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Towards elite mildew resistant selections suitable for industry use



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1. Abstract

This project evaluated 1200 field-planted downy and powdery mildew resistant vines to identify elite selections with superior agronomic performance and desirable wine attributes. Vines were assessed for a number of agronomic traits including yield, berry maturity date and mildew resistance over multiple seasons. Elite selections with a range of flavour styles were identified. The potential for robust flavours at low sugar levels for low alcohol wine production was also investigated. As part of the selection process, methods for field evaluation were developed and small-scale winemaking methods developed for assessment of wine made from single vines. No mildew infection was observed on the unsprayed vines during the term of the project and sensory evaluation of the white wines identified aromatic wines and diverse flavour profiles for highly coloured red wines. Twenty red and 20 white berry vines were selected for further evaluation in regional trials. Unique wines with robust flavour styles, produced with low input costs from mildew resistant vines, may assist the Australian industry to reduce future risks.

2. Executive summary

The breeding of new disease resistant wine grape selections has become a priority in a number of countries (Dry et al. 2010). These new varieties, with resistance to downy and powdery mildew, offer the opportunity to substantially reduce the use of fungicide sprays and lower the cost of production. A previous Wine Australia project (Thomas and Dry, CSP 0904) described the use of DNA markers in a marker-assisted selection (MAS) process to identify 1200 new mildew selections that were planted in the field for evaluation. This project report describes the screening process used to identify the 20 best red and 20 best white berry individuals from those 1200 selections.

Strategies to evaluate new selections require both field evaluation and wine evaluation over a number of years to account for the different response of plants to seasonal variation. This is especially important for the screening of selections of diverse genetic background. The 1200 vines were assessed in the field for a large number of traits from budburst until harvest and it was found that there was large variation between individuals for all traits measured. A large diversity in berry chemistry was also found. Of all the traits measured in the field, the most important for selection was final yield and harvest (berry maturity) date. Selections were discarded if they had low yield and/or had a late harvest date because the berries did not reach maturity until late in the season.

Wine chemistry and sensory was an important part of the screening process and small-scale winemaking methods were developed for single vines of both red and white berry selections. Wine sensory panels included industry winemakers to ensure that wines of commercial interest were identified. The results showed that small-scale wine made from single vines was an effective screen to identify superior selections. Red wines favoured by the panel had high colour and a diversity of flavours that ranged from like Cabernet Sauvignon to Shiraz as well as some unique styles. The highly coloured wines had red flesh parentage and these were found to also have high antioxidant potential. The white wines most favoured by the sensory panel were aromatic and floral. An examination of the parentage of these selections showed that they had a Muscat or Riesling parent. The 20 superior selections for both reds and whites captured a range of wine styles and flavour and aroma profiles.

Low alcohol wines were also made from selected red and white berry vines by harvesting early at low sugar levels. The objective was to see if it was possible to make attractive low alcohol styles without the need for additional manipulations in the winery. The sensory results indicated that the reds showed the most promise, producing a Beaujolais style, but further research was required.

The diversity of aromatic and floral flavours and aromas observed from wine of selections with Muscat parentage indicated that it would be a good parent for future breeding. To improve future selection processes, this study developed a SNP DNA marker so that progeny at the seedling stage could be screened for muscat flavour. This SNP marker has been incorporated into a SNP panel that can be used to rapidly screen seedlings for the following traits, before field planting; powdery and downy mildew resistance, berry colour, flower sex (fruitfulness), stature and muscat flavour.

Another important outcome of this research is the finding that, for future screening efforts, it is not necessary to collect data on all of the traits measured in this study, and that only a subset of traits needs to be measured for an effective selection process that includes yield data, harvest date, wine chemistry, pH of must and wine sensory.

Unique wines with robust flavour styles produced with low input costs from mildew resistant vines may assist the Australian industry reduce future risk. Larger scale regional field trials will assist in determining the suitability of the 20 best red and white berry vines to produce acceptable yields under different management treatments and different climates. It is also expected that regional trials will assist with industry adoption by providing the opportunity for regional industry to assist in the evaluation of the wines.

This project was aligned with Theme 1.2 (Grapevine germplasm) of the (then) GWRDC Strategic RD&E Plan 2012-17 and had aspects relevant to other Themes in Program 1 (climate adaptability, vineyard profitability) and Program 3 (improving products and processes).

3. Acknowledgments

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4. Background

World agriculture will be forced to undergo dramatic and rapid changes to address the challenges of climate change, limited resources (water, land, energy) and population growth, well before the projected population peak in 2050 (The Royal Society 2009). This can only be achieved by sustainable intensification of agriculture using fewer inputs and higher outputs per unit land. For the Australian wine industry, reducing inputs will be a key factor in maintaining the competitiveness of the industry, in addition to having unique desirable products.

A 2007 GWRDC report (AHA viticulture, RD 05/03-3) indicated that average costs per hectare for pest and disease control across a number of regions was $95-$1849. A 2010 GWRDC report (Scholefield Robinson Horticultural Services Pty Ltd and EconSearch Pty Ltd., GWR 08/04) stated that powdery ($76m per annum) and downy mildew ($63m per annum) had the largest negative economic impact of all pests and diseases. Reducing inputs remains a key factor in vineyard profitability but product differentiation and uniqueness are also important for having a desirable competitive product in the market.

The breeding of new winegrape selections has become a priority in a number of countries (Dry et al., 2010). However, strategies to evaluate new selections still require both field and wine evaluation. A previous project supported by Wine Australia (CSP 0904), established in the field 1200 new selections, from a CSIRO marker-assisted selection (MAS) breeding program, that showed strong resistance to powdery mildew and reduced susceptibility to downy mildew. Preliminary results indicated that there was high diversity observed for many agronomic traits and wine attributes across these selections that may equal or exceed the existing diversity present in existing Australian winegrape germplasm collections.

This project evaluated 1200 field planted downy and powdery mildew resistant vines to identify elite selections with superior agronomic and wine attributes. The data obtained during the evaluation process will provide genetic insight into trait diversity within the population of 1200 vines and information on the best methods for breeding and evaluating second generation disease resistant selections that have additional powdery and downy mildew resistance loci to improve the durability of resistance in the vineyard. Vines were assessed for yield performance and mildew resistance over multiple seasons. Sensory and chemical analysis of berries and wine was done to identify elite material with desirable flavours and winemaking properties. As part of the project, low alcohol wine production was also investigated, and DNA markers linked to genes responsible for flavour were investigated for marker-assisted selection (MAS) studies. An outcome of the project will be a mother vine source block of both white and red elite selections that could be used for further evaluation and to provide material for nurseries and for second generation breeding.

5. Project Aims and Performance Targets

The objective of this study was to evaluate the first generation of powdery and downy mildew resistant vines, to identify elite individuals that are high yielding and produce robust, desirable wine styles under Australian conditions.

This was to be achieved through:

(a) the collection of agronomic performance and yield data

(b) sensory and chemical analysis of berries and wine

(c) investigation of the flavour potential of wines from early harvest low sugar berries

(d) the development and assessment of DNA markers for muscat and methoxypyrazine. Note: After a joint review of the project by CSIRO and Wine Australia in year 3, it was agreed to stop the research on DNA markers for methoxypyrazines and investigate antioxidant levels in the grapes and red wine from some of the selections.

(e) the establishment of a mother vine planting to investigate yield potential and provide a source of material for industry, larger scale winemaking and second generation breeding.

This research allowed us to address the following questions:

- How many vines in each style class satisfy the criteria of an elite plant suitable for industry release?
- To what extent can the genetic diversity within the 1200 mildew resistant plants be used to select additional value traits that contribute to further reducing inputs and producing robust flavour profiles across a range of berry sugar levels?

The answers to these questions have both short and long-term effects on the number of elites that might be suitable for industry regional trials and how best to plan new research to take advantage of genetic diversity to maximise and combine as many desirable traits as possible.

6. Methods

The project assessed the performance of 1200 new selections that were resistant to powdery mildew and show reduced susceptibility to downy mildew. Establishment of the vines was previously described in the Final Report for project CSP 0904. The vines were not sprayed to control powdery or downy mildew, to allow evaluation of resistance levels to pathogen pressure in the Barossa Valley.

6.1 Vine management

Vine management was similar for both red and white selections, fertiliser was applied once in spring and near the end of the season. Vines were irrigated to a level to avoid water stress. Mature vines had four arms and were pruned to a maximum of 120 buds. The vines were sprayed against mites in spring. Weed control was conducted during the season with herbicide sprays in the vine row and the mid row, and frequent mowing.

The climatic conditions were collected by a nearby BOM weather station at Nuriootpa. The monthly cumulative thermal line was calculated using the following formula:

Monthly cumulative thermal time $= \sum_{last\ day}^{first\ day} \frac{Tmax+Tmin}{2} - Tbase$

T *base* is the mean base temperature for grapevine.

6.2 Phenotyping and evaluation of field grown plants

Single vines were evaluated each season for a number of important agronomic traits; inflorescence number per cane, fruitfulness of spur pruned canes, bunch number, berry colour, date of berry maturity (22 °Bx (degrees Brix) for whites and reds), bunch type, bunch weight and total yield. Vine vigour was measured using trunk girth at the irrigation line.

For some selections, a laboratory-based powdery mildew detached leaf assay similar to that previously described (Donald et al., 2002) was used to determine resistance to powdery mildew.

6.3 Berry and wine chemical analysis

6.3.1 Berry chemical analysis

Ten berries were randomly picked from bunches collected from three randomly selected plants from each treatment. The berries were crushed in a plastic bag and 2 mL of must was transferred to a 2 mL Eppendorf tube and centrifuged for 1 minute at 13,000 rpm. Approximately 0.6 mL of the supernatant was analysed using an OenoFoss™ analyser for Brix, pH, total acidity, volatile acidity, alpha amino nitrogen, ammonia, tartaric acid, malic acid and density.

6.3.2 Determination of phenolics in red berries

Thirty berries selected at random from red berry plants were weighed and frozen in liquid nitrogen. The frozen berries were ground to a fine powder using a motorised grinder cooled with liquid nitrogen. The ground powder samples were stored in a -20° C freezer until analysed for phenolics. For phenolic analysis, one gram of frozen powder was weighed and 10 mL of 50 % (v/v) ethanol (pH 2.2) was added and incubated for 1 hour in a 10 mL falcon tube. The tubes were inverted every 10 minutes. The mixture was then centrifuged for 5 minutes at 3,500 rpm. One mL of the supernatant was then pipetted into 10 mL HCl, mixed thoroughly and allowed to stand for 3 hours. 300 µL of this

sample was read at 700 nm, 520 nm and 280 nm for absorbance using a UV/VIS (FLUOstar Omega) spectrometer (BMG Labtech, Germany) against a blank sample. All measurements were carried out in triplicate and averaged.

6.3.3 Wine chemical analysis

About 1 mL of final wine was collected during winemaking and transferred into a 2 mL Eppendorf tube and centrifuged for 1 minute at 13,000 rpm. Approximately 0.6 mL of the supernatant was analysed using an OenoFoss^{TM} analyser for ethanol, pH, total acidity, volatile acidity, malic acid, wine density and glucose/fructose.

6.3.4 Analysis of total phenolics, monomeric anthocyanins and tannins

Red wines were analysed for total phenolics and tannins using the method in Harbertson et al., 2003 which was modified to allow for reading to be done on the UV/VIS (FLUOstar Omega) spectrometer (BMG Labtech, Germany). This is a colorimetric assay where the wine is dissolved in a solution of SDS 5%(w/v), and 5%(w/v) triethanolamine, pH 9.4 and an optical density OD_{510nm} reading taken before and after the addition of ferric chloride. Tannins were initially precipitated with protein. Total phenolics and tannins were calculated from the difference in optical density measured as mg/L catechin equivalents. Monomeric anthocyanins were extracted in a 23 % maleic acid and 9.93 % sodium chloride high acid solution (pH 1.8). Extracted anthocyanins were measured at optical density $OD_{520nm,}$ adjusted for background measurement. Anthocyanins were calculated as mg/L using the anthocyanin extinction coefficient for malvidin-3-glucoside.

6.3.5 Antioxidant analysis

The Orac (Oxygen radical antioxidant capacity) assay was used to measure antioxidant potential of the grape, grape marc and wine marc, and wine samples. The samples were sent to CSIRO Food and Biosecurity to perform the Orac assay. This assay is based on the oxidation of a fluorescent probe by the peroxyl radical via hydrogen atom transfer. The test sample was diluted and mixed with the fluorescence probe and the free radical initiator which provided the free radicals, quenched the fluorescence probe. The antioxidant potential was measure by the rate at which the fluorescence dye was quenched. The higher the antioxidant potential of the sample, the longer it took to quench the probe. The antioxidant standard Trolox was assayed at varying known concentrations from which a linear standard curve was generated. Trolox equivalents mMol/g were calculated for each sample.

6.4 Berry sensory analysis

6.4.1 Berry sampling and preparation for sensory analysis

The genotypes selected for berry sensory analysis were from those selected for winemaking and had a 22 °Bx value or greater. Samples were taken over the three vintages 2014, 2015 and 2016. The day after collection, all the berries were carefully removed from bunches with harvesting snips, leaving a piece of the stalk (~5 mm) attached. Each white berry genotype tasting sample was between 3-5 berries and 4.2 g and 5.2 g. Each red berry genotype tasting sample was between 3-4 berries and 3.4 g and 3.9 g. Each sample was analysed in triplicate and each sample was placed in an individual patty pan in a clear gridded box in a randomised order, generated using Design Express. The boxes containing the white and red berries were kept at 4^{o}C until the formal evaluation which was conducted no later than five days after harvest. The samples were equilibrated at room temperature for one hour before being analysed by the panellists.

6.4.2 Sensory evaluation of grape berries

Sensory evaluation of the white and red berry selections was conducted using the principles of Descriptive Analysis with a berry sensory applied methodology called BSA. All the sessions were conducted at The University of Adelaide, Waite Campus Sensory Facilities. Sensory sessions to evaluate the red and white berry selections were divided into two stages: initial training in the open plan focus group room and final training and evaluation sessions in computerised, individual booths under red light with a light temperature of 6500^{o}K, in the sensory laboratory. Before commencing the berry analysis, panellists smelled the aroma standards which linked to the characters being assessed. Tasting quality preparations for acidity and sweetness and astringency and bitterness were also available from low to high intensity, to serve as scale anchors prior to evaluation. The assessment of the red and white berries was conducted in the same two hour session. The white berries were presented first to the assessors and after a break of 20 minutes, all the assessors continued with the red berries assessment. Five weeks of 2 hr sessions were required to evaluate the berries from 2014 and 2015, as all of the samples did not reach the predetermined levels of Brix at the same time, whereas six weeks of 2 hr sessions were required to assess the berries in 2016. Great care was taken to make sure that as many matching genotypes were assessed over the three seasons. Sensory ratings were recorded using Fizz software, version 2.47b (Biosystèmes, Couternon, France). Breaks of 45 seconds between each sample and three minutes after every five samples were enforced and panellists used citrus pectin solution (1g/L, Sigma-Aldrich Co., St Louis, MO, USA) and plain water crackers (Home Brand, Woolworths Limited, SA, Australia) to alleviate palate fatigue, as described in Olarte et al. (2013). Table 1 shows all the attributes assessed in both white and red grapes.

6.4.3 Statistical analysis of berry sensory data

Statistical analysis was done to evaluate sensory attributes and also compare them with the OenoFoss data for both white and red berries. HS-SPME-GCMS monoterpenes were measured for the white berries that had Muscat Gordo Blanco or White Frontignac parentage.

A two-way mixed model analysis of variance (ANOVA) was conducted to identify the berry sensory attributes significantly different between the genotypes. PCA was performed on all the berry sensory attributes loaded as main variables and the OenoFoss data loaded as supplementary variables for each of the seasons with the panellists as the random factor. Cluster Analysis was used to identify groups associating together based on the berry sensory attributes scores for the white and red berry hybrids respectively. Pearson's correlation was conducted to identify relationships between berry sensory attributes and OenoFoss measurements for the white and red grapes respectively. PCA was also conducted to evaluate the relationship between aroma volatile analysis and berry sensory attributes for 12 of the white berry hybrids that had Muscat Gordo or White Frontignac parentage. All statistical analyses were performed using the statistical package, XLSTAT version 16.06 (24/11/2014) (Addinsoft SARL, Paris, France). Each sample was given a short code for the statistical analysis.

HS-SPME-GCMS monoterpenes data and the sensory attributes were analysed by PCA. Berry monoterpene analysis was conducted in white berries of 12 genotypes collected in 2015 from genotypes that had a White Frontignac parent (W3, W4, W5, W6 and W18) and genotypes with a Muscat Gordo Blanco parent (W21, W22, W23, W24, W25, W26 and W28). The five monoterpenes analysed were linalool, cis-rose oxide, nerol, beta-citronellol and geraniol.

6.5 Winemaking

6.5.1 Small-scale red winemaking

Grape bunches were harvested at 22 °Bx or greater for standard wines and at 17-19 °Bx for low alcohol wines. The berries were removed from the rachis by hand and 1.3 kg were collected in a plastic bag (305 mm x 405 mm) with a tablespoon of dry ice and 850 µL of PMS (100 mg/mL) which equates to 50 ppm SO_2 for 850 mL of recovered juice (70 % of 1.3 kg). The berries were crushed and gently massaged in the bag for juice extraction. The total contents of the bag were placed into fermentation vessels (modified Chambord Bodum 1.5 L coffee press). Must parameters of the juice were measured with the OenoFoss and sugars were analysed using the Megazyme D-fructose/D-glucose assay kit (Deltagen Catalogue # KFRUGL). The pH was adjusted to 3.5 with tartaric acid. The juice was allowed to temperature adjust to 22°C for 1 to 2 hours. YAN (yeast assimilable nitrogen) (DAP - diammonium phosphate) was added at 200 ppm (1.7 mL of at 0.476 g/mL to 850 mL must) and 17 mL of an overnight yeast culture (2 % of the must volume) to the ferment.

The must was plunged and then pulled up just below the liquid surface to avoid exposure to oxygen. The plunger was sealed with Parafilm. The must was plunged on a daily basis to mix the cap with the liquid. Sugars were monitored every day. When sugar concentration reached 2.5 g/L the wine was racked by pushing the plunger down to separate the solids and then pouring the wine into a clear wine bottle, and then left to settle at 4°C for 24 hours. The wine was then racked into another bottle with PMS to obtain SO_2 at 50 ppm using a siphoning device under argon pressure and left at 4°C for 14 days. After cold settling, the wine was filtered through a 0.45µm autoclaved ground water filter (Air-Met FTH-45) by using argon gas to push it into a combination of 200 mL, 100 mL and 50 mL amber bottles (Cospak). The bottles were sealed with Tampertell cap cello wadded caps (Cospak) and then sealed with wax. Finished wine analysis was done for each wine using the OenoFoss. Wines were stored at 16°C.

6.5.2 Small-scale white winemaking

Grape bunches were harvested at 22 °Bx or greater for standard wines and at 17-19 °Bx for low alcohol wines. Exactly 2 kg of grape bunches were transferred to a press seal bag (305 mm x 405 mm) with a tablespoon of dry ice and 1.2 mL PMS 100 mg/mL solution (based on 50 ppm for 60 % juice recovery). Berries were squeezed until all were disrupted and free running juice was visible. The juice was strained through a kitchen sieve and centrifuged for 2 minutes at 3197 RCF to remove solids. Exactly 500 mL of juice was removed from the centrifuge bottle and transferred to a 500 mL Schott bottle with an airlock and silicon sampling septum. The juice was temperature adjusted for 1-2 hours for before adding yeast, DAP and PVPP. The juice pH was measured and adjusted to pH 3.5 with tartaric acid. Must parameters were measured with the OenoFoss and sugars were analysed using the Megazyme D-fructose/D-glucose Assay kit (Deltagen Catalogue # KFRUGL). The juice was inoculated with 10 mL of an overnight yeast culture (Maurivin PDM Yeast), 1 mL of 476 mg/mL DAP stock (200 ppm YAN**)** and 1 mL of PVPP 130 mg/mL (260 ppm PVPP). The juice was fermented at 18°C, with 2 min shaking at 100 rpm every two hours. Total sugars were measured every day. When the sugar concentration reached 2.5 g/L the wine was racked by transferring into a clear wine bottle, using a siphoning device under argon pressure with 500 µL copper sulfate 1 mg/mL and 500 µL PMS (100 mg/mL) and left to settle for 7 days at 4°C. The headspace was minimised by filling to the top with marbles. After cold settling, the wine was filtered through a 0.45 µm autoclaved ground water filter (Air-Met FTH-45) by using argon gas to push it into a combination of 200 mL, 100 mL and 50 mL

amber bottles (Cospak). The bottles were sealed with Tampertell cap cello wadded caps (Cospak) and then sealed with wax. Finished wine analysis was done for each wine using the OenoFoss. Wines were stored at 16°C.

6.6 Wine sensory analysis

Red and white wines were tasted and evaluated by a panel comprising individuals from the wine industry, university academics and students. Each panellist was given an iPad or computer from which they could access a database to record their findings. Each wine was evaluated for how it compared to the corresponding reference control wine sample. The panellists gave each wine a score based on how much they liked it, using a 9 point hedonic scale, 1 (dislike extremely) to 9 (like extremely). Each wine was also given a technical quality score from 1 to 20 and any winemaking faults were also be recorded. Wine dominant flavour and potential use could be selected from drop down menus. Wine interest level was given a score from 1 (no interest) to 9 (to very high interest). Panellists could also record any other comments for each wine. Wines were assessed for the vintages 2013, 2014, 2015 and 2016. Results of the wine assessments from the previous year were used to determine which genotypes would be selected for winemaking in the following year. Both standard and low alcohol wines were assessed.

For the Analysis of Interest Level Scores and Hedonic Scale, the scores were averaged for each wine. An overall average was calculated across all wines. Each wine was scored as either being above or below this average. The percentage of wines above the average was calculated.

6.7 DNA marker development for muscat flavour

6.7.1 HS-SPME-GCMS for the detection of monoterpenes in white berry genotypes

Berry monoterpene targeted analysis was conducted in berries collected in 2015 from all plants that had a Muscat Gordo or White Frontignac parent. The five targeted monoterpenes selected were linalool, cis-rose oxide, nerol, beta-citronellol and geraniol. They have been reported as being detected in high concentrations in Muscat cultivars (Gunata et al., 1985, Ribereau-Gayon et al., 1975). Negative (white berry variety) and positive (Muscat Gordo Blanco and White Frontignac) control berry samples were included in the targeted monoterpene analysis. Berry monoterpenes were analysed from 2.5 g of frozen ground berry material by headspace solid phase micro extraction with gas chromatography–mass spectrometry (HS-SPME-GCMS). The frozen material was added to 2.5 mL 4mM zinc sulfate and 2.5 g NaCl into a 20 mL amber glass vessel. Deuterated (D3) linalool was added to each sample (10 µL for 0.173 mg/L). Volatile compounds were detected using the Agilent Technologies 5975C inert XL-MSD Triple – Axis Detector with the 2 cm DVB/CAR/PDMS SPME fibre for GC with the ZB – WAX column (Zebron). The MS was operating in selective ion monitoring mode to identify monoterpenes based on the standard. Monoterpene concentration was calculated by converting the area under the curve values to mg/L using the internal standard. Standard curves were generated for linalool, citronellol, nerol, rose oxide and geraniol (normalised area peak across concentration (mg/L)) to check for linearity.

6.7.2 Muscat SNP detection in white selections

The Muscat single nucleotide polymorphism (SNP) is localised in the candidate gene associated for Muscat flavour *VvDXS*, which codes for 1-deoxy-D-xylulose 5-phosphate synthase. The SNP is localised within exon 6 of the gene with a G-T base substitution associated with Muscat flavour. The polymorphism was converted into a Sequenom marker by developing flanking gene specific

amplification primers and an extension primer at the site of the polymorphism with the last base at the mismatch location. The grapevines were genotyped using the Sequenom MassArray on a Compact Spectrometer with iPlex GOLD Chemistry, at the Australian Genome Research Facility (AGRF) in Brisbane. The genotyping analysis was conducted on selections that had a Muscat Gordo Blanco or White Frontignac parent.

Validation of the muscat SNP marker was done on test crosses (Mm x mm) to determine whether the dominant muscat allele segregated in a Mendelian fashion with berry muscat flavour and the presence of monoterpenes.

7. Results and discussion

7.1 Vine performance in the field

Field data from the seasons 2013-14 to 2016-17 were collected to determine the diversity within the selections and determine vine performance in the field for the 1200 selections. During these years, a culling process was started, to remove vines that did not meet selection criteria based on field performance and wine attributes. As a result of this culling process, in the final year, data were only gathered from 124 selections.

Weather data such as daily maximum and minimum temperature, daily rainfall and daily sunshine hours recorded at Nuriootpa weather station were obtained from the Bureau of Meteorology for the period October 2013 to April 2017. These data were used to generate Figure 1 which depicts the monthly cumulative sunshine hours, monthly average rainfall, monthly average maximum and minimum temperature and monthly cumulative thermal time (degree days), respectively, for the four grape growing seasons.

7.1.1 Anthesis

Anthesis was monitored in spring on a weekly basis for all selections. Anthesis was defined as the opening of at least 5% of flowers of 5% of inflorescences. Anthesis dates were recorded across the four seasons (Fig. 2a). In general, anthesis occurred from the last week of October until the middle of November except in the 2016-17 season. In 2016-17, anthesis started in mid-November and was complete by the end of November. Figure 3 shows percentages of plants from each cross undergoing anthesis on a weekly basis. The majority of the plants from all crosses underwent anthesis within a two week time frame. Most of the selections (80%) had anthesis in the last two weeks of November in 2016-17. In 2016-17 onset of anthesis was delayed by about three weeks (Fig. 2a). In addition to genetic differences, temperature acts as a strong determinant for the period from the breaking of dormancy to flowering (Martínez-Lüscher et al., 2016). A lower than usual cumulative thermal time in October and November of 2016 (Fig. 1d) contributed to this delay in the anthesis.

There appears to be a genetic component to anthesis date with some selections from crosses C10, C19, C20 and C25 having an early anthesis date across all four seasons (Fig. 3). Similarly, some progeny from some crosses (C21, C22, C18) showed late anthesis dates. The data indicate that the genetics of anthesis date is not simple and is likely to be multigenic. The anthesis date of a selection may contribute to harvest date but the climate during the berry ripening period from fruit set will have a role as well as genetic factors that determine the pre-veraison period and rate of sugar accumulation at the post-veraison berry stage.

7.1.2 Veraison

Berry development was monitored weekly from the start of veraison until all vine selections completed veraison. Initiation of veraison was determined by the softening of berries on 5-10 bunches for white varieties and the appearance of colour tinges in several berries on 5-10 bunches in case of red varieties. Figure 2b shows the time of veraison for 2013-14 to 2016-17 seasons. Except for 2013-14, veraison across the selections occurred over 5-6 weeks. The percentage of progeny selections from each cross undergoing veraison at successive time points from 2013-14 season to 2016-17 season was also calculated (Fig. 4). In the 2013-14 season all vines went through veraison in just four weeks of January. Temperature records showed that the 2014 January average was more than a degree higher than other years (Fig. 1). There were two December heatwaves with

temperatures reaching nearly 45 °C and three January heatwaves reaching up to 38.5, 44 and 46 °C respectively. It is not uncommon in South Australia to experience 4-5 heatwaves in December and January. However, the heatwaves in the 2013-14 season were relatively more intense and lasted longer than in other years. The time between flowering and veraison can be affected by abiotic factors like water deficits (Martinez-Luscher et al., 2015) and has a rather weaker correlation with temperature (Duchene et al. 2010). However, it has been reported that water deficits increase ABA levels in the xylem stream. During the December-January prolonged heatwave, the plants might have been exposed to episodes of water stress, even though the irrigation regime applied was intended to prevent water stress. Also, during the heatwaves, plants were exposed to higher levels of UV B which promotes ABA biosynthesis in grapevine (Berli and Bottini, 2013). It has been demonstrated that elevated level of UV B hastened onset of ripening of grapes (Berli et al., 2011). Thus, either water stress and/or increased level of UV B irradiation could have contributed to the shortening of the veraison time in 2013-14. At high temperatures, grapevines show reduced photosynthetic capability and this may also have contributed to a stress that shortened the pre-veraison period. An alternative explanation is that the higher temperatures contributed to a shorter veraison period due to a faster metabolism and ripening rate.

Progeny selections from most crosses completed veraison in 2.5 weeks in 2013-14 and in 3 weeks in other years (Fig. 4). For example, in 2013-14 almost 84% of the selections went through veraison between day 1 and day 19 (2nd to 4th week of January). While in 2014-15 and 2015-16 almost 81% and 89% selections respectively, went through veraison between 8-28 days (2nd to 4th week of January). In 2016-17 with a delayed anthesis, veraison was also delayed by two weeks. In that season, the veraison progressed a bit slower and 84 selections went through veraison between day 8 and 35 (3rd week of January to 2nd week of February). Veraison for progeny from the C10 cross were found to be spread across the entire veraison period in all years.

Similar to anthesis, the veraison date for berries is affected by the climate for a particular season. However, genetics does also appear to have a role with some selections from cross C10 consistently showing early veraison dates across the seasons (Fig. 4). Dissecting the genetics of early veraison will require further investigation as the data suggest it is not a simple trait. Selections with an early veraison date combined with an early maturity date may be of interest to industry as it would allow the crop to be harvested early and reduce inputs. For future breeding, the parents of C10 would be candidates to include in a breeding program to produce the second generation of mildew resistant grapevines.

7.1.3 Berry maturity and harvest

Berries were monitored for sugar accumulation about 75-90 days after the beginning of anthesis, using a handheld refractometer. Five to six berries were randomly picked from six bunches from different parts of the vine, once a week. The juice was squeezed together and 22 °Bx was used as a benchmark for berry ripeness and harvesting.

There are a number of factors that can influence the ripeness or sugar accumulation in the berries, apart from genetics. Since carbohydrate synthesis is largely dependent on sunlight and ambient temperature, these factors are major contributors to berry ripening. Heatwaves during the latter part of the post-veraison stage can stop sugar accumulation for a period or cause a surge in the

sugar content in grapes as levels of acids fall abruptly. Volume and frequency of rains during the ripening process can also affect berry condition and Brix.

Figure 5 shows the period most selections reached 22 °Bx. About 14% and 12% of the selections failed to reach 22 °Bx by late March when harvest was completed in 2014-15 and 2015-16 season, respectively. In 2016-17, a little over 15% of the selections did not reach 22 °Bx. The berry ripening season in 2015-16 was comparatively longer than in 2014-15. The January average maximum and minimum temperature for these two seasons was similar (Fig. 1c). However, March in 2015-16 was much wetter than other years (Fig. 1a and b) with increased duration of cloud cover. This might have contributed to the delay in fruit ripening. In 2016, like anthesis and veraison, fruit ripening was also delayed and continued up to the first week of April. Figure 5 shows the percentage of selections from different crosses that reached 22 °Bx for 3 seasons. It can be seen that there is variation within and between crosses on the date that a selection reached a sugar level suitable for harvest. Progeny from some crosses (e.g. C10, C19, C25) were the first to ripen across the seasons while some crosses (e.g. C2, C23, C22, C18) had a larger proportion of progeny that failed to reach 22 °Bx during the harvest period. This suggests that there is complex genetic control of the period from fruit set to harvest that is influenced by the environment and also management (e.g. date of pruning).

7.1.4 Yield

Harvest: Berries were harvested when the Brix of the sampled berries reached 22 °Bx. Therefore, the time of harvest shown in Fig. 2c is closely matching to that of 22° Brix. However, in 2013-14 and 2014-15 not all vines were harvested as soon as they hit 22° Brix due to labour shortages. Certain selections were earmarked for winemaking based on agronomic and sensory qualities and only those varieties were harvested as soon as they hit 22 °Bx. The rest were left until the end for harvesting. Figure 6 shows the percentage of vines from each cross that were harvested at different time points. It is clearly seen in Fig. 6 that large percentages of vines from most crosses were harvested at the last harvest time point in 2013-14 and 2014-15 season. Figures 7 and 8 show the harvest dates for selected early and late selections.

Yield components: Various yield attributes were recorded for the vines, including, total bunch weight, total bunch count and mean single berry weight (averaged from 30 randomly sampled berries). Although variations in yield components are mainly governed by genetic factors in the grapevine genotype, temperature has a great influence on various yield components too (Keller et al., 2010; Petrie and Clingeleffer, 2005). Elevated day temperature has been reported to influence berry set, number of berries/bunch and berry size (Keller et al., 2010).

Total Bunch Count: Total bunch number varied widely between varieties and between seasons. Fig. 9a represents ranges of bunch number and their proportions from 2012-13 to 2016-17 season. Fig. 9b shows the mean bunch weight of variety selections for last five seasons. As the vines aged, the total bunch number increased (Fig. 9). In 2012-13 over 90% varieties produced up to 100 bunches/vine. The majority of the vines produced less than 50 bunches. As the years progressed, more varieties produced greater than 100 bunches. It is worth remembering here that the process of selections also progressed through these years and plants that produced greater number of bunches were favoured. The combination of these two factors determined the proportion of bunch number/vines for each successive year. In the final years of selection, 48% of the selections produced 101-150 bunches, while 38% produced 151-200 bunches. The next largest group (23%)

produced 51-100 bunches. It is worth noting that the proportions of bunch number categories of the final variety selection (2016-17) closely reflected that of the previous year (2015-16). The number of bunches per cane varied from 0-3 across the selections. In general, there were 1-2 bunches per cane.

Mean Berry weight: Mean berry weight, a measure of berry size, is considered as an important trait of grapes that affects wine quality, and larger berries are the least desirable (Matthews and Nuzzo 2007). Mean berry weight is typically a genotypic trait. However, increased day temperature has been reported to reduce the number of berries per bunch but proportionately increases the berry size in Danlas grapes to compensate for yield (Ezzahouani, 2003). Water deficits and shading of bunches has been reported to reduce berry size (Matthews and Nuzzo, 2007). Figure 10 shows the proportions of different weight categories of mean berry weight observed over the five seasons. Most selections had berries with a mean berry weight between 0.5-1.5 grams. In the final year, over 91% of the selections produced berries in this range, with the rest being in the higher weight categories. It is evident that the mean berry weight of the selections varied across the years probably due to seasonal effects (Fig. 10b).

Mean Bunch weight: Mean bunch weight was obtained by dividing the yield by total bunch count. Figure 11 shows the proportions of different weight categories of mean bunch weight observed across seasons. Mean bunch weight varied widely among lines, reaching up to 350 grams for some lines in some years. However, the majority of the lines produced bunches between 50-100 grams. In the 2015-16 season the mean bunch weight rarely exceeded 150 grams. The cumulative thermal time (GDD) was highest (2190.35 hrs) that season (Fig. 1d) among the last five seasons. The next highest GDD was observed in 2012-13 amounting to 2025.75 hrs and the mean bunch weights of that season were closely matched to that of 2015-16. Figure 11b shows that the mean bunch weight of the selections in 2015-16 and 2012-13 was lower than other years, demonstrating a seasonal effect.

Vine yield: Figure 12 shows the proportions of different weight categories for yield observed across the seasons. In general, yield increased over time as the vines matured. In 2015-16 most lines yielded 5-15 kg berries. It can be seen from Fig. 12b that lines producing less than 5 kg were all but eliminated from the final selection and higher yielding vines were retained. In the last year of selection, 90% of selections fell in the categories of 5-10 kg, 10-15 kg and 15-20 kg and roughly representing equally (Fig. 12b).

7.1.5 Vine vigour

There was a range in vine vigour across the selections (Figs. 13 & 14). Indirect measures of vine vigour were collected for trunk circumference and pruning weights. It was found that there was a low correlation (R-squared value 0.3268) between trunk circumference and pruning weights (Fig. 14).

7.1.6 Summary of field performance

7.1.6.1 Components of time to sugar berry ripeness and harvest

The time components of berry sugar ripeness and harvest date can be thought of as the dates of budburst, anthesis (fruit set), veraison and harvest. Each component is influenced by GEM (Genetics x Environment x Management) and driven by developmental and physiological processes.

Budburst data were not collected for the selections, however, we can look at the other components to see if there is any relationship between anthesis date, veraison date and harvest date. It can be seen that there was variation in the period between anthesis to veraison and between veraison and harvest across seasons for selected individuals (Figs. 7 and 8). There appeared to be greater variation between veraison and harvest than between anthesis and pre-veraison. This indicates that there is a genetic component to berry ripening and that the control of the pre-veraison period is different to the post-veraison period. The genes responsible for this control are unknown but further genetic research may provide information that could allow the selection of vines that either mature during the early part of the season or later in the season. There would be an advantage to early maturing selections as they would require fewer inputs such as water and the early harvest date may reduce the risk of weather events later in the season that could damage the crop.

7.1.5.3 Field evaluation and selection outcomes

The main components of yield are bunch number, bunch size (berry number) and berry weight with bunch number the major component (Clingeleffer et al., (2001) GWRDC Project CSH 96/1). There was large variation across the selections for these components and seasonal influences were evident (Figs. 9,10 & 11). During the selection process, across the different seasons, greater weight was placed on selecting vines with higher total yield (Fig. 12) for wine evaluation. High yielding vines would be of benefit to the Australian industry if these vines produced desirable wine.

Figure 1. BOM weather station data near the experimental site during four grape growing seasons. (a) Monthly cumulative sunshine hours, (b) Monthly average rainfall, (c) Monthly average maximum and minimum temperature and (d) Monthly cumulative thermal time (degree days)



Figure 2. Timelines for monitoring (a) anthesis, (b) veraison and (c) harvest

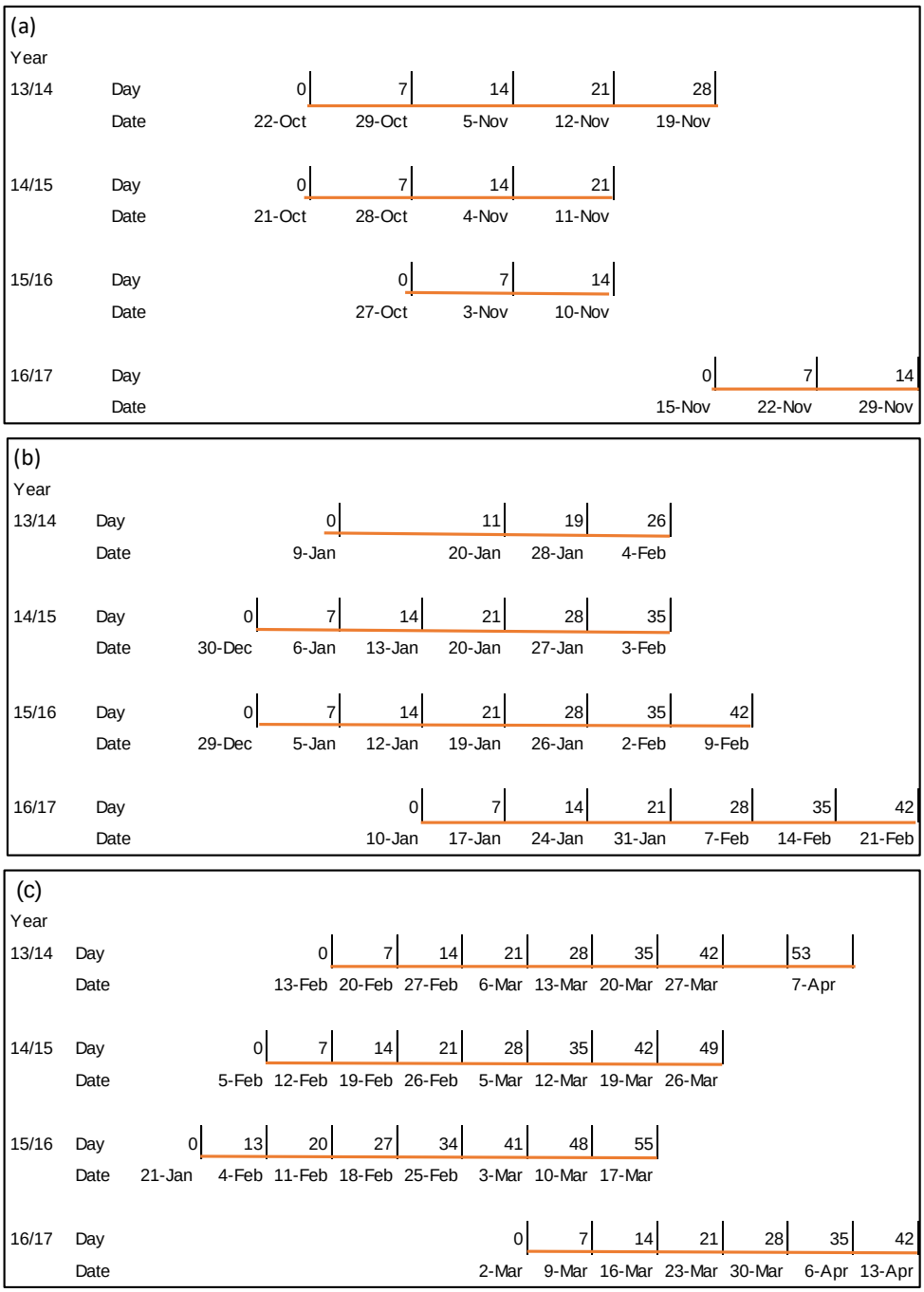


Figure 3. Percent progeny of selections from various crosses undergoing anthesis. (a) 2013/14, (b) 2014/15, (c) 2015/16 and (d) 2016/17

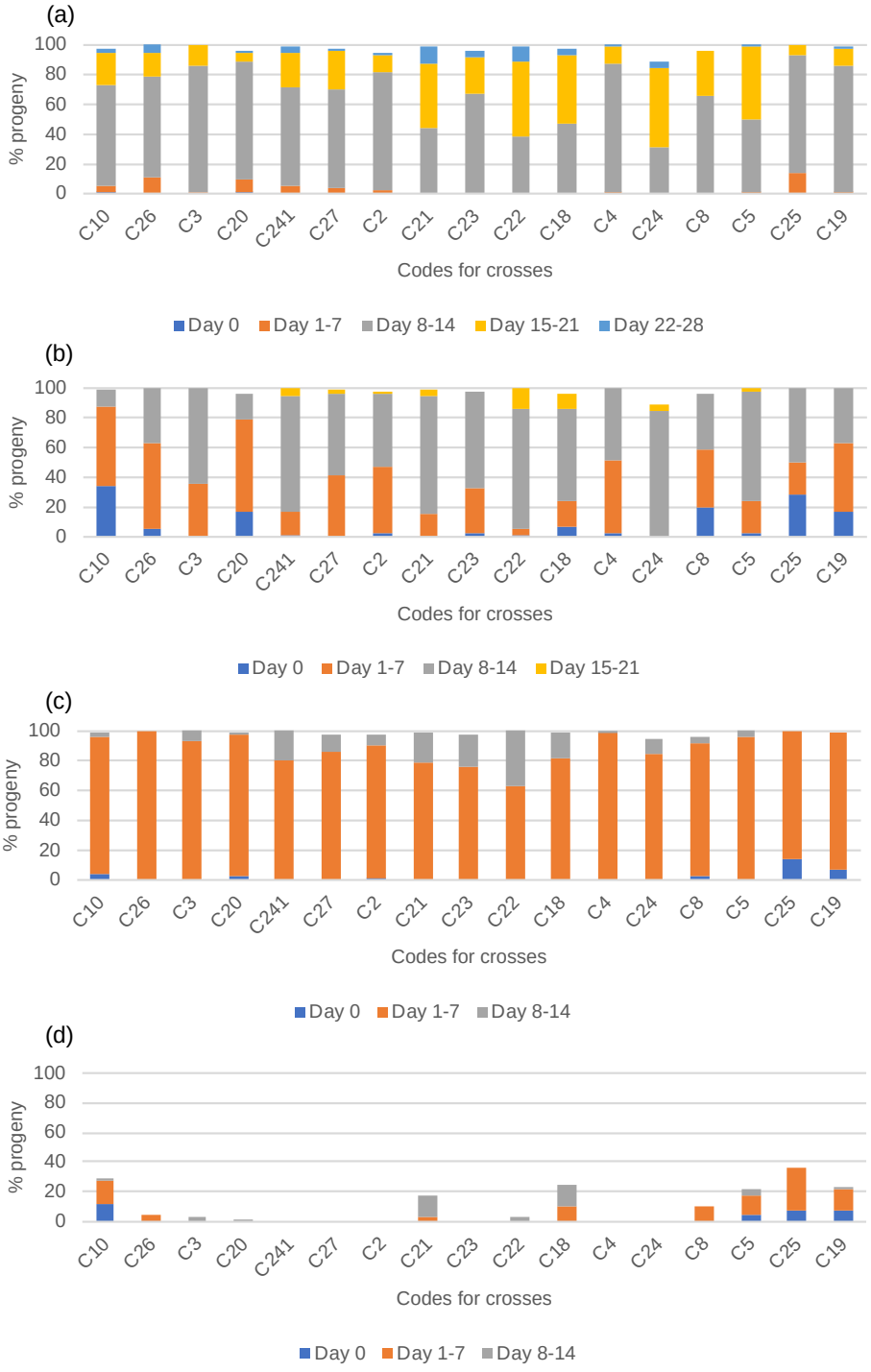


Figure 4. Percent progeny of selections from various crosses undergoing veraison. (a) 2013/14, (b) 2014/15, (c) 2015/16 and (d) 2016/17

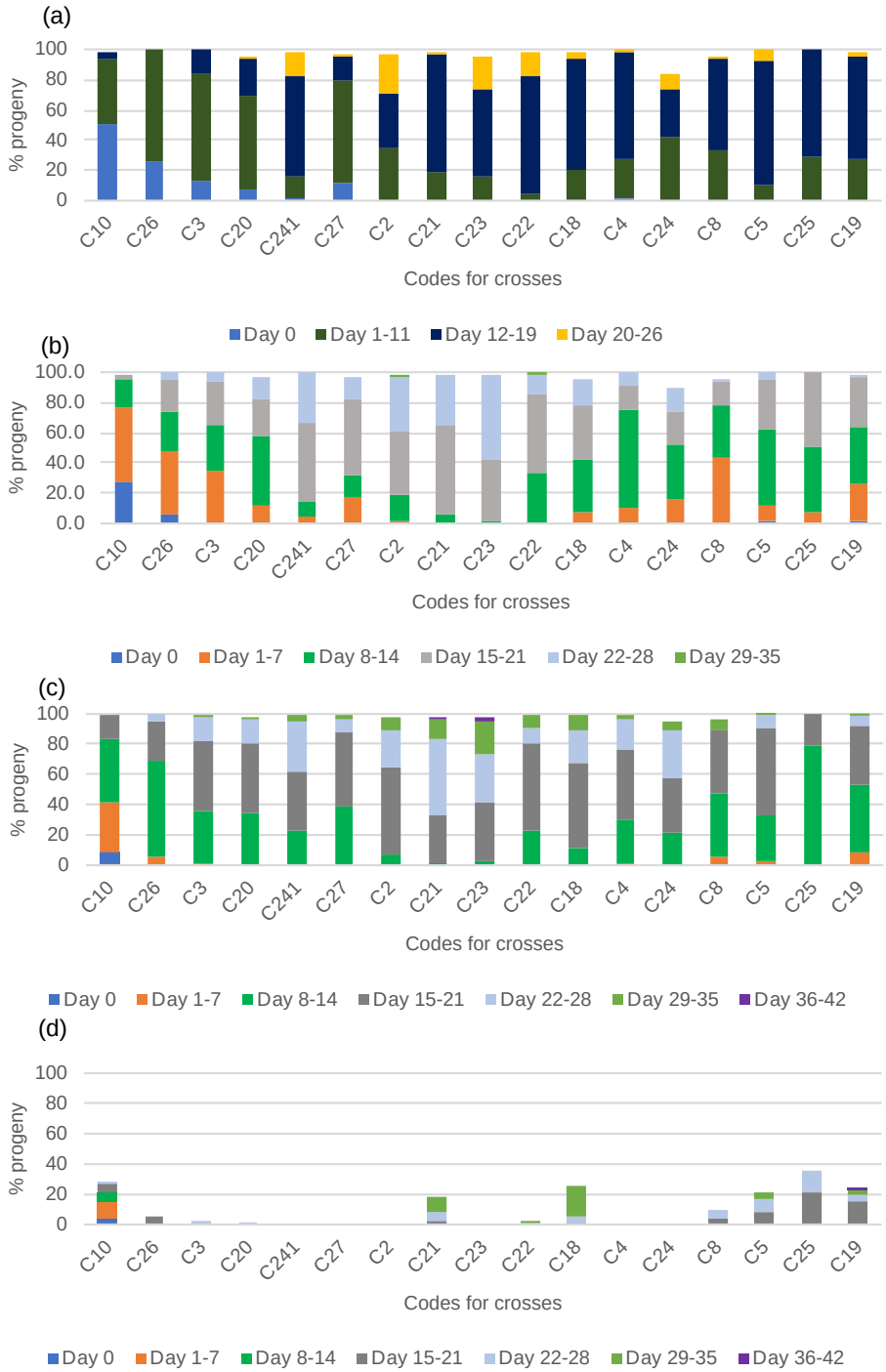


Figure 5. Percent progeny of selections from various crosses that reached 22 °Bx during the harvest period. (a) 2014/15, (b) 2015/16 and (c) 2016/17. Some progenies from different crosses failed to reach 22 °Bx during the harvest period in all years and has been shown in black.



Figure 6. Percent progeny of selections from various crosses harvested at different time points. (a) 2013/14, (b) 2014/15, (c) 2015/16 and (d) 2016/17

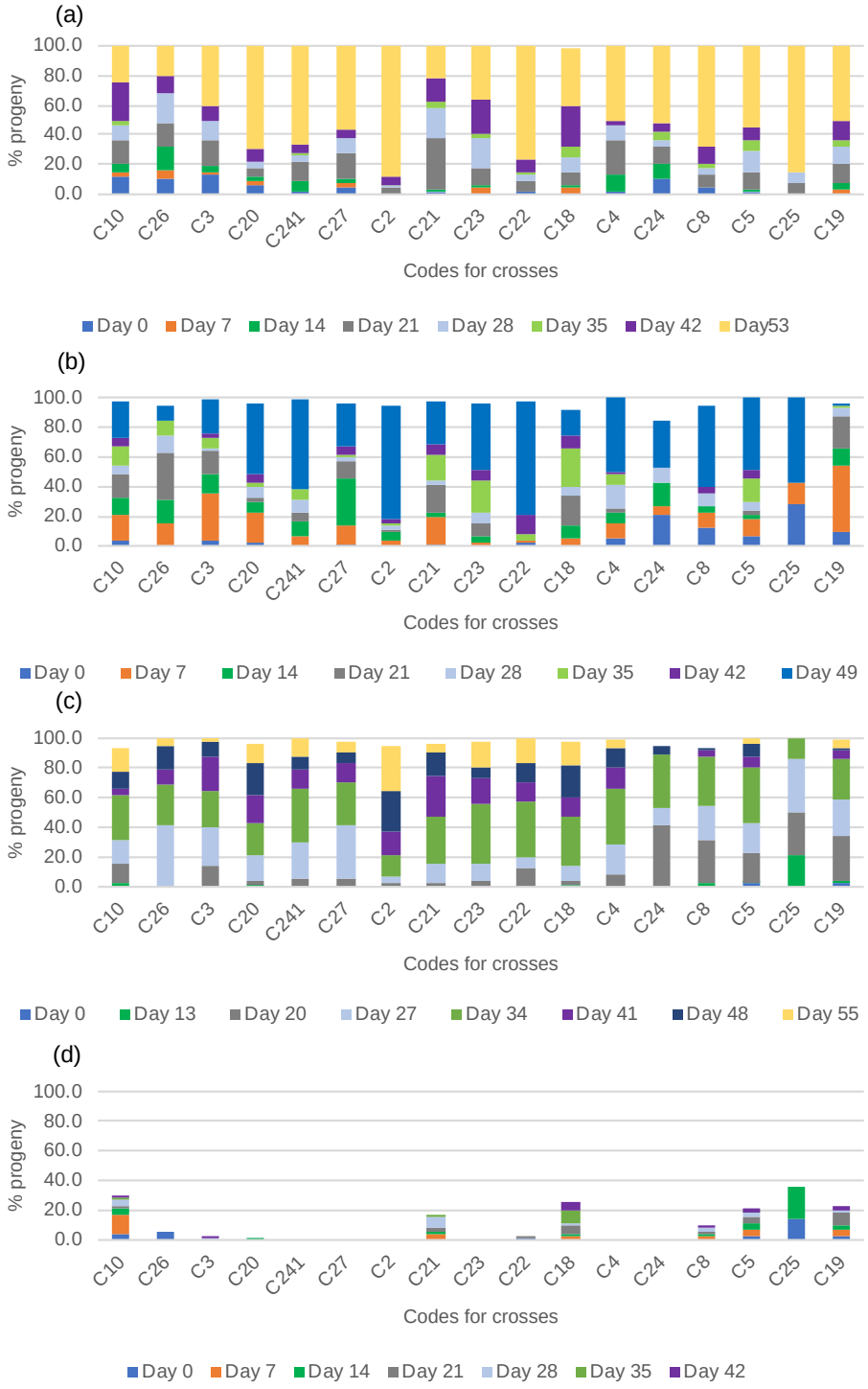


Figure 7. Berry sugar ripeness and anthesis date (A), veraison date (V) and harvest date (H). Selected early and late (based on 2016/17 anthesis dates) red selections originating from different crosses. (a) 2016/17, (b) 2015/16 and (c) 2014/15

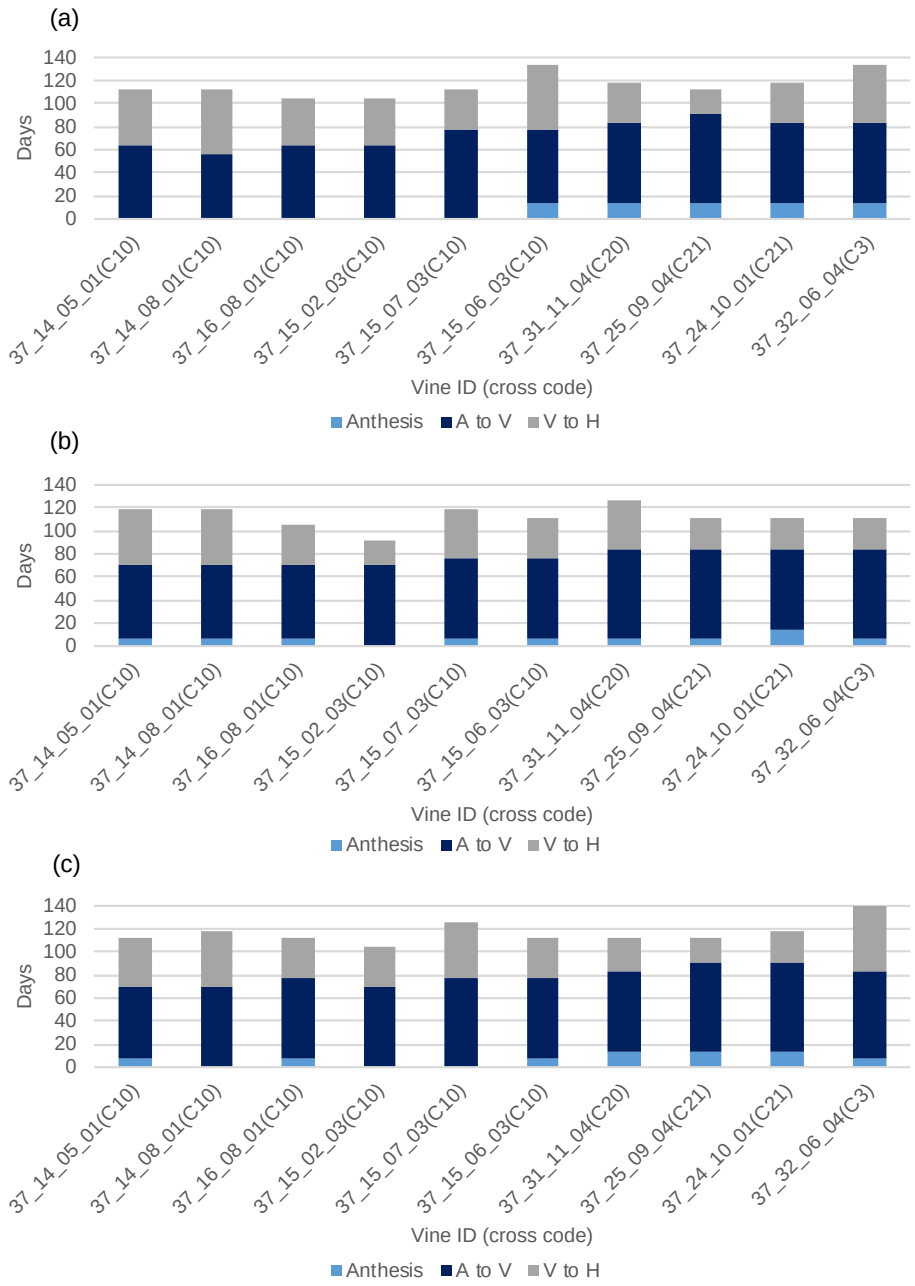


Figure 8. Berry sugar ripeness and anthesis date (A), veraison date (V) and harvest date (H). Selected early and ate (based on 2016/17 anthesis dates) white selections originating from different crosses. (a) 2016/17, (b) 2015/16 and (c) 2014/15

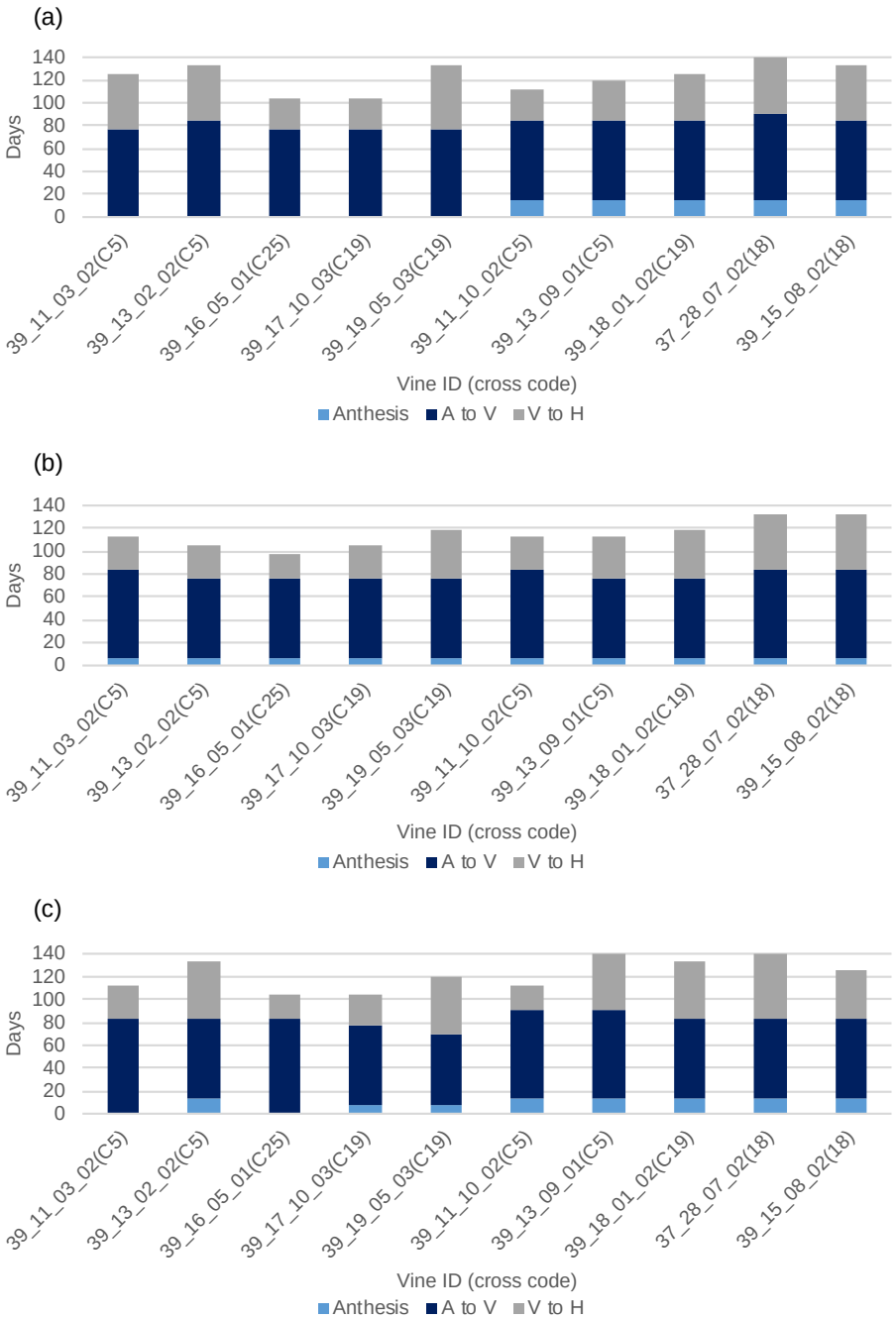


Figure 9. Total bunch count of selections (a) and proportion of bunch count number categories as selection progressed through four consecutive seasons (b).

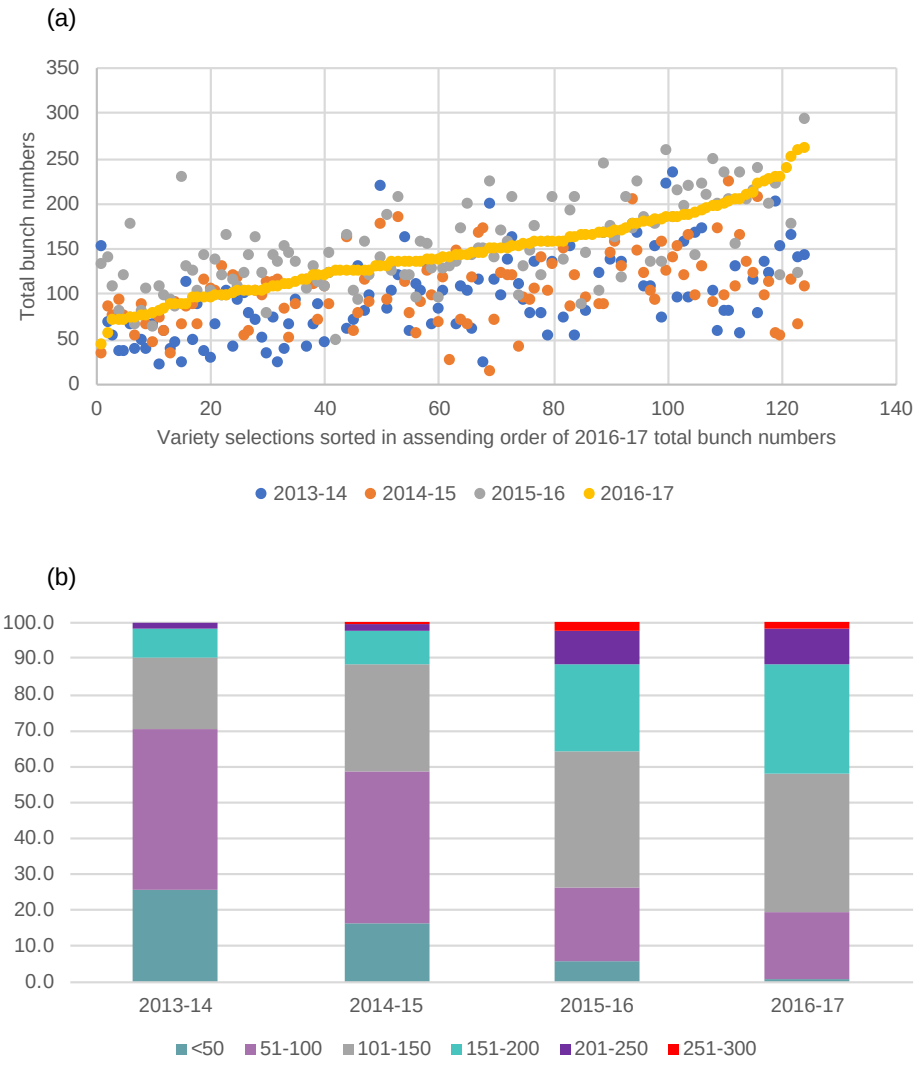


Figure 10. Mean berry weight of selections (a) and proportion of mean berry weight categories as selection progressed through four consecutive seasons (b).

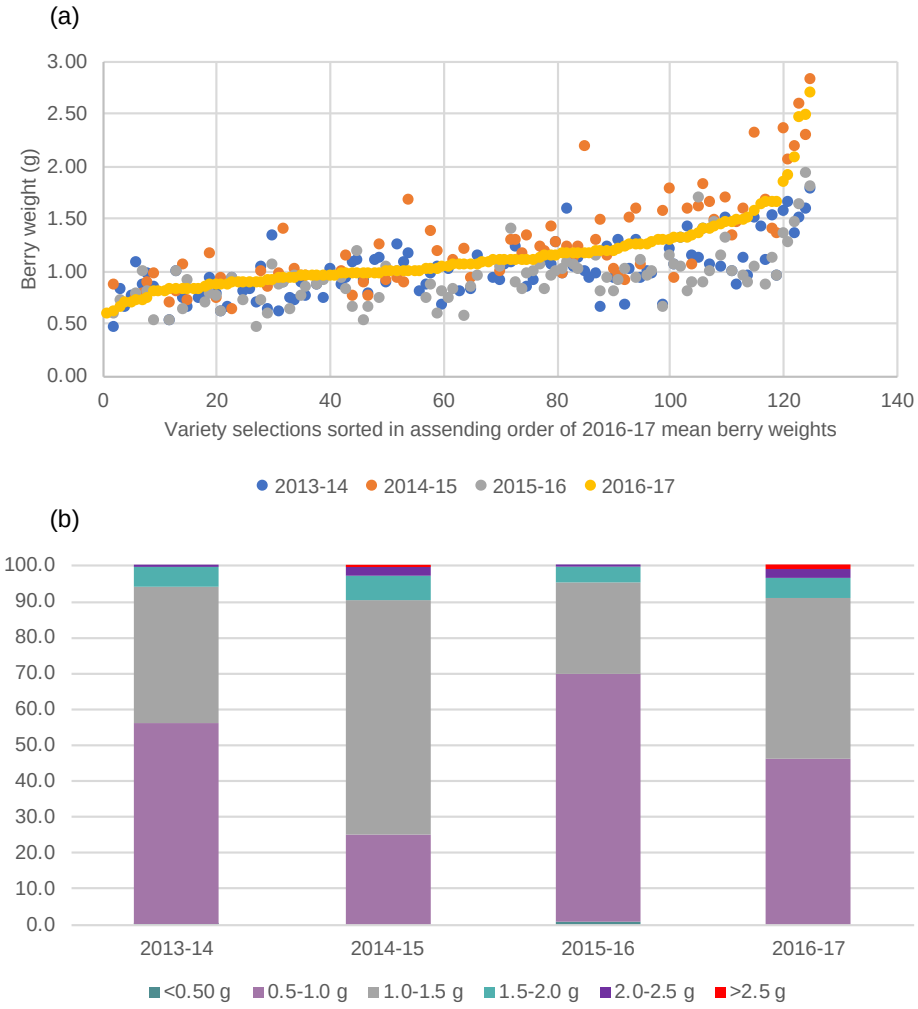


Figure 11. Mean bunch weight of selections (a) and proportion of mean bunch weight categories as selection progressed through four consecutive seasons (b).

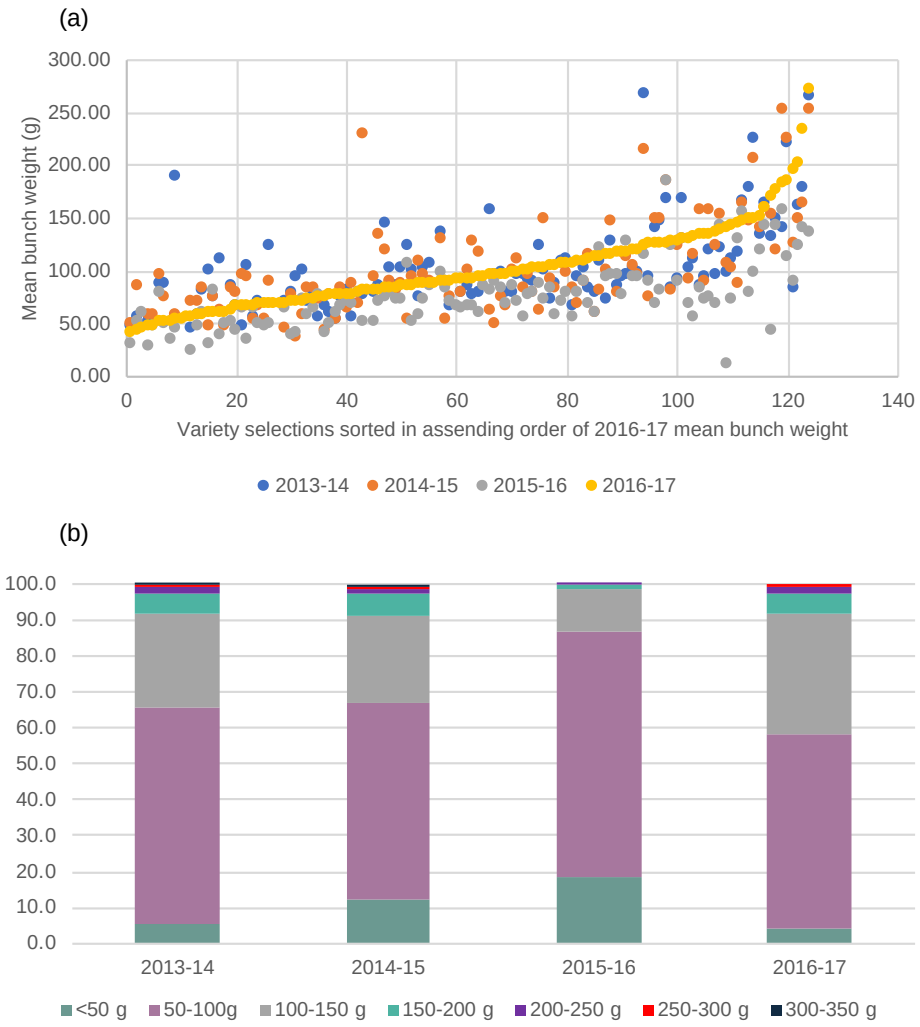


Figure 11. Mean bunch weight of selections (a) and proportion of mean bunch weight categories as selection progressed through four consecutive seasons (b).

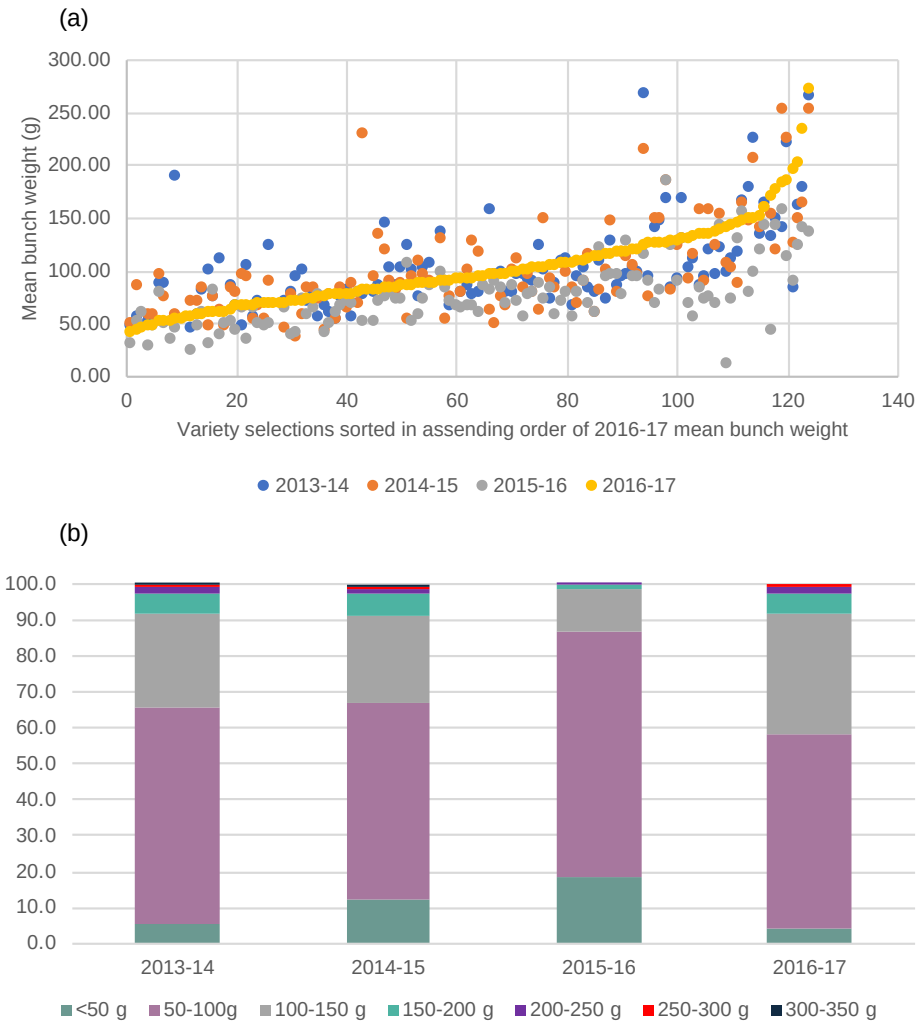


Figure 12. Total bunch weight (yield) of selections (a) and proportion of total bunch weight categories as selection progressed through four consecutive seasons (b).

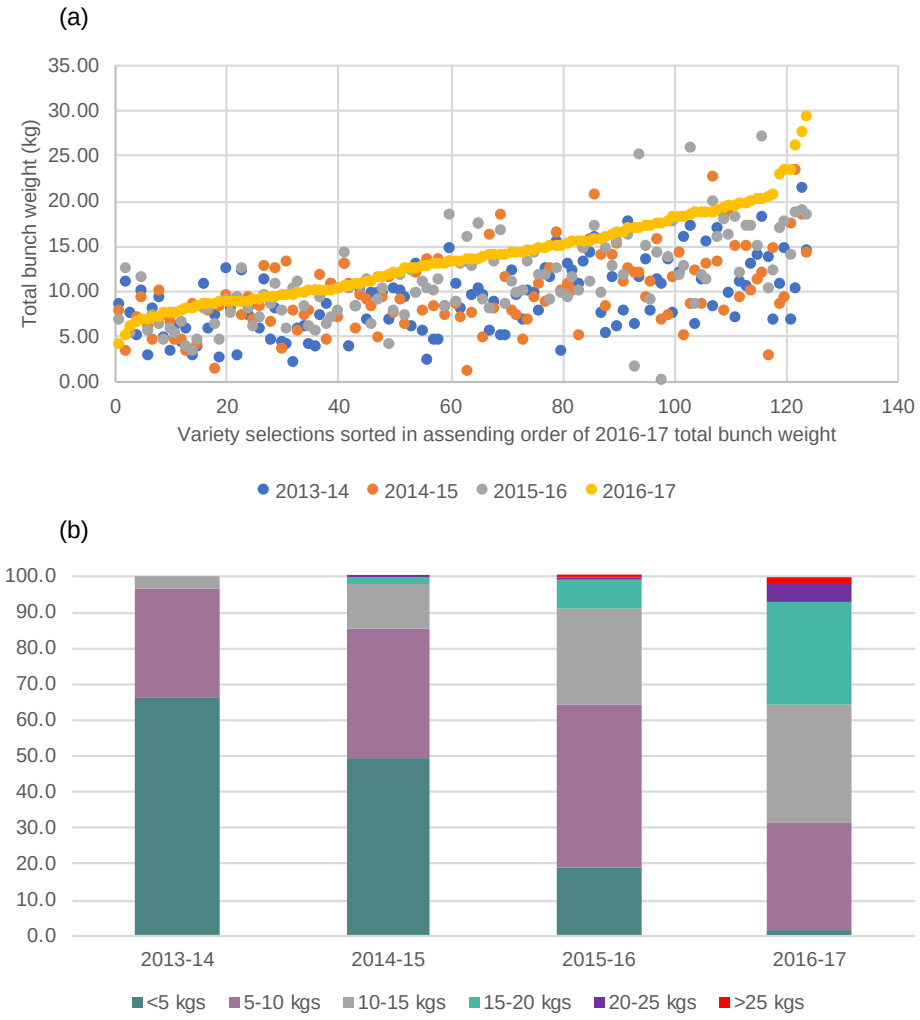


Figure 13. Trunk circumference of selections from 2012-13 to 2014-15

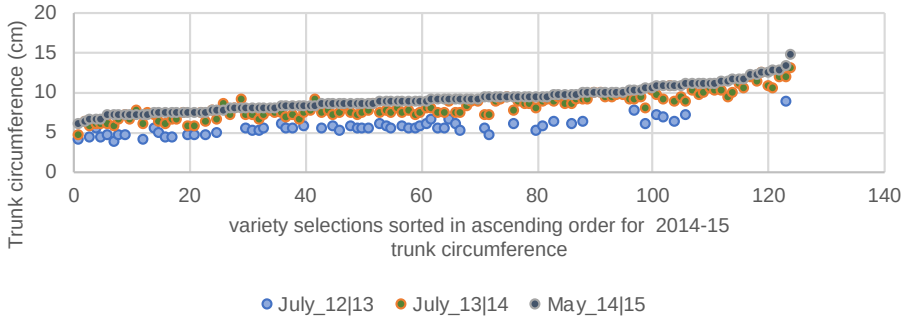
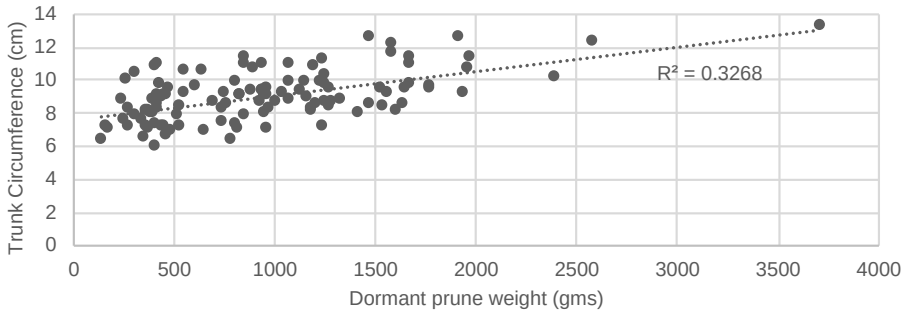


Figure 14. Relationship between vine trunk circumference and dormant prune weight of variety selections



7.2 Chemical analysis of berries

Results from the analysis of chemical composition of the grape must from white and red varieties are presented below.

7.2.1 Anthocyanins

The anthocyanins that are usually accumulated in the skin of berries determine the colour of red and black grapes. The colour of grape berries at harvest is an important factor that influences wine colour and quality. Anthocyanin content in berries of the red grape selections studied (Fig. 15a), ranged from less than 1 mg/berry to the highest level of 5.24 mg/berry. Plants were clustered based on berry anthocyanin content (Fig. 15b). There were only a few plants that yielded more than 4 mg/berry over the period of study (four plants in 2015-16 and three in 2016-17). A very large proportion of the selections screened had less than 1 mg/berry anthocyanin content in the early years of the study (2012-13 and 2013-14). As the screening process progressed over the years, the proportion of plants with low anthocyanin content also decreased. Anthocyanin concentration in berries was also measured as mg/g and the results are presented in Figure 16. The highest berry anthocyanin concentration was 5.87 mg/g and it was observed in 2012-13. Only a few plants (one in 2012-13, five in 2014-15 and one in 2016-17) had higher than 4 mg/g berry concentration and the majority of the varieties screened, had less than 2 mg/g of berry anthocyanin. As in the case of berry content, the larger proportions of plants with less than 1 mg/g anthocyanin concentration that was seen in the early period of the study (2012-13 and 2013-14) decreased towards the later years due to the selection process for plants with higher anthocyanin content. The differences between selections is not surprising as it has previously been shown that anthocyanin content in grape juices varies with the cultivar, ripening and climate characteristics (Boas et al., 2014; Mazza and Francis, 1995).

7.2.2 Phenolic compounds

Phenolic compounds present in grape berries, depending on their chemical structure, are directly responsible for special characteristics of grapes and their derivatives (Mattivi et al., 2006; Montealegre et al., 2006). Phenolic compounds present in grape juice may also play a role in defining the sensory characteristics of its products (Recamales, 2006).

Phenolic content of berries (Fig. 17) was measured as absorbance/berry and three plants showed absorbance values greater than 3, with the highest value of 3.44 present in a plant in 2014-15. The proportion of plants with an absorbance value <1/berry was higher in the first two years and their number decreased towards the later years of the study. Similarly, in the case of phenolics concentration measured as absorbance/g, most of the plants fell into the cluster with an absorbance value less than 2, except in 2014-15 when the largest cluster had 2-3 absorbance/g (Fig. 18). Phenolic content in grape juices also varies with the cultivar, ripening and climate characteristics (Boas et al., 2014).

7.2.3 Alpha amino nitrogen

Grapes contain a variety of nitrogenous compounds. Nitrogen metabolism contributes to the pool of aroma and flavour compounds. Because nitrogen metabolism also regulates other pathways, including sugar and sulfur metabolism, nitrogen availability has a significant impact on the production of many flavour-active metabolites. Hence, the nitrogen content of a must affects wine flavour as well as influencing yeast growth and the fermentation of sugars in winemaking

(Leonardelli, 2014). Nitrogenous compounds are essential macronutrients for yeast used in winemaking, for cell growth, multiplication, and activity and alpha amino nitrogen, is a measurement of primary amino acids usable by yeast and it does not include proline, which is not utilised by yeast, or ammonia (ETS Laboratories, 2018).

The highest values for alpha amino nitrogen in white must were observed in 2014-15 with ten plants yielding > 300 mg/L alpha amino N. Most of the plants grouped in the cluster with 100-200 mg/L alpha amino N in the must of white grape varieties in all five years of the study. Most of the plants had higher alpha amino N in 2014-15 in grapes of red selections than all other years. The highest value of alpha amino N (387.1 mg/L) in the must of red selections over the entire period of study was also obtained in 2014-15. Except for 2013-14, the largest cluster of red grapevine plants was the group with 100-200 mg/L alpha amino N values. In 2013-14 the largest cluster of red grape selections had 200-300 mg/L alpha amino N in must.

7.2.4 Ammonia

Ammonia content in the must of white grape selections was generally higher in 2012-13 than the other years of study. White grapevine plants clustered largely with the group with 50-100 mg/L ammonia in 2012-13, 2015-16 and 2016-17 while the biggest cluster was with <50 mg/L ammonia in the must in 2013-14 and 2014-15.

The highest values for ammonia in the must of red selections was seen in 2012-13 with nine plants yielding >150 mg/L ammonia. The largest cluster of red grape varieties was the one with 50-100 mg/L ammonia in the must in all five years of the study.

Ammonia is the preferred form of nitrogen for yeast nutrition and wineries routinely supplement nitrogen deficient musts with diammonium phosphate at the start of fermentation to provide adequate nitrogen levels (ETS Laboratories, 2018).

7.2.5 Yeast assimilable nitrogen

Yeast assimilable nitrogen (YAN) includes both alpha amino nitrogen and ammonia. Analysis of only alpha amino nitrogen or only ammonia nitrogen does not provide an accurate indication of total nitrogen status for a given must (ETS Laboratories, 2018).

In 2012-13 and 2013-14, the highest YAN values were observed in the must of white grape varieties. Seven plants in these years yielded >400 mg/L YAN. In 2012-13 and 2014-15, the largest number of white grape varieties studied fell into the cluster with 100-200 mg/L YAN while in all the other years, the highest number of plants had 200-300 mg/L YAN in the must.

Most of the red grape varieties had generally higher YAN in must in 2013-14 than in other years but the highest value of 559 mg/L in the must for red grape selections (Plant ID 37-26-05-03) was obtained in 2012-13. Red grapevine plants with 200-300 mg/L YAN in the must was the largest group in all five years of the study.

The difference between years for the various measures of nitrogen in grapes (YAN, ammonia, alpha amino nitrogen) may be due to the season and the age of the plants, as well as management treatments (irrigation and fertigation). Overall the values for yeast assimilable nitrogen were in a

range suitable for winemaking that would avoid stuck ferments, however, for winemaking, DAP was added for consistency across all ferments.

7.2.6 Total soluble solids

The total soluble solids (TSS) in wine grapes is normally 99% sugars (glucose and fructose). It is therefore a good assessment of berry ripeness. Sugar content is measured and expressed as Brix (°Bx). The amount of sugar in wine grapes influences the potential alcohol content after fermentation, and the amount of residual sugars remaining in the wine. During the evaluation period a 22 °Bx value for whites and reds was chosen as being the harvest value and as outlined in a previous section (6.1.3 Berry maturity and harvest) the harvest date varied between selections.

7.2.7 pH

Must has an acid composition comprising free hydrogen ions, acids, acid salts and cations. Levels of these factors and their interactions influence many winemaking parameters. Just as sugars, organic acids present in grape juices also contribute to its sensory quality, because they ensure a balance between sweet and acid flavours, which confers palatability (Gurak et al., 2010). The pH of the juice/must affects the taste, sugar/acid balance and stability of wines (Yara, 2018).

The lowest (pH 2.84) and highest pH value (pH4.35) over the study period were observed in white grape must in 2012-13. In all years of the study, the largest number of plants had a must pH between 3.5-4.0. The number of plants with a must pH >4 (29 in 2012-13 and 40 in 2013-14) decreased to 1, 2 and 4 in the later years.

For red selections, must pH was generally higher in 2013-14 compared to other years. The highest value (pH 4.68) over the entire study period for red must was observed in 2013-14 while the lowest (pH 2.91) was noted in2014-15. Red grape selections mostly had a pH of 3.5-4.0 in all the five years of study.

7.2.8 Malic acid

Malic acid accumulates early in berry development and declines during ripening due to dilution and respiration. Viticultural practices and grape cluster environments may directly affect respiration rates of malic acid. Malic acid levels affect pH and titratable acidity. Malic acid concentration can range from 4 – 6.5 g/L in grapes from cool climates, to 1 – 2 g/L in musts from warmer climates. (ETS Laboratories, 2018).

The highest level of malic acid in the must from white grape selections was 7.5 g/L, found in a sample from 2014-15 (Fig. 19). However, must from the period 2012-13 generally had the highest levels of malic acid. In white grape varieties clustered based on malic acid in must, the greatest number of plants from 2012-13 had malic acid levels of <1 g/L. In 2013-14, 2015-16 and 2016-17, the greatest numbers of plants had malic acid levels of 1-2 g/L. Comparatively, the lowest number of plants had 1-2 g/L malic acid in must in 2015-16; the greatest number of plants in that year had 2-3 g/L malic acid in must.

The highest level of malic acid in must of red grape selections (Fig. 20) was 7.2 g/L, found in a sample from 2014-15. Must from the period 2013-14 generally had the highest levels of malic acid. In red grape varieties clustered based on malic acid in must, the greatest number of plants (149) from

2013-14, (23) from 2014-15 and (19) from 2016-17 had malic acid levels of 2-3 g/L. In 2012-13 and 2015-16, the greatest numbers of plants (68 and 45 respectively) had malic acid levels of 1-2 g/L.

The ranges of malic acid observed agreed with earlier observations that malic acids are present at concentrations ranging from 1.0 to 8.0 g/L in unfermented grapes (Amerine and Ough, 1980).

7.2.9 Tartaric acid

Like malic acid, tartaric acid is also a major organic acid found in grapes. It accumulates in grape tissue early during development and is not usually metabolised in grapes.

In general, 2015-16 had the highest levels of tartaric acid recorded across all years. Must from 2012-13 showed the general trend of highest levels of tartaric acid. In white grape selections, the greatest number of plants from 2012-13 and 2013-14 had tartaric acid levels of 5-6 g/L. The greatest numbers of plants in 2014-15, 2015-16 and 2016-17 had tartaric acid levels of 4-5 g/L (Fig. 21).

In red grape selections the greatest number of plants (102) from 2013-14 had tartaric acid levels of 5-6 g/L. In 2012-13, the greatest number of plants (46) had tartaric acid levels of 8-9 g/L. In 2014-15, the greatest numbers of plants (17) had tartaric acid levels of 6-7 and 7-8 g/L. In years 2015-16 and 2016-17, the greatest numbers of plants (52 and 49 respectively) had tartaric acid levels of <5 g/L (Fig. 22).

The ranges observed agreed with earlier observations that tartaric acid is present at between 2.0 to 10 g/L in unfermented grapes (Amerine and Ough, 1980).

7.2.10 Titratable acidity

Titratable acidity (TA) measures total available hydrogen ions in solution. It reflects complex interactions between the hydrogen ions, organic acids, organic acid-salts, and cations in solution. Often TA and pH may not be directly correlated and musts with similar titratable acidity may vary in pH (ETS Laboratories, 2018).

Must from 2012-13 had overall higher amounts of titratable acids compared to all other years with a range from 1.4 to 10.5 g/L. In white grape selections the greatest number of plants (202, 32 and 27 respectively) from 2012-13, 2015-16 and 2016-17 had TA levels of 3-4 g/L (Fig. 23). Comparatively, in 2013-14, the greatest number of plants (119) had must TA levels <3 g/L. In 2014-15, the greatest numbers of plants (28) had must TA levels of 4-5 g/L.

The highest amount (12.2 g/L) of titratable acids in must of red grape varieties was found in a sample from 2014-15. This period contained the top three results for titratable acids in must. However, must from 2012-13 contained most of the highest amounts of titratable acids out of all periods from 2012-2017, with a range from 2.5-9.6 g/L.

In red grape selections, the greatest number of plants (27 and 17 respectively) from 2015-16 and 2016-17 had TA levels of 3-4 g/L (Fig. 24). Comparatively, in 2012-13, the greatest number of plants (42) had must levels of 4-5 g/L. In 2014-15, the greatest numbers of plants (14) had TA levels of 5-6, 6-7 and >7 g/L.

7.2.11 Summary of chemical analysis of berries

The data showed that there was a large range of values for the chemicals measured in berries collected at harvest. For winemaking, YAN, organic acids and pH are important and many of the selections had suitable levels not requiring additions in the winery. In the winery, DAP is added if YAN is low, to prevent stuck ferments and tartaric acid is added to adjust the pH if it is too high. Plant selections that have good levels of YAN across seasons and environments may help reduce winery inputs. Likewise, if a winery does not need to add tartaric acid to adjust pH and organic acids in a hot climate, then savings can be made through reduced inputs. The data also indicate that the genetic control of these measured chemicals is complex and is likely under multigenic control rather than under the control of a single gene.

Figure 15. Anthocyanin content in berries of red grape selections. (a) all plants and (b) number of plants in different berry anthocyanin yield categories as selection progressed through four consecutive seasons

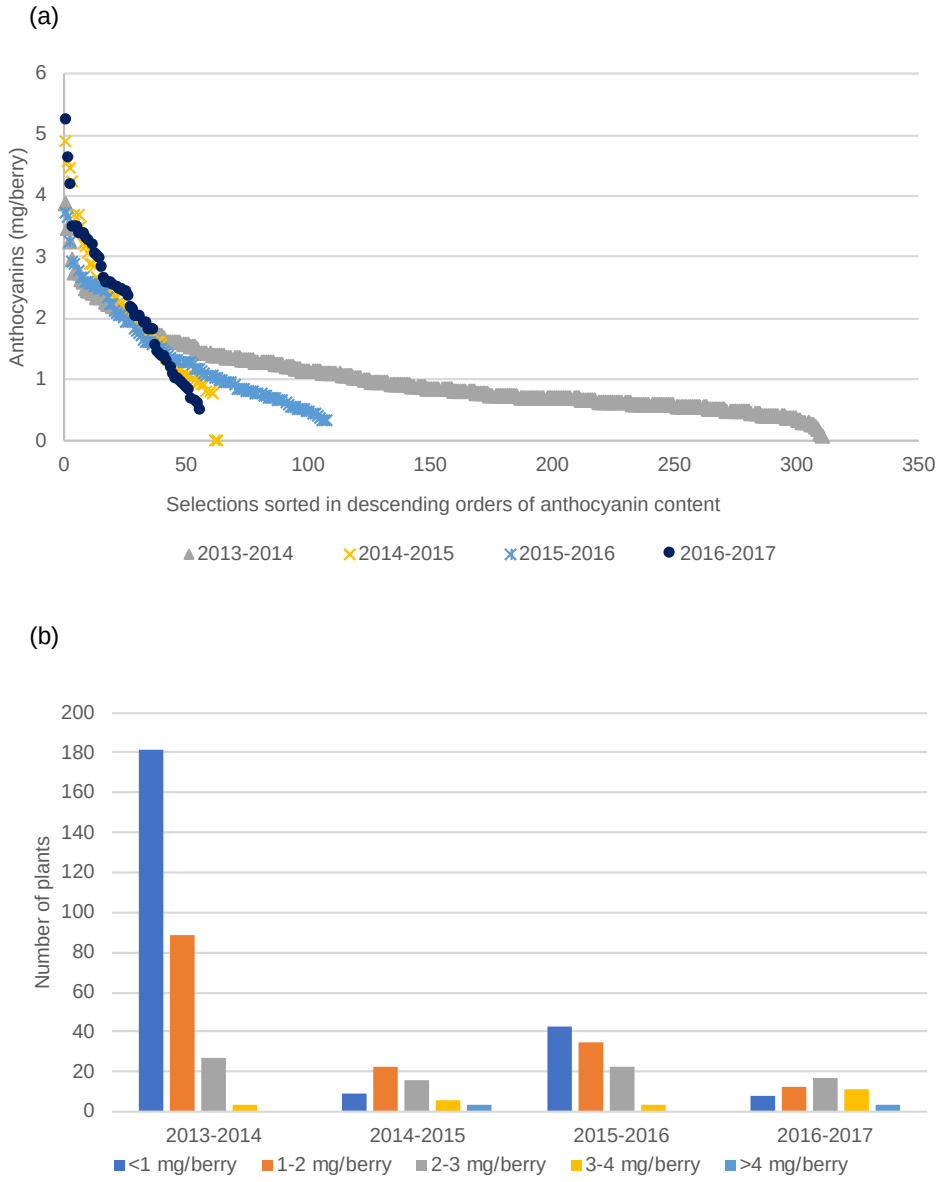


Figure 16. Anthocyanin concentrations in berries of red grape selections. (a) all plants and (b) number of plants in different berry anthocyanin concentration categories as selection progressed through four consecutive seasons

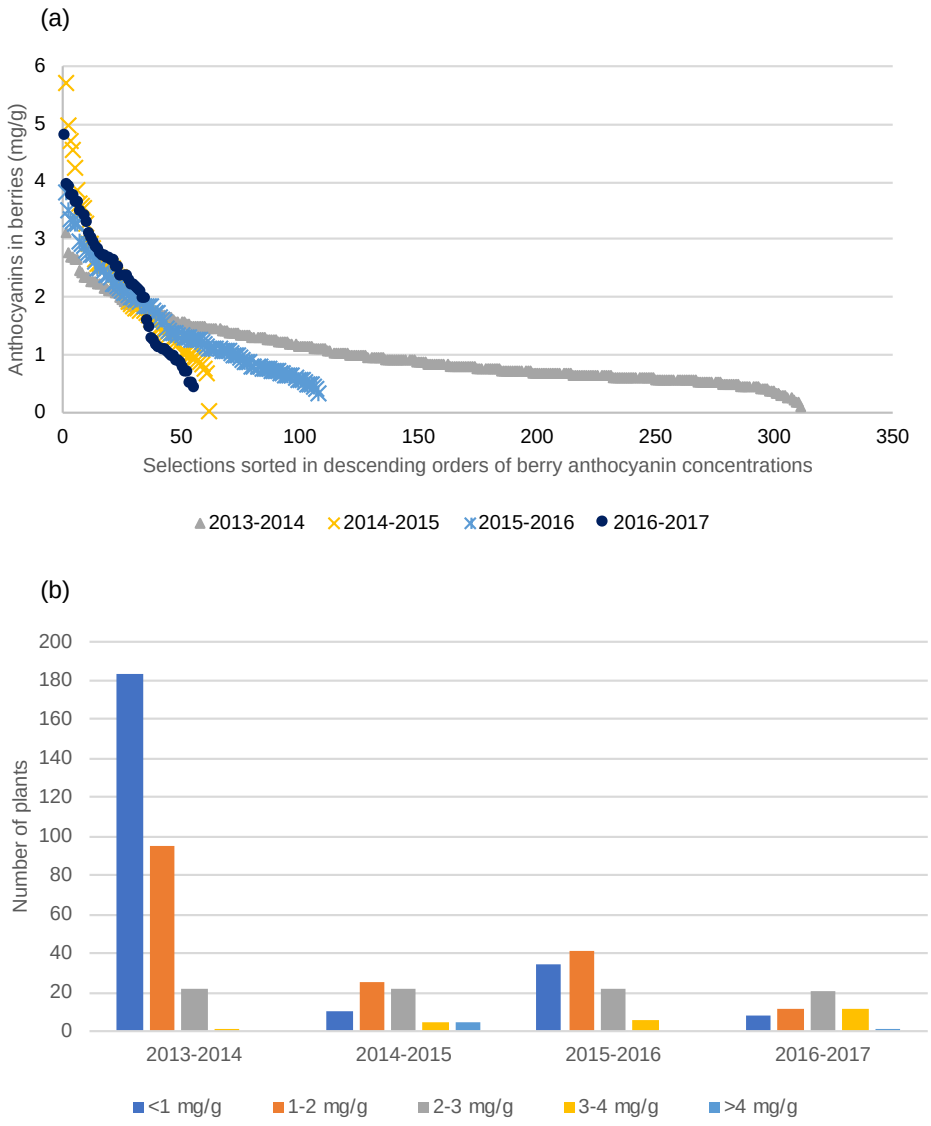


Figure 17. Phenolic content in berries of red grape selections. (a) all plants and (b) number of plants in different berry phenolic content categories as selection progressed through four consecutive seasons

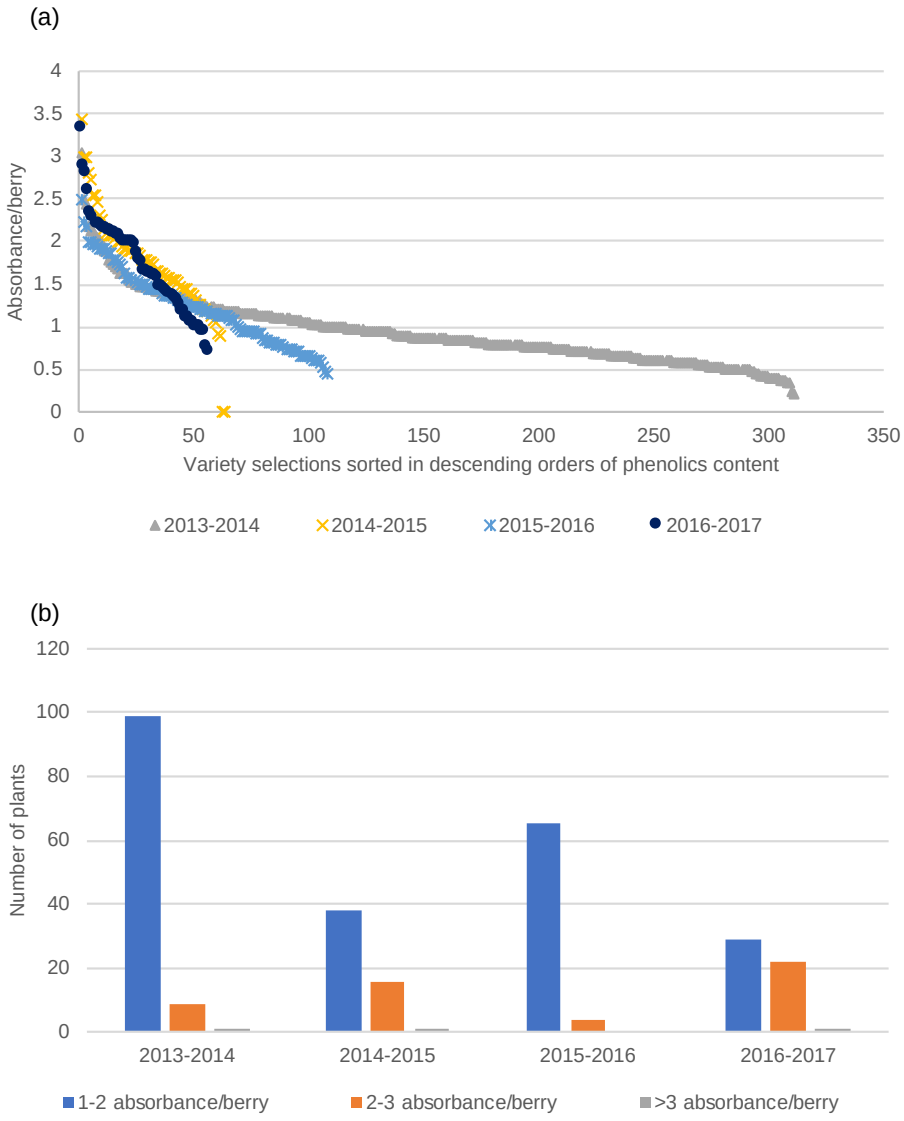


Figure 18. Phenolic concentrations in berries of red grape selections. (a) all plants and (b) number of plants in different berry phenolic concentration categories as selection progressed through four consecutive seasons

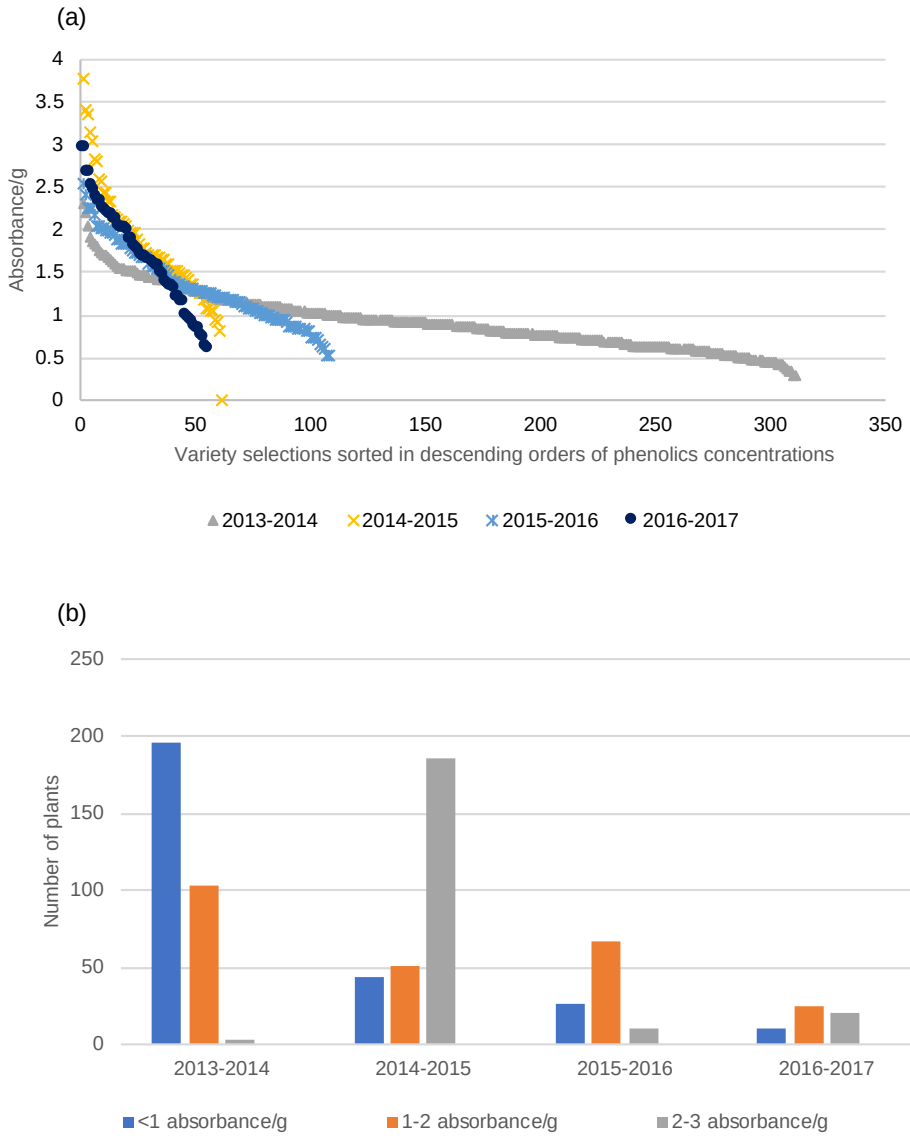


Figure 19.Concentration of malic acid in must of white grape selections. (a) all plants and (b) number of plants in different must malic acid concentration categories as selection progressed through four consecutive seasons

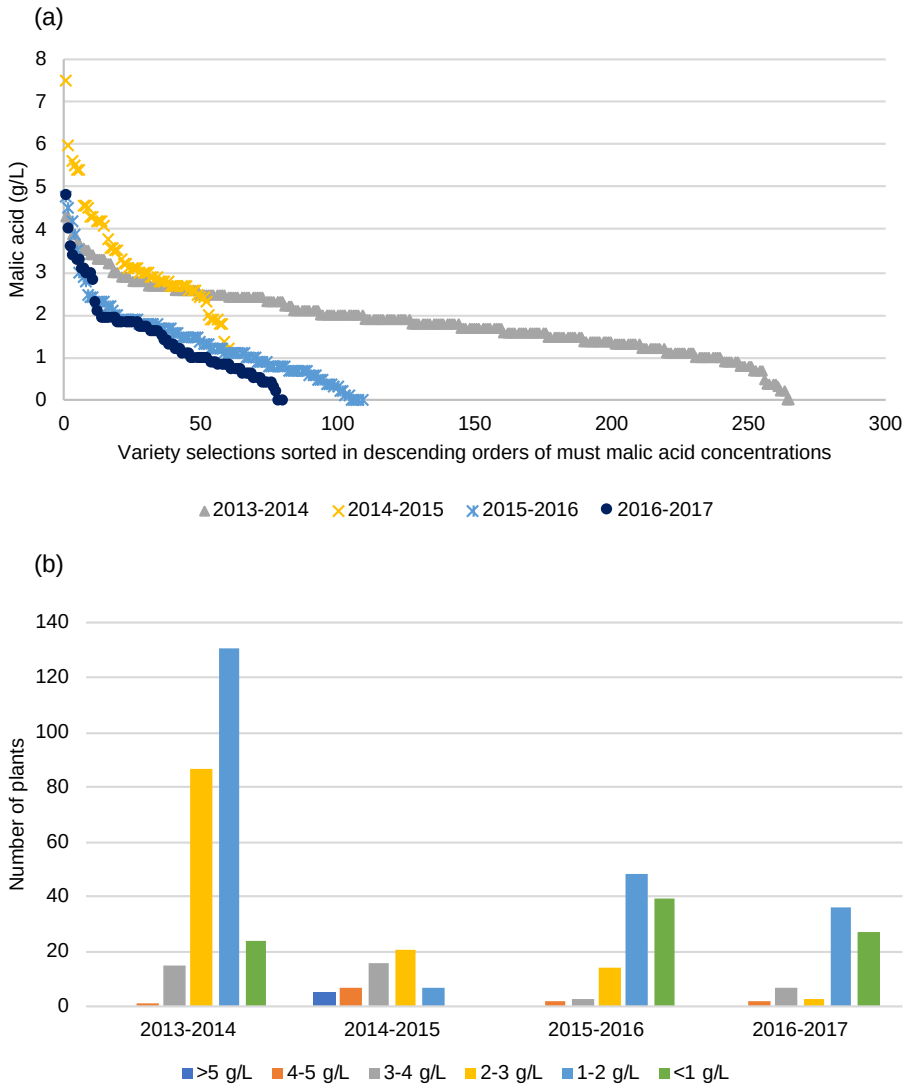


Figure 20.Concentration of malic acid in must of red grape selections. (a) all plants and (b) number of plants in different must malic acid concentration categories as selection progressed through four consecutive seasons

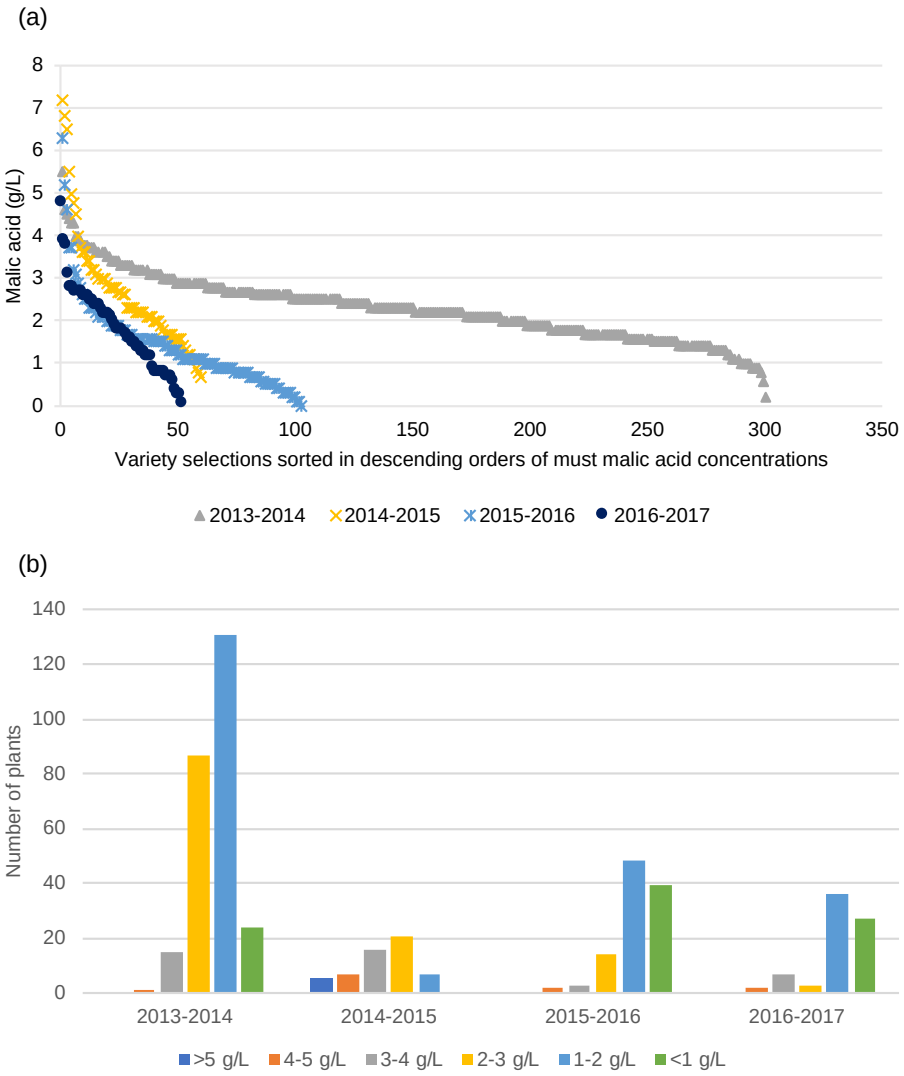


Figure 21.Concentration of tartaric acid in must of white grape selections. (a) all plants and (b) number of plants in different must tartaric acid concentration categories as selection progressed through four consecutive seasons

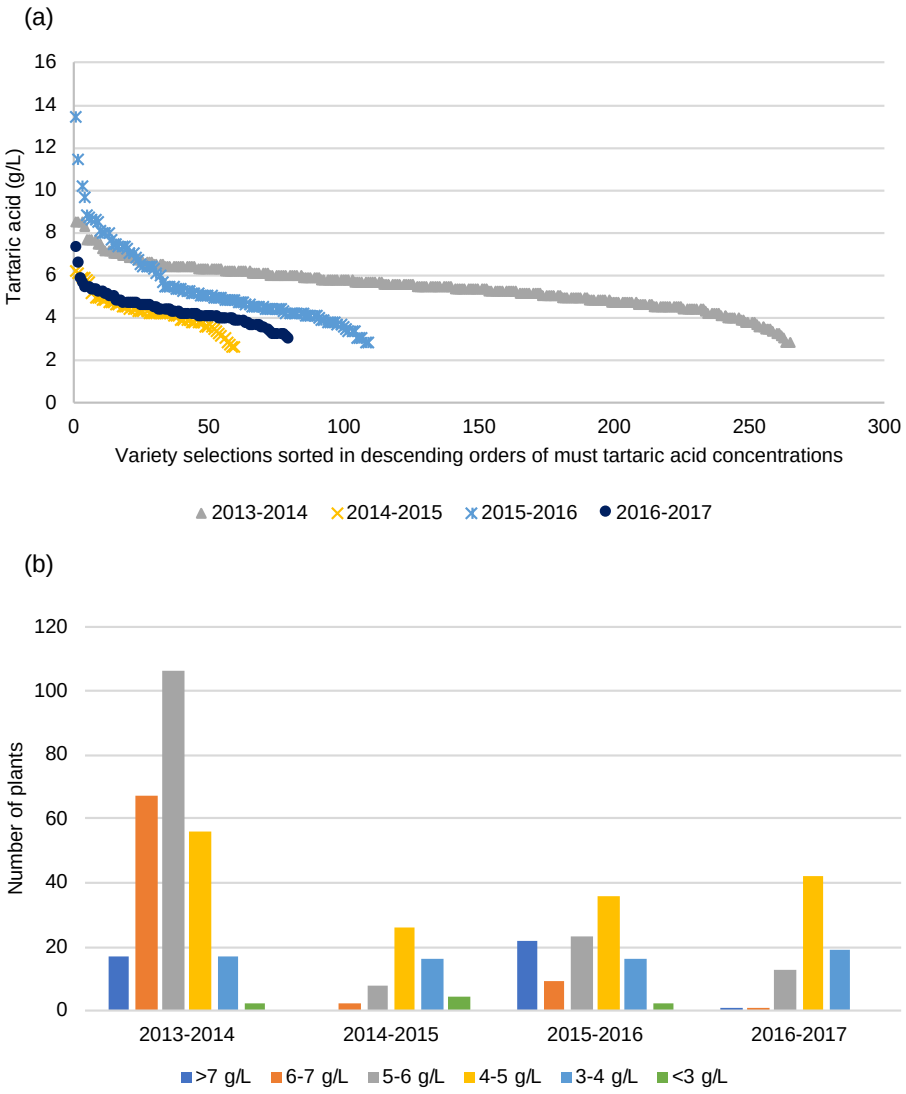


Figure 22.Concentration of tartaric acid in must of red grape selections. (a) all plants and (b) number of plants in different must tartaric acid concentration categories as selection progressed through four consecutive seasons

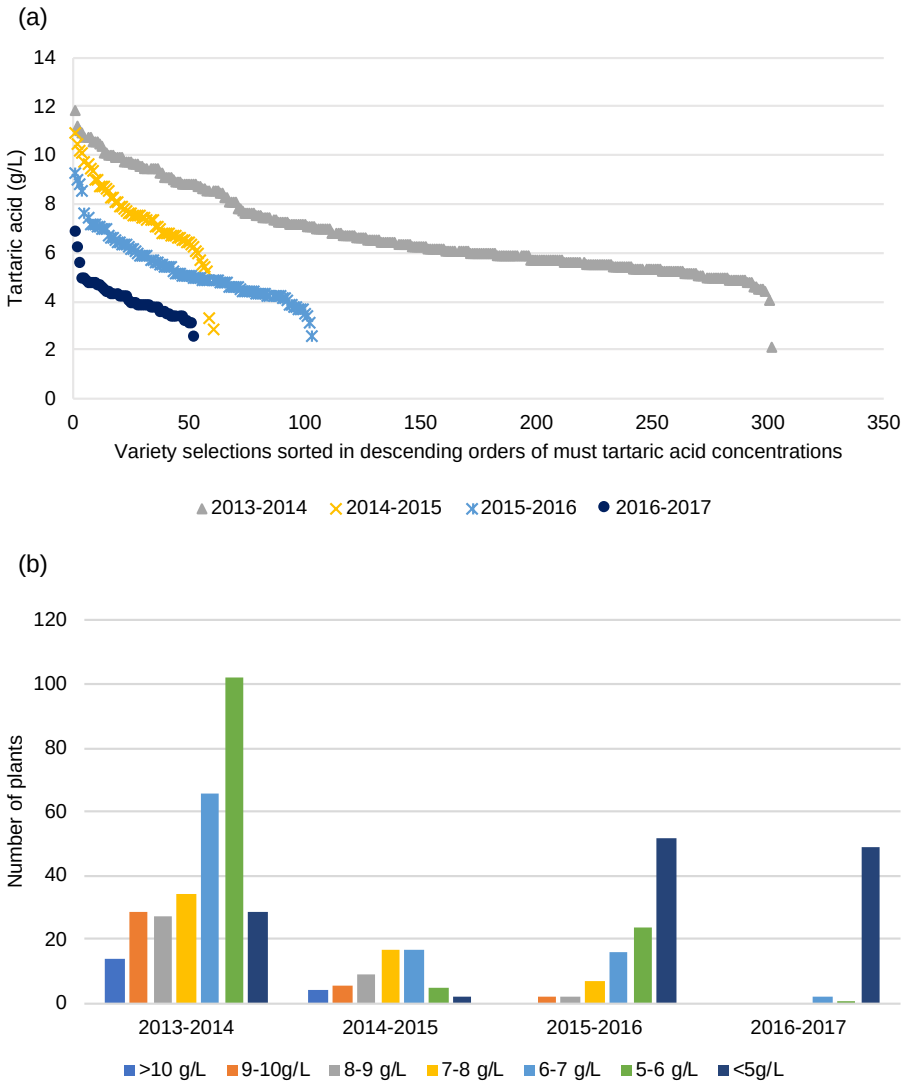


Figure 23.Concentration of titratable acids in must of white grape selections. (a) all plants and (b) number of plants in different must titratable acids concentration categories as selection progressed through four consecutive seasons

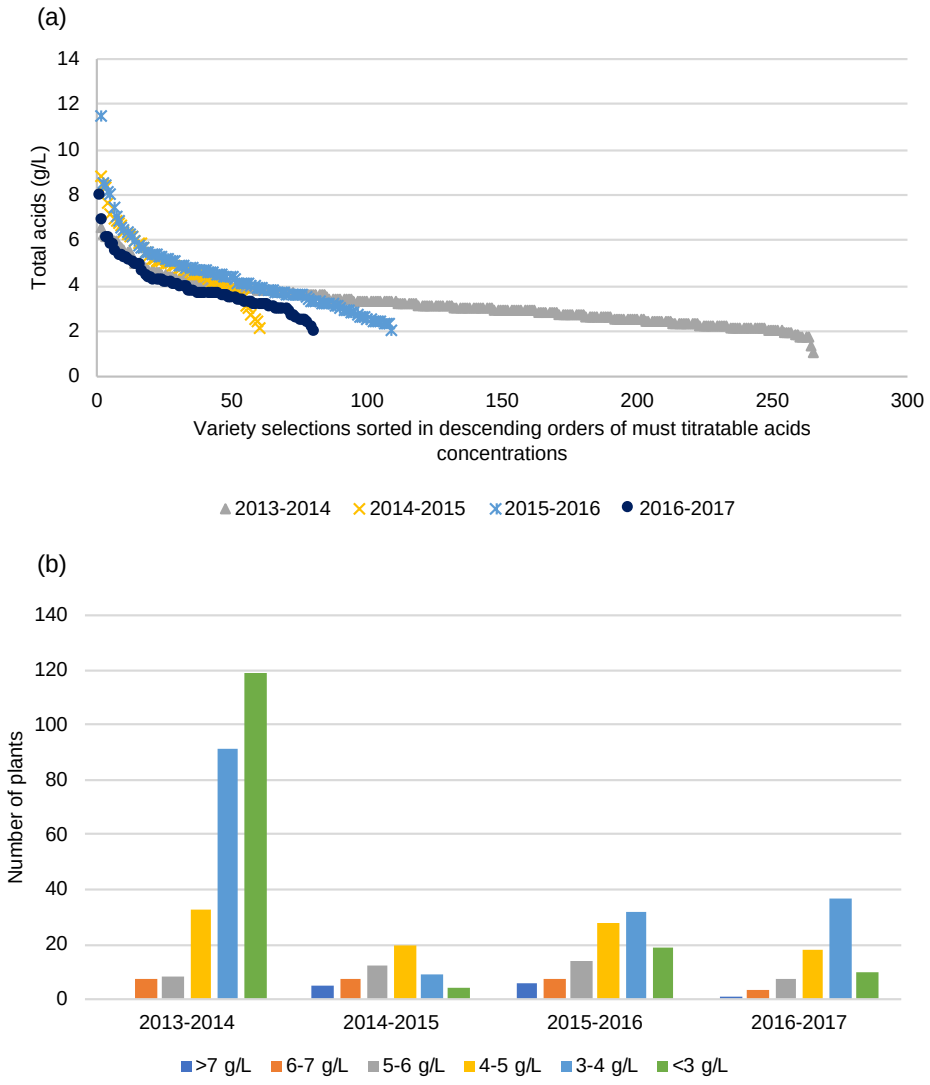
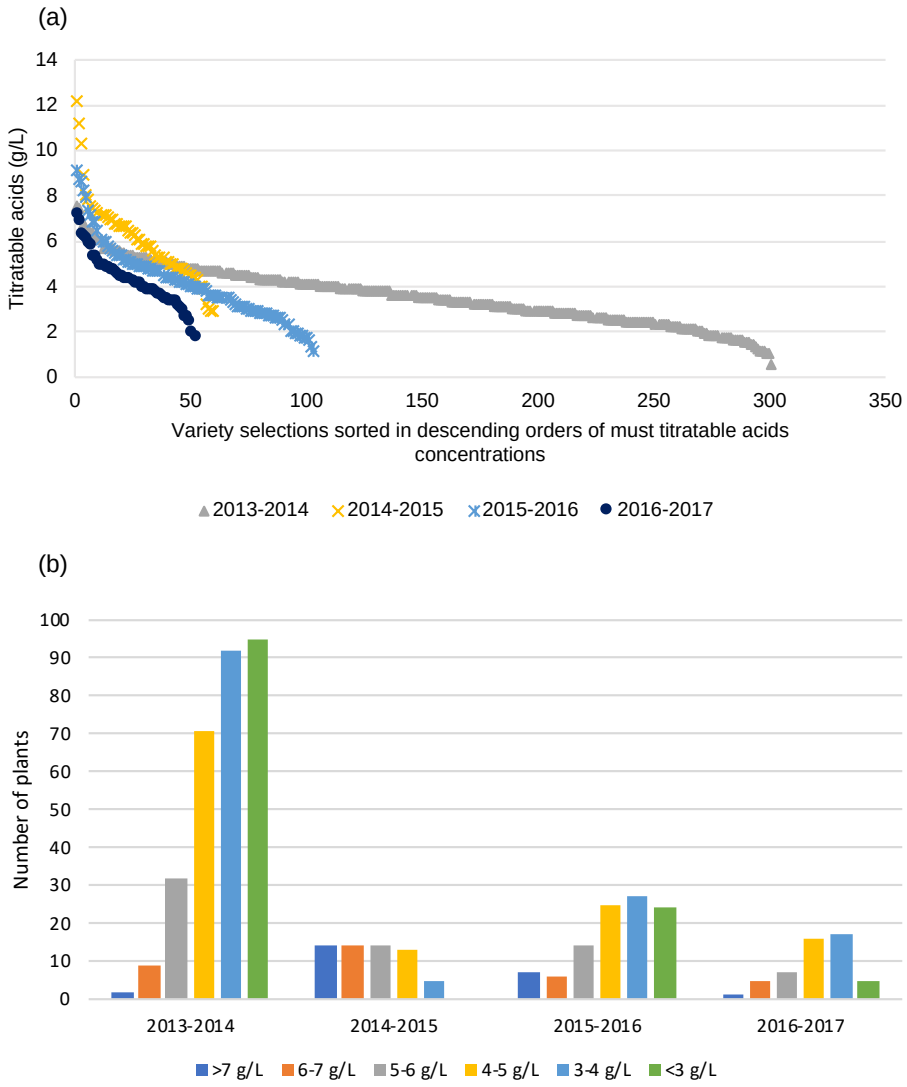


Figure 24.Concentration of titratable acids in must of red grape selections. (a) all plants and (b) number of plants in different must titratable acids concentration categories as selection progressed through four consecutive seasons



7.3 Chemical analysis of wine

Small-scale winemaking was done on berries collected from selections over several seasons. Over the course of the project selections were culled due to poor field performance and/or poor results from sensory trials. Red selections were also culled for poor wine colour (anthocyanins), phenolics and tannin content.

7.3.1 Anthocyanins in wine

Colour differences between grapes and the resultant wines are influenced by the anthocyanins. The changes from the purple red of young wines to the tawny hue observed in older red wines are attributed to the conversion of grape anthocyanins to new, more stable polymeric pigments (Oberholster, 2003). Anthocyanins in the wines are also affected by aging of wine and fermentation conditions such as temperature, duration, sulfur dioxide and alcohol concentrations. Anthocyanins also contribute to the organoleptic and chemical qualities of wine because of their interaction with other phenolic compounds as well as with proteins and polysaccharides (Mazza and Francis, 1995).

In wine from the red selections studied, the anthocyanin concentration varied between selections and seasons (Fig. 25). Season 2016-17 gave higher wine anthocyanin concentrations with the highest being 1703 mg/L for the year. Clusters based on wine anthocyanin concentration showed that most of the varieties screened fell into the group with 500-1000 mg/L of anthocyanins in the wine in 2012-13, 2014-15 and 2016-17 while in 2013-14 and 2015-16, most plants fell in the cluster with >2000 mg/L anthocyanins.

Wine hue was also measured, and the data are presented in Figure 26. Again, season 2013-14 had a larger range than later years with the final selection year having most wines in the range of 0.4-0.6 absorbance.

Oberholster (2003) had previously reported that the anthocyanin content can vary in young wines from 100 mg/L in Pinot Noir to 1500 mg/L in varieties such as Shiraz and Cabernet Sauvignon, to a minimum of 50 mg/L or less during aging. However, in the present study the highest values for anthocyanins in wine were <2000 mg/L. The wines in this study were not aged, with measurements taken shortly after bottling.

7.3.2 Phenolics in wine

Phenolic compounds are major wine constituents that affect the colour and astringency of wine. Their health contribution has also attracted considerable interest. Oberholster (2003) observed that the total amount of phenols ranged from approximately 1000 to 5000 mg/L in a light to full bodied red wine.

In the current study, wine phenolic concentration was also highest in 2012-13 with a few plants yielding phenolics more than 3000 mg/L and the highest value being >5000 mg/L (Fig. 27). In 2012-13 a higher number of plants fell in the cluster of 1000-2000 mg/L phenolics in wine. In 2013-14 and 2014-15, most of the plants studied fell in the cluster yielding <1000 mg/L phenolics in wine. For 2015-16, there were only two groups with phenolics concentration of 1000-2000 and <1000 mg/L.

7.3.3 Tannins in wine

Tannins in grape berries are located mainly in the skin and seeds. A good harmonisation of tannins from both sources gives tannic balance to young wines (Oberholster, 2003). There is a high risk of excessive astringency if seed tannins dominate, while bitterness is typical of too much extract from the skins, especially if the berries have not ripened enough. Astringency is generally accepted as developing when lubricating proteins in the saliva are precipitated as a protein-tannin complexes, losing the lubricating effect (Oberholster, 2003).

Over the study, tannin concentration in red wine ranged from <200 mg/L to >1000 mg/L (Fig. 28). Generally, in 2012-13 plants gave higher tannin concentration in wine compared to the other years. In all five years of the study, most plants fell into the group with <200 mg/L tannin concentration in wine.

The levels of tannin observed were less than the levels of 2 to 4 g/L condensed tannin that was generally reported to be derived from grapes (Herderich and Smith 2005).

7.3.4 Ethanol content in wine

Selections were harvested when they reached 22 °Bx so that sensory analysis could be carried out on wines of approximately the same alcohol content. It was found that most of the wines had alcohol levels between 12-14% which was in the expected range for grapes of that sugar content.

Stead et al. (2013) had estimated that between 1990 and 2007, the average strength of commercial wine had increased by 13%, from 11.2% to 12.6% alcohol by volume (ABV) while Robinson (2012) observed that while wines with more than 14% v/v alcohol were hard to find a few decades ago, wines with more than 16% v/v alcohol were not rare in the present times, resulting from response to consumer preferences for rich, full-bodied and ripe fruit flavour profiles. Increasing grape maturity before harvest enhances ripe fruit wine flavours and wine colour intensity. Greater maturity, however, increases sugar concentration, which in turn leads to wines with elevated ethanol concentration (Varela et al., 2015). It was decided to harvest both red and white selections at 22 °Bx instead of at a higher sugar content as there is now a world-wide trend to reduce the alcohol content of drinks.

7.3.5 Wine pH

Acids are an important factor in winemaking, influencing the colour, balance and taste of the wine as well as the activity of yeast during fermentation. They also protect the wine from bacterial spoilage. Acidity is measured according to pH, with most wines having a pH between 2.9 and 3.9 (Mathew et al., 2017). Wine with low pH will taste sour and high pH wine tastes flat and lack freshness. A minor change in wine pH (0.05 units), along with changes in total acidity (TA) of 0.2 to 0.5 g/L, significantly influences the organoleptic perception of wine (Margalit, 1997). The requirement for chemical additives such as SO_2 is reduced in low pH wines due to the added protection against oxidation and microbial spoilage provided by the low pH (Volschenk et al., 2006).

For the winemaking process, tartaric acid was added to adjust the pH at the start of the fermentation for those selections with high pH to a pH of 3.5. If the pH was below this, then no pH adjustment was made.

For white wines, across the seasons the pH values ranged from 2.34 to 3.5 (Fig. 29a). For red wines, higher pH values were found from 3.09 to 4.1 (Fig. 29b). The small-scale red winemaking procedure resulted in an increasing pH from start to finish as no pH adjustments were made during or at the end of the fermentation process. In a commercial winery, finer control of pH would be possible but due to the high number of small-scale fermentations it was decided to only do one tartaric acid addition at the start of the fermentation to maintain consistency across all wines.

7.3.6 Malic acid in wine

Acidity in wine originates mainly from two sources. The first is the organic acids extracted from grapes into the must during harvesting and crushing; L-tartaric, L-malic and citric acids are the predominant acids (Boulton et al., 1996).

For white wines, the highest level malic acid (4.7 g/L) was found in wine of white grape varieties from 2014-15 (Fig. 30). Overall, wine from this season had a significantly higher level of malic acid compared to all other years. The season with the next highest level of malic acid (3.3 g/L) was 2012-13. In white grape selections clustered based on malic content in wine, the greatest number of plants had malic acid content of 1.2 g/L across all years 2012-2017.

In red winemaking, malolactic fermentation (MLF) is often used after the alcoholic fermentation, whereby lactic acid bacteria, *Oenococcus oeni*, de-acidify the wine by converting malic acid to lactic acid. This gives the wine a softer mouthfeel (Nielsen and Richelieu, 1999). In addition, the MLF also affects the final aroma and taste balance by modifying fruit-derived aromas and producing aroma-active compounds (Laurent et al., 1994). In this study, no MLF treatment was done during the red winemaking process as the volumes were too small. However, in the final year (2016-17) a trial was done where the red wine was co-inoculated with both lactic acid bacteria and yeast, with promising results.

7.3.7 Titratable acids in wine

Titratable acid (TA) is a measure of the sum of all the organic acids in juice or wine. In healthy wine, the major acids are tartaric and malic, but all wines contain small quantities of citric, succinic, acetic, butyric, lactic and other organic acids. The taste of wine is strongly related to titratable acidity. When wine contains too much acid, it will be very tart or even sour tasting. When too little acid is present, the wine often lacks freshness and tastes flat.

The highest level of titratable acids of 11.8 (g/L) was found in a white wine sample (Fig. 31) from 2015-16. Despite this single finding, wine from season 2013-14 almost consistently had the highest levels of titratable acids for all plants sampled. In white grape selections clustered based on titratable acids in wine, the greatest number of plants in 2012-13 (48 plants) and 2016-17 (22 plants) had titratable acids levels of 6-7 g/L. However, the greatest number of plants (60) in 2013-14 had a titratable acidity of >7 g/L. In 2014-15, the greatest number of plants (39) had titratable acidity of <6 g/L.

As no malolactic fermentation was carried out during red winemaking, TA values were high except for the 2016-17 season where lactic acid bacteria co-inoculation was carried out on the wines (Fig. 32).

It is clear from the results for both white and red wine, and despite the addition of tartaric acid to adjust the pH, the seasonal effect is still obvious in the results reflecting an environmental effect on the organic acid levels in the grapes.

7.3.8 Volatile acids in wine

Volatile acids (VA) in wine can lift wine aromas and add to the complexity of the flavour components. However, in quantities higher than the optimum, it has spoilage properties. The predominant volatile acid is acetic acid.

In white grape varieties clustered based on volatile acids in wine, the greatest numbers of plants from 2012-2014 had volatile acids levels of 0.25-0.30 g/L. In 2014-15, greatest numbers of plants (19) had 0.15-0.20 g/L and 0.20-0.25 g/L of volatile acids levels. The greatest number of plants in 2015-16 had 0.20-0.25 g/L volatile acids levels while in 2016-17, the greatest number of plants had 0.15-0.20 g/L volatile acids.

In red grape varieties clustered based on volatile acids in wine, the greatest number of plants in 2012-13 had volatile acids of <0.15 g/L and 0.15-0.20 g/L. Similarly, in 2014-15, the greatest number of plants had volatile acids of <0.15 g/L and 0.15-0.20 g/L. In 2013-14 and 2015-16, the greatest numbers of plants had 0.15-0.20 g/L volatile acids. Data from 2016-17 was unique as it showed that the greatest number of plants had volatile acids of >0.30 g/L and this might be explained by the co-inoculation treatment done in that year.

Figure 25. Anthocyanin concentrations in wines made from red grape selections. (a) all plants and (b) number of plants in different wine anthocyanin concentration categories as selection progressed through four consecutive seasons

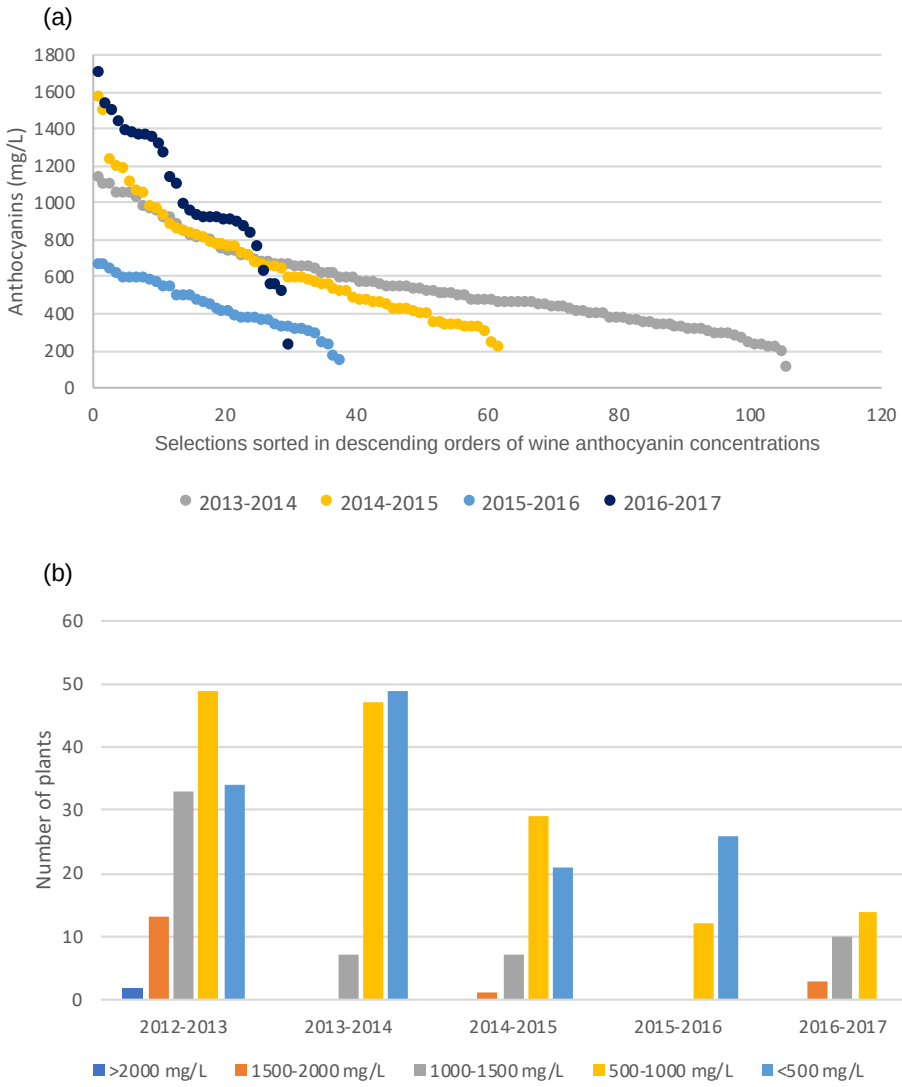


Figure 26. Wine hue of red grape selections. (a) all plants and (b) number of plants in different wine hue categories as selection progressed through four consecutive seasons

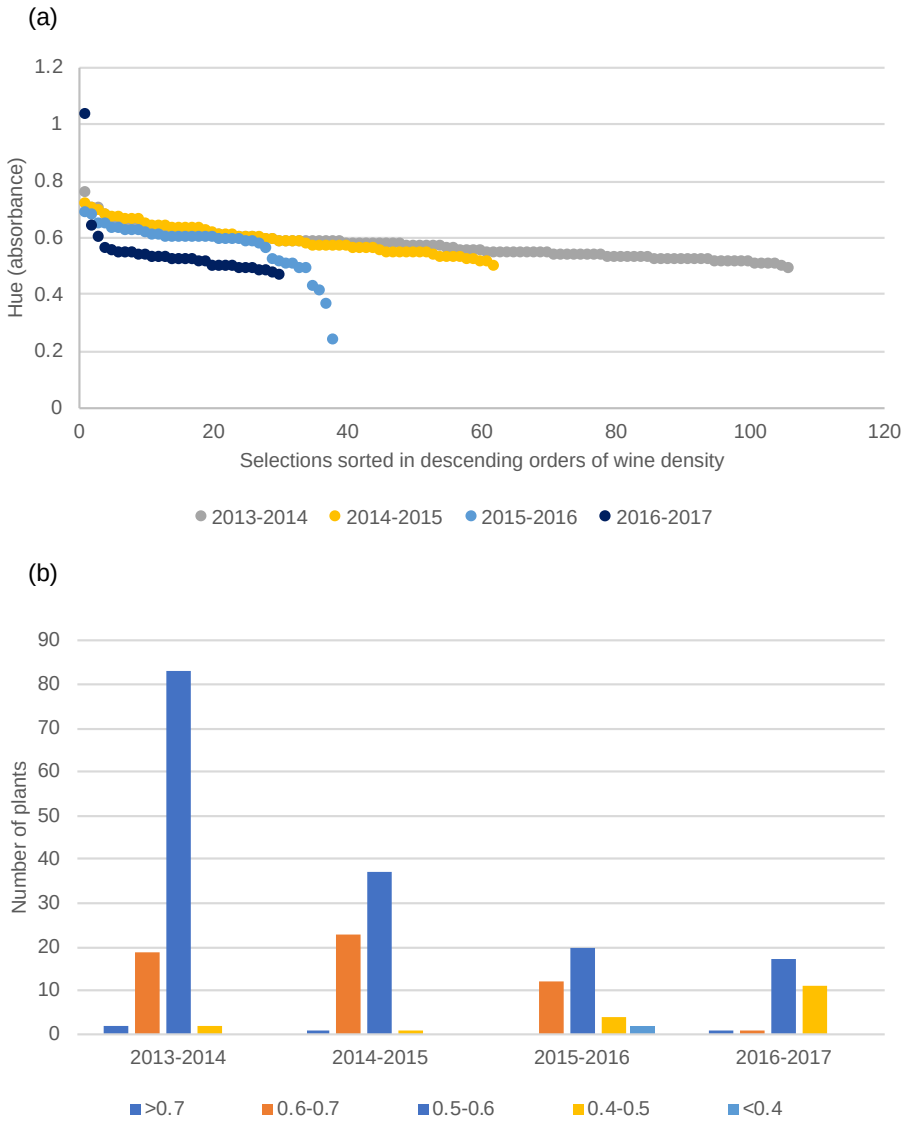


Figure 27. Phenolic concentrations in wines made from red grape selections. (a) all plants and (b) number of plants in different wine phenolic concentration categories as selection progressed through four consecutive seasons



Figure 28. Tannin concentrations in wines made from red grape varieties. (a) all plants and (b) number of plants in different wine tannin concentration categories as selection progressed through four consecutive seasons

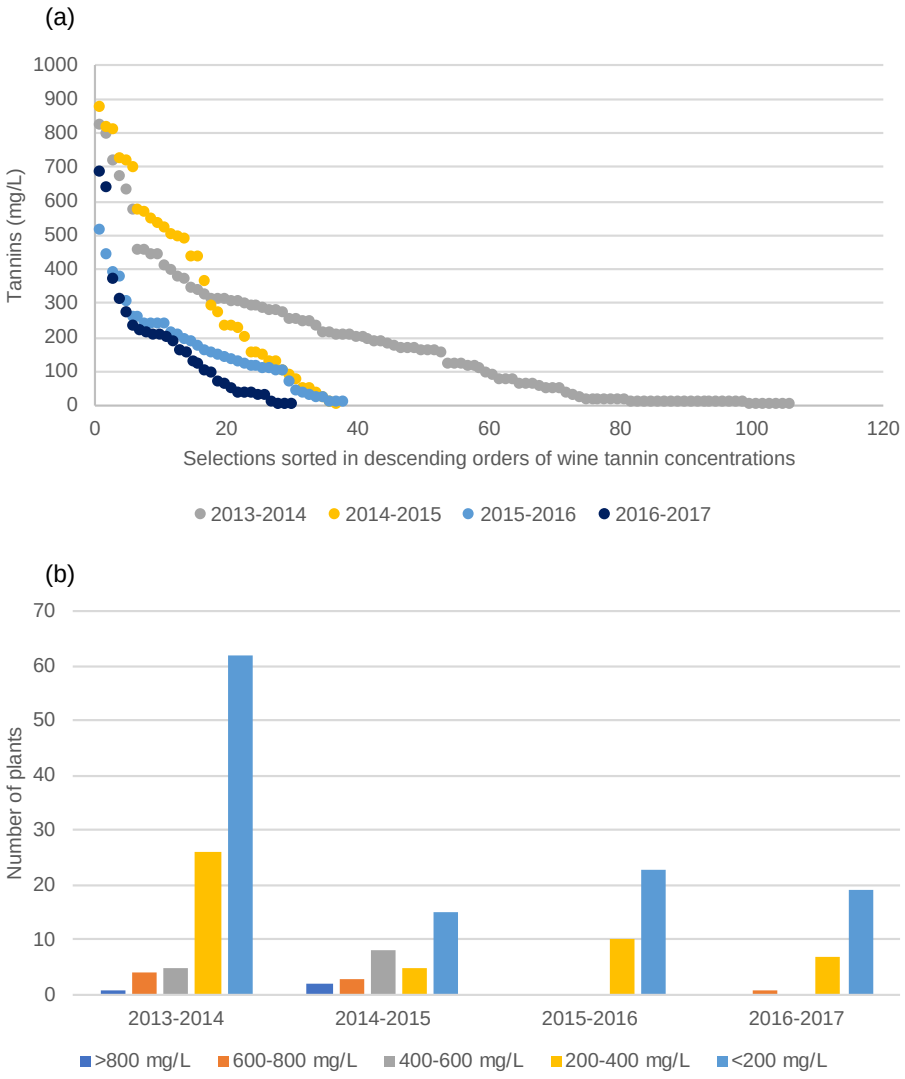


Figure 29. pH of wines made from white and red grape selections. (a) white grape selections and (b) red grape selections

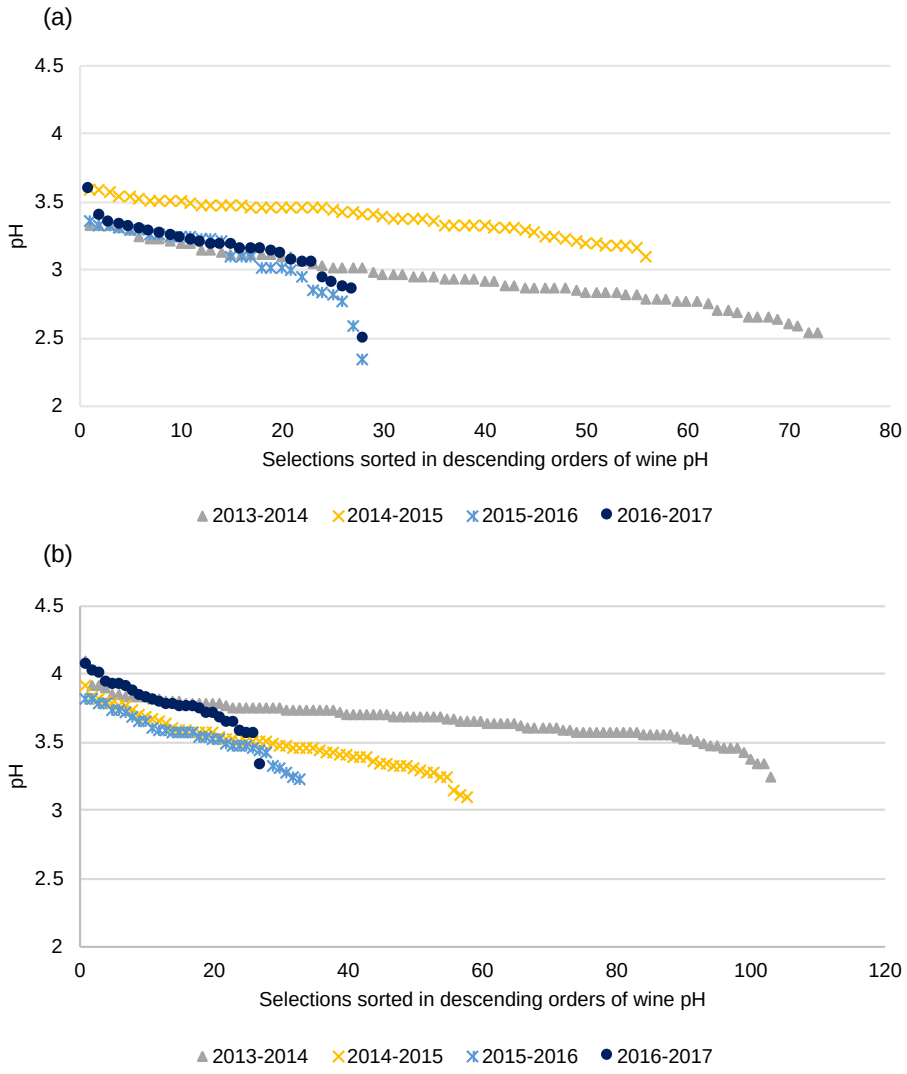


Figure 30. Malic acid content in wines made from white grape selections. (a) all plants and (b) number of plants in different wine malic acid content categories as selection progressed through four consecutive seasons

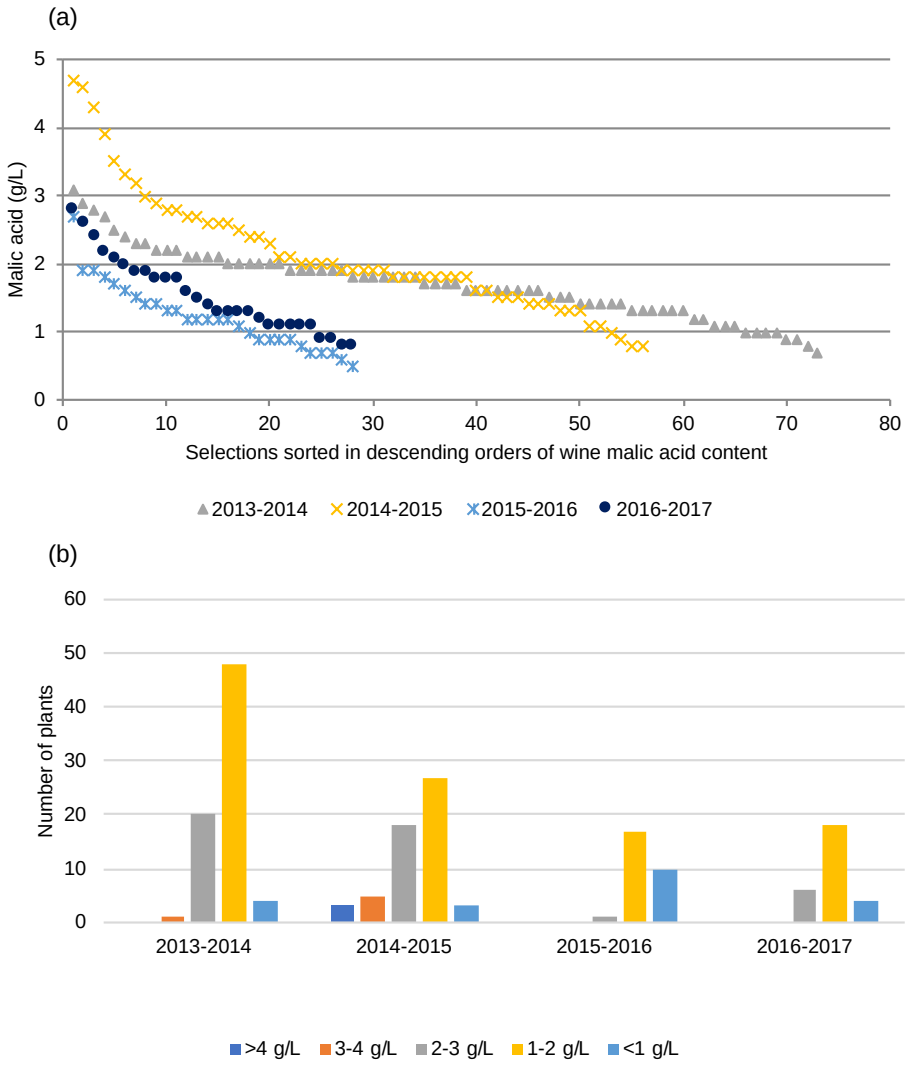


Figure 31.Concentration of titratable acids in wines made from white grape selections. (a) all plants and (b) number of plants in different wine titratable acids concentration categories as selection progressed through four consecutive seasons

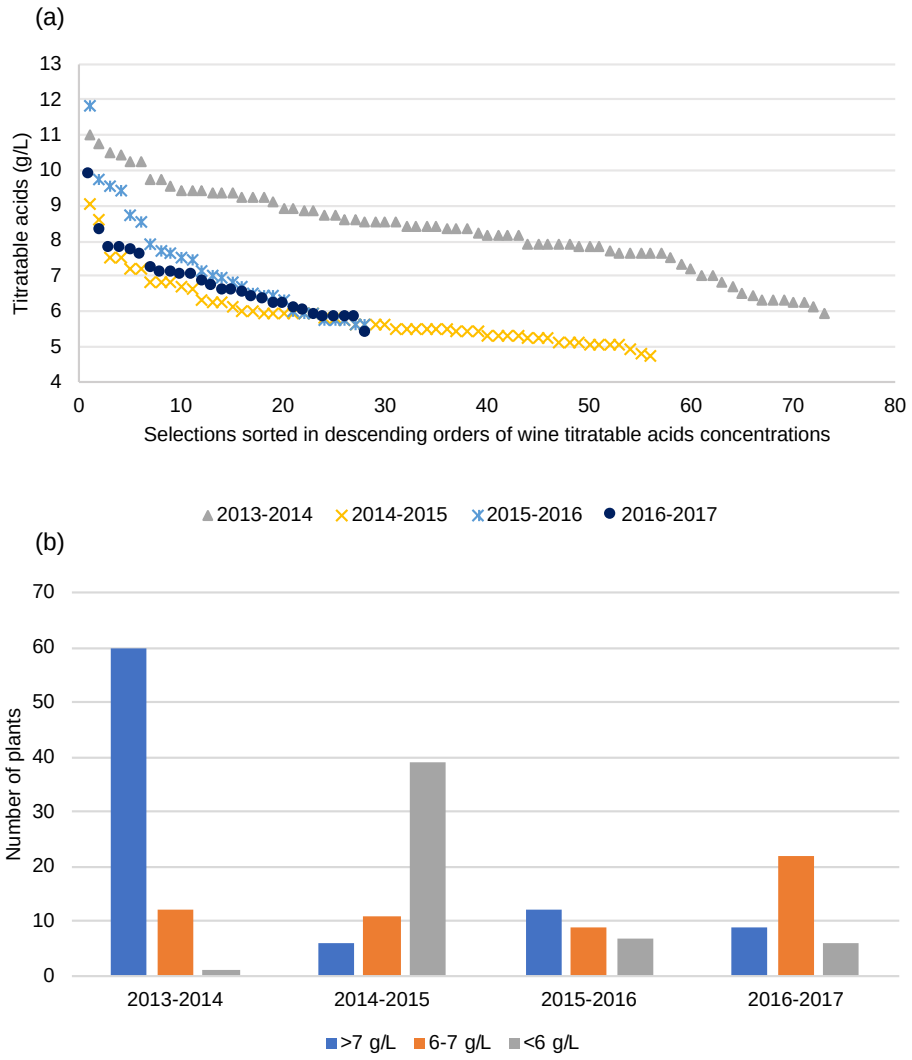
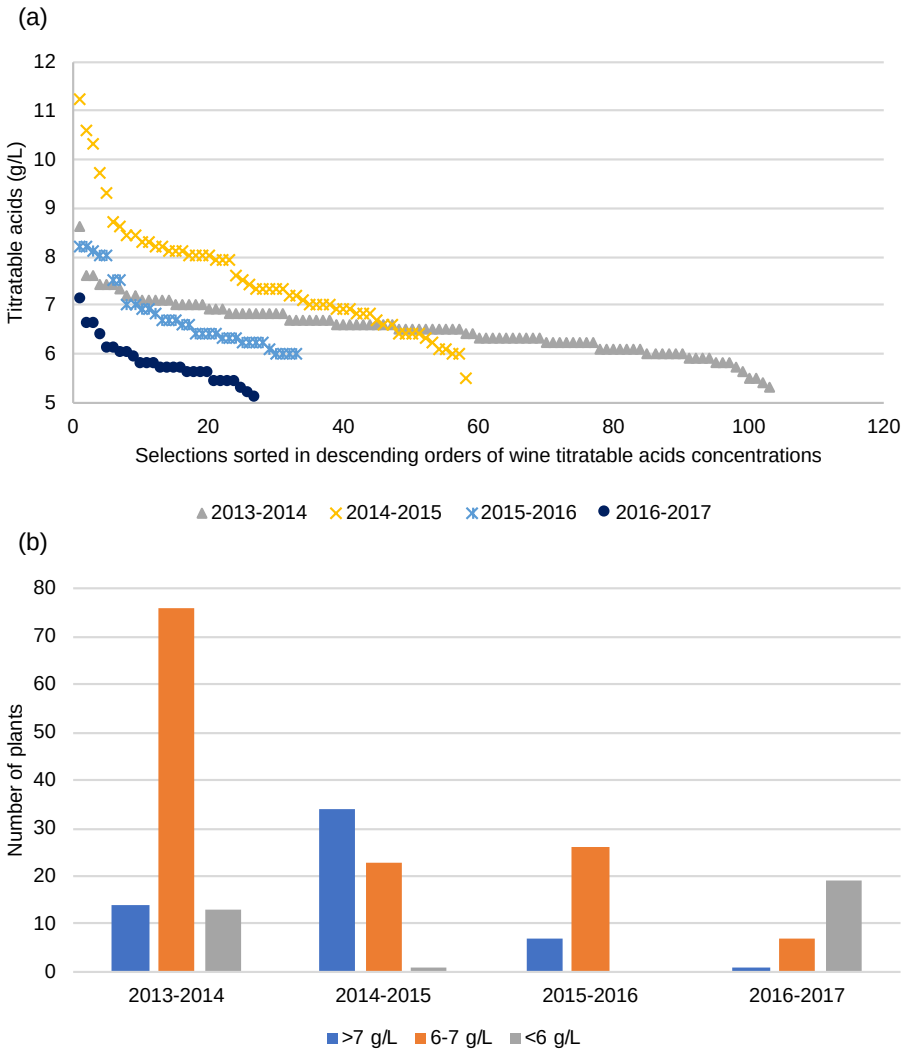


Figure 32.Concentration of titratable acids in wines made from red grape selections. (a) all plants and (b) number of plants in different wine titratable acids concentration categories as selection progressed through four consecutive seasons



7.4 Sensory evaluation of berries

Sensory evaluation of berries was undertaken to determine whether it was a faster method for evaluating berry attributes and wine style than small-scale winemaking.

7.4.1 Sensory evaluation of white berries

Twenty attributes were evaluated for each season (Table 1). A total of 28 white grape genotypes were evaluated in all. From those 28, 16 were evaluated across the three seasons (Table 2). Twenty genotypes were evaluated in 2014 and 25 were evaluated each year in 2015 and 2016. ANOVA of 2014 assessment showed that 19 attributes were significantly different ($P<0.1$) while significant differences could not be seen for the attribute of pulp green capsicum flavour. In 2015 and 2016 all 20 attributes were significantly different.

Principal component analysis (PCA) showed that must variables measured by OenoFoss could explain over 60% of the variability in each of the three seasons. A total of six clusters was identified (Fig. 33). The PCA plots for all seasons showed that the white berries were made up of two main groups. The left hand side comprising genotypes that had floral, sweet, tropical and Muscat attributes whereas the right hand side comprised genotypes with acid, green grassy, citric and other related attributes. In each of the three seasons, a cluster of genotypes showed higher astringency and bitterness. A second cluster across all seasons was characterised by Muscat and floral flavours in the pulp. In 2014 it was cluster 3 and 6 and in 2015 and 2016 it was cluster 2. The genotypes within cluster 2 had either Muscat Gordo Blanco or White Frontignac parentage. In each of the seasons there was a cluster of hybrids that had higher ratings for tannic and astringent skin. It was cluster 1 in 2014, cluster 5 in 2015, and cluster 3 in 2016. Pearson Correlation Coefficients were calculated across Principal Components, sensory attributes and OenoFoss values. Table 3 shows the significant correlations. In 2014 and 2015, OenoFoss pH and TA showed consistent correlations to sensory attributes and other correlations were also found for these seasons. In 2014, TA ranged from 1.1 and 4.7 g/L for the 20 hybrids. The eight hybrids with higher TA (between 4.7 and 3.1 g/L) ranked in decreasing order were W25, W15, W1, W4, W6, W10, W2, W19. All of these eight selections were located on the right side of the PCA bi-plot comprised of genotypes with acid, green grassy, citric and other related attributes. TA was correlated positively with pulp acidity, pulp citrus flavour, skin astringency, skin tannic intensity, skin grain size of tannins, skin re-salivation and negatively with pulp sweetness, pulp floral flavour and pulp tropical flavour.

The pH range for the white berry genotypes in 2014 was between 3.5 and 4.2. The pH was correlated positively with pulp sweetness and negatively with pulp acidity, pulp citrus flavour, skin astringency, skin tannic intensity, skin grain size of tannins and skin re-salivation. In 2015, the range of TA between the hybrids was between 2.2 and 8.3 g/L with nine hybrids giving higher TA – between 8.3 and 5.0 g/L. TA was positively correlated with pulp acidity, pulp citrus flavour, pulp green grassy flavour, skin disintegration, skin acidity, skin green flavour, skin grain size of tannins and negatively correlated with pulp sweetness. The pH range for the white berry selections in 2015 was between 3.3 and 4.0 and pH was correlated positively with pulp sweetness and negatively with the other attributes.

The range of VA in the white hybrid samples was 0.06 to 0.19 g/L in 2014, 0.05 to 0.17 g/L in 2015 and 0.03 to 0.17 g/L in 2016. VA was correlated positively with pulp tropical flavour. The range of TSS of the 25 hybrids was between 17.3 and 26.2 °Bx. TSS was correlated positively with pulp sweetness.

Table 1. Attributes evaluated for the berry sensory analysis

Whole Berry Attributes	Skin Attributes	Pulp Attributes
Firmness	Disintegration	Sweetness
Stalk of Removal	Acidity	Acidity
	Green flavour	Detachment of pulp
	Dark grape flavour*	Juiciness
	Bitterness	Green grassy flavour
	Astringency	Green capsicum flavour
	Tannin intensity	Floral flavour
	Grain size of tannins	Tropical flavour
	Re-salivation	Citrus flavour
		Muscat flavour

*was not evaluated for white varieties

Table 2. White berry genotypes evaluated for sensory analysis over three seasons (2014-16)

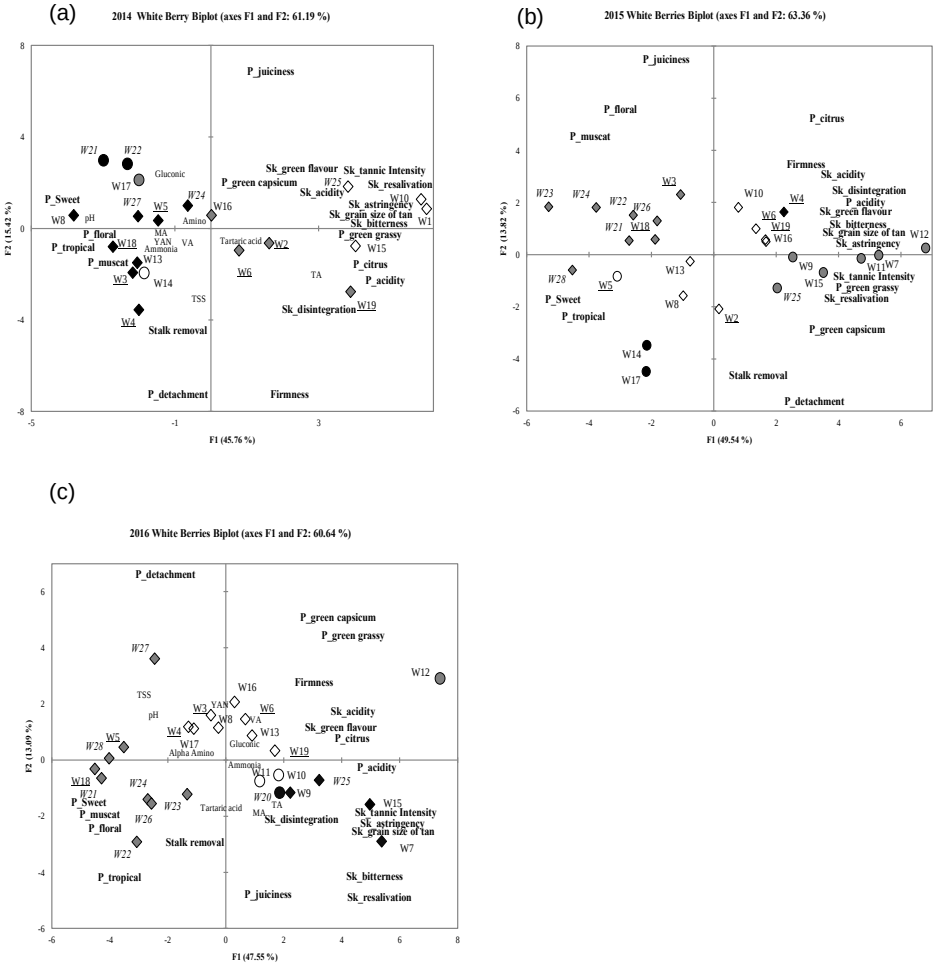
Short Codes	Selections
W1†‡	37_27_01_04
W2‡	37_28_05_01
W3	37_28_06_02
W4	37_28_07_03
W5	37_28_10_01
W6	37_28_11_01
W7*	37_30_03_02
W8	37_30_05_03
W9*	37_30_05_04
W10	37_32_08_02
W11*	37_32_08_04
W12*	37_32_11_01
W13	37_32_12_02
W14‡	39_08_05_01
W15	39_11_04_02
W16	39_11_10_02
W17	39_14_04_02
W18	39_15_04_03
W19	39_15_10_01
W20*†	39_15_10_02
W21	39_17_07_02
W22	39_17_09_01
W23*	39_17_10_03
W24	39_18_03_01
W25	39_18_03_02
W26*	39_18_04_01
W27†	39_18_10_01
W28*	39_19_02_01

* not evaluated in 2014, † not evaluated in 2015, ‡ not evaluated in 2016

Table 3. Significant Pearson Correlation Coefficients demonstrating associations between Principal Components, OenoFoss measurements (e.g. titratable acidity (TA), pH, volatile acidity (VA) total soluble solids (TSS)), and various sensory attributes for white berries over three seasons (2014-16)

	2014		2015			2016
	TA	pH	TA	pH	VA	TSS
PC1	0.739	-0.695	0.779	-0.850	-0.596	-0.538
Pulp acidity	0.711	-0.667	0.713	-0.746		
Pulp citrus flavour	0.658	0.646	-0.831	-0.860		
Pulp sweetness	-0.727	0.695				0.658
Pulp floral flavour	-0.606	-				
Pulp tropical flavour	-0.635	-		-0.753		
Skin astringency	0.673	-0.646		-0.682		
Skin tannic intensity	0.676	-0.644				
Skin re-salivation	0.696	-0.663				
Pulp green grassy flavour			0.740	-0.668		
Skin disintegration			0.680	-0.763		
Skin acidity			0.787	-0.811		
Skin green flavour			0.669	-0.685		
Skin bitterness				-0.608		

Figure 33. PCA of berry sensory attributes (**bold**), and berry compositional measures mean values (n=3), from white berry hybrids assessed in seasons 2014(a), 2015(b) and 2016(c), Barossa Valley, South Australia. White berry hybrids name are coded by letter and a number which is the same for each hybrid for the three seasons. White hybrid names in italic have grape variety White Frontignac parentage and white hybrid names underlined have Muscat Gordo Blanco parentage. Round markers represent the location of the white berry hybrids in the PCA space and their distribution from the cluster analysis of the berry sensory ratings: cluster one (cleat diamond), cluster two (grey diamond), cluster three (black diamond) cluster four (clear circle), cluster five (grey circle), cluster six (black circle).



7.4.2 Sensory evaluation of red berries

Twenty seven red berry genotypes were evaluated during the same three seasons as the white grape berry genotypes. Twenty of the 27 selections were evaluated in the 2014, 24 in 2015 and 26 in 2016. Sixteen of the 28 selections were evaluated in all three seasons. (Table 4). ANOVA of the sensory evaluation of red berry hybrids revealed that most of the 20 berry sensory attributes were significantly different (P<0.100) between the 27 genotypes in the three seasons. Sixteen attributes were significantly different in the three seasons (Table 5). Pulp confectionary flavour and pulp green capsicum flavour were not significantly different in any of the seasons. Pulp dark berry flavour was significantly different (P<0.100) only in 2014 but not in the other two seasons. Pulp red berry flavour was significantly different (P<0.100) in the seasons 2014 and 2015, but not in 2016.

Principal component analysis (PCA) showed that OenoFoss must variables could explain over 60% of the variability in 2014 and 2015 but only 48% for 2016. (Fig. 34). Four main clusters for the attributes were found across the seasons. The first cluster of attributes was for pulp detachment, pulp dry fruit flavour, pulp sweetness and pulp berry flavour. However only four genotypes (R3, R6, R8 and R9) were in this group for all three seasons. The second cluster had the following five attributes in common across the three seasons: skin bitterness, skin astringency, skin tannic intensity, skin grain size of tannins and skin re-salivation. However only R13 was consistently located in this group in all three seasons. There was a third cluster with the same attributes (pulp acidity, pulp juiciness, pulp red berry flavour, pulp green capsicum flavour, pulp green grassy flavour and skin green flavour) across each season. Two genotypes (R20 and R25) were located in this group across the three seasons. A fourth cluster contained firm berry and juicy pulp. Three genotypes R18, R19 and R24 were a part of this group across the three seasons. Pearson correlation analysis was able to find various relationships among the sensory attributes, however only a few were consistent across the three seasons.

Pearson Correlation Coefficients were calculated for Principal Components, sensory attributes and OenoFoss values. Tables 6, 7 & 8 show the significant association. Among those consistent relationships was the positive correlation between pulp dark berry flavour and pulp sweetness and negative correlation between pulp dark berry and pulp acidity. Positive correlations between pulp dry fruit flavour and pulp sweetness were also found in the three seasons and negative correlations with pulp acidity. A negative correlation was found in the three seasons between pulp detachment from the skin and pulp juiciness. As expected, berry sensory attributes rating bitterness, astringency, tannic intensity and grain size of tannins of the skins were positively correlated for the three seasons, but they were not correlated with any other berry sensory attributes.

Table 4. Red berry selections evaluated for sensory analysis over three seasons (2014-16)

Short Codes	Selections
R1	37_14_05_02
R2	37_14_05_03
R3	37_14_08_01
R4*‡	37_14_09_01
R5	37_14_10_04
R6	37_15_01_01
R7	37_15_02_03
R8	37_15_04_01
R9	37_15_04_02
R10*	37_15_05_04
R11*	37_15_07_01
R12*	37_15_08_02
R13	37_15_11_03
R14	37_15_12_01
R15*	37_15_13_02
R16	37_16_04_04
R17	37_16_11_02
R18†	37_20_01_04
R19	37_23_03_04
R20	37_24_07_01
R21	37_24_09_01
R22†	37_24_10_01
R23†	37_25_03_02
R24	37_25_09_04
R25	37_25_10_04
R26†	37_26_04_01
R27*	37_26_12_01

* not evaluated in 2014, † not evaluated in 2015, ‡ not evaluated in 2016

Table 5. Significant Pearson Correlation Coefficients for sensory pulp attributes in red berries over three seasons (2014-16)

Pulp attributes		Dry fruit flavour	Dark berry flavour	Juiciness
Sweetness	2014	0.869	0.735	
	2015	0.630	0.735	
	2016	0.652	0.798	
Acidity	2014	-0.884	-0.615	
	2015	-0.738	-0.700	
	2016	-0.600	-0.636	0.786
Detachment	2014			0.940
	2015			0.945
	2016			
Firmness	2014			
	2015			0.640
	2016			

Table 6. Significant Pearson Correlation Coefficients demonstrating associations between Principal components, OenoFoss measurements (e.g. titratable acidity (TA), pH, yeast assimilable nitrogen (YAN), free alpha amino nitrogen (FAN) and total soluble solids (TSS)), and various sensory attributes for red berries for 2014

	TA	pH	YAN	FAN	TSS
PC1	0.785	-0.838	-0.720	-0.773	
Pulp sweetness	-0.835	0.894	0.599	0.644	-0.683
Pulp dark berry flavour	-0.608	0.599			
Pulp dry fruit flavour	-0.898	0.909	0.633	0.694	
Pulp acidity	0.871	-0.918	-0.648	-0.721	
Pulp red berry flavour	0.727	-0.678			
Pulp green grassy flavour	0.743	-0.739		-0.708	
Skin astringency			0.615	0.637	

Table 7. Significant Pearson Correlation Coefficients demonstrating associations between Principal Components, OenoFoss measurements (e.g. alpha amino nitrogen (FAN), titratable acidity (TA), tartaric acid, pH, volatile acids (VA) and yeast assimilable nitrogen (YAN)), and various sensory attributes for red berries for 2015

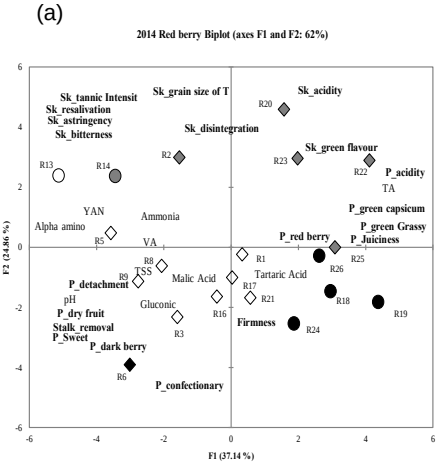
	FAN	TA	Tartaric acid	pH	VA	YAN
PC1	-0.712					
PC2		0.649	-0.669			
Pulp dry fruit flavour	0.760				0.609	
Pulp acidity				-0.633		
Pulp red berry flavour				-0.615		
Pulp green capsicum				-0.615		
Pulp juiciness	-0.742					0.695
Pulp detachment	0.760					0.712
Pulp astringency		0.716				
Pulp tannic intensity		0.671				
Skin grain size of tannins		0.68				
Skin re-salivation		0.604				
Firmness	-0.758				-0.636	-0.619

Table 8. Significant Pearson Correlation Coefficients demonstrating associations between Principal Components, OenoFoss measurements (ammonia, pH, titratable acidity (TA), and yeast assimilable nitrogen (YAN)), and various sensory attributes for red berries for 2016

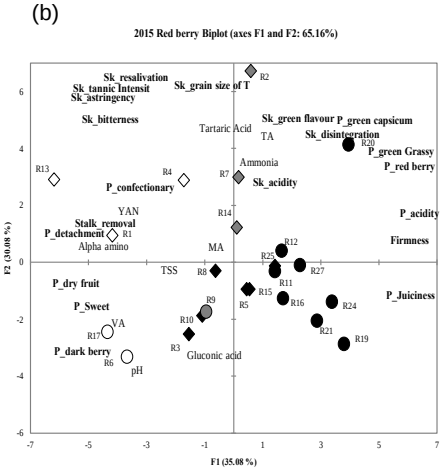
	Ammonia	pH	TA	YAN
PC3	0.642			
Pulp dry fruit flavour				0.621
Pulp acidity		-0.65	0.682	

Figure 34. PCA of berry sensory attributes (**bold**), berry compositional measures (*italic*) mean values (n=3), from red berry hybrids assessed in seasons 2014(a), 2015(b) and 2016(c), Barossa Valley, South Australia. Round markers represent the location of the red berry hybrids in the PCA space and their distribution from the cluster analysis of the berry sensory ratings: cluster one (clear diamond), cluster two (grey diamond), cluster three (black diamond) cluster four (clear circle), cluster five (grey circle), cluster six (black circle).

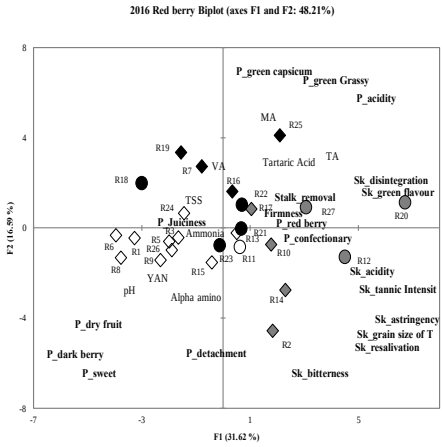
(a)



(b)



(c)



7.4.3 Summary of berry sensory results

It could be concluded from the berry sensory results that PCA plots were effective at separating and grouping the selections based on sensory attributes, with significant differences found between selections. However, the requirement for a trained panel and the limit to the number of samples the panel could process each week meant that this approach was not suitable for screening large numbers of selections. Until berry sensory procedures can be better streamlined to allow higher numbers of berry samples to be assessed, small-scale winemaking appears to be a better approach.

7.5 Wine sensory analysis

For small-scale red winemaking, a total of 137, 107, 63 and 48 small-scale wines were prepared from individual selections for 2013, 2014, 2015 and 2016 vintages respectively. Low alcohol wines were made in 2015 and 2016. The amount of wine obtained from 1.3 kg of destemmed berries ranged from 500 to 750 mL. For small-scale white winemaking a total of 101, 77, 68 and 41 small-scale wines were prepared from individual genotypes for 2013, 2014, 2015 and 2016. Low alcohol wines were made in 2015 and 2016. The amount of wine obtained from 2 kg of white grape bunches was fixed to 500 mL. Yeast fermentation usually took around 14 days. For the vintages of 2013, 2014, 2015 and 2016 there were 6, 5, 5 and 7 panellists respectively assessing the wines.

7.5.1 White wine sensory analysis

The genotypes selected for winemaking in 2013 were based on yield data and berry tasting before harvest. The results from the 2013 sensory data were used to select genotypes for winemaking for 2014. The results from 2014 and 2013 were used to determine the selection of genotypes for 2015 vintage. The results from 2015, 2014 and 2013 were used to determine which genotypes were to be selected for winemaking for 2016. This resulted in an overlap in similar genotypes being assessed across all vintages. Figure 35 shows the plots of the average interest level score and average hedonic score against the number of wines for each season. Quite a few wines scored 4 or greater for interest level across all seasons. The panellists tended to give higher hedonic scores with a score of 5 or greater across all seasons. Two genotypes also scored greater than 5 on the hedonic scale indicating that the panellist favoured these across seasons. Another 18 white wines scored greater than 5. The majority of these had Muscat Gordo Blanco or White Frontignac heritage (77%). The remaining had Riesling heritage and one had Chardonnay heritage. The overall average interest level score was calculated across all the wines evaluated for a particular season (Tables 9, 10). The percentage of wines with an interest score greater than the average was calculated in order to identify wines of consistently above average interest level and approximately half of the wines evaluated gave above average scores over the three seasons. Four white wines were found to give above average interest scores for three or more seasons. They all had the Muscat SNP genotype.

Figure 35. Rounded average scores of hedonic scale (a) and interest level (b) for white wines over four seasons.

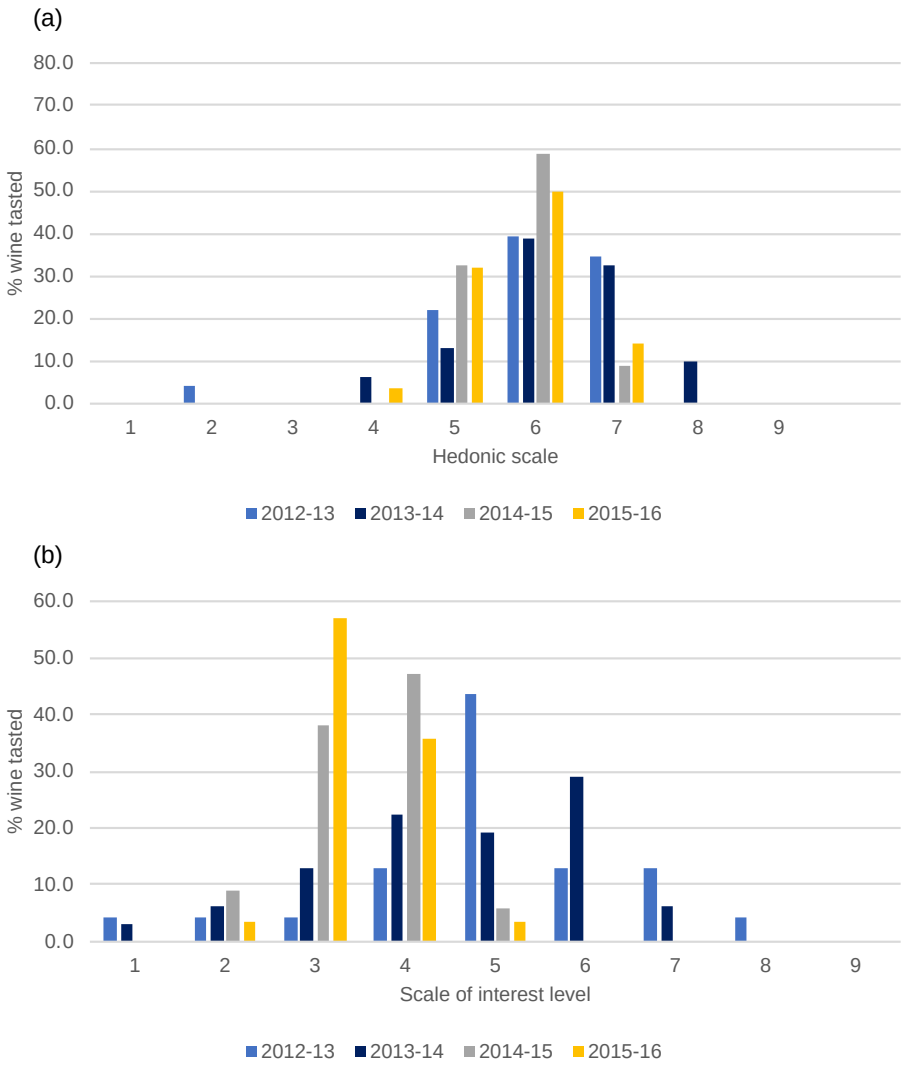


Table 9. White Wine Average Interest Levels across seasons

Year	Average Interest Level	% of Wines
2013	4.88	56.52
2014	4.59	54.84
2015	3.48	52.94
2016	3.42	53.57

Table 10. White Wine genotypes with above average interest level over four seasons

2013	2014	2015	2016
37_28_07_02		37_28_07_02	37_28_07_02
37_28_07_03	37_28_07_03	37_28_07_03	37_28_07_03
39_15_04_03		39_15_04_03	39_15_04_03
	39_18_06_02	39_18_06_02	39_18_06_02

7.5.2 Red wine sensory analysis

Red wine sensory analysis was done the same way as for the white wine and results are presented in Tables 11 and 12. Figure 36 shows plots of the average interest level score and average hedonic score against the number of wines for each season. Most wines scored 4 or greater for interest level across all seasons however some wines did score higher indicating they had greater potential. The panellists tended to give higher hedonic scores to the red wines across all the seasons indicating preference for red wines (compare Figs. 35, 36). A total of 22 genotypes scored greater than 5 on the hedonic scale over at least 3 seasons (Fig. 36a).

Around half of the wines evaluated gave above average scores over the three seasons. Of these, seven consistently scored above average on interest level (Table 12).

Figure 36. Rounded average scores of hedonic scale (a) and interest level (b) for red wines over four seasons.

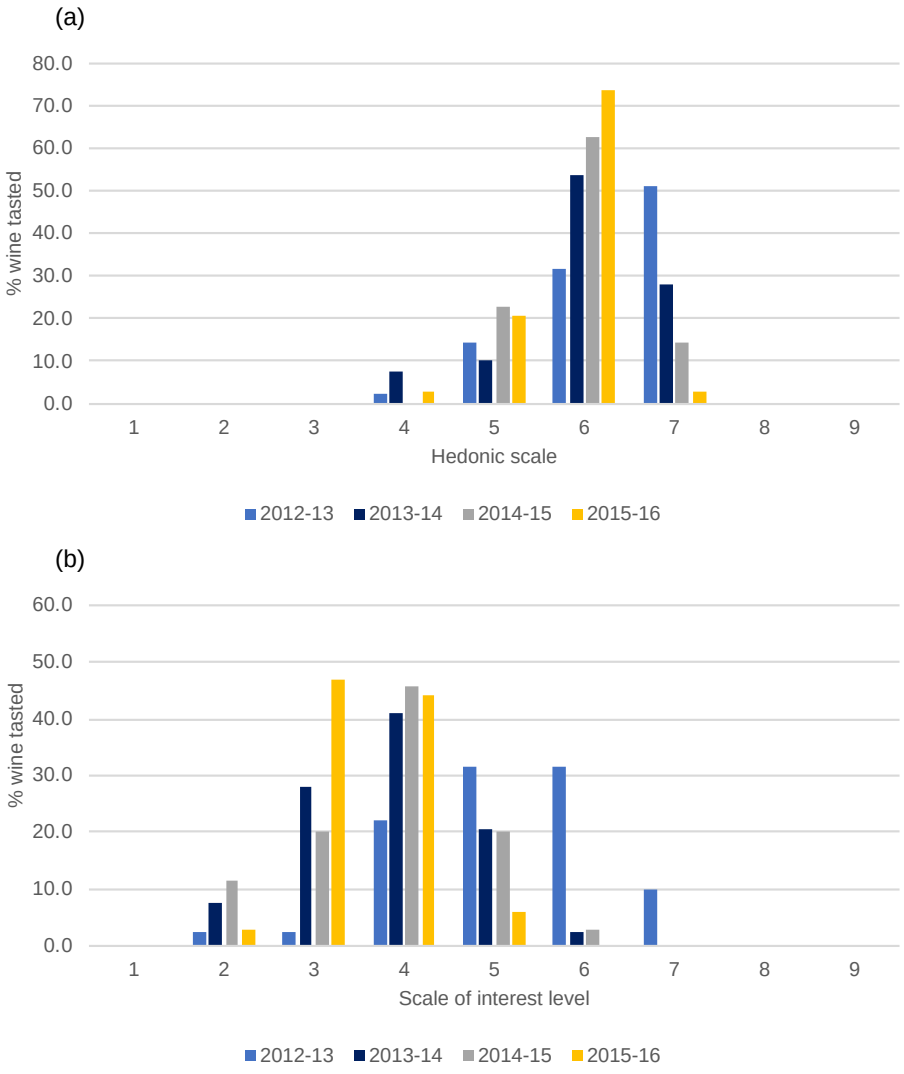


Table 11. Red Wine Average Interest Levels across seasons

Year	Average Interest Level	% of Wines
2013	5.02	52.4
2014	3.77	48.0
2015	3.77	48.0
2016	3.47	50.0

Table 12. Red Wine genotypes with above average interest level over four seasons

2013	2014	2015	2016
37_14_08_01		37_14_08_01	37_14_08_01
37_14_12_02	37_14_12_02		37_14_12_02
	37_14_11_04	37_14_11_04	37_14_11_04
37_15_04_01	37_15_04_01	37_15_04_01	37_15_04_01
37_15_06_04	37_15_06_04	37_15_06_04	
37_16_07_01	37_16_07_01	37_16_07_01	37_16_07_01
	37_15_11_03	37_15_11_03	37_15_11_03

7.6 Low alcohol wines

Of late, health and wellness are becoming a focus in food and beverage consumption in the western world. This has given rise to a new category of wine known as 'low calorie and low alcohol wines', which by definition contain less than 12% alcohol. Low alcohol wines are favoured also for their lightness and flavour. A number of white and red genotypes were selected for making small-scale low alcohol wines based on the hedonic scores the normal wines of these genotypes received in previous years.

Tables 13 and 14 show the white and red genotypes respectively, selected for LA (low alcohol) wine in 2014-15, 2015-16 and 2016-17. The tables also show 'days to harvest from anthesis' for LA wine and at 22 °Bx for these selections. Tables 15 and 16 show the ranges of various parameters measured by OenoFoss analysis in white and red grape musts respectively, of low alcohol collections over three seasons. Table 17 shows ranges of anthocyanin and phenolics measured in red grape musts of low alcohol collections over two seasons.

For wine, Tables 18 and 19 show the ranges of various parameters measured by OenoFoss in low alcohol wines made from white and red grapes respectively over three seasons. Table 20 shows the ranges of anthocyanin, phenolics and tannins measured in wines made from red grapes of low alcohol collections over two seasons.

7.6.1 pH and TSS in must

The must pH of white grape varieties sampled for low alcohol winemaking ranged from 3.12 to 3.49 in 2014-15, 3.01 to 3.58 in 2015-16 and 2.93 to 4.0 in 2016-17. The pH of low alcohol must of red grape selections ranged from 2.99 in 2014-15 to 3.72 in 2016-17.

Brix values for white varieties ranged from 12.1 in 2014-15 to 19.8 in 2016-17 while the range was between 11.2 in 2015-16 to 20 in 2016-17 in the case of red grape must.

7.6.2 Anthocyanin and phenolics in must

Anthocyanin concentration ranged from less than 0.5 mg/g to 3 mg/g in red grape berries sampled for low alcohol winemaking. Most of the plants sampled had a berry phenolic concentration between 1 and 2 mg/g. Brix values did not show any consistent relationship with berry anthocyanin concentration or phenolics concentration and this may be due to the small number of samples collected or other factors.

7.6.3 Malic acid in must

Malic acid levels in the must of white grape selections ranged from 1.1 g/L in 2016-17 to 8.2 g/L in 2014-15. Samples collected in 2014-15 generally had higher malic acid values than those collected in the other years. Malic acid levels of red grape selections ranged between 0.6 g/L to 8.6 g/L over the three years of study. Malic acid levels from both white and red varieties did not show any consistent relationship with Brix values when plotted against Brix.

7.6.4 Tartaric acid in must

The lowest levels of tartaric acid in must of white grape selections used for low alcohol winemaking was recorded in 2014-15. Samples from 2015-16 recorded the highest values of tartaric acid levels. Tartaric acid values ranged from 1.6 g/L in 2014-15 to as high as 11 g/L for one sample in 2016-17. In the case of red must, highest values were recorded in 2014-15 while the lowest values were

recorded in 2016-17. Values of tartaric acid ranged from 4.1 to 11.9 g/L over the entire period of study. As in the case of malic acid, tartaric acid also did not show any consistent relationship with Brix values for red grape and white grape must.

7.6.5 Titratable acids in must

Titratable acids in low alcohol collections ranged from 4.7 g/L to 13.3 g/L in white grape must and 5.5 g/L and 15.4 g/L in red grape must. Titratable acids also did not show any consistent relationship with Brix values.

7.6.6 Yeast assimilable nitrogen in must

Yeast assimilable nitrogen varied widely in white grape must over the three years of study of low alcohol collections from as low as 15.4 mg/L to as high as 309.4 mg/L. Generally, low levels of yeast assimilable nitrogen were recorded by the plants in 2014-15 while the highest levels were recorded in 2015-16. For low alcohol red must, yeast assimilable nitrogen levels ranged from 137.9 mg/L to 425.1 mg/L.

7.6.7 Ethanol content in wine

Ethanol content in white wine of low alcohol collections ranged from 7.4% to 12% and for red wine the range was from 6.5% to 11.9%. There was, as expected, a positive linear relationship between ethanol content in wine and Brix values.

7.6.8 Anthocyanin and phenolics in red wine

Anthocyanin levels ranged from 80 mg/L to 1260 mg/L in red wine of low alcohol wine. The phenolics concentration ranged from 262 mg/L to 1885 mg/L. Tannin concentration in red wine of low alcohol wines varied widely. Most wine had a tannin concentration ranging from 27 - 268 mg/L in the red wine of low alcohol selections.

7.6.9 pH and glucose:fructose ratio in wine

White wine of low alcohol collections had a pH range of 2.53 to 3.27 while the red wine had a nearly similar pH range of 2.92 to 3.75. Most of the white grape varieties had a glucose fructose level of 0.8 to 4.4 while the red wine glucose fructose levels ranged from1.3 to3.3.

7.6.10 Sensory analysis of low alcohol wine

Preliminary sensory analysis was carried out on the low alcohol wines with the method used for standard wines as described previously. In 2015, eight low alcohol whites were made and in 2016, nine were made. Interest level was low for 2015 but the wines scored higher on the hedonic scale for both vintages. The most common remark was that they were more suitable for a light bodied white wine style.

In 2015, 11 low alcohol reds were made and in 2016, seven were made. Interest level was moderate for both vintages and wines scored higher on the hedonic sale. Higher scoring wines were thought by the sensory panel to be more suitable to the Beaujolais style.

The approach taken to make low alcohol wines was to do early harvests at low Brix values to determine if any of the selections showed promising wine styles and flavours. The results suggest that further research is required to determine if any of the selections have suitable berry flavour

profiles at low sugar levels for making low alcohol wine without the need for additional manipulations in the winery.

Table 13. White grape genotypes selected for low alcohol wine for three different seasons

Year	Plant ID (Cross code)	Anthesis to Harvest for LA (Days)	Anthesis to Harvest at 22 °Bx (Days)	°Bx at Harvest for LA	% alcohol in LA wine
2014-15	37_28_05_04 (C18)	100	135	12.2	7.4
	37_28_07_02 (C18)	100	128	13.4	8.1
	37_28_11_01 (C18)	100	114	14.6	9.2
	37_32_06_04 (C3)	107	135	12.1	7.5
	39_11_09_01 (C5)	100	114	15.3	10
	39_15_02_03 (C18)	100	128	14.3	8.7
	39_15_04_03 (C18)	100	135	14.4	9.5
	39_15_05_01 (C18)	100	128	14.7	9.3
2015-16	37_28_08_02 (C18)	93	114	14.1	8.7
	37_28_11_01 (C18)	93	114	12.7	8.3
	39_15_02_03 (C18)	86	114	12.6	7.6
	39_17_09_01 (C19)	86	100	12.8	9.9
	39_17_10_03 (C19)	93	100	16.9	10.4
	39_18_04_02 (C19)	93	100	17.6	10.7
	39_19_01_01 (C19)	100	107	16.3	9.8
	39_19_02_01 (C19)	93	107	15.1	9.7
2016-17	37_28_05_04 (C18)	114	128	13.3	9
	37_28_08_02 (C18)	100	114	16.9	10.2
	37_32_06_04 (C3)	100	121	18.1	10.2
	39_11_04_02 (C5)	107	135	16.4	9.7
	39_15_05_01 (C18)	114	135	15.9	10
	39_17_09_01 (C19)	93	100	17.8	11.1
	39_17_10_03 (C19)	100	107	19.8	12
	39_18_01_02 (C19)	93	114	17.5	10.6

Table 14. Red grape genotypes selected for low alcohol wine for three different seasons

Year	Plant ID (Cross code)	Anthesis to Harvest for LA (Days)	Anthesis to Harvest at 22 °Bx (Days)	°Bx at Harvest for LA	% alcohol in LA wine
2014-15	37_15_05_02 (C10)	107	128	14.9	8.9
	37_15_05_04 (C10)	114	128	15.1	9.3
	37_15_09_04 (C10)	114	142	14	8.7
	37_15_10_01 (C10)	100	128	12.2	7.3
	37_15_11_01 (C10)	108	128	16.5	9.4
	37_15_13_02 (C10)	107	128	14.6	8.5
	37_16_03_01 (C10)	107	135	12.1	6.5
	37_16_04_04 (C10	114	142	13.7	7.8
	37_16_10_01 (C26)	107	135	14.6	8.6
	37_25_03_04 (C21)	100	128	14.4	8
	37_25_05_01 (C21)	100	128	14.7	8.6
2015-16	37_15_04_02 (C10)	86	114	13.7	8.2
	37_15_05_02 (C10)	93	107	13.5	8.1
	37_15_05_04 (C10)	100	128	11.8	7.5
	37_15_10_01 (C10)	86	114	13.9	8.4
	37_15_11_01 (C10)	93	107	15.9	9.1
	37_15_12_01 (C10)	86	114	13.5	7.4
	37_15_13_02 (C10)	93	114	11.2	7.3
	37_16_04_04 (C10)	86	114	12.4	11.3
2016-17	37_14_05_01 (C10)	107	114	18.4	11.9
	37_15_04_01 (C10)	107	114	20	10.5
	37_15_05_02 (C10)	100	114	17.2	11.8
	37_15_07_03 (C10)	107	114	19.4	9.3
	37_15_08_02 (C10)	100	121	16.4	9.3
	37_15_11_01 (C10)	93	107	16.0	8.6
	37_15_12_01 (C10)	93	114	17.7	-

Table 15. Ranges of various parameters measured by OenoFoss in white grape must of low alcohol collections over three seasons.

	TSS (°Bx)	Malic acid (g/L)	Tartaric acid (g/L)	TA (g/L)	VA (g/L)	pH	YAN (mg/L)
2014-15	12.1-15.2	3.0-8.2	1.6-4.5	4.5-11.2	0.03-0.15	3.12-3.49	15.4-98.0
2015-16	12.6-17.6	1.0-4.8	5.4-11.0	4.7-13.3	0.02-0.18	3.01-3.58	136.3-309.4
2016-17	13.3-19.8	1.1-4.7	3.1-11.0	5.5-10.3	0.14-0.23	2.93-4.00	139.4-298.4

Table 16. Ranges of various parameters measured by OenoFoss in red grape must of low alcohol collections over three seasons.

	TSS (°Bx)	Malic acid (g/L)	Tartaric acid (g/L)	TA (g/L)	VA (g/L)	pH	YAN (mg/L)
2014-15	12.1-16.5	0.9-8.6	8.5-11.9	6.6-15.4	0.02-0.12	2.99-3.38	143.7-283.5
2015-16	11.2-15.9	0.6-4.8	5.3-8.8	6.7-11.8	0.03-0.10	3.06-3.38	148.3-365.2
2016-17	16.0-20.0	0.7-6.6	4.1-5.3	5.5-8.4	0.09-0.38	3.13-3.72	137.9-425.1

Table 17. Ranges of anthocyanin and phenolics measured in red grape musts of low alcohol collections over two seasons.

	Anthocyanin (mg/g)	Anthocyanin (mg/berry)	Phenolics (absorbance/g)	Phenolics (absorbance/berry)
2014-15	0-2.98	0-3.59	0-2.21	0-2.66
2015-16	0.64-2.49	0.36-2.51	0.99-1.9	0.82-1.75

Table 18. Ranges of various parameters measured by OenoFoss in wines made from white grapes of low alcohol collections over three seasons.

	Glucose:Fructose ratio	Ethanol %	Malic acid (g/L)	TA (g/L)	VA (g/L)	pH
2014-15	1.6-3.1	7.4-10.0	1.9-5.7	5.8-11.1	0.19-0.47	2.93-3.30
2015-16	0-2.3	7.6-10.7	0.5-3.5	6.5-13.0	0-0.36	2.53-3.19
2016-17	1.6-4.4	9.0-12.0	0-3.1	5.5-10.3	0.14-0.23	2.6-3.27

Table 19. Ranges of various parameters measured by OenoFoss in wines made from red grapes of low alcohol collections over three seasons.

	Glucose:Fructose ratio	Ethanol %	Malic acid (g/L)	TA (g/L)	VA (g/L)	pH
2014-15	1.9-3.3	6.5-9.4	1.3-5.9	6.5-13.2	0.10-2.37	2.92-3.47
2015-16	1.3-2.3	7.3-9.1	1.0-4.7	7.0-10.1	0.14-0.34	3.21-3.44
2016-17	2.0-2.7	8.6-11.9	0-0.9	5.5-8.4	0.30-0.38	3.35-3.75

Table 20. Ranges of anthocyanin, phenolics and tannins measured in wines made from red grapes of low alcohol collections over two seasons.

	Anthocyanin (mg/L)	Phenolics (mg/L)	Tannins (mg/L)
2014-15	119.61-766.60	261.88-1885.23	
2015-16	80.16-214.25	320.83-837.59	0-237.38
2016-17	390.49-1259.77	412.82-1366.03	26.73-268-.11

7.7 Antioxidants

The Orac (Oxygen radical antioxidant capacity) assay was used to measure antioxidant potential of the grape, grape marc and wine marc, and wine samples from red selections. The antioxidant standard Trolox was assayed at varying known concentrations from which a linear standard curve was generated. Trolox Equivalents mMol/g was calculated for each sample. As some of the selections had red flesh and had increased anthocyanin levels, it was decided to compare some of the selections to Cabernet Sauvignon. It was found that the selections had higher antioxidant potential than Cabernet Sauvignon and this was found for both fruit and wine (Table 21). The levels found in the juice were lower, as expected, than the whole grape. The highest levels were found in the marc which was composed of skin and seeds.

Table 21. Antioxidant potential of various berries and berry products. Antioxidant potential is measured as Trolox equivalents TE mMol/g.

Samples	TE (mmol/g)
Cabernet Sauvignon ripe grapes (22 °Bx)	31.9
Selection 37_14_05_01 ripe grapes (22 °Bx)	53.4
Selection 37_14_05_01 grapes juice only (22 °Bx)	20.7
Selection 37_15_04_02 low sugar grapes (18 °Bx)	58.6
Pooled red Selections wine marc (est. 11.6%)	118.7
Selection 37_14_05_01 marc from fresh juice (22 °Bx)	106.4
Selections 37_14_06_01 & 37_15_07_03 wine 2016 (11.9% ethanol)	41.8
Cabernet Sauvignon wine 2016 (12.2% ethanol)	20.1

7.8 DNA markers and genetics of muscat flavour

Previous studies in grapevine identified the gene 1-deoxy-D-xylulose 5-phosphate synthase 1 (VvDXS1), the first enzyme of the methylerythritol phosphate pathway for isoprenoid precursor biosynthesis as a candidate gene involved in muscat flavour (Battilana et al., 2009; Emanuelli et al., 2010). DNA SNP markers were developed for polymorphic regions in the gene and evaluated to determine if SNP markers were a good predictor of muscat and floral flavours.

It was found that one DNA SNP marker in the gene VvDXS1 was linked to muscat flavour and Mendelian segregation was demonstrated by test crosses and back crosses with plants possessing the Muscat allele (M). Chi squared values were calculated to determine goodness of fit. None of the crosses had significant p-values which indicate that the marker was inherited in a Mendelian fashion (Table 22). A strong association was observed between muscat flavour detection and the muscat allele (M) indicating that it was a dominant allele. All plants that were homozygous for the muscat allele (MM) had berries with muscat flavour. Nearly all plants having the heterozygous genotype (Mm) had muscat flavour, however, no muscat flavour was detected in a small proportion of these plants. Virtually all plants without the muscat allele had no muscat flavour in their berries.

The development of this SNP marker for muscat flavour will allow it to be used in future marker-assisted breeding (MAS) efforts to select plants at the seedling stage for individuals with muscat flavoured berries.

7.8.1 Linking sensory analysis to muscat flavour in white berries

White selections (Table 23) having the Muscat SNP genotype (Mm) were used to investigate the role of terpene compounds in sensory studies. PCA was conducted for the five terpene compounds quantified (linalool, *cis*-rose oxide, nerol, citronellol and geraniol) on white berry selections and loaded as main variables. Ratings for the ten berry sensory attributes of the same selections evaluated for flavour and taste were included as supplemental variables of the PCA. Dimensions one and two of the PCA explained 73.61% of the variability of the selections' volatile composition (Fig. 37). Component F1 accounted for 52% of the variability, mainly driven on the positive direction by content of nerol and citronellol in selection W24. In the negative direction, F1 was driven by the sensory attributes skin green grassy flavour and skin green flavour ratings, mainly from selection W5 but also from selections W21 and W25. Component F2 explained 21% of the variability mainly driven by high concentrations of geraniol particularly in selection W18. Linalool and cis-rose oxide also were important at explaining the variability in components F1 and F2, due to the higher concentrations of these two compounds in selections W22, W23, W24 and W26. Pearson correlation analysis between the five aroma compounds and the twenty berry sensory attributes revealed important relationships. Sensory attributes such as pulp floral flavour and pulp tropical flavour helped to explain the variability of the selections in component F2 and pulp muscat flavour to a lesser level. Geraniol content was positively correlated to berry firmness (r=0.712), the more geraniol the firmer the white hybrids were. *Cis*-rose oxide was positively correlated to pulp juiciness (r =0.699) and negatively correlated to pulp detachment (r=-0.647). Linalool was positively correlated to pulp floral flavour (r=0.661) and muscat pulp flavour (r=0.590). *Cis*-rose oxide was another aroma compound that had a positive correlation, although not very high, with muscat pulp flavour (r=0.551) and pulp floral flavour (r=0.492).

The conclusion from this study is that while all selections had the muscat allele, there were distinct differences in the sensory results between the selections and that this difference could in part be explained by the levels of some terpene compounds. However, other sensory attributes also play a part in differentiating the selections.

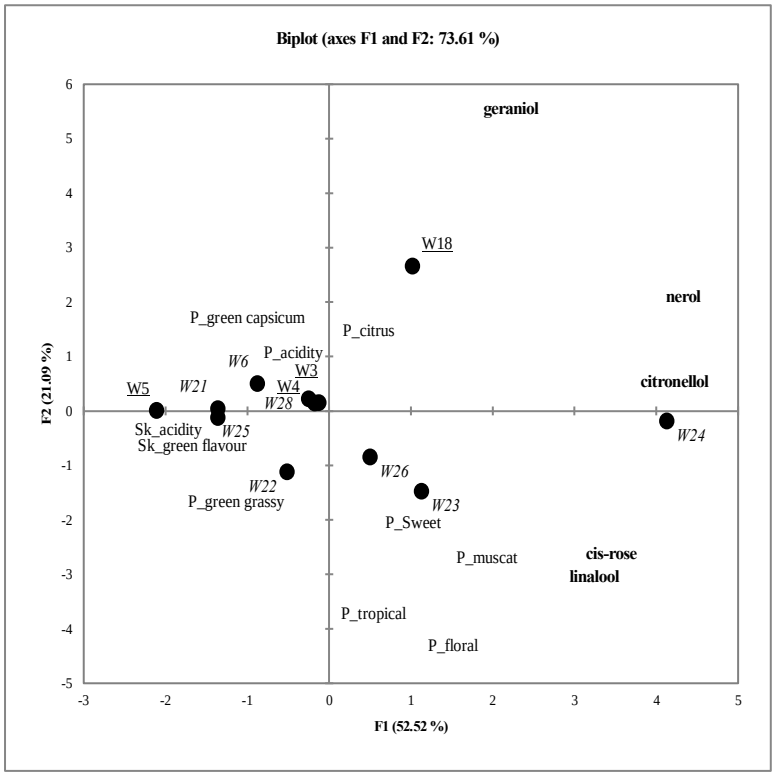
Table 22. Chi squared values and p-values to test goodness of fit to Mendelian inheritance (cut off p-value less than or equal to 0.05). The genotypes of progeny from both test crosses and F1 selfed crosses were scored.

Crosses	Female parent genotype	Male parent (genotype)	Chi square value	p-value
Test Cross 1	mm	Mm	1.286	0.257
Test Cross	mm	Mm	1.064	0.302
Test Cross	mm	Mm	0	1.000
Test Cross	mm	Mm	0	1.000
Test Cross	mm	Mm	1.600	0.206
Test Cross	mm	Mm	1.778	0.182
F1 self cross	Mm	Mm	2.872	0.238
F1 self cross	Mm	Mm	2.000	0.368

Table 23. Muscat SNP genotype of white berry selections analysed for monoterpenes

Short Codes	Hybrid selections	Muscat SNP
W3	37_28_06_02	Yes
W4	37_28_07_03	Yes
W5	37_28_10_01	No
W6	37_28_11_01	Yes
W18	39_15_04_03	Yes
W21	39_17_07_02	Yes
W22	39_17_09_01	Yes
W23	39_17_10_03	Yes
W24	39_18_03_01	Yes
W25	39_18_03_02	No
W26	39_18_04_01	Yes
W28	39_19_02_01	Yes

Figure 37. Principal component analysis (PCA) of volatile aroma compounds. Berry sensory attributes data from 11 white berry genotypes for the season 2015



8. Outcomes and conclusions

This project investigated methods for the screening of 1200 new mildew-resistant winegrape selections to identify individuals that had both superior viticultural performance and wine attributes. The evaluation process was done over several seasons as it is well known that phenotype is a function of genotype, environment and management (P=GEM) and differences across seasons is due to the environmental conditions, mainly driven by the climate, when management is consistent.

It was found that there was large variation in agronomic traits measured in the field, berry chemistry, wine chemistry and wine sensory. The large variation observed for the traits measured is likely due to the genetic differences between the individual plants. The plants were obtained by conventional breeding and screened at the seedling stage using DNA markers to select for those with powdery and downy mildew resistance and berry colour before planting in the field (Wine Australia report CSP 0904). Nine different crosses were undertaken to produce progeny with red grapes and 12 different crosses were done to produce progeny with white grapes. Grapevine varieties have highly heterozygous genomes as very little breeding and backcrossing has occurred for cultivar development, unlike for other crops like cereals. The high genetic difference in the genome and subsequent breeding would result in genetically diverse grapevine progeny that would have high variation in traits.

It was found that the best criteria for identifying superior selections were yield data, harvest date, wine chemistry and wine sensory results. The need for acid addition to lower pH for winemaking was also looked at during the selection process to avoid selections that had high berry pH. From the 1200 plants, 20 red selections and 20 white selections were selected to multiply for regional trials and future larger scale winemaking trials. The 20 selections for both reds and whites captured a range of wine styles and flavour and aroma profiles. This suggests that future breeding and selection strategies should focus on measuring these factors and that some of the traits measured in this study need not be measured resulting in a saving of time and resources. Berry sensory studies were undertaken to investigate whether this approach would allow rapid selection and avoid the need for small-scale winemaking, however, it was found that the use of trained tasting panels limited the number of samples that could be assessed in a season.

Sensory evaluation of small-scale wines involved industry winemakers and experienced research winemakers and sensory scientists. Red wines favoured by the panel had high colour and a diversity of flavours that ranged from like Cabernet Sauvignon to Shiraz as well as some unique styles. The highly coloured wines had red flesh parentage and these were found to also have high antioxidant potential. Many of the 20 red selections carried forward for regional trials have red flesh. The white wines most favoured by the sensory panel were aromatic and floral. An examination of the parentage of these selections showed that they had a Muscat or Riesling parent. Most of the 20 white selections carried forward for regional trials had either Muscat, Riesling or Chardonnay parentage. The data suggests that fewer parents are necessary for future breeding experiments and that instead of conducting 10 red crosses and 10 white crosses it would be possible to reduce the parents to 2-3 red and 3 white parents. The results also indicate that for future breeding it will not be necessary to plant and screen up to 1200 plants and that due to the reduced number of parents and increased DNA markers used, it should only be necessary to plant 250-500 individuals for field assessment and wine evaluation to identify superior selections for industry.

Low alcohol wines were also made from selected red and white berry selections by harvesting early at low sugar levels. The objective was to see if it was possible to make attractive low alcohol styles without the need for additional manipulations in the winery. The sensory results indicated that the reds showed the most promise producing Beaujolais style, but further research is required.

The diversity of aromatic and floral flavours and aromas observed from wine of selections with Muscat parentage indicated that it would be a good parent for future breeding. To improve future selection processes, this study developed a SNP DNA marker so that progeny at the seedling stage could be screened for muscat flavour. This SNP marker has been incorporated into a SNP panel that can be used to rapidly screen seedlings before field planting for the following traits; powdery and downy mildew resistance, berry colour, flower sex (fruitfulness), stature and muscat flavour.

9. Recommendations

This study completed the first large-scale screening of mildew resistant vines in Australia to identify the best 20 red and 20 white mildew-resistant selections for regional field trials. A new SNP DNA marker was developed that can be used in new breeding efforts using marker-assisted selection (MAS). The results also showed that small-scale wine made from single vines is effective to screen for superior selections when the wine sensory panel consists of both industry winemakers and researchers. The research also found that in future studies it is not necessary to collect data on all of the traits measured in this study and that only a subset of these traits needs to be measured for an effective selection process. It is recommended for future MAS breeding that a reduced number of parents identified in this study be used, that the SNP marker for muscat flavour be used in MAS and that the traits measured be reduced to collect data only for yield, harvest date, wine chemistry, must pH and wine sensory. Digital technologies for rapidly measuring traits in the field are under development in a number of countries and it is suggested that these technologies be used in future research to increase the number of traits measured if the technologies reduce the time and resources involved in assessment of the trait as the major issue with collecting trait data is the time and resources required.

Larger scale regional field trials are essential to determine the suitability of the vines to produce acceptable yields under different management treatments and different climates. These regional trials will assist with industry adoption by providing the opportunity for larger-scale winemaking and for the regional industry to assist in the evaluation of the wines. Four regional field sites that cover the main climates where grapes are grown (cool to hot irrigated) have been established in the Barossa Valley SA, Irymple VIC, Riverina NSW, and Orange NSW and it is recommended that the Australian industry continues to support regional trials as a means not only to evaluate selections, but as a mechanism to engage researchers and industry to enable adoption.

Appendix 1: Communication

Grapevine genetic research delivers disease resistance and quicker results. (2013) Australian & NZ Grapegrower & Winemaker p.24

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Appendix 2: Intellectual Property

The IP produced by this project is in the form of new winegrape varieties that could be adopted by industry after further regional evaluation and a SNP DNA marker for muscat flavour. In addition, new knowledge was obtained about methods for field evaluation of new varieties and small-scale winemaking methods for assessing wine from new varieties. The information obtained may help industry make informed decisions about the suitability of new disease mildew resistant varieties for Australian conditions and the type of wine produced and yields that might be achieved with fewer sprays to control mildew.

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Appendix 5: Other material and raw data

Raw data are retained at CSIRO