-gulonic acid and cofactor NADH, as specified by Burbidge (2011). The absorbance of the assays was visualised spectrophotometrically, to monitor the conversion of NADH to NAD^+. I also ran an assay with some protein that had been denatured by boiling. I freeze-dried the enzyme assay products, then extracted and ran through the GC-MS according to the method outlined by Foito et al. (2013). Results are shown in Figure 2. The result from the boiled enzyme assay was very similar to the result from the substrate standard, indicating that none of the substrate was metabolised in the absence of native enzyme. The result from the active enzyme assay was very similar to the L-idonic standard. In the latter there was also still a small amount of substrate present, indicating that the reaction had not gone to completion. Overall, this provides evidence that

the gene candidate encodes an enzyme that can convert 2-keto-L-gulonic acid to L-idonic acid *in vitro*, and may therefore be involved in the tartaric acid biosynthesis pathway.

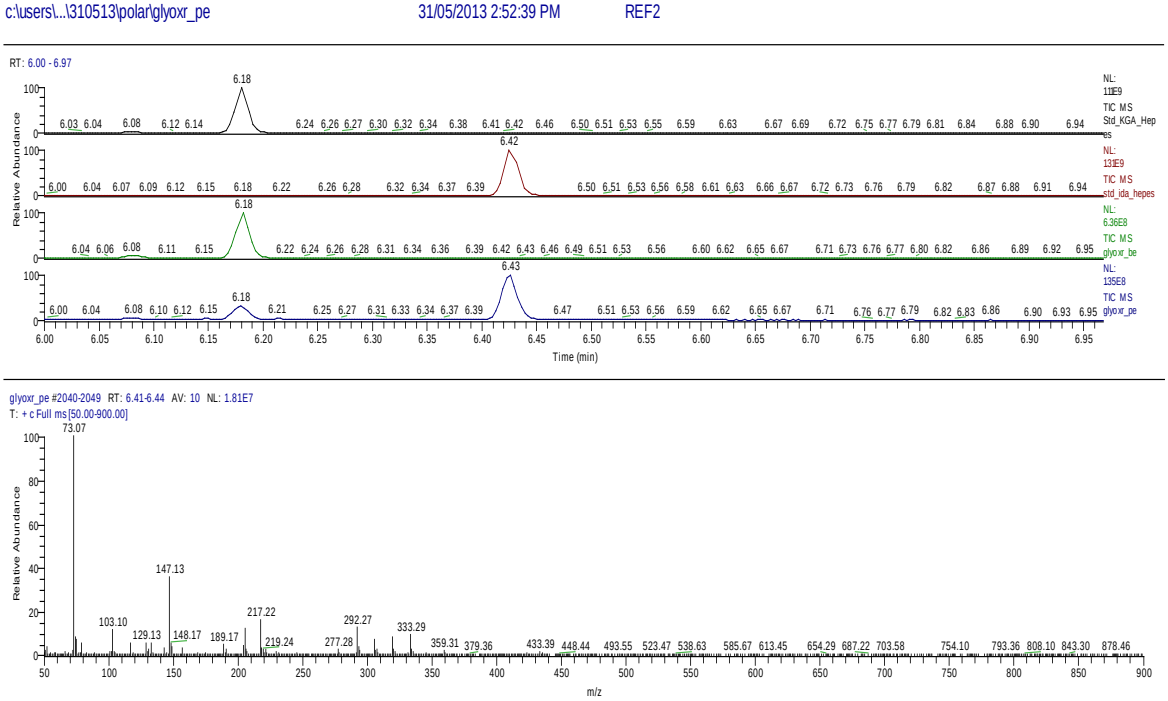


Figure 2: Characterisation of products from enzyme assays with recombinant 2-keto-L-gulonate reductase (2-KGR) from *E. coli*. The top panel shows four elution profiles. The first (black) is that of the substrate, 2-keto-L-gulonic acid, which elutes at 6.18 mins. The second (red) is that of the expected product, L-idonic acid, which elutes at 6.42 mins (the small peak at 6.04 mins cannot be seen due to the scale of this figure). The third (green) is a product from the 2-KGR enzyme assay using boiled enzyme, showing the substrate peak at 6.18 mins. The fourth (blue) is a product from the 2-KGR enzyme assay using native enzyme, showing a peak at 6.43 mins that is consistent with that of the expected product, L-idonic acid, as well as some residual substrate in a small peak at 6.18 mins. The bottom panel shows the fragmentation pattern of the 6.43 min peak from the native enzyme assay, which gives a pattern identical to that of the L-idonic acid standard in Figure 1.

3.4.2. Project 2: Metabolic analysis of grape tissues with differing malic acid levels

In the second project, I used the same GC-MS technique to run a global metabolic analysis of grape berry extracts from various experiments (Foito et al. 2013). The first was a heat-treatment experiment, where potted Shiraz vines were exposed to "control" (25:10°C day:night) and "heated" (35:15°C day:night) treatments in controlled-environment (Conviron) growth cabinets at The Plant Accelerator, The University of Adelaide. Another experiment involved analysis of four *Vitis vinifera* varieties that have naturally differing malic acid accumulation and degradation patterns during development. The aim of these two experiments was to identify compounds that are differentially regulated in these varieties and heat treatments, and which may therefore be linked to changes in the metabolism of malic acid during ripening.

Seeing as this method was now to be utilised for quantitative purposes, some quality control measures were taken. The repeatability of the GC-MS technique was validated through the analysis of several replicate extractions and replicate runs of "reference material" made up of a mixture of many of the grapevine samples used in the analysis. Results showed high similarity and low variability (Figure 3), confirming that the extraction procedure and separation technique were both repeatable. Only compounds that gave repeatable results with the reference material were used in the analysis of the experimental samples.

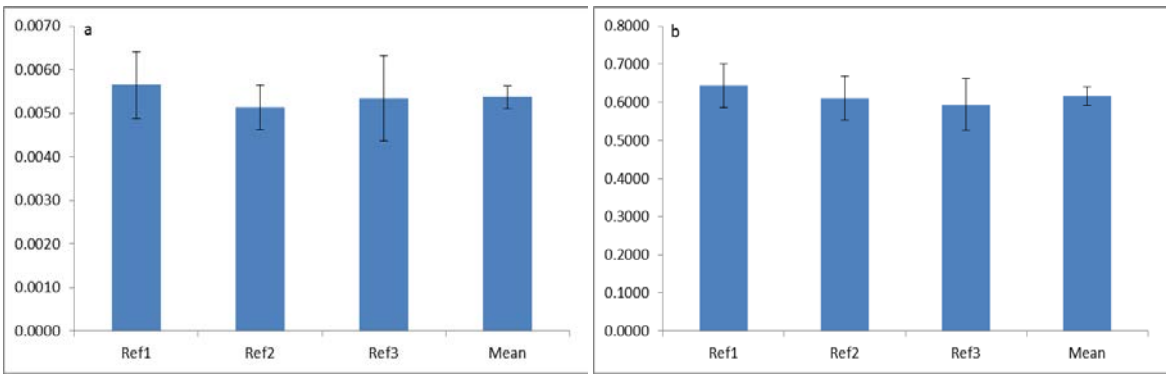


Figure 3: Repeatability of GC-MS technique for two representative compounds (a) isoleucine and (b) malic acid. Ref1, Ref2 and Ref3 indicate three separate extractions from the same reference material. References samples were run in the same sequence as experimental samples. Each Ref sample was run three times through the GC-MS, once at the beginning of the sequence, once in the middle, and once at the end. Therefore each column represents mean data from three separate GC-MS runs with the same extract. The Mean column represents the mean value calculated from Ref1, Ref2 and Ref3 data. (n=3 ± S.D.)

3.4.2.1. Heat-exposed samples

A metabolic map of changes in response to elevated temperature (Figure 4) demonstrated up-regulation of several amino acids including the stress-inducible proline and GABA and down-regulation of some organic acids, most notably malic acid. There were no changes in sugar levels, while glucose-6-phosphate and inorganic phosphate levels were temporarily higher in the heated fruit. These data will be incorporated into a manuscript, along with existing information regarding changes in gene transcript levels and enzyme activities that may be involved in regulating malic acid metabolism in response to elevated temperature.

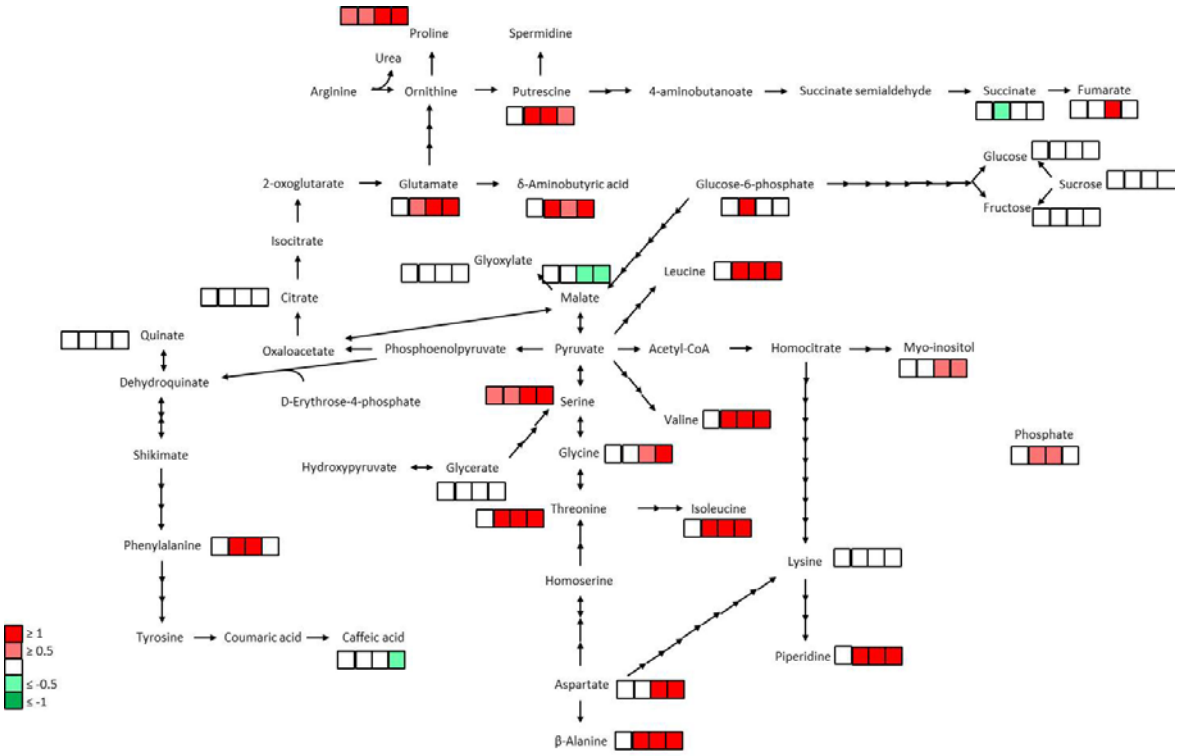


Figure 4: Grape berry metabolite differences in response to Conviron elevated temperature treatments with potted Shiraz vines. Coloured boxes indicate a significant difference ($p \leq 0.05$, according to 2-tailed t-tests) between berries from heated and control treatments, according to the scale shown. Data were calculated as log(2) of the ratio between heated and control berries, such that an increase in metabolite level with heating could be represented as a positive fold-change, while a decrease in metabolite level could be represented as a negative fold-change. Boxes represent time-points throughout berry ripening, with the first box representing berries collected during the heat treatment, the second box representing berries collected at the end of the heat treatment, the third representing berries collected three days after the treatment had ended, and the final box representing ripe berries. (n=4)

3.4.2.2. Varieties differing in acid levels

Metabolic data were also collected from four grape varieties that differ in malic acid (MA) level. Fruit from two white varieties, Chenin Blanc (high MA) and Palomino (low MA), and two red varieties, Tinta Cao (high MA) and Cabernet Sauvignon (low MA) were collected throughout development, and the levels of malic acid were determined in our laboratory at The University of Adelaide, South Australia, using a routine reversed-phase HPLC technique. The same samples were then taken to the James Hutton Institute and analysed using GC-MS. It should be noted that the HPLC technique utilises standard curves and therefore gives absolute levels of the acid, whereas the GC-MS technique provides levels relative to an internal standard, ribitol. Nevertheless, results for malic acid level obtained from both of these techniques (Figure 5) show very similar patterns, thereby providing another level of validation for this technique with these grape berry samples.

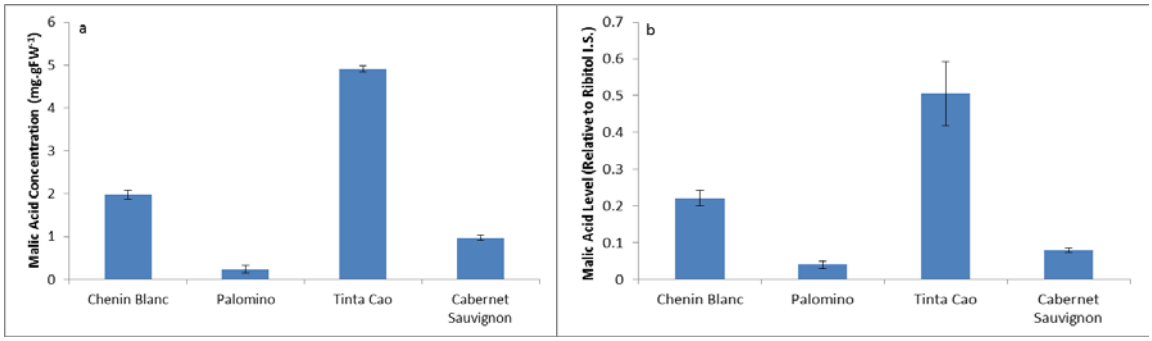


Figure 5: Malic acid levels in ripe berries of two white grape varieties (Chenin Blanc and Palomino) and two red grape varieties (Tinta Cao and Cabernet Sauvignon), determined using (a) a reversed-phase HPLC method at The University of Adelaide, South Australia, and (b) a GC-MS method at the James Hutton Institute, Dundee, Scotland. Both techniques gave similar results, although (a) demonstrates absolute quantities in $mg.gFW^{-1}$, while (b) provides a value relative to the internal standard, ribitol. (n=3 ± S.E.M.)

Some interesting preliminary data from the four-varietal trial are shown below. These are compounds with levels that may be positively (Figure 6) or negatively (Figure 7) correlated with levels of malic acid in the fruit, including some non-polar compounds (Figure 8). Further analyses with these samples, along with samples from other developmental stages are currently being conducted, and it is expected that these data will be published in a manuscript along with information on berry physiology and gene transcript levels from these varieties.

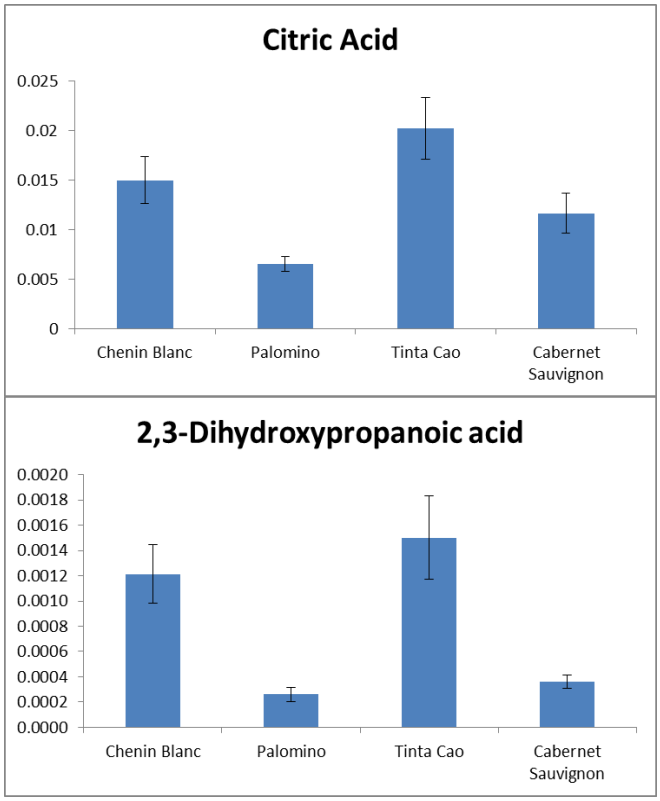


Figure 6: Polar compounds that may be positively correlated with levels of malic acid between the four varieties, Chenin Blanc, Palomino, Tinta Cao and Cabernet Sauvignon, measured at The James Hutton Institute using GC-MS. Data are presented relative to polar internal standard, ribitol. (n=3 ± S.E.M.)

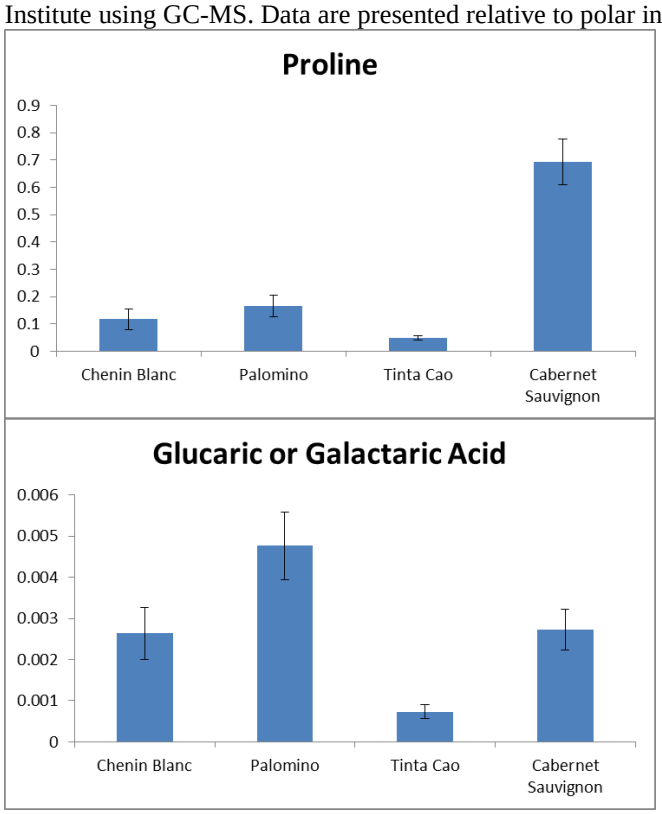


Figure 7: Polar compounds that may be negatively correlated with levels of malic acid between the four varieties, Chenin Blanc, Palomino, Tinta Cao and Cabernet Sauvignon, measured at The James Hutton Institute using GC-MS. Data are presented relative to polar internal standard, ribitol. (n=3 ± S.E.M.)

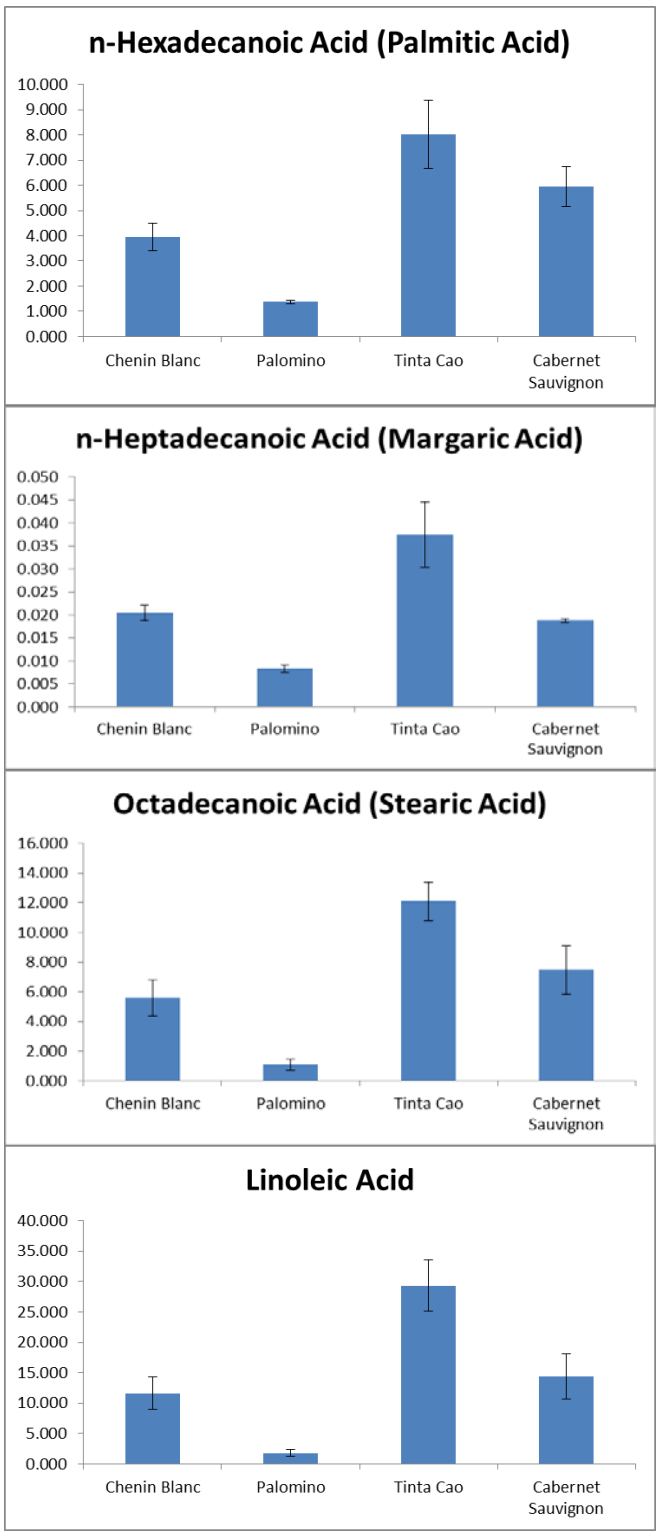


Figure 8: Non-polar compounds that may be positively correlated with levels of malic acid between the four varieties, Chenin Blanc, Palomino, Tinta Cao and Cabernet Sauvignon, measured at The James Hutton Institute using GC-MS. Data are presented relative to non-polar internal standard, n-nonodecanoic acid. (n=3 ± S.E.M.)

3.4.3. Project 3: Grapevines lacking L-idonate dehydrogenase

I extracted and ran leaf samples from some transgenic RNAi microvine lines designed to knock down the expression of an L-idonate dehydrogenase gene, as well as berry samples of a wild Chinese grape species called *Ampelopsis aconitifolia*, which naturally lacks the L-idonate dehydrogenase gene and does not produce tartaric acid (Debolt et al. 2006). It was expected that there may be an increase in levels of L-idonic acid or other tartaric acid precursors in these latter tissues, however L-idonic acid was found to co-elute with a glucose molecule and therefore the peak could not be identified in these samples. Nevertheless, many other compounds, both polar and non-polar, were identified from these experiments and the data are currently being analysed. Some preliminary results follow.

3.4.3.1. Transgenic lines

Figures in this section include the following nomenclature: “WT” (wildtype), which is the mean of three leaves from plants that do not contain the RNAi construct, as well as “KO1”, “KO2” and “KO3”, which are leaves from three putatively transgenic lines. It should be noted that gene expression studies at the time of this project had not yet been carried out to determine which of the lines had reduced expression of the L-idonate dehydrogenase gene. It should also be noted that the transgenic lines were represented by tissue from a single leaf only, and need to be replicated. Data presented below indicated that two of the three transgenic lines (“KO2” and “KO3”) showed some reduction in the level of tartaric acid in leaves, when compared to the control. These leaves also showed a reduction in citric acid and 2,3-dihydroxypropanoic acid, and an increase in glycerol and inorganic phosphate.

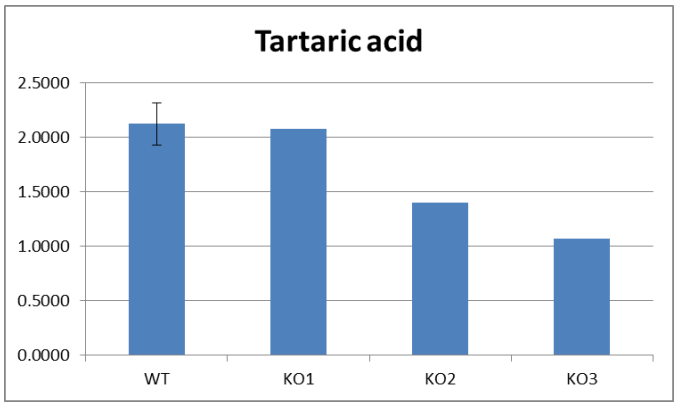


Figure 9: Levels of tartaric acid in transgenic microvine lines, measured at The James Hutton Institute using GC-MS. Data are presented relative to polar internal standard, ribitol. (for WT, n=3 ± S.E.M.)

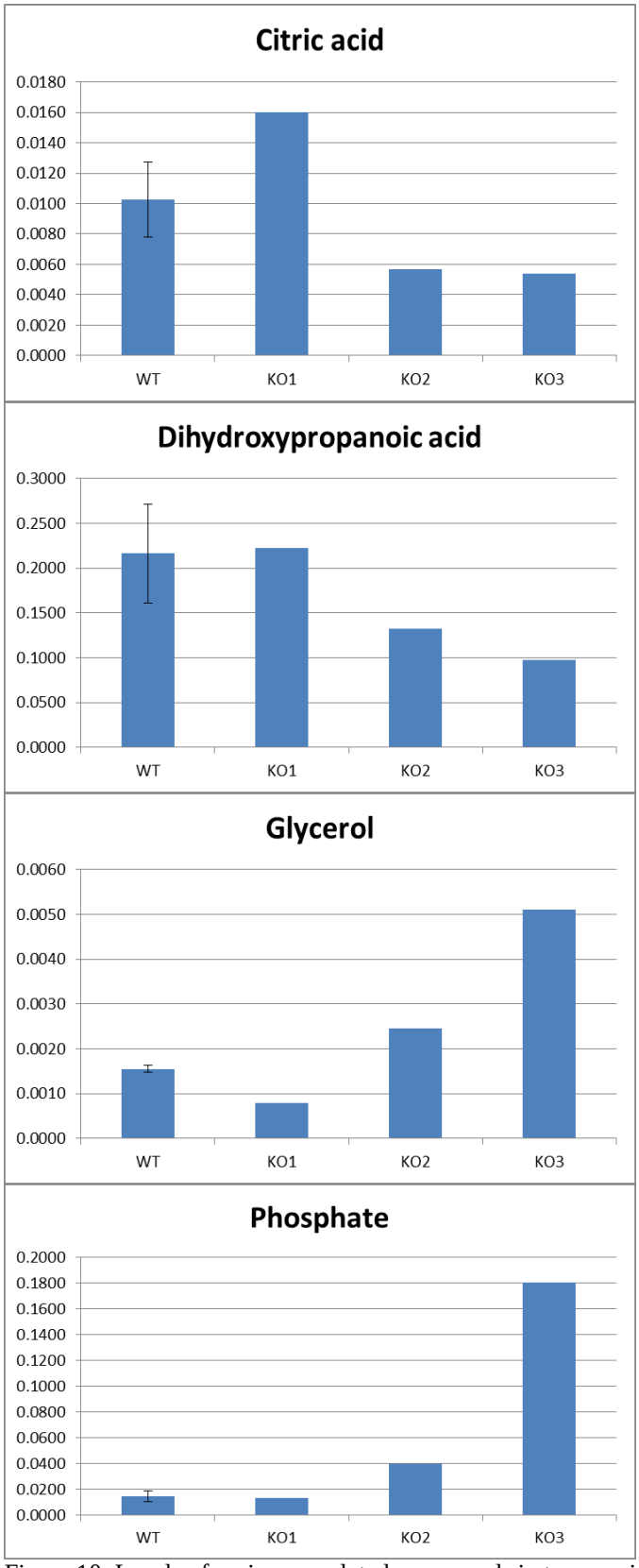


Figure 10: Levels of various regulated compounds in transgenic microvine lines, measured at The James Hutton Institute using GC-MS. Data are presented relative to polar internal standard, ribitol. (for WT, n=3 ± S.E.M.)

3.4.3.2. Natural Mutants

The *A. aconitifolia* berries contained no tartaric or malic acid. These data can be seen in Figure 9, alongside data from the four *V. vinifera* varieties that were previously discussed. They did contain slightly higher levels of succinic and citric acids, and dramatically higher levels of quinic acid. The fruit also displayed higher levels of inorganic phosphate than the *V. vinifera* species tested (Figure 10).

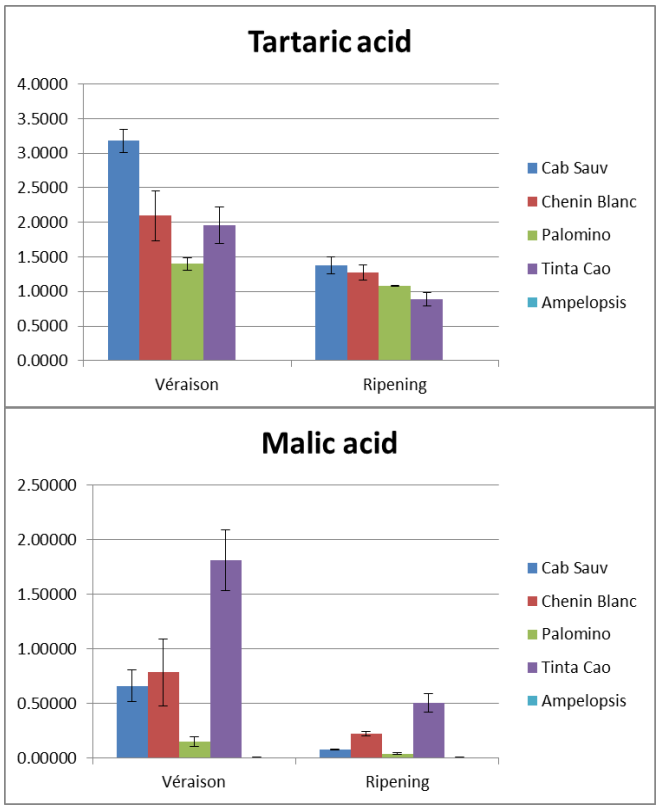


Figure 9: Levels of tartaric acid and malic acid in berries of four *Vitis vinifera* varieties and in *Ampelopsis aconitifolia*, measured at The James Hutton Institute using GC-MS. Data are presented relative to polar internal standard, ribitol. (n=3 ± S.E.M.)

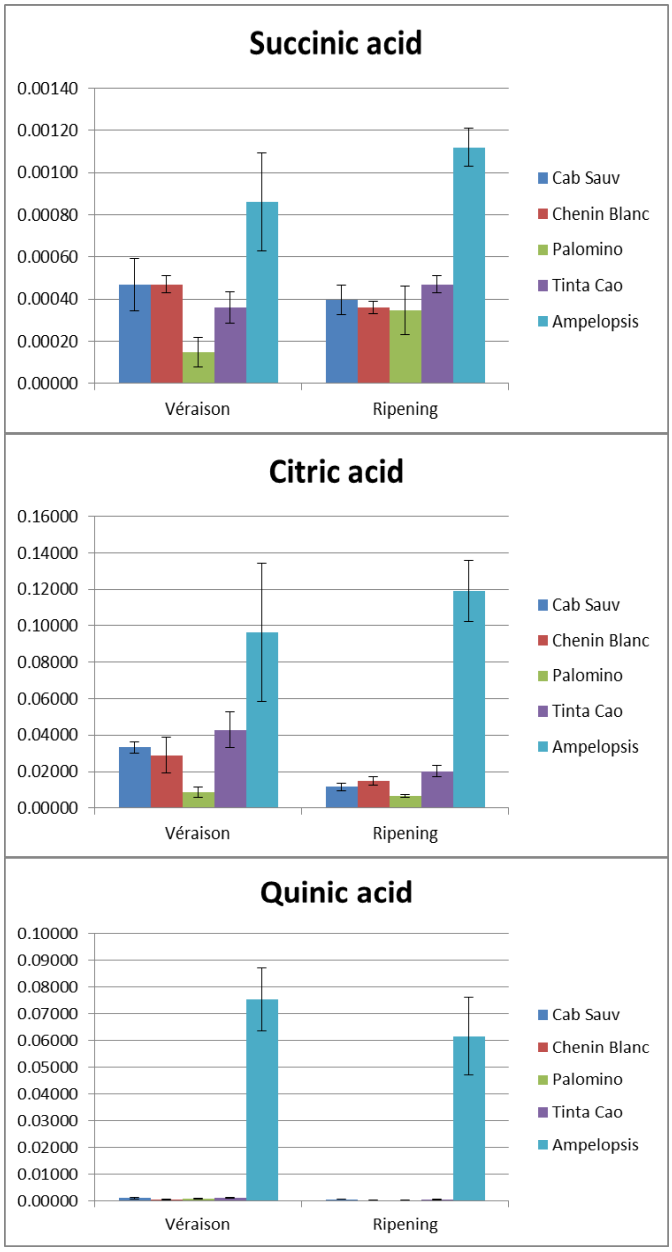


Figure 10: Levels of other compounds in berries of four *Vitis vinifera* varieties and in *Ampelopsis aconitifolia*, measured at The James Hutton Institute using GC-MS. Data are presented relative to polar internal standard, ribitol. (n=3 ± S.E.M.)

3.5. Universidad de La Rioja, Logroño, Spain

After I had completed my work at the JHI, I moved on to visit some researchers at various institutes in Spain and France.
First I travelled to Logrono, Spain, where I was hosted by Dr Jerome Grimplet and Prof. Jose Martinez Zapater at the Science Institute of Vine and Wine (ICVV), located on campus at Universidad de La Rioja. I gave my seminar on Monday 3rd June, and Dr Grimplet spoke to me of a grapevine genome annotation project that he is initiating. He hopes to set up a large-scale collaboration to annotate genes of the grapevine genome that are currently unknown or incorrectly annotated, but is currently tackling with a method to collect these data from researchers. This could be a very beneficial project to the grapevine research community. I also met with Dr Pablo Carbonell-Bejerano, author of a recently published manuscript related to temperature stress and changes in the transcript profile of Muscat Hamburg berries (Carbonell-Bejerano et al., 2013). We discussed the potential for changes in transcript levels of cation transporters in influencing malic acid metabolism in response to elevated temperature. I also accompanied Dr Carbonell-Bejerano to the experimental vineyard to collect samples for a bunch structure project.
I met with Prof. Javier Ibañez, who told me of some 1600 clones that are present in the experimental vineyard, as well as hundreds of progeny from crosses between Tempranillo and a wild grapevine species. The researchers currently use these various clones and progeny to identify which characteristics are responsible for bunch compactness, and are trying to identify genes that regulate aspects of bunch structure and could therefore be used in gene-assisted breeding for optimising characteristics of table grape bunches. He also spoke of his work in genotyping wild grapevine species and cultivars, where they have identified 240 SNPs that they can use to compare to domesticated cultivars in Spain, for use in phylogeny and determining ancestry.



Photos 5 & 6: Experimental vineyard plot at Logrono, with Dr Carbonell-Bejerano collecting samples for bunch structure project (right).

Just outside of Logrono, I also had the opportunity to meet Dr Juan Carlos Sancha at his winery in Baños de Rio Tobia, where he and his wife use collections of rare varieties to make small batches of wines from the region of Rioja. I was also very lucky to have the opportunity to try wine made from the recently discovered Tempranillo Blanco; a variety cultivated from a single cane that had demonstrated a natural pigment mutation, giving white berries instead of red. The wine had a great acid:sugar balance. Along with the

Universidad de La Rioja, Dr Sancha aims to collect as many varieties as possible from the Rioja region, to preserve the germplasm and take advantage of the natural variation in potential future breeding studies.



Photo 7: Myself with Dr Juan Carlos Sancha and his wife in their winery in Logroño.

3.6. INIA, Bordeaux, France

Next, I went to INRA, Bordeaux, where I was hosted by Dr Eric Gomes on Monday 10th June. I was very keen to meet with Dr Gomes, as he had just published a paper investigating the effect of microclimate on berry transcriptome. He showed me the experimental set-up, whereby fruiting cuttings were grown in a glasshouse, with bunches subjected to elevated temperature, but leaves remaining un-treated. This meant that there was no effect on photosynthesis of the plant, and therefore no effect on sugar accumulation. Eric also took me to see the experimental vineyard plot, which contains 52 *V. vinifera* varieties and 120 wild Vitis species, as well as progeny from various crosses. Dr Gomes has data on malic and tartaric acid levels from the 52 *V. vinifera* varieties and we spoke of a project where we might sequence candidate genes involved in regulating berry acidity, and determine whether there are alleles that correlate with levels of acidity in the 52 varieties. This would be a good opportunity to further our current GWRDC-funded research.
I also met the director of the institute, Professor Serge Delrot. Professor Delrot gave me a full tour of the new facility, which housed an impressive array of equipment, including an HPLC-NMR, which can be used to separate and identify the structures of novel compounds, and is being used to analyse grape and wine samples for aroma research (among other things). We also spoke of potential collaborations for heat stress and metabolomics research that could be operated through the tri-lateral agreement, which involves collaboration between three institutions; INRA Bordeaux, Geisenheim University, Germany and AWRI, Adelaide. Serge suggested that we could try to include The University of Adelaide in the agreement. He is keen to set up a project dealing with the metabolic effect of temperature stress. Following this, I also had the opportunity to give my seminar and meet with many other interesting scientists, including Dr Cecile Cabasson, who was interested to collaborate, and spoke on a project about hexose transporters in grape berries, although a project proposal for this collaboration is due August 2013.

3.7. INRA Colmar, France

At INRA Colmar, I was warmly welcomed by Dr Pere Mestre on Wednesday 12th June. I spent the day meeting with people of the institute, and was given a demonstration of a grapevine leaf agroinfiltration technique that can be used to produce transient transformations in grapevine leaves. As yet, researchers in Australia have had limited success with this technique, which would be useful for a relatively quick test of gene function in grapevine. For example, it could be used to knock down or overexpress candidate genes of the grapevine tartaric acid biosynthesis pathway, to confirm their role *in vivo*. Dr Mestre and a student of his showed me how to set up and maintain the grapevine plantlets in tissue culture, and what is the best leaf age to use for the experiments. We spoke about the grapevine cultivars that are more suitable for use, as well as the best Agrobacterium strains, and how to prepare the bacterial cultures. They then demonstrated the vacuum infiltration technique, and showed me how to tell that the leaf has been successfully infiltrated by the bacterial culture. Dr Mestre and I discussed potential applications of this technique to our research in Adelaide.



Photo 8: Demonstration of grapevine leaf agroinfiltration technique at INRA, Colmar

On Wednesday 12th June I also met with Eric Duchene, a grapevine geneticist who has identified two potential QTLs for acidity driven by tartaric acid, on chromosome 6 and 8, using mapped progeny from a cross between Riesling and Gewurtztraminer. He has used this method to successfully identify genes involved in terpenol synthesis. Dr Duchene has also recently published a manuscript proposing the use of thermal time to select dates for sampling fruit (e.g. degrees after véraison), rather than chronological time (e.g. days after flowering), as this gives a lower level of variability in TSS levels (Duchene et al 2012). He strongly encouraged me to use this sampling strategy in future research. He has also used this information to put together a simple model for malic acid degradation during berry ripening. Some of our research at The University of Adelaide also agrees with Dr Duchene's findings. Dr Duchene was very open with his research findings and keen to collaborate in finding genes that may be involved in determining grape berry acidity. Finally, I returned to INRA Colmar on Thursday 13th June to give my seminar and to meet with a couple more researchers, whom I hadn't had time to see the previous day.

4. Conclusion

In summary, this trip has facilitated our GWRDC-funded research via multiple avenues. Firstly, the opportunity to attend an international conference focussed solely on grapevine physiology and biotechnology enabled me to share our current research findings with other experts in our field, and to gain valuable feedback from these experts. This also opened opportunities for me to meet one-on-one with grape scientists, some of whom agreed to host and meet with me at their own institutes to discuss our research in more detail, and which resulted in the establishment of potential future collaborative ties. My visit to the James Hutton Institute in Dundee, Scotland has progressed two practical aspects of our current research, in the form of enzyme characterisation and metabolomics analyses, both of which go some way toward the understanding of organic acid metabolism in grape berries, and the influences of environmental changes. Further analyses of these data are underway, and the results will be used to enhance manuscripts that are currently under construction.

5. Acknowledgements

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