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Using in-canopy misters to mitigate the negative effects of heatwaves in grapevines (UA 1502)



FINAL REPORT TO WINE AUSTRALIA

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Abstract

Heatwaves in South Australian viticultural regions are becoming more frequent and lasting longer. To address this environmental challenge, a study was undertaken in the Riverland and Adelaide regions on Sauvignon Blanc and Cabernet Sauvignon grapevines, respectively, during the 2016-17 and 2017-18 growing seasons. Grapevine canopies were cooled using water-based evaporative cooling either in the canopy with fine mist applications (ICM) when temperatures exceeded 35 °C, or with undercanopy sprinklers (UCS) or supplemental drip irrigation (SI) during warm nights preceding heatwaves. Canopy temperatures were 5 °C cooler with lower vapour pressure deficits (VPD) than ambient in all cooling treatments. Cooling resulted in improved vine water status, physiological performance (gas exchange), and yield in many of these treatments, but especially in the UCS treatment at the Riverland site for Sauvignon Blanc. During heatwaves, the SI treatment with additional irrigation maintained respective undervine and midrow temperatures approx. 2 °C and 7 °C lower than controls. SI and UCS vines consistently had superior water status and gas exchange than controls, and maintained these levels irrespective of prevailing environmental conditions. SI vines also had the highest yields and crop loads in both seasons. Differences between treatments were not as evident at the Adelaide site with Cabernet Sauvignon. The Riverland fruit under misting had greater levels of volatile compounds but had lower wine polyphenols compared to controls. UCS and SI treatments had higher juice and wine acidity. A simulation model of canopy cooling based on misting accurately predicted canopy, leaf and bunch temperatures resulting from mist applications under various environmental conditions, and can be incorporated in the future into automated mist controllers in the vineyard. It was found that the method of water application was critical for effective cooling and improved vine performance. Future work in this area should investigate the use of undercanopy sprinklers, in particular the role that root temperatures play in improving vine performance during heatwaves.

1 Executive Summary

Climate change in South Australian viticultural regions has prompted much research in the areas of drought stress mitigation, and, more recently, heat stress mitigation in vineyards. With an increasing incidence, severity and duration of heatwaves over the years, practical strategies are being sought by the industry to manage grapevines under these adverse conditions and to improve their resilience to future similar events. Very few studies have investigated the efficacy of water-based evaporative cooling in vineyards and the effects of cooler/warmer grapevines on wine quality. Strategies such as overhead sprinklers are currently being utilised in vineyards in Australian inland regions, and although effective, they are large consumers of water and much of the water is lost through evaporation. We aimed to evaluate similar but more efficient approaches of water-based cooling in vineyards based on in-canopy misting and undervine sprinklers, and to compare these approaches to additional water applications prior to and during heatwaves.

Heatwaves are defined by SA-BOM as being five or more days above 35 °C or three or more days above 40 °C, but this definition was not specific to winegrapes or agriculture. Key physiological processes such as photosynthesis become compromised beyond 37 °C. This threshold led to us re-defining the temperature threshold for grapevines for the purposes of this study to 35 °C, and heatwaves being defined as three or more consecutive days at or above this temperature. Prolonged periods of even moderate temperatures (30-32 °C or higher) can result in impaired performance as heat stress, often in conjunction with water stress, can impair vine performance. With this new threshold defined, our objectives in the present study were to assess the efficacy of in-canopy misting (ICM), undercanopy sprinklers (UCS), and supplemental irrigation with drippers (SI) on: (i) canopy microclimate, (ii) vine physiology, (iii) fruit development, (iv) yield and vine vigour, and (v) juice and wine composition. The study used two sites: (i) Yalumba Oxford Landing Estates in Qualco, SA (Riverland) planted with mature Sauvignon Blanc vines grafted on Ramsey rootstock, and (ii) Alverstoke vineyard, Waite Campus, Adelaide, SA planted with own-rooted Cabernet Sauvignon vines. Canopy microclimate, vine performance and fruit composition was monitored over two growing seasons, 2016-17 and 2017-18. Wines made at the end of each season underwent compositional analysis including flavour and aroma compounds.

In both seasons, ICM had a dramatic effect on canopy and leaf temperatures, decreasing temperatures by between 5-12 °C compared to control (uncooled) vines. Bunch temperatures were only about 3 °C cooler due to their high thermal mass. Vine water status and physiology were not altered as a result of mist applications. With misting, yields and pruning weights were slightly higher in the Riverland site in the first season but not in the second season. No changes in fruit and wine composition, or cell vitality were observed due to the misting with the exception of increases in certain volatile compounds such as linalool, isoamyl acetate and β-damascenone, which contribute fruity aromas to the wine. Fruit from the misted vines also had lower polyphenols compared to controls. In terms of water application rates, misting accounted for approx. 3-4% of total water used for irrigation in the Riverland block and 5-17% in the Waite block.

Undercanopy water applications were conducted at night for two hours preceding a heatwave. Each treatment – sprinkler and supplemental irrigation – accounted for 7-11% of the total season irrigation, or between 0.5-1.1 ML/ha. Both treatments were effective in reducing canopy temperatures and VPDs making conditions more favourable for vine physiology during heatwaves. While soil moisture levels were unaltered with the undercanopy water applications, vine water status improved compared to uncooled treatments. This resulted in higher net photosynthesis and stomatal conductance values

even after the heatwave indicating better vine recovery with these treatments. In many cases, no decreases in gas exchange were observed even on the warmest days (T > 40 °C) calling into question the temperature thresholds for grapevine that were previously proposed. It therefore appears that a favourable canopy microclimate with high humidity and even perhaps cooler soils and roots may allow vines to continue performing at their optimum even on the warmest days. Undercanopy cooling with the SI treatment resulted in slightly higher yields in both seasons, but no changes were observed in juice and wine composition except for higher TA and lower polyphenols. In Cabernet Sauvignon (Alverstoke), the undervine cooled vines were more balanced with Ravaz Index values between 4-5 compared to the control vines that were undercropped (Ravaz Index ~ 1.2).

In comparing the Riverland and Waite vineyards, the Riverland site tended to be more responsive to the cooling treatments compared to the Waite location, with a number of reasons possibly explaining this. First, the Riverland experiences more heatwaves that tend to be warmer and longer in duration as warm air moves south from the centre of Australia. Second, the Waite vineyard is organically managed with a permanent fescue on the floor that likely moderates temperatures and retains soil moisture allowing roots to stay cooler than the Riverland site, which has a bare floor. Finally, vine size and crop load differences between the two vineyards could have contributed. The Waite Cabernet Sauvignon vines were undercropped (Ravaz Index ≤ 1.5) while the Riverland Sauvignon Blanc vines were overcropped (Ravaz Index > 16).

One of our goals was to develop an automated system to trigger and control the mister-based cooling system that would regulate the canopy temperature to 35 °C or below. In order to achieve this, a simulation model was developed using the Scilab programming language. The model was successfully developed and validated versus real vineyard data of canopy, leaf and bunch surface temperatures. Based on the mister settings used in the Riverland location, the model accurately predicted a temperature drop of the canopy (air) by 10-15 °C, leaf temperature drops of 20 °C and bunch temperature drops by 5 °C. A scenario analysis of various mister settings and environmental parameters determined that the efficacy of cooling is greater when (i) the frequency of misting is increased, and (ii) misting is applied until the leaves and bunches are wet thereby allowing greater evaporative cooling from those surfaces to draw out sensible heat. The model is now fully deployable on a portable Raspberry Pi microcontroller with capability to communicate with future control systems for automated operation of misting solenoids.

The project has successfully met all the objectives and delivered:

- New heatwave (high temperature) mitigation strategies for vineyards based on evaporative cooling of water;
- Evaluation of the efficacy of different cooling strategies including in-canopy misting and undervine sprinklers and drip irrigation;
- Data on the responses of vine performance (water status, gas exchange, productivity) based on various cooling approaches in two cultivars, Sauvignon Blanc and Cabernet Sauvignon, and at two contrasting locations, Riverland and Adelaide;
- Quantification of the effects of cooling on fruit and wine composition including flavours and aroma compounds;
- A new simulation model to predict canopy, leaf and bunch temperatures in response to misting; this model is ready for vineyard deployment on a microcontroller; and,
- Knowledge dissemination to the industry via seminars and articles.

2 Background

The increasing severity and duration of high temperature events and freshwater scarcities are implications of climate changes faced by the wine industry, especially for the Mediterranean and semi-arid viticultural regions of Australia. Heatwaves – defined in this case as daily maximums reaching 35°C or greater for 3 or more days – can be detrimental to vine physiological performance, reproductive biology, yield, and wine composition (Greer and Weedon 2014). High temperatures, particularly during key phenological stages of development such as flowering, have been shown to have a negative effect on fruit set and ultimately on yield (Pagay and Collins 2017). Based on a limited number of studies, there appear to be differences between cultivars of grapevine as well. For example, Shiraz appears to have a higher tolerance to high temperature events compared to Semillon (Greer and Weedon 2017, Soar et al. 2009).

Canopy temperatures can be lowered by using overhead shade cloth, canopy-skirting netting, sprinklers, reflective sprays based on particle films, increased irrigation to promote transpirational cooling, adjusting row orientation and vine training system, and various cultural practices. However, evaporative cooling is the most effective technique to lower canopy temperatures during heatwaves since it takes advantage of the high latent heat of vaporisation of water to convert sensible heat to latent heat.

In California, Aljibury et al. (1975) found that temperatures above 38.8°C negatively affected grape berry development by resulting in sunburn, desiccation, and reduced yield. Use of overhead sprinklers in their experiment resulted in delayed ripening by 2-3 weeks and 20% higher berry weights. Gilbert et al. (1971) also used overhead sprinklers in vineyards for 3 min cycles on 15 min intervals and observed temperature decreases of 4 °C and 8 °C in the air and leaves, respectively. Overhead sprinklers reduced berry temperatures by 5 °C and increased berry sizes in potted table grape and wine grape cultivars when ambient temperature exceeded 30°C (Kliewer and Schultz 1973). Greer and Weedon (2014) tested overhead sprinklers on Semillon canopies and found a 7-8 °C canopy air temperature reduction when ambient temperature was above 40 °C. Additionally, the fruit in the overhead sprinkler treatment had higher soluble solids at harvest compared to the control. Studies using sprinkler cooling have also been done on various other horticultural crops including oranges (Brewer et al. 1979), potato and bush beans (Hobbs 1973), apples (Gindaba and Wand 2005, Iglesias et al. 2005), strawberries (Chesness et al. 1970), pears (Dussi et al. 1997), lettuce (Baasiri and Ryan 1984), corn (Saadia et al. 1996), cranberry (Pelletier et al. 2016), and snap beans (Kemp et al. 1974). In general, these studies observed air temperature decreases of 4-8 °C, higher yields, and few changes to fruit composition.

Under Australian conditions, Caravia et al. (2017) have shown that in-canopy sprinkler cooling on Cabernet Sauvignon grapevines was highly effective in reducing air temperatures and had a positive effect on berry size and yield (25-30% increase) without altering grape composition. In their trial, only a few cooling events when temperatures exceeded 38 °C were sufficient to have a significant effect on berry ripening. Noticeable decreases in berry cell death (or increased cell vitality) were also observed under sprinkler cooling compared to uncooled fruit. In Riverina (NSW), Greer and colleagues found that Semillon grapevines also responded positively to hydrocooling, where cooled fruit had a higher rate of ripening over the season as well as higher soluble solids compared to uncooled fruit (Greer and Weedon 2014).

In this study, various forms of evaporative cooling were investigated on two grapevine cultivars – Sauvignon Blanc in the Riverland (SA), and Cabernet Sauvignon in Adelaide. The evaporative cooling

methods consisted of in-canopy misting or cooling (ICM) and under-canopy cooling based on either broadcast sprinklers (UCS) or supplemental irrigation (SI). The cooling treatments were applied during heatwaves to mitigate heat stress effects on grapevines. We assessed the effects of evaporative cooling on canopy and vineyard floor microclimates, vine physiological performance, berry development, yield, and fruit and wine composition over two seasons, 2016-17 and 2017-18.

3 Project Aims

Target Date – FY1	Outputs	Activities
31/7/2016	Deliver finalised project plan	Meet with project personnel to finalise project design, plan, and timeframe of data collection, and individual responsibilities
30/9/2016	Canopy cooling simulation model (v1) built	Build canopy cooling models and obtain simulation results of various cooling scenarios, survey literature on topic
31/10/2016	Sites checked and vines marked for use; mister system designed and installed	Identify and purchase misting system parts and environmental sensors; verify trial sites (3) and vines (2 cultivars x 3 reps x 6 vines/rep); install and test misting system
1/2/2017	Submit mid-year progress report	Generate initial progress report via CIMS
31/5/2017	Initial treatment efficacy assessed (November-May)	Measurements of canopy temperature, soil and vine water status, leaf gas exchange, berry development (size, weight), berry cell vitality, root and xylem ABA, juice/must composition (sugars, acids, YAN, glutathione, polyphenols, aromas and precursors), mister water use (volume); small-scale winemaking, wine composition analysis (polyphenols, aroma compounds and their precursors)
1/8/2017	Submit year-end progress report	Collate and analyse data from FY1; generate FY1 progress report via CIMS

Target Date – FY2	Outputs	Activities
30/9/2017	Canopy cooling simulation model (v2) built	Canopy cooling model v2 built based on refinements of v1 and Y1 field validation of canopy temperatures
1/2/2018	Submit mid-year progress report	Generate progress report via CIMS
31/5/2018	Y2 treatment efficacy assessed (December-May)	Implement improved (v2) canopy cooling model; Measurements of canopy temperature, soil and vine water status, leaf gas exchange, berry development (size, weight), berry cell vitality, root and xylem ABA, juice/must composition (sugars, acids, YAN, glutathione, polyphenols, aromas and precursors), mister water use (volume); small-scale winemaking, wine composition analysis (polyphenols, aroma compounds and their precursors)
30/6/2018	Factsheets, journal articles, field day seminars	Write fact sheets, articles, scientific papers; present data at grower field days and scientific conferences in 2018
30/6/2018	Final report	Collate and analyse data from FY2; write comprehensive report by June 30, 2018

4 Materials and Methods

4.1 Vineyard site, plant material and experimental design

The trials were conducted at Yalumba Oxford Landing Estate (OLE), a commercial vineyard in the Riverland, South Australia (34°06'06.29" S and 139°50'39.21" E) over two seasons, 2016-2017 and 2017-18. The trial used mature Sauvignon Blanc on Ramsey rootstock planted at 1.8 m between vines and 3 m between rows (1,852 vines/ha). Row middles were resident vegetation with an herbicide strip maintained under the vines. The vines were mechanically box pruned and trained to a four-cordon sprawl canopy. The in-canopy cooling (ICC) trial was a randomized block design replicated three times with ten vines per treatment within each twenty-vine replication, plus three buffer vines. The two treatments were misting (M) and control (unmisted, C). The undercanopy cooling (UCC) trial was a Latin squares design replicated three times with twenty-three vines per treatment within each sixty-nine-vine replication. The treatments were undercanopy sprinklers (UCS), supplemental irrigation (SI), and control or un-cooled vines (C). Conventional irrigation and viticultural practices typical for the region were applied at the cooperating grower's discretion.

Canopy cooling was also assessed on mature, own-rooted Cabernet Sauvignon vines at Alverstoke vineyard (ALV) of the Waite Campus, The University of Adelaide (34°58'04.06"S and 138°38'13.15" E). The north-south oriented vines were planted at a spacing of 2.0 × 3.3 m (vine × row), 1,515 vines/ha, cordon-trained and spur pruned to two bud spurs for a shoot density of approx. 10-12 shoots per m of cordon, with a sprawling canopy. Irrigation and viticultural practices typical for the region were applied over the season. During the ripening period, vines were irrigated for six hours twice per week with an effective irrigation of approx. 8.5 mm per week.

Oxford Landing Cooling Setup

In-canopy misting (ICM) and control (un-misted) treatments were set up in three rows (blocks) with each block (row) consisting of 23 vines with 11 vines used per treatment and one vine left as buffer. The misters were Netafim CoolNet Pro™ with a "tee" configuration (Netafim Australia Pty Ltd. Adelaide, Australia) with a nominal water application rate of 11 L/h per sprinkler. Due to vine architecture, a metal post and wire was installed 25 cm above the top cordon (Figure 1). Two sets of misters were installed: a first set of short length (15 cm) sprinklers was installed between the two cordons; these were hanging downward from a 25 mm irrigation pipe. This poly pipe was installed along the top cordon, approximately 1.65 m from the ground. The mister spacing for this line to mist the lower sub-canopy was 0.8 m. A second line of long misters (65 cm) was installed 25 cm above the top canopy. The misters were supported on an additional top wire and were oriented upwards and located 15-20 cm above the upper canopy (with full length shoots). The system was operated automatically through a "Frostie" Mist Guard Thermal Damage Controller (Netafim Australia Pty Ltd. Adelaide, Australia), which was set to operate when the ambient (canopy) temperature exceeded 35° C. Mist was applied for 20 s every five minutes.

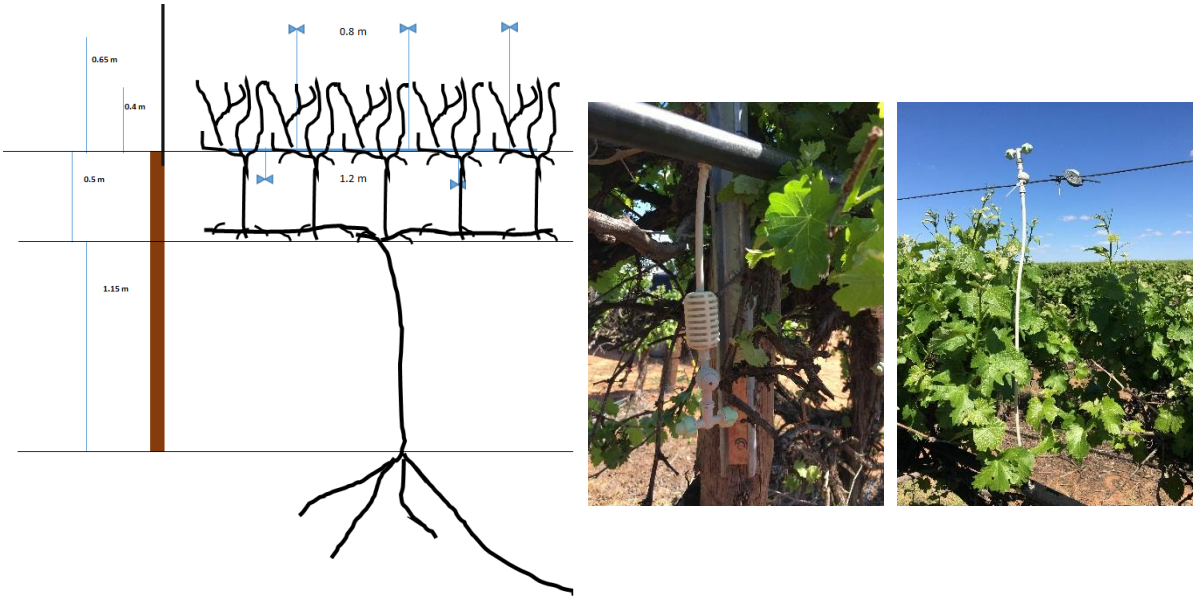


Figure 1: Setup of the in-canopy misting system at Oxford Landing Estates, Riverland. Photos depict the misters deployed in the lower (left) and upper (right) sub-canopies.

Sprinkler setup for the undercanopy cooling (UCC) trial's UCS treatment consisted of a separated pipe along every two rows of each UCS treatment replication with radial sprinklers every 7 m in a triangular pattern (Figure 2A). The water flow rate was established at 27 L/h/vine with a target application of 57 L/vine/night (two hours of water applied). The system was controlled with a telemetry controller (Siemens), which was triggered to turn on during the night preceding a heat event (when next day's high temperature was predicted to be at or above 35 °C and when the night minimum temperature was over 20 °C). The sprinklers turned on for 30 min every 2 h, for a total of four cycles during the night (total duration: 2 h per night).

The supplemental irrigation (SI) treatment consisted of a separated irrigation line with two drippers per vine (flow rate: 8.1 L/h), which was set on six vines per row in each replication (Figure 2B). Seven cycles of 30 minutes on/30 min off between the hours of 22:00 and 04:00 was triggered at night under the same forecast conditions as UCS and with the same controller system. The target flow rate was also 57 L/vine/night as in the UCS treatment. For this part of the trial, a set of control (non-cooled) vines was also measured. In each of the three blocks consisting of three treatments each, three representative vines were measured over the season (total: nine vines per treatment).



(A) **(B)**

Figure 2: **(A)** Setup of the undercanopy sprinkler system, and **(B)** supplemental irrigation system at Oxford Landing Estates, Riverland.

Alverstoke Vineyard Cooling Setup

The experimental design was a randomised complete block with three blocks each consisting of four treatments: misting (M), UCS, SI and control (C). The ICC/misting set up was similar to the OLE vineyard (Riverland): an evaporative cooling system using two CoolNet Pro™ sprinklers per panel with a water use rate of 11 L/h per sprinkler unit (22 L/h per panel of four vines) were located inside the canopy at bunch height (30 cm above the cordon arm). Potable water from the mains water supply was stored in a 1,000 L plastic tank and a pump provided the 0.5 MPa pressure required in the system. The system was operated automatically as in the OLE vineyard using a "Frostie" controller. Water was applied for 15 s every 10 min.

The UCS treatment consisted of undercanopy microsprinklers with adjustable nozzles (Hunter Pro, Model 6A, Hunter Industries, San Marcos, CA, USA) with 360° coverage and a nominal spray diameter of 3.6 m. The water output per sprinkler was approx. 5.3 L/min at 2.5 bar pressure. Three sections of the vineyard block, each with four panels of three vines each, were used for this treatment as shown in Figure 3 below (light blue regions are the UCS treatment). The sections were allocated at the beginning, middle and ends of the rows, and due to pressure and flow requirements, the sections were split into two: east and west covering nine vines each with 6-7 sprinklers per section. The system was operated at night when the forecast states that the maximum temperature the next day will exceed 35°C. A target flow rate of 58 L/vine/night was set. Water was delivered in four cycles: on for 5 minutes and paused for 55 min in each cycle between the hours of 22:30 and 01:55. Each of the six sections were activated by a Hydrawise Wi-Fi-enabled controller (Hunter Industries, San Marcos, CA, USA).

The SI treatment (red sections in Figure 3) consisted of an independent irrigation line with two drippers per vine (flow rate: 8.1 L/h) applied to six vines per row in triplicate. This system was operated

at night similar to the UCS treatment and under the same conditions. The system was operated in seven cycles of 30 minutes on and 30 minutes off starting from 22:00 h until 04:00 h. The system was controlled and pre-programmed through the Hydrawise controller. The flow rate target was 57 L/night/vine, approx. the same as the UCS treatment. A control, un-cooled treatment (green regions in Figure 3) consisting of a set of six contiguous vines per row was used for comparison with the three cooling treatments.

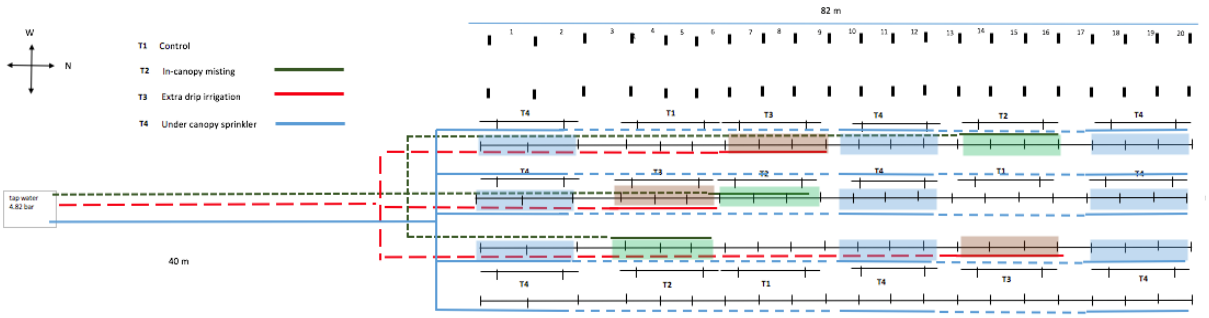


Figure 3: Experimental design at Alverstoke vineyard.

4.2 Environmental parameters

In each treatment, one TinyTag sensor (Model: TGP 4500, Hastings Data Loggers, Port Macquarie, NSW, Australia) was placed in the canopy interior to continuously record ambient temperature and relative humidity every 15 min over the growing season. The sensors were protected by a shell made of foil bubble wrap following the design described in Tarara and Hoheisel (2007). Seasonal weather data was obtained from the on-site automatic weather station (Qualco, SA) maintained by NRM Weather Network. Vapour pressure deficit (VPD) in each canopy and treatment was calculated using the in-canopy canopy temperature and relative humidity from the TinyTag sensors. Prior to deployment in the field, the sensors were tested on the lab over four days. A minimum variation between them was observed: the maximum difference between the means of all of them were ±0.16°C and ±1.35% RH.

4.3 Physiological measurements

4.3.1 Soil and vine water status

Soil matric potential was estimated as the pre-dawn leaf water potential (Ψ_{pd}) using a leaf pressure chamber (Model: 1505-EXP, PMS Instruments, OR, USA). Two leaves per vine were measured between 03:00-05:00 h. Midday stem water potential (Ψ_s) was measured using the same instrument by bagging two leaves per vine with opaque bags for at least one hour prior to measurement. Measurements were done between 11:00-14:00 h except on the warmest days (> 40 °C) when measurements were done between 10:00-13:00 h.

4.3.2 Canopy temperature

A handheld thermal infrared (IR) camera (Model B400, FLIR Systems; IR resolution: 320 × 240 = 76,800 pixels; spectral range: 7.5-13 µm) was used to record IR and RGB photographs simultaneously of each of the measurement vines' canopies. Images were captured obliquely to the canopy to fit the entire canopy area into the camera's field of view of 25°. The IR camera's microbolometer sensors had a

thermal sensitivity of 0.05 °C across their full range of temperatures from -20°C to +350°C with an accuracy of ±2% as per the manufacturer's specifications. Thermal images were processed using 'FLIR Tools' image processing software (v.5.4, FLIR Systems). Minimum, maximum and average canopy temperatures were extracted from each canopy's thermal image. Using the same approach, temperatures of the vineyard floor (interrow and undervine regions) were also captured for the various undercanopy cooling treatments.

Detailed temperature measurements of the leaves and bunches (internal and surface) were made using fine wire thermocouples connect to a datalogger (Model CR1000, Campbell Scientific, Queensland, AU) and solid state thermocouple multiplexer (Model AM25T, Campbell Scientific, Queensland, AU). Using reflective tape, thermocouple junctions were attached to the upper surfaces of leaves and bunches, as well placed in the interiors of bunches near the rachis in the ICC trial. Leaves and bunches were preferentially selected, if they had direct contact with mist in the inner canopy of the ICM treatment. Of the 24 thermocouples installed, only18 successfully logged data from three vines in each treatment.

4.3.3 Leaf gas exchange

Leaf gas exchange was measured on two full-expanded leaves per vine, three vines per treatment in each block and vineyard, using a open-system portable infrared gas analyser (Model: LI-6400XT, LI-COR, Lincoln, NE, USA) over the season before, during, and after heatwaves. Leaf cuvette (area: 6 cm^2) environmental conditions were: reference CO_2 – 400 ppm, saturating light – 1500 µmol/m^2/s, relative humidity – 35-50%, temperature – ambient (uncontrolled), flow rate: 500 µmol/s. From the measured parameters, net CO_2 assimilation rate (A_n), transpiration rate (E), stomatal conductance (g_s), and intrinsic water use efficiency (A_n/g_s) were calculated by the instrument. Measurements were performed simultaneously with midday stem water potential measurements and on the same leaves.

4.3.4 Xylem ABA

On January 30th, 2017, during a heatwave, sap was collected from vines in the UCC trial or analysis of abscisic acid (ABA) concentration by LC-MS. After measuring stem water potential, extra pressure was applied to the leaf in the pressure chamber to force sap out through the petiole. Sap was collected with a 1 ml pipette into vials, frozen in the field with liquid nitrogen, then stored at -80°C until LC-MS analysis using methods described by Speirs et al. (2013). Briefly, the volume of each sample was measured using a pipette for normalisation. Each sample was mixed with 30 µL of deuterated standard (Plant Biotechnology Institute, Saskatoon, SK, Canada) containing deuterium-labelled analogues of ABA, phaseic acid (PA), dihydrophaseic acid (DPA) and the glucose ester of ABA (ABA-GE) at a concentration of 100 ng/mL each. Solids were precipitated in a centrifuge at 12,470 × g for 5 min. From each sample, 20 µL supernatant was transferred to a LC/MS tube and analysed by liquid chromatography/mass spectrometry (Agilent 6410 Triplequadropole LC-MS/MS with Agilent 1200 series HPLC, Agilent Technologies Inc. Santa Clara, USA). A Phenomenex C18 column (75 mm × 4.5 mm, 5 µm; Phenomenex, Torrance, CA, USA) was used at 40 °C and samples were eluted with a 15 min linear gradient of 10 % to 90 % acetonitrile. Nanopure water and acetonitrile were both mixed with 0.05% acetic acid. Compounds were identified by retention times and mass/charge ratio. This measurement was conducted only in the first season.

4.3.5 Berry development

From two weeks post-fruitset until harvest, 10 bunches from 5 randomly-selected vines per treatment were sampled for berry development assessment. The fruit was split into two groups: fresh analysis and volatiles analysis. The fresh batch of grapes was placed in a cool box with ice packs and transported to the lab where they were stored overnight at 4°C until analysis the next day. A sample of 50 berries from all bunches were obtained for determination of size, colour and composition. The batch of grapes for volatiles was snap frozen at harvest in liquid nitrogen and transported to the laboratory over dry ice, then stored at -80°C until analysis by HS-SPME-GC-MS (see section below on wine volatiles analysis).

4.3.6 Berry Cell Vitality

Fluorescence microscopy was used to quantify berry cell vitality at harvest in the ICC trial. A total of twelve berries were collected from four vines in each treatment. Berries were preferentially selected from clusters that had direct contact with mist in the inner canopy of the ICM treatment. Berries were refrigerated overnight and analysed the next day using the staining method described by Tilbrook and Tyerman (2009). This measurement was conducted only in the first season.

4.4 Yield components and pruning weights

The Sauvignon Blanc vines were harvested on February 13, 2017 and February 5, 2018 at Oxford Landing Estates (OLE), and the Cabernet Sauvignon vines were harvested on March 13, 2017 and March 10, 2018 at Alverstoke Vineyard (ALV). Yield components were measured including yield per vine and bunch number per vine. Average bunch weight was calculated from these two numbers. 50 berries were later sampled from each lot to calculate average berry weight and average berries per bunch.

Three grapevines per treatment and block were pruned and pruning weights were measured in 2016-17 and 2017-18. In the OLE vineyard in 2017-18, the vines were pre-pruned early and therefore only ancillary pruning data was available: basal cane diameter, internode lengths of three internodes on a cane and their weights.

4.5 Winemaking

The fruit was crushed using a small electrical crusher-destemmer, and the musts sampled for later chemical analyses. The Sauvignon Blanc musts were pressed using a basket press (10 L capacity). Both cultivars were inoculated with Lalvin EC-1118 'prise de mousse' yeast (*Saccharomyces cerevisiae bayanus*, Lallemand) at the manufacturer's recommended rate. The ferment size was approx. 4 L. Diammonium phosphate was added at 100 ppm prior to inoculation with yeast. Skins of Cabernet were punched down twice per day and fermentations were conducted at 22 °C. The Sauvignon Blanc juice was fermented to dryness at 14 °C. After approx. two weeks of fermentation, the dry red wine was pressed off using a basket press. Wines were stored in carboys for 2-3 days prior to racking. Red wines underwent malolactic fermentation, induced by inoculation with lactic acid bacteria (*Oenococcus oeni*) and checked at the end of MLF for the absence of malic acid using an enzymatic assay. Sulfur dioxide (30 ppm) was added and the wines were bottled in 375 mL amber colour bottles for later analysis.

4.6 Fruit, juice and wine composition

4.6.1 Basic chemical composition

Juice samples were analysed for total soluble solids (TSS), pH, titratable acidity. Red wines were additionally assayed for colour and hue, total anthocyanins (pH Shift method) and total polyphenols (Folin assay). Wines were analysed similarly except for TSS as they were fermented to dryness. Berry samples were analysed for skin and seed tannins as per Iland (2013). Yeast assimilable nitrogen was measured using a Chemwell Autoanlyser instrument as per the manufacturers protocol.

4.6.2 Total anthocyanins and polyphenols

Anthocyanin measurements were completed on all juice and wine samples using the pH shift method by measuring the differential absorbance at 520 nm between wines at pH 1.0 and pH 4.5. Two 1 L buffer solutions of pH 1.0 and pH 4.5 were prepared as follows: pH 1.0 buffer prepared using 0.2 M KCl + 0.2 M HCl (1:2.68); pH 4.5 buffer prepared using 1 M NaOAc + 1 M HCl + distilled water (1.67:1:1.5). pH was corrected using HCl and NaOH, as needed. Samples were diluted ten-fold in test tubes using the pH 1.0 and 4.5 buffers, vortexed, and placed in the dark for one hour at room temperature for the reaction to complete. Absorbance was measured at 520 nm wavelength against blanks (the respective pH buffers) using a UV/Vis spectrophotometer (Shimadzu BioSpec). Total anthocyanin concentration was calculated as malvidin chloride 3,5-diglucoside using the following formula:

$$\text{Total Anthocyanins (mg/L)} = (A_{pH1.0}^{520} - A_{pH4.5}^{520}) \times 255.75$$

Phenolic analysis was done on all juice and wine samples by the phenolics reduction method as per Slinkard and Singleton (1977) using Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, MO, USA). A phenol standard (gallic acid) stock solution was prepared at a concentration of 5 g/L by dissolving 0.5 g of gallic acid in 100 mL of distilled water at room temperature. 10 mL of ethanol was added to the gallic acid powder to help dissolve the powder followed by topping up to 100 mL with distilled water (Waterhouse, 2001). Calibration standards were prepared from the phenol stock solution by adding 0, 1, 2, 3, 5, and 10 mL of stock solution to 100 mL volumetric flasks and diluting to volume with distilled water to obtain concentrations of 0, 50, 100, 150, 250 and 500 mg/L, respectively.

Sodium carbonate solution was prepared by dissolving 200 g of anhydrous sodium carbonate (Na_2CO_3) in 700 mL of distilled water, with heating until the sodium carbonate was dissolved, followed by topping up to 1 L with distilled water. The solution was allowed to cool, seeded with 2-3 g of sodium carbonate to induce crystallization and left for 24 hours prior to vacuum filtration through Whatman No. 2 filter paper. A 1 mL aliquot of wine or juice sample was diluted ten-fold. Into 100 mL volumetric flasks, 1 mL of diluted sample (1 mL water for blank) or standard were pipetted, along with 60 mL distilled water, and 5 mL of Folin reagent, and flasks were shaken by hand for ca. 30 seconds. 15 mL of sodium carbonate was then added within 8 minutes of adding the Folin reagent and shaken for ca. 30 s by hand. The volume was brought up to mark with distilled water and placed in the dark for 2 hours at room temperature (20°C). Absorbance was measured at 765 nm against a blank using a UV/Vis spectrophotometer (Shimadzu BioSpec) in 10 mm plastic cuvettes. A calibration curve was constructed from the phenol standards and multiplied by the dilution factor (10:1) to obtain the total phenol concentration in the original sample, expressed in mg/L of gallic acid equivalents (GAE).

Berry total anthocyanins and polyphenols, and skin and seed tannins were quantified using the method of Iland (2013).

4.6.3 Skin and seed tannins

The tannins in seeds or skins of grape berries were measured using the methyl cellulose precipitable (MCP) tannin assay as described by Sarneckis et al. (2006). Seeds and skins from 50 grape berries were collected and homogenized separately. For all seed or skin samples, approximately 1 g of homogenate was placed in a 15 mL centrifuge tube and the mass was accurately determined. 10 mL of the 50% (v/v) ethanol solution was added and the tube was vortexed for one hour, then centrifuged at 4,000 rpm for 5 min. Subsequently, two 1 mL aliquots of seed or skin extracts were transferred into separate 10 mL centrifuge tubes. One tube containing 1 mL extract was used for the 'treatment' test, while the second tube was used for the 'control' test. In the 'treatment', 3 mL of 0.04% methyl cellulose solution was added and mixed with 1 mL extract by gently inverting the tube several times. The tube was allowed to stand at room temperature for 2-3 min before 2 mL of saturated ammonium sulfate solution and 4 mL of deionised water were added. In the 'control' test, methyl cellulose polymer was replaced by water. The tubes were left at room temperature for 10 min, followed by centrifugation at 4,000 rpm for 5 min. The absorbance of the supernatant from both control and treatment tests were measured at 280 nm in 10 mm cuvettes using a Biospec Mini UV–vis spectrophotometer (Shimadzu, Japan). Epicatechin was used as a standard, and a standard curve was generated with epicatechin concentrations of 0, 10, 25, 50, 75, 100, 150, 200 and 250 mg/L. For calculation of tannin content, the difference of the absorbance between control and treatment read at 280 nm was substituted in the linear equation generated from the epicatechin standard curve. The results were corrected for any dilutions by using the appropriate dilution factor. The tannin content was then reported on epicatechin equivalent basis (mg/L epicatechin equivalents).

4.6.4 Individual anthocyanins and polyphenols

Analyses of individual anthocyanin and phenolic compounds in wines were performed using an Agilent 1100 Series HPLC and ChemStation software (Agilent Technologies, Palo Alto, CA). Separation was carried out using an InertSustain C18 reverse-phase column (250 mm x 4.6 mm, 5 µm; GL Science Inc., Japan) with an InertSustain C18 guard column (33 x 4.6 mm, 5 µm; GL Science Inc., Japan). The column temperature was maintained at 30 °C. A flow rate of 1 mL/min was used. The binary mobile phase used were buffer A (water/5% FA) and buffer B (ACN/5% FA). The gradient was as follows: 5-9 % B (0-10 min), 9-20 % B (10-43 min), 20-60 % (43-73 min), 60-90 % (73-75 min), 90 % B (75-80 min), 90-5 % B (80-81 min), 5 % B (81-85 min). All wine samples were filtered through 0.45 µm polytetrafluorethylene (PTFE) syringe filters (CHROMAFIL® O-45/15 MS, Germany) and 70 µL of filtered wine was injected into the HPLC. Individual anthocyanins and phenolics were detected at 280 nm, 320 nm, 365 nm and 525 nm. Identification of compounds was carried out by comparing their retention times with the standards (gallic acid, cinnamic acid, caffeic acid, quercetin, epicatechin, malvidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside). The concentration of each compound was calculated based on the peak area and calibration curves. All reagents and solvents were of analytical grade or higher. Formic acid (FA) and standards were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile (ACN) of HPLC grade was purchased from RCI Labscan (Thailand). MilliQ water was obtained from a MilliQ water purification system (Milli-Q plus, Adelab, SA).

4.6.5 Volatiles analysis

Wine volatiles were analysed for Sauvignon Blanc and Cabernet Sauvignon using headspace solid phase microextraction gas chromatography and mass spectrometry. C6 alcohols and aldehydes, monoterpenes, and methoxypyrazines were analysed using published methods (Dennis et al. 2012, Kalua and Boss 2009).

Briefly, aliquots of the wines (5 mL) were analysed at two different concentrations: 1 in 100 or 1 in 2, diluted with H_2O to a final volume of 10 mL. In all cases, 3 g of NaCl was added to each SPME vial (20 mL) prior to sample addition. Samples were spiked with five internal standards before SPME-GC-MS analysis: d_{13}-hexanol (9.2 µg for 1:2 dilution, 0.92 µg for 1:100 dilution; C/D/N Isotopes, Pointe-Claire, Canada); d_{11}-hexanoic acid (9.3 µg for 1:2 dilution, 0.93 µg for 1:100 dilution; C/D/N Isotopes); d_{16}-octanal (0.821 µg for 1:2 dilution, 82.1 ng for 1:100 dilution; C/D/N Isotopes); methyl nonanoate (61.2 ng for 1:2 dilution, 6.12 ng for 1:100 dilution; Sigma-Aldrich, Sydney, Australia); and d_3-linalool (17.3 ng for 1:2 dilution and 1.7 ng for 1:100 dilution; C/D/N Isotopes). Compounds were quantified across the fermentations using either the 1 in 100 dilution or the 1 in 2 dilution for all analyses.

SPME-GCMS was carried out using an Agilent 6890 gas chromatograph equipped with a Gerstel MP2 autosampler and using an Agilent Technologies 5973N mass spectrometer for peak detection and compound identification. The autosampler was operated in SPME mode utilizing a divinylbenzene-carboxen-polydimethylsiloxane fibre (DVB-CAR-PDMS, 2 cm, 23-gauge, 50/30 µm, Supelco, Bellefonte, PA) for extraction. Volatile compounds were extracted using agitation (250 rpm) at 40 °C for 30 min. Chromatography was performed using a ZB-Wax column (length 30 m, 0.25 mm i.d., film thickness 0.25 µm) using helium as a carrier gas at 1.2 mL/min (constant flow). Volatiles were desorbed from the fibre in the GC inlet (220 °C) for 1 min and separated using the following temperature program: 40 °C for 1.5 min, increasing at 7 °C/min to 245 °C, and held isothermally at 245 °C for 4.5 min. The temperature of the transfer line was held at 250 °C. Positive-ion electron impact spectra (70 eV) were recorded in scan mode (range, m/z 35−350; scan rate, 4.45 scans/s).

The identity of detected volatiles was determined by comparing mass spectra with those of authentic standards and spectral libraries. A laboratory-generated library (328 compounds) as well as the U.S. National Institute of Standards and Technology-05a (NIST-05a) and the Wiley Registry 7th ed. mass spectral libraries were used for identification purposes. Compounds were considered positively identified after matching of both mass spectra and linear retention indices (LRI) with that of authentic samples. The LRI was calculated from a compounds retention time relative to the retention of a series of n-alkanes (C8−C26).

4.7 Canopy cooling modelling

A canopy cooling simulation model was developed using the Scilab 5.5.0 programming language. This model is a dynamic simulation that predicts ambient temperature around a virtual grapevine canopy that is sprayed with a known volume of mist and being cooled from mist evaporation. The simulation model introduces a fixed volume and number of droplets in a short timestep into the canopy volume taking into account factors including canopy volume, mist droplet size, ambient temperature and relative humidity, wind speed (both external to and within the canopy), solar radiation, vine transpiration and flow rate through the mister nozzle. The model determines the thermodynamics of evaporative cooling resulting from the mist application, as well as droplet trajectory through the canopy volume to determine whether droplets either evaporate in-flight or reach a surface such as a leaf or bunch.

4.8 Statistical analysis

Statistical analysis and graphing of all data was performed using GraphPad Prism software (v.7.02, GraphPad Software, Inc., La Jolla, CA, USA). One-way and two-way analysis of variance (ANOVA) was performed on the datasets followed by a post-hoc analysis of treatment means using Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$.

5 Results and Discussion

5.1 Climate, weather, and water inputs

Seasonal weather data of the heat units or degree days during the growing season indicated that both years of the trial were similar in terms of heat accumulation (Table 1). The 2016-17 season (Season 1) was slightly cooler than 2017-18 (Season 2) at OLE, the Riverland site. The same general trend was observed at the Alverstoke vineyard (Waite) with Season 2 being slightly warmer than Season 1. Interestingly, in comparing the two sites, ALV was slightly warmer over the season than OLE – this could be explained by low night temperatures observed at OLE despite the higher daily maximum temperatures at that site. The implication of this on the canopy cooling trial was that undercanopy cooling treatments were not activated on cool nights that were under 20 °C irrespective of the day time high temperatures. January was the warmest month during the growing season with over 400 heat units accumulated at both sites and in both seasons.

Table 1: Monthly growing degree day accumulation (base 10°C) during the 2016-2017 and 2017-18 seasons. Data collected from Qualco weather station (Riverland) and on-site weather station at ALV.

	Oct	**Nov**	**Dec**	**Jan**	**Feb**	**March**	**Seasonal Sum**
OLE							
2016-17	184.7	261.5	357.3	440.1	333.1	365.8	**1942.7**
2017-18	235.0	324.3	356.5	450.4	371.6	290.6	**2028.7**
ALV							
2016-17	179.5	252.4	372.8	446.5	359.6	413.4	**2024.8**
2017-18	236.5	355.2	341.9	461.8	391.0	350.5	**2136.7**

At OLE, comparing the two individual growing seasons with recent historical average seasonal heat units, it was observed that 2016-17 was a relatively warmer year compared to the 5-year average (Figure 4) while 2017-18 was relatively cooler compared to the 5-year average (Figure 5). In particular, in 2016-17, the early part of the season was characterised by cooler than average conditions that later became warmer than average. In comparison, 2017-18 had similar conditions to the historical average.

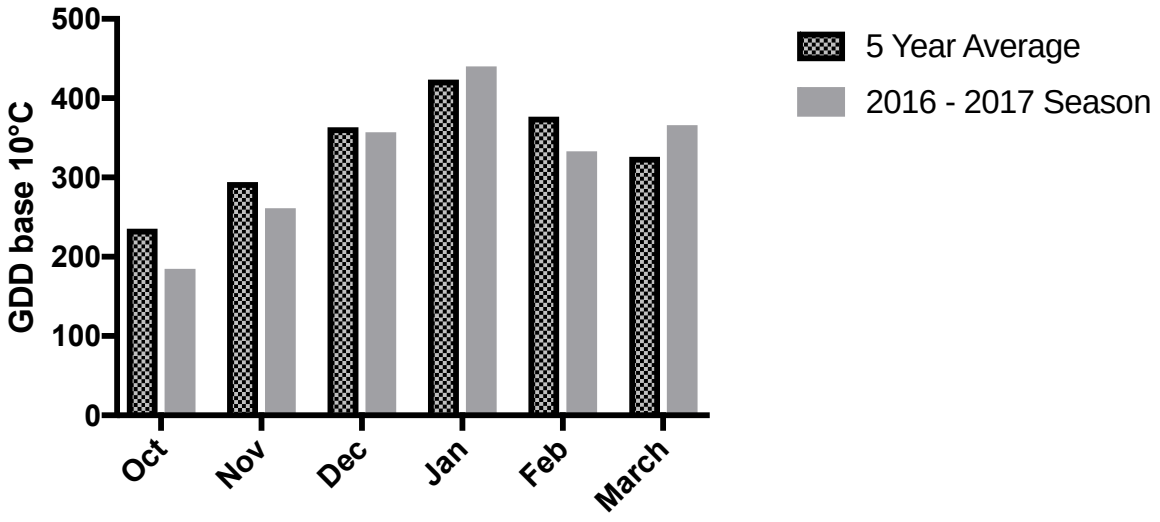


Figure 4: Growing degree days from October 1 to March 31 in 2016-17 compared to the five-year average. Data collected from Qualco weather station.

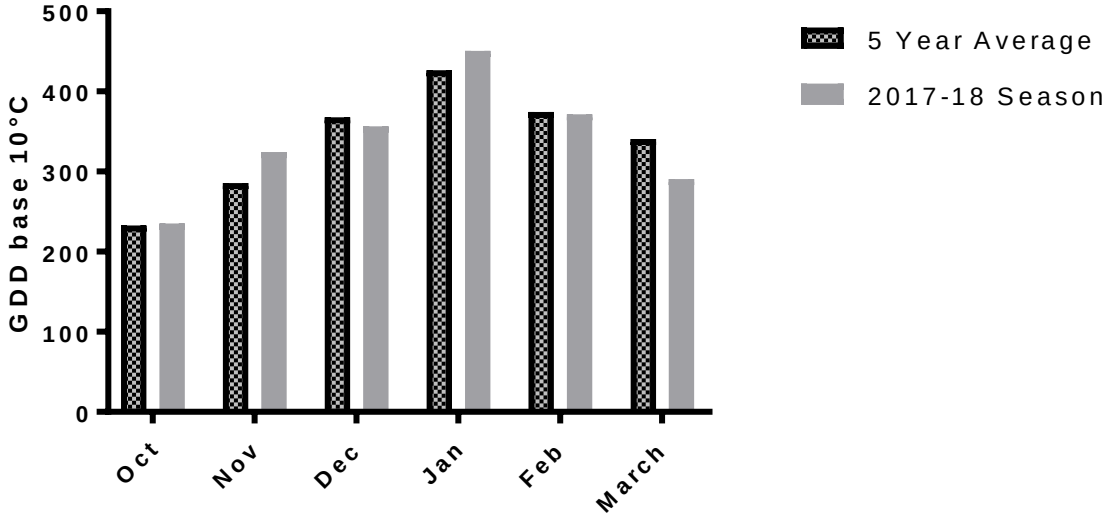


Figure 5: Growing degree days from October 1 to March 31 in 2017-18 compared to the five-year average. Data collected from Qualco weather station.

At ALV (Waite), the two individual growing seasons with recent historical average seasonal heat units, it was observed that 2016-17 was a relatively warmer year compared to the 5-year average (Figure 6) while 2017-18 was relatively cooler compared to the 5-year average (Figure ***7***). In particular, in 2016-17, the early part of the season was characterised by cooler than average conditions that later became warmer than average. In comparison, 2017-18 had similar conditions to the historical average heat units.

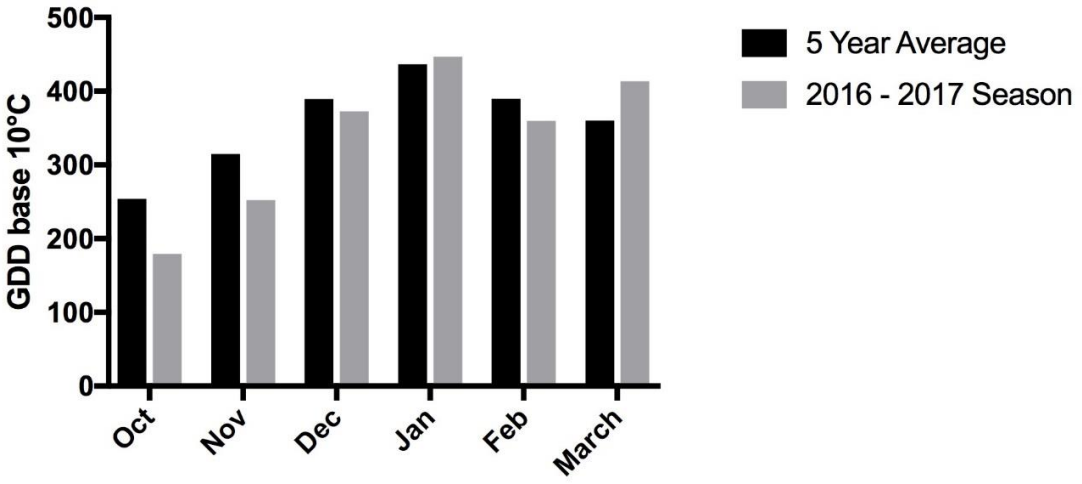


Figure 6: Growing degree days from October 1 to March 31 in 2016-17 compared to the five-year average. Data adapted from Kent Town weather station.

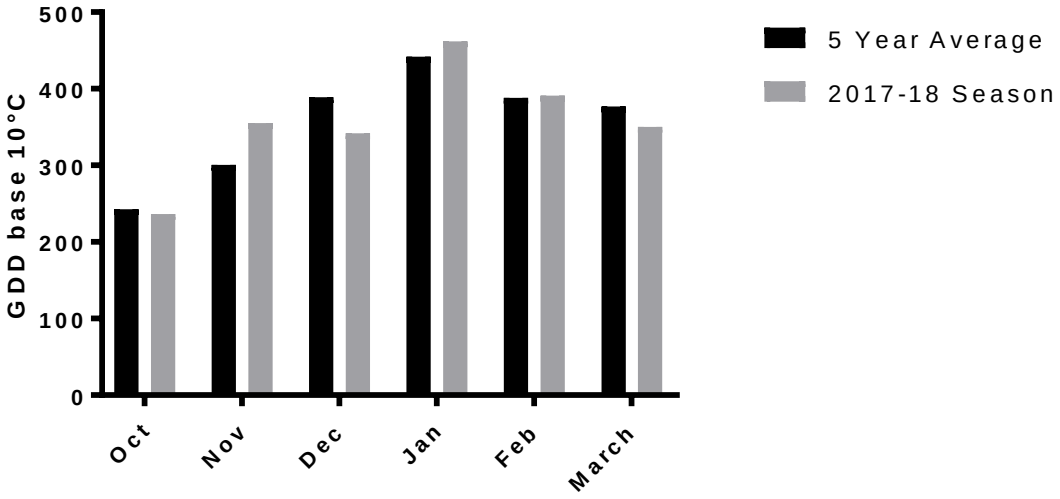


Figure 7: Growing degree days from October 1 to March 31 in 2017-18 compared to the five-year average. Data adapted from Kent Town weather station.

Table 2: Heatwave and cooling water application information from the two growing seasons, 2016-17 and 2017-18. Data shown only for the pre-harvest period and collected from Qualco weather station (OLE-Riverland). Heatwaves defined as three or more days with maximum daily temperatures 35 °C or higher.

Heatwave Dates	Duration (days)	T_{max} (°C)	Time > 35 °C (h)	ICM Volume (L/vine)	SI Volume (L/vine)	UCS Volume (L/vine)
23/12/2016 - 25/12/2016	3	41.6	18	43	99	168*
04/01/2017 - 07/01/2017	4	40.9	26	74	132	224*
08/02/2017 - 11/02/2017	4	45.2	23	49	132	168*
TOTAL	**11**		**67**	**166**	**363**	**560**
28/11/2017 – 30/11/2017	3	37.6	9	22	N/T	N/T
04/01/2018 – 06/01/2018	3	44.6	16	38	69	69
17/01/2018 – 21/01/2018	5	45.0	44	105	195	189
TOTAL	**11**		**69**	**165**	**264**	**258**

* values estimated based on nominal sprinkler flow rates
N/T – not triggered due to low minimum night temperatures (< 20°C)

At OLE (Riverland), the 2016-17 season (Season 1) witnessed three heatwaves between October and harvest on February 10, 2017 (Table 2). Heatwaves were defined as daily maximum temperatures at or exceeding 35 °C for three or more days consecutively. The duration of these heatwaves ranged from three to four consecutive days in December, January and February with maximum temperatures ranging from 40-45 °C. A total of 11 days of heatwaves were experienced. During these three heatwaves, the total time above the critical threshold temperature of 35 °C was 67 hours.

The amount of water applied for evaporative cooling based on all three methodologies – misting, supplemental irrigation, and undercanopy sprinklers – ranged from 166 L to 560 L. In setting up the experiment, we aimed to match the water application rates of both SI and UCS treatments but were unsuccessful in the first season; the UCS treatment received approx. 54% more water than the SI treatment, however, it should be noted that the UCS water was distributed evenly in both the midrow as well in-row (undervine) areas of the vineyard. Therefore, it is quite possible that the actual water availability to the vines was similar between the two treatments (see soil moisture data in Section 6.3.1). The in-canopy misters used only 166 L of water to cool the canopies over the season. This was a significantly lower volume of water compared to the other two undervine cooling approaches, using less than half of SI and one-third of UCS. As a fraction of total water incident on the vineyard (precipitation + irrigation), these water inputs represented 8% (SI) and 11% (UCS), whereas ICM represented only 3% of the total (Figure 8).

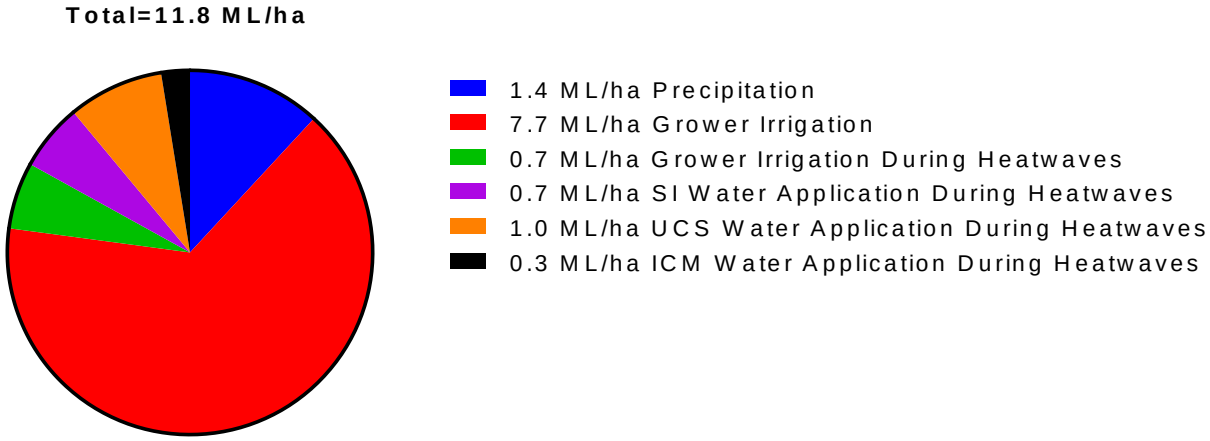


Figure 8: Water incident in OLE vineyard in the 2016-17 season. Values in the legend indicate water volume applied in ML/ha.

During the second season, 2017-18, three heatwaves totalling 11 days and 69 hours above 35 °C were experienced at OLE in the Riverland (Table 2). The heatwaves ranged from three to five days in duration with daily maximum temperatures ranging from 37 °C to 45 °C. not dissimilar to the previous season (Season 1). During these heatwaves, the volume of water used for cooling ranged from 165 L (ICM) to 264 L (SI) with the UCS treatment using a similar volume of water to SI (258 L). The water application techniques of the undercanopy cooling treatments were optimised in the second season reflected by the closely matched volumes of the SI and UCS treatments. In contrast, the misting treatment used approx. 40% less water (165 L/vine) than either undercanopy cooling treatment (258-264 L/vine). As a fraction of total water incident on the vineyard (precipitation + irrigation), these water inputs represented 7% (SI) and 7% (UCS), whereas ICM represented only 4% of the total (Figure 9: Water incident in OLE vineyard in the 2017-18 season. Values in the legend indicate water volume applied in ML/ha.).

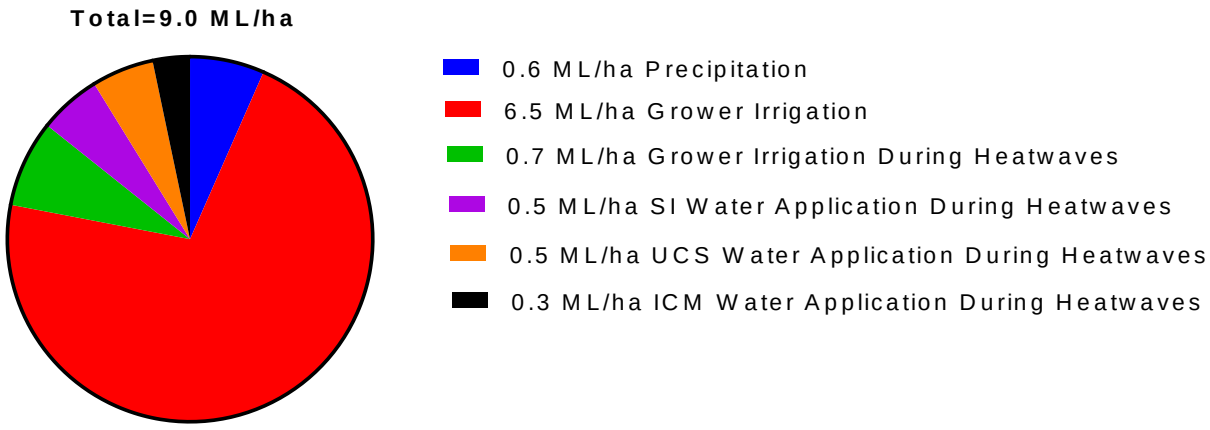


Figure 9: Water incident in OLE vineyard in the 2017-18 season. Values in the legend indicate water volume applied in ML/ha.

Table 3: Heatwave information from the two growing seasons, 2016-17 and 2017-18. Data collected from the on-site weather station at ALV (Waite) and shown only for the pre-harvest period.

Heatwave Dates	Duration (days)	T_{max} (°C)	Time > 35 °C (h)	ICM Volume (L/vine)	SI Volume (L/vine)	UCS Volume (L/vine)
23/12/2016 - 25/12/2016	3	39.6	10	-	141	206
05/01/2017 - 07/01/2017	3	39.5	17	-	141	206
08/02/2017 - 10/02/2017	3	41.2	27	45	141	206
27/02/2017 - 02/03/2017	4	38.1	15	51	188	275
TOTAL	**13**		**69**	**96**	**611**	**893**
18/01/2018 - 21/01/2018	4	42.6	32	176	130	228
05/02/2018 - 09/02/2018	5	40.5	30	165	162	285
08/03/2018 – 10/03/2018	3	36.2	5	28	97	171
TOTAL	**15**		**67**	**369**	**389**	**684**

* values estimated based on nominal sprinkler flow rates

N/T – not triggered due to low minimum night temperatures (< 20 °C)

At the second site, Alverstoke vineyard (Waite), the first season witnessed four heatwaves ranging in duration from three to four days, totalling 13 days over the season (Table 3). The heatwaves generally coincided with the Riverland heatwaves, and were experienced in late December, early January, February and early March leading up to harvest. During these heatwaves, the total duration where temperatures inside the canopy were over 35 °C was 69 hours. This resulted in a water consumption of 96 L/vine for the misting treatment, 611 L/vine for the SI, and 893 L/vine for the UCS treatment.

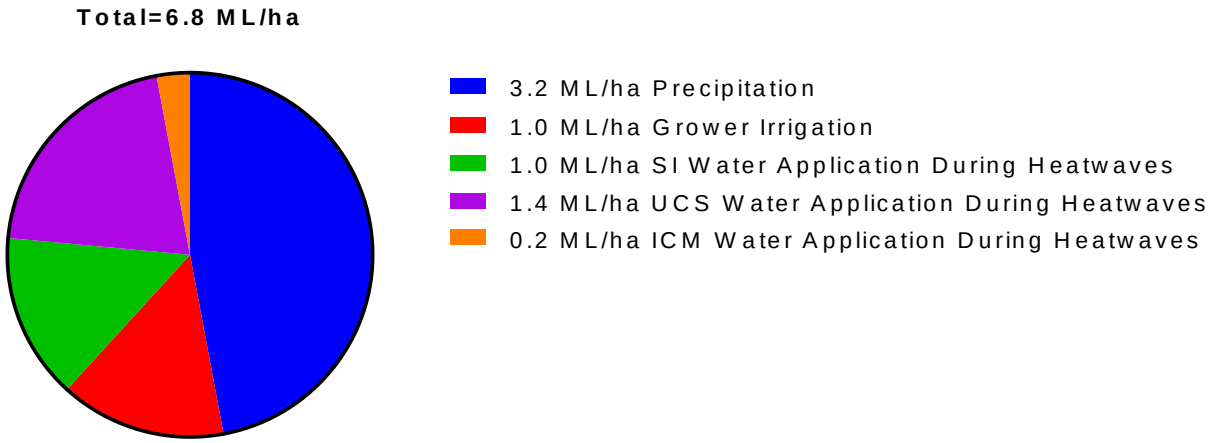


Figure 10: Water application rates in the 2016-17 season at ALV. No additional irrigation was applied during heatwaves. Values in the legend (brackets) indicate water volume applied in ML/ha.

Like the OLE site, UCS treatment received the highest rates of water followed by SI in both seasons. As a fraction of total water incident on the vineyard, these water inputs represented approx. 24% (SI) and 33% (UCS) of the total whereas misting represented only 5% of the total (Figure 10).

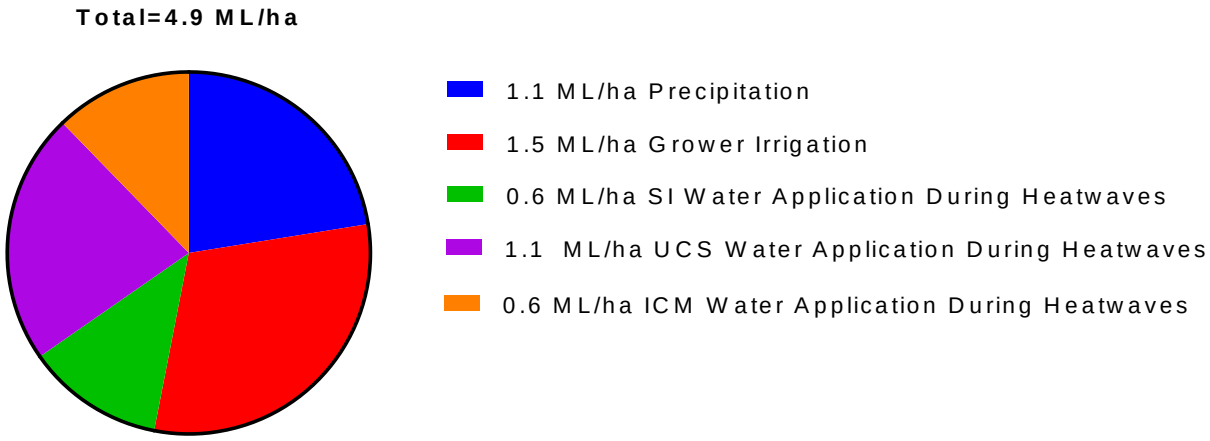


Figure 11: Water application rates in the 2017-18 season at ALV. No additional irrigation was applied during heatwaves. Values in the legend indicate water volume applied in ML/ha.

In the second season, 2017-18, three heatwaves were received in the latter half of the season in January, February and March totalling 15 days. During this time, 67 hours of temperatures over 35°C were experienced by the vines, similar to Season 1. The first two heatwaves in January and February were prolonged with 30 hours or more above 35 °C during each event. The water required for canopy cooling by mist was 369 L/vine with more than 92% of this consumed during the first two heatwaves. For the two undercanopy cooling treatments, SI used about 389 L/vine while UCS used 684 L/vine. As a fraction of total water incident on the vineyard (precipitation + irrigation), these water inputs represented 23% (SI) and 42% (UCS), whereas ICM represented 17% of the total (Figure 11).

Although there were several occasions where daily maximum temperatures were at the 35°C temperature threshold or higher, only three events listed in the table were three days or more. Therefore, the cumulative heat stress experienced by the vines over the growing season could be considerably higher than that reflected by the data presented here.

5.2 Canopy microclimate

5.2.1 Temperature, relative humidity, VPD

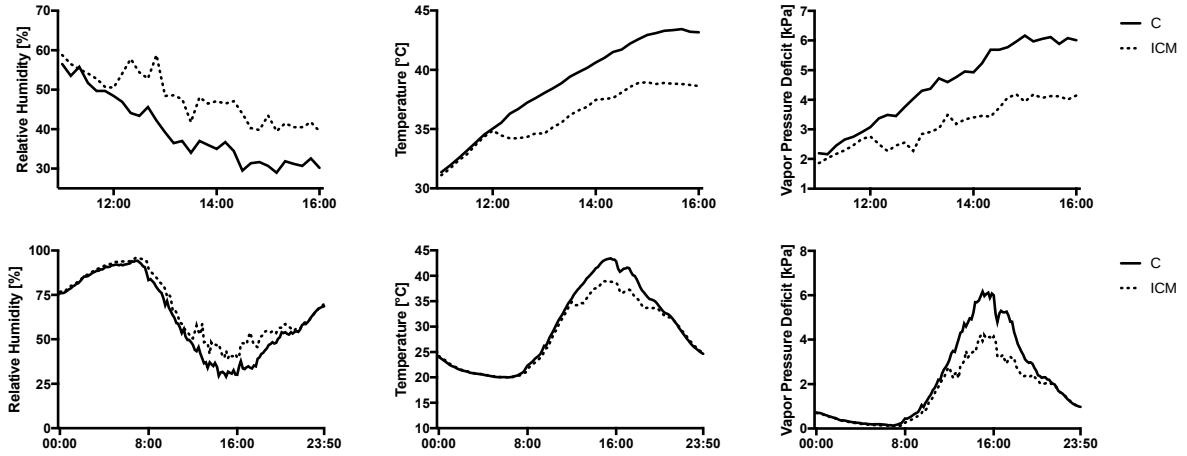


Figure 12: Canopy microclimate at OLE with the ICC (ICM) treatments, 2016-17. Internal canopy relative humidity, temperature, and vapour pressure deficit of the ICC trial during a heatwave on February 10th, 2017. The lower panel is a diurnal plot of the same data as the detailed upper panel. Data logged using Tinytags.

Detailed microclimatic measurements inside the grapevine canopies during a heatwave day in 2016-17 showed that ambient temperatures of control or un-cooled vines approached 45 °C (Figure 12 – middle panel). The VPD during this period was in excess of 6 kPa (Figure 12 - third panel) indicating a high environmental demand on the leaves, specifically driving transpiration. These conditions have been previously shown to induce stomatal closure due to large water losses from the leaf and sensitivity of stomatal guard cells to high VPD via changes in their turgor. This is especially exacerbated during periods of water stress under low soil moisture availability. Misting applications to the canopy had a significant impact on canopy microclimate: in contrast to the control vines, the misted vines had temperatures around 35 °C, at least for a short period after which the ambient temperatures were much too high for the misting rates to continue to hold down the canopy temperature. However, even during the warmest time of the day, around 16:00 h, misting lowered the canopy temperature by approx. 4-5 °C compared to ambient, un-cooled canopies as well as lowered VPD by 2 kPa.

In the second season, 2017-18, at OLE, similar responses to misting were observed as in Season 1. On a heatwave day, ambient temperatures inside the canopy were over 43 °C while the misted canopies were 2-3 °C below that (Figure 13 – middle panel). Although our goal was to maintain canopy temperatures below 35 °C with misting, the intensity of the heatwave required us to further optimise the mister settings, specifically timing and duration of the mist applications. At higher temperatures, the evaporation rates of water are faster, and a greater cooling is achieved, but once the leaf is dry, there is rapid heating of the leaf. More is discussed about this based on the thermocouple temperatures (see below).

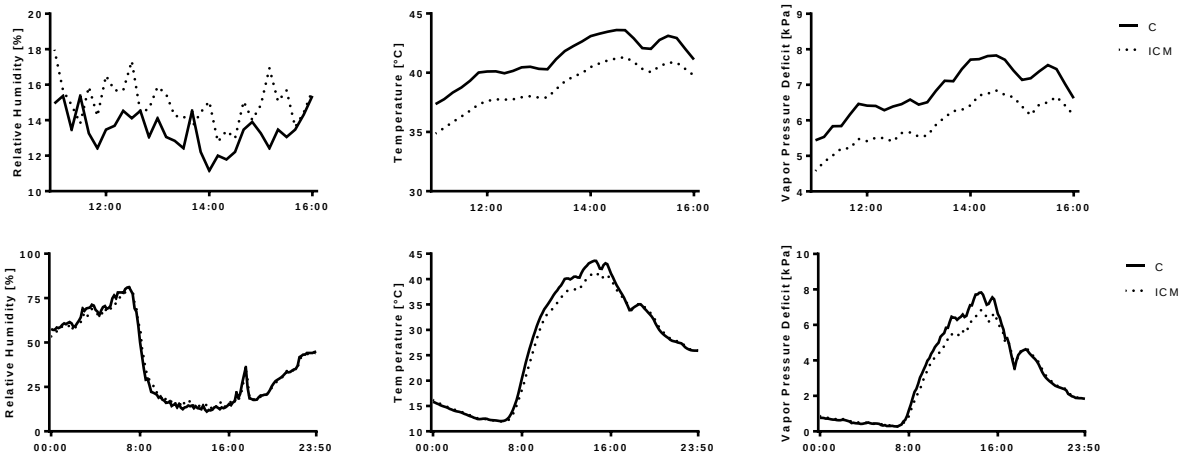


Figure 13: Canopy microclimate at OLE with the ICC (ICM) treatments, 2017-18. Internal canopy relative humidity, temperature, and vapour pressure deficit of the ICC trial during a heatwave on December 27th, 2017. The lower panel is a diurnal plot of the same data as the detailed upper panel. Data logged using Tinytags.

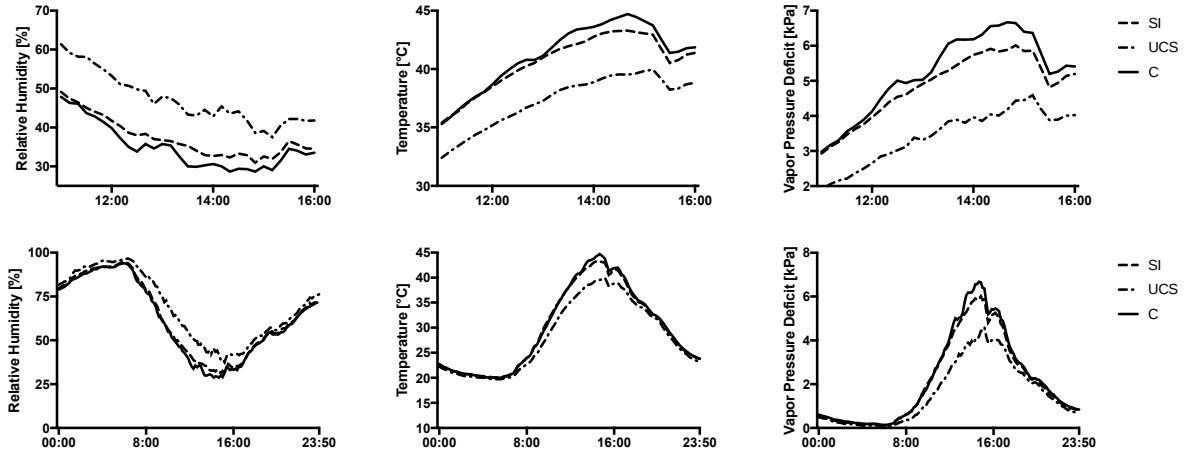


Figure 14: Canopy microclimate at OLE with the UCC treatments, 2016-17. Internal canopy relative humidity, temperature, and vapour pressure deficit during a heatwave on February 10th, 2017. Data logged using Tinytags.

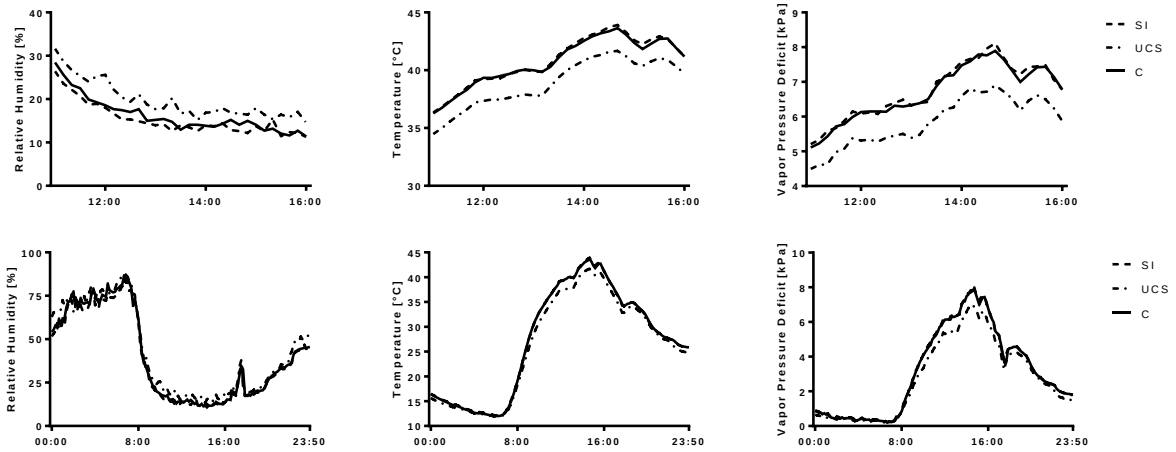


Figure 15: Canopy microclimate at OLE with the UCC treatments, 2017-18. Internal canopy relative humidity, temperature, and vapour pressure deficit during a heatwave on February 10th, 2017. Data logged using Tinytags.

In the undercanopy cooling treatments (UCC), canopy temperatures were also observed to be lower as a result of only two hours of night-time applications of water to the vineyard floor. In the first season, 2016-17, control vines had in-canopy (shade) temperatures of 45 °C while the SI and UCS cooled vineyard floors had temperatures 2 °C and 5 °C lower, respectively (Figure 14). Differences between control and SI vines was not marked in the second season, 2017-18, but UCS vines remained significantly cooler (Figure 15 – middle panel). The VPD inside these canopies was also lower compared to control: while control vines had VPD levels around 7 kPa, SI and UCS vines had VPD levels of 6 kPa and 4.5 kPa, respectively, in the first season, and similar differences in the second season with the UCS treatment.

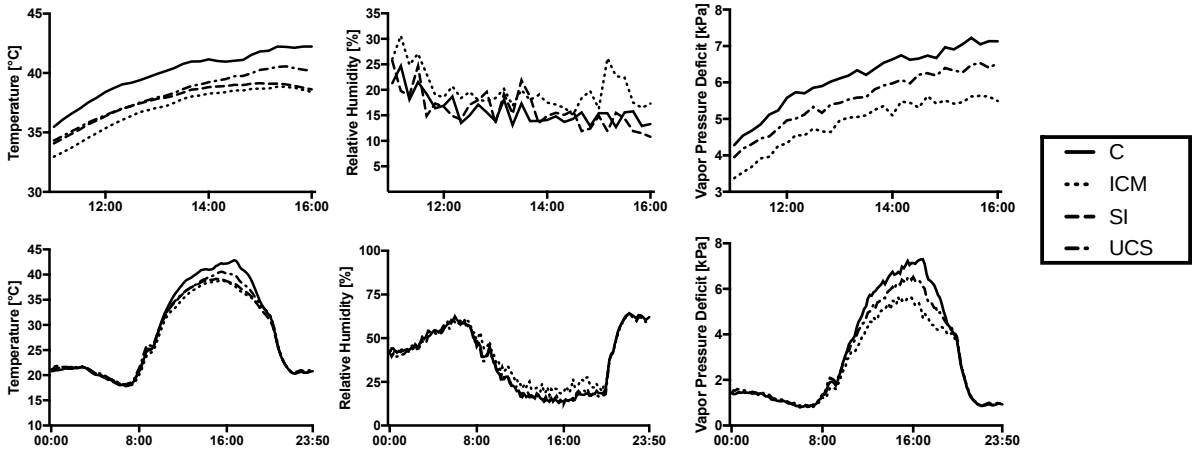


Figure 16: Canopy microclimate at ALV in 2016-17. Internal canopy relative humidity, temperature, and vapour pressure deficit during a heatwave on January 17th, 2017. Data logged using Tinytags.

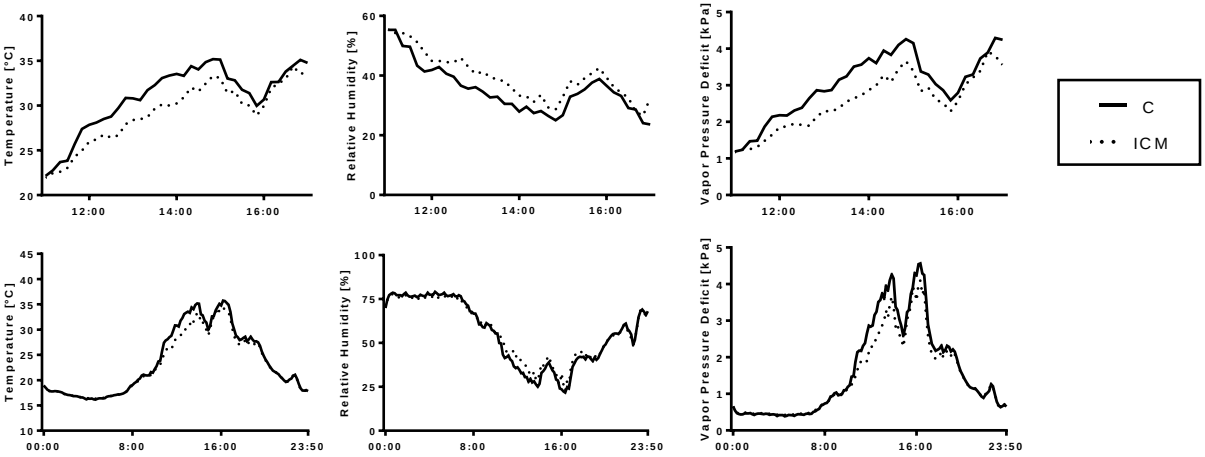


Figure 17: Canopy microclimate at ALV in 2017-18. Internal canopy relative humidity, temperature, and vapour pressure deficit during a heatwave on December 14th, 2017. Data logged using Tinytags.

At Alverstoke vineyard in the first season, temperatures on a heatwave day reached 43°C in the control vines while the misting vines were approx. 5 °C cooler (Figure 16 - left panel). In comparison, the UCS and SI vines were between 2-4 °C cooler than the control vines indicative of the effectiveness of night time application of water on the vineyard floor. During the same period, the VPD levels on the un-cooled canopies were in excess of 7 kPa in the mid-afternoon while the misted canopies were just above 5 kPa. The two floor cooling treatments, SI and UCS, were around 6 kPa.

During the second season at ALV, misting also lowered canopy temperatures, as expected, by 2-3 °C and increased relative humidity by approx. 5% (Figure 17). Concomitantly, canopy interior VPD decreased by almost 1 kPa, slightly less than observed in the first season. These small changes in canopy microclimate could have large effects on vine performance, particularly if vines are already at their thresholds of temperature stress.

High resolution temperature measurements of grape leave and bunches during a heatwave day in 2016-17 highlighted the temperature profiles as a result of misting. During the warmest period of the day, un-cooled leaves appeared to maintain temperatures around 42-43 °C while the misted leaves cycled between cool and warm (Figure 18). Misted leaves were as much as 12 °C cooler than un-cooled leaves with the full effect of evaporative cooling; upon drying, however, their temperatures increased to approx. 3-5 °C lower than the un-cooled leaves. In no situation were the cooled leaves warmer than the un-cooled leaves.

Bunch surface temperatures were also lower with the misting compared to un-misted fruit. Temperature differences of 2-3 °C were observed, much less than the difference between leaves, which is not surprising given the larger thermal mass (and buffering of temperature changes) of the bunches compared to the leaves. Grape bunches were observed to be above 40 °C and only a couple of degrees cooler than ambient temperatures, which is likely to have implications on fruit composition and wine quality.

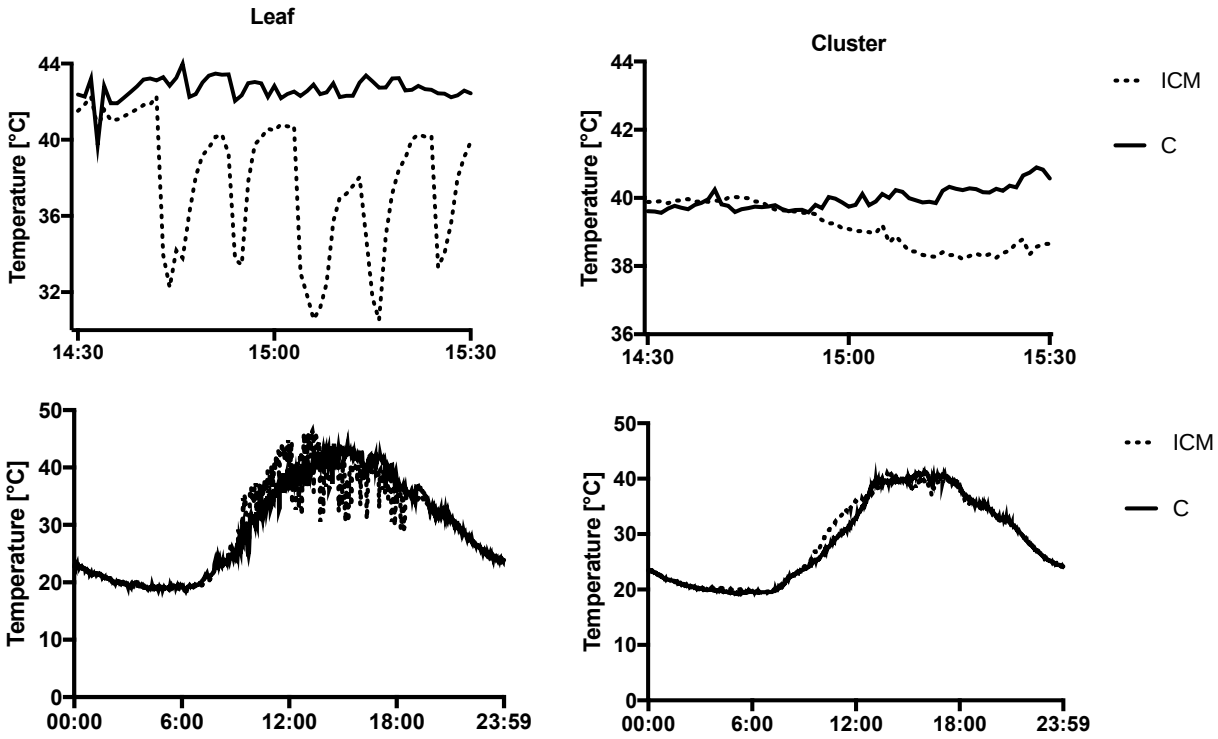


Figure 18: Leaf and bunch surface temperatures (from thermocouples) from the ICC trial at OLE on February 10th, 2017 during a heatwave.

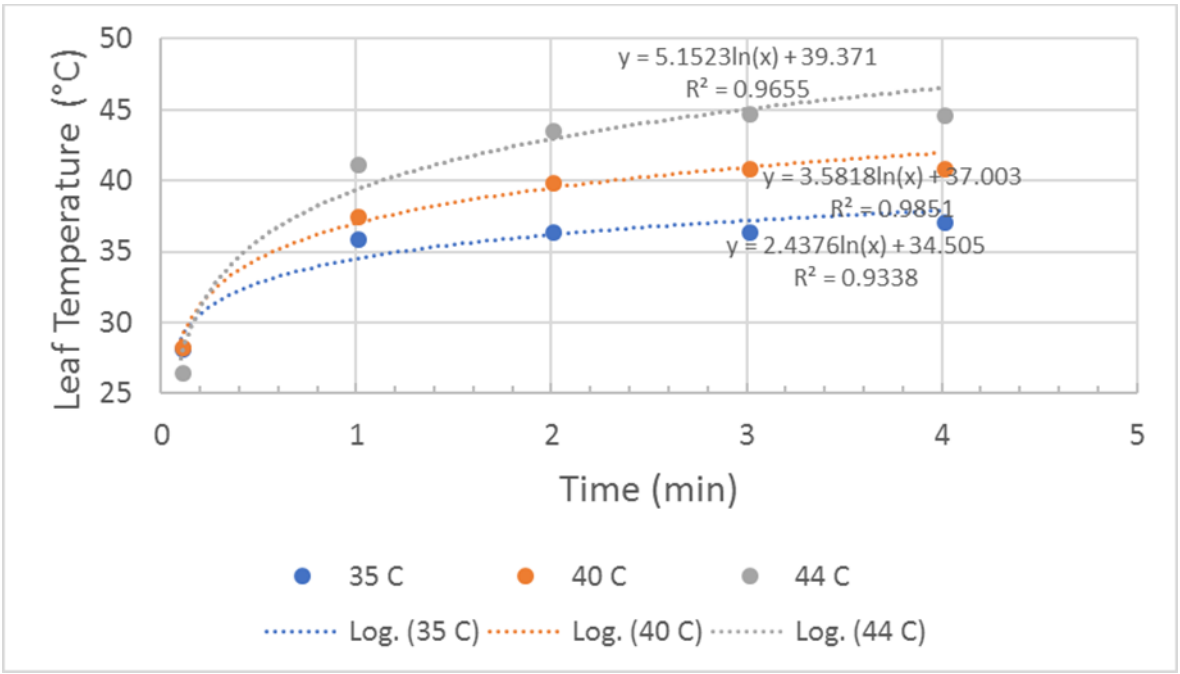


Figure 19: Leaf temperature increases after misting at various ambient temperatures: 35°C, 40°C, 45°C. Data collected from thermocouples at OLE on February 10th, 2017.

To study the dynamics of heating and cooling of grapevine leaves, detailed temperature measurements with the thermocouple were analysed. Temperature data of leaves under misting were collected on three heatwave days with different temperatures: 35, 40 and 44 °C. The leaves were cooled by mist to approximately the same (low) surface temperature of 27-28 °C. Upon drying, the leaves were observed to heat up to different maximum temperatures and at different rates as shown in Figure 19. During the coolest heatwave day at 35 °C, leaves heated back up to 35 °C, our threshold temperature, within one minute. At a higher temperature, 40 °C, leaves heated back up to 35 °C within 0.7 min, while at 44 °C, leaves heated back up to 35 °C within 0.5 min. These temperature transients provide valuable guidance to misting frequencies (intervals) required to maintain canopy temperatures under a certain threshold under specific environmental conditions.

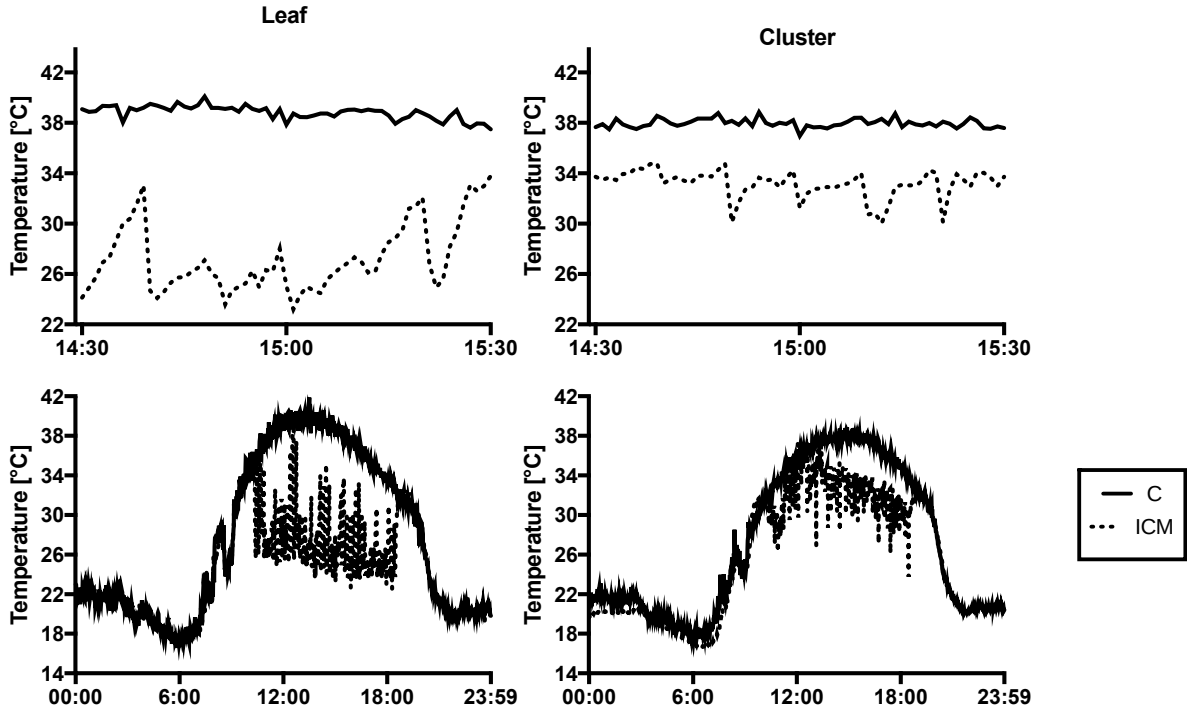


Figure 20: Leaf and bunch surface temperatures (from thermocouples) from the ICC trial at ALV during a heatwave on January 17th, 2017.

At Alverstoke, ambient leaf temperatures were around 40 °C under heatwave conditions in 2016-17 (Figure 20). Under these conditions, misting resulted in leaf surface temperatures approx. 12 °C cooler, similar to OLE, while bunch temperatures of 38 °C were cooler in misted vines by approx. 4 °C. Temperature cycling (amplitudes) was not as evident in the Cabernet vines as in the Sauvignon Blanc canopies; this could be attributed to the greater heat exchange in the larger canopies at OLE while the more shaded canopies at ALV would have less of a temperature effect due, for example due to reduced convective heat transfer from lower air flow through these canopies.

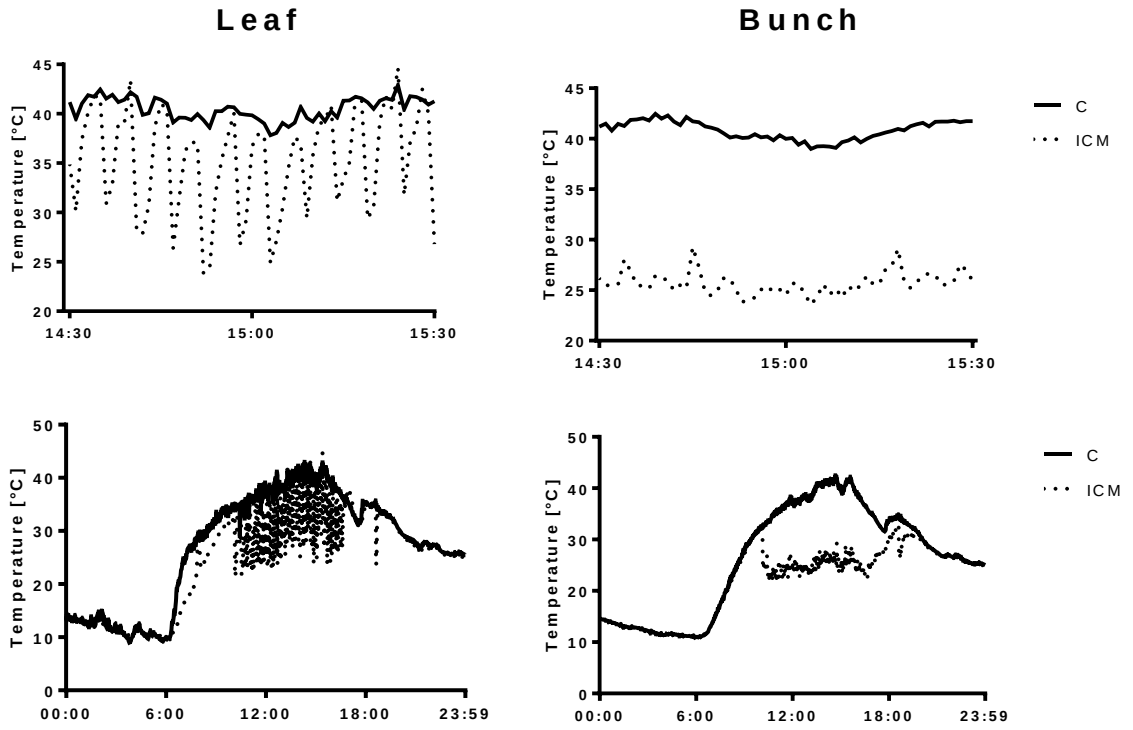


Figure 21: Leaf and bunch surface temperatures (from thermocouples) from the ICC trial at OLE on December 27th, 2017 during a heatwave.

In the second season, 2017-18, at ALV, similar cooling was observed as in the first season. Canopy (leaf) temperatures were approx. 15 °C cooler under misting compared to control vines in the hottest period of the day, mid-afternoon (Figure 21). The ability of the misting system to maintain leaf temperatures lower than the control (uncooled) vines was limited by the high ambient temperatures that were above 40°C. Bunch temperatures were also markedly cooler in the misted vines by approx. 15°C at around 25-27°C compared to the control vines that were over 40°C. These large temperature differences, if sustained over longer parts of the season, would likely compromise grape acidity, as well as flavour and aroma compounds that are determinants of wine quality.

5.2.2 Thermal images of vineyard floor

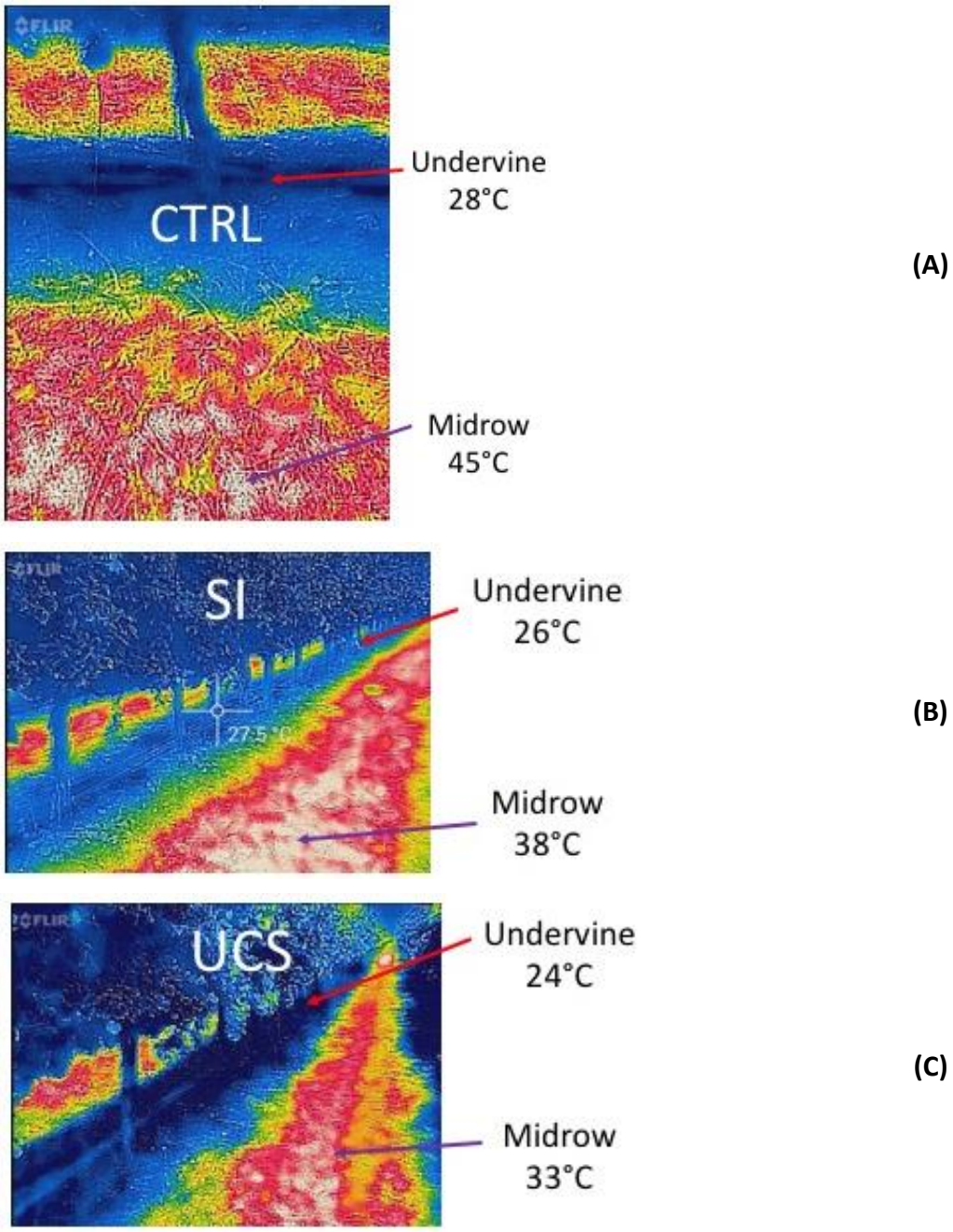
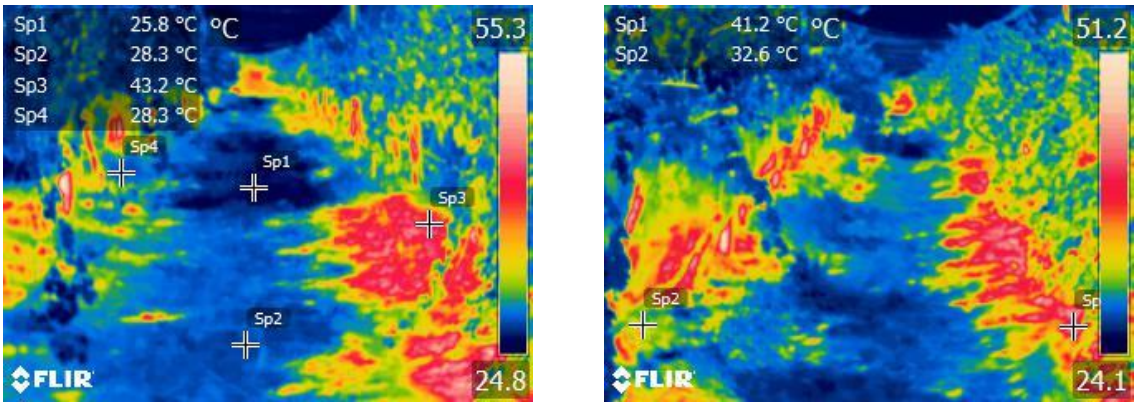


Figure 22: Thermal images of the vineyard floor showing undervine and midrow temperatures on a heatwave day at 10:00 h from the UCC trial at OLE in 2016-17. **(A)** Control vines, **(B)** Supplemental irrigation, **(C)** Undercanopy sprinklers.

Thermal images obtained from a handheld infrared camera at OLE (Riverland) in Season 1 show the spatial patterns of temperature across the vineyard floor in response to various undervine cooling treatments compared to uncooled areas (control) (Figure 22). During the middle of the heatwave, in the morning around 10:00 h, the control vines had undervine temperatures around 28 °C, cooler than the ambient air temperatures at the time around 32 °C, while the bare midrow temperatures were

around 45 °C (Figure 22A) The cooler undervine areas likely result from the additional irrigation applied by the grower in advance of the heatwave and also greater moisture uptake and redistribution in different soil horizons by the vine roots. In comparison, the SI treatment with additional water applied the previous night for two hours only to the undervine area had undervine temperatures of around 26 °C and midrow temperatures around 38 °C, both cooler than the control vines (Figure 22B). Finally, the UCS treatment with water broadcast for two hours the preceding night to the entire vineyard floor had the coolest vineyard floor temperatures: 24 °C in the undervine region and 33 °C in the midrow region (Figure 22C). The humidity of these sections of the block were also noticeably higher as a result of the distributed water application.



(A) **(B)**

Figure 23: Thermal images of the canopies and vineyard floor showing undervine and midrow temperatures on a heatwave day (27/01/2018) at 10:00 h from the UCC trial at ALV. **(A)** Control vines in the foreground, UCS vines in the background; **(B)** SI vines on left, Control vines on right.

Thermal images taken on the vineyard floor at ALV during the middle of heatwave on January 27, 2018 showed contrasting differences in floor temperatures, both undervine and inter-row. In the uncooled or control vines, shown in the foreground of Figure 23A, the undervine region was approx. 43 °C (position Sp3 in the thermal image) while the midrow region was around 28 °C (position Sp2) although this was an artefact of having another cooling treatment on the left row in the image. In contrast, the UCS cooled vines had undervine and midrow temperatures approx. 17 °C cooler, around 26 °C (position Sp1).

Looking at the same block and conditions, undervine supplemental irrigation also had a positive effect on lowering floor temperatures as seen on the left side of Figure 23B. These regions had undervine temperatures around 33 °C (position Sp2) compared to the control vines on the right side of the image, which had undervine temperatures approx. 10 °C higher at around 41 °C (position Sp1). These high temperatures were surprising considering the vineyard is organically managed with a permanent sward on the floor that would have been expected to moderate high temperatures, something that was not seen based on these thermal images. The possibility that the cover crops were not transpiring and therefore cooling their leaves could explain this observation.

5.3 Vine physiology

5.3.1 Soil and vine water status

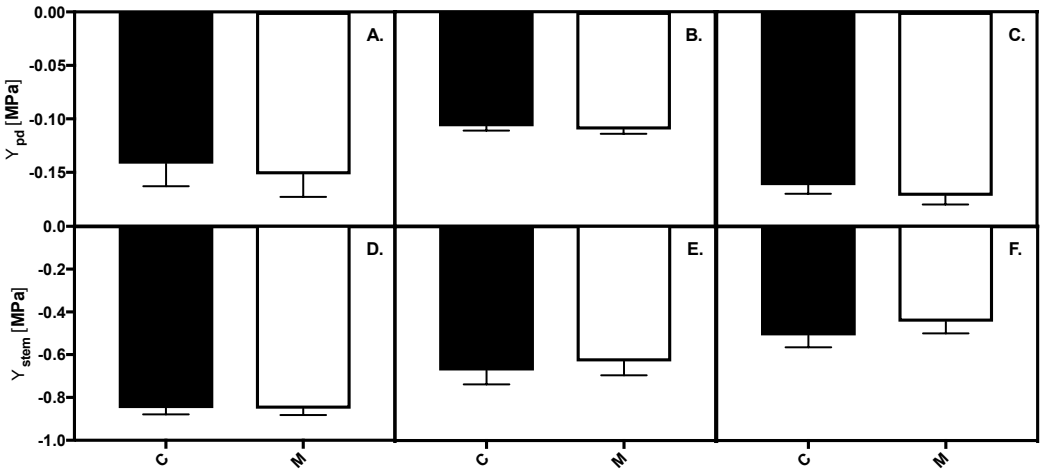


Figure 24: ICC trial at OLE. Predawn (Ψ_{pd}) and mid-day stem (Ψ_{stem}) water potentials. **A**. Pre-heatwave Ψ_{pd} on 02/01/2017, **B**. Mid-heatwave Ψ_{pd} on 08/01/2017, **C**. Post-heatwave Ψ_{pd} on 11/01/2017, **D.** Pre-heatwave Ψ_{stem} on 02/01/2017, **E**. Mid-heatwave Ψ_{stem} on 08/01/2017, **F.** Post-heatwave Ψ_{stem} on 11/01/2017.

Soil moisture was estimated as leaf water potential, measured at pre-dawn between the hours of 03:00 and 05:00. This estimate of soil moisture is based on the low to zero transpiration rates of the canopy resulting in an equilibration of water potentials between the soil and plant. Measurements of soil and vine water status were made pre-, mid-, and post-heatwaves as shown in the three panels in Figure 24. Soil moisture or Ψ_{pd} was similar for vines that were either cooled or un-cooled at all three timepoints. Therefore, we can conclude that misting did not have an effect on soil moisture. Vine water status, as indicated by midday stem water potential (Ψ_{stem}), was also similar between the two treatments, indicating that misting did not alter the water status of the vines. In some cases, during the mid-heatwave and post-heatwave, slight increases in Ψ_{stem} were observed but these were statistically non-significant.

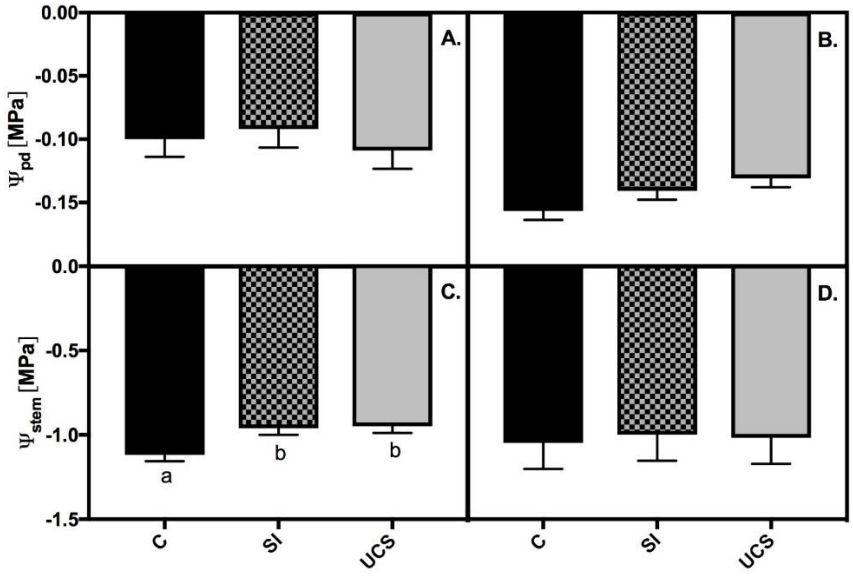


Figure 25: UCC trial at OLE, 2016-17. Predawn (Ψ_{pd}) and mid-day stem (Ψ_{stem}) water potentials. **A.** Mid-heatwave Ψ_{pd} on 07/01/2017, **B**. Post-heatwave Ψ_{pd} on 11/01/2017, **C**. Mid-heatwave Ψ_{stem} on 07/01/2017, **D**. Post-heatwave Ψ_{stem} on 11/01/2017.

The same soil and vine water status parameters were measured in the undercanopy cooling trial at OLE in 2016-17; results are shown in Figure 25. Soil moisture was similar during and after the heatwave between treatments. Ψ_{pd} was lower after the heatwave likely resulting from the increased evapotranspiration from the vineyard. Ψ_{pd} was found to be higher in the cooled vines despite the lack of water application after the heatwave (only night time applications of water were done during the heatwave). Vine water status was significantly lower in the control (un-cooled) vines during the heatwave compared to both the cooled treatments, SI and UCS (Figure 25C). This positive result from the cooling could be due to lower canopy VPD levels as well as lower root and soil temperatures (data collected but not presented here). Following the heatwave, all vines had a similar water status.

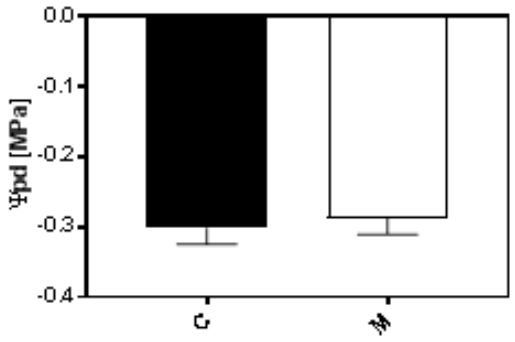


Figure 26: ICC trial at OLE, 2017-18, predawn leaf water potential, mid-heatwave Ψ_{pd} on 13/12/2017.

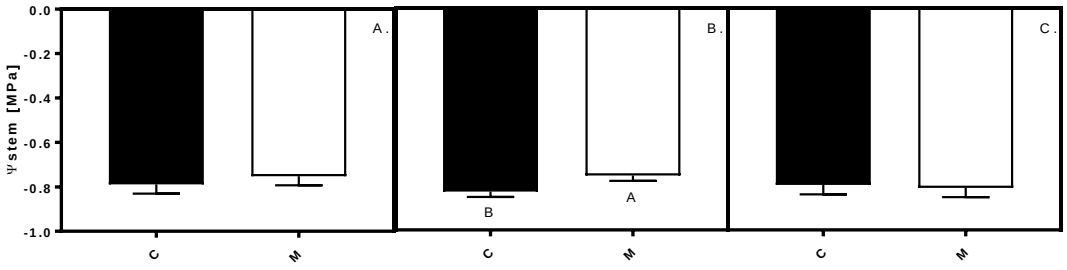


Figure 27: ICC trial at OLE, 2017-18, midday stem water potential, **A**. Pre-heatwave Ψ_{stem} on 9/12/2017, **B**. Mid-heatwave Ψ_{stem} on 13/12/2017, **C.** Post-heatwave Ψ_{stem} on 16/12/2017.

In the ICC trial at OLE in the second season, soil moisture as indicated by Ψ_{pd} was virtually the same in both misting and control vines (Figure 26), which is consistent with the first season. We did not expect changes in soil moisture due to mist application, primarily due to the small volumes of water applied only to the canopy. Ψ_{pd} was around -0.3 MPa in both treatments, lower than in the first season (-0.15 to -0.10 MPa), likely due to the early arrival of heatwaves in Season 2 that would have depleted soil moisture.

Vine water status was not significantly different between treatments with values around -0.8 MPa prior to the heatwave in December 2017 (Figure 27A). In the midst of the heatwave, control vines had significantly higher stress as indicated by their lower Ψ_{stem} values (Figure 27B). Following the heatwave, both groups of vines had similar water status similar to prior to the heatwave.

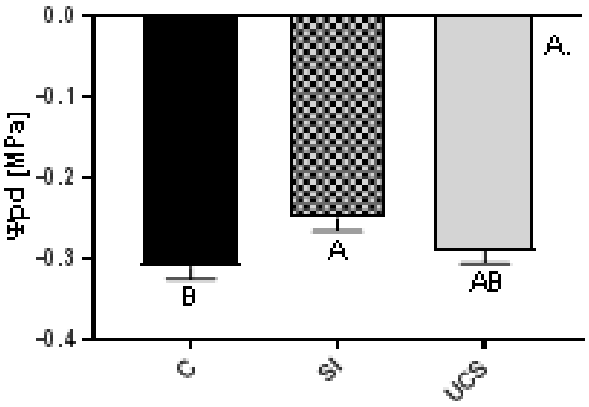


Figure 28: UCC trial at OLE, 2017-18. Predawn leaf water potential (Ψ_{pd}) during the mid-heatwave timepoint on 13/12/2017.

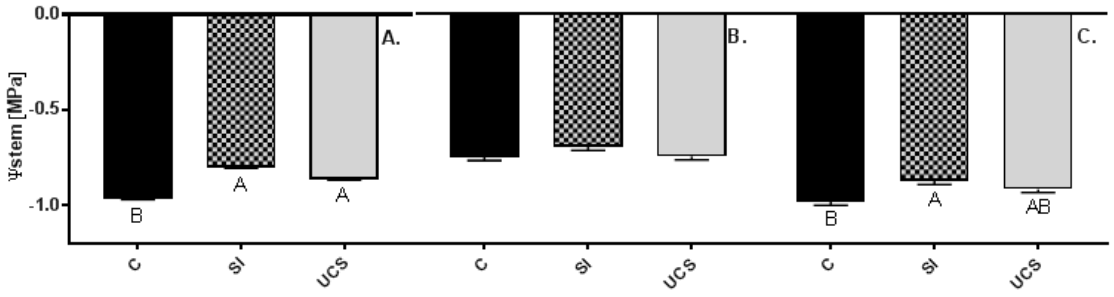


Figure 29: UCC trial at OLE, 2017-18. Midday stem (Ψ_{stem}) water potentials. **A.** Pre-heatwave Ψ_{stem} on 09/12/2017, **B**. Mid-heatwave Ψ_{stem} on 13/12/2017, **C.** Post-heatwave Ψ_{stem} on 16/12/2017.

Similar results were obtained in the second season at OLE. Ψ_{pd} was observed to be lowest in the control vines while SI and UCS vines had higher values indicating more soil moisture availability (Figure 28). Vine water status was also higher pre-heatwave and post-heatwave in those treatment, likely reflecting the moisture availability in the soil. Ψ_{stem} values approached -1.0 MPa before the heatwave, indicating moderate stress, but increased to -0.7 MPa during the heatwave, which could reflect a degree of stomatal closure as the heat-stressed vines attempt to minimise water loss (Figure 29). The reduction of Ψ_{stem} following the heatwave could reflect a re-opening of stomata and increased transpiration.

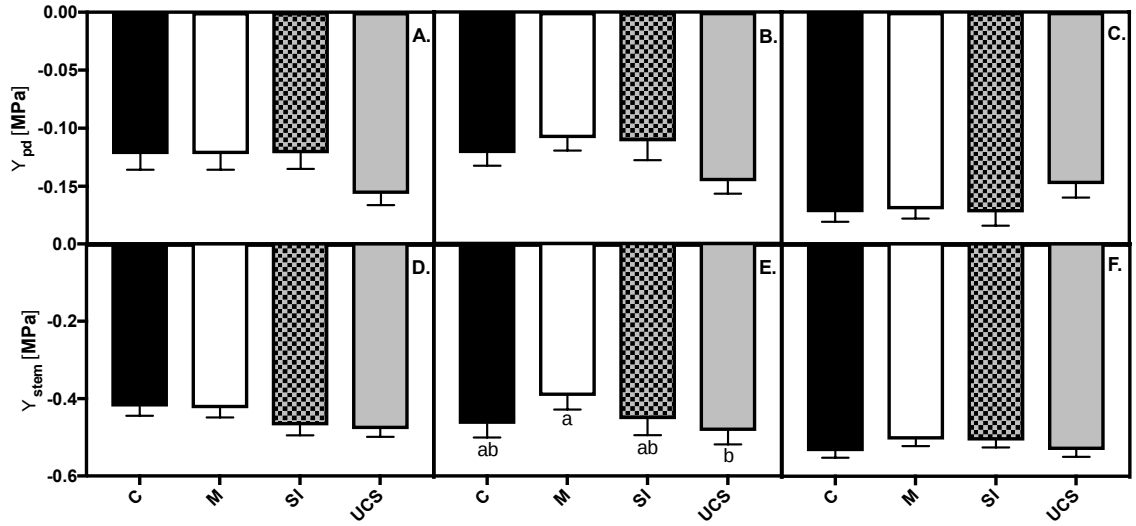


Figure 30: Predawn (Ψ_{pd}) and mid-day stem (Ψ_{stem}) water potential on Cabernet Sauvignon vines at ALV, 2016-17. **A**. Pre-heatwave Ψ_{pd} on 15/01/2017, **B**. Mid-heatwave Ψ_{pd} on 17/01/2017, **C.** Post-heatwave Ψ_{pd} on 21/01/2017, **D**. Pre-heatwave Ψ_{stem} on 15/01/2017, **E**. Mid-heatwave Ψ_{stem} on 17/01/2017, **F.** Post-heatwave Ψ_{stem} on 21/01/2017.

At Alverstoke vineyard in the first season, soil moisture as indicated by Ψ_{pd} was similar at all timepoints surrounding a heatwave event (Figure 30-A,B,C). Values of Ψ_{pd} were higher than at OLE (Riverland),

likely due to the higher clay content in the soil and also higher organic matter and midrow fescues providing greater water retention in the soil. Vine water status was also similar pre-heatwave but difference mid-heatwave; the misting treatment had the best water status or highest Ψ_{stem} (-0.4 MPa) while the UCS treatment had the lowest Ψ_{stem} at around -0.5 MPa (Figure 30-E). After the heatwave, all vines had the same water status.

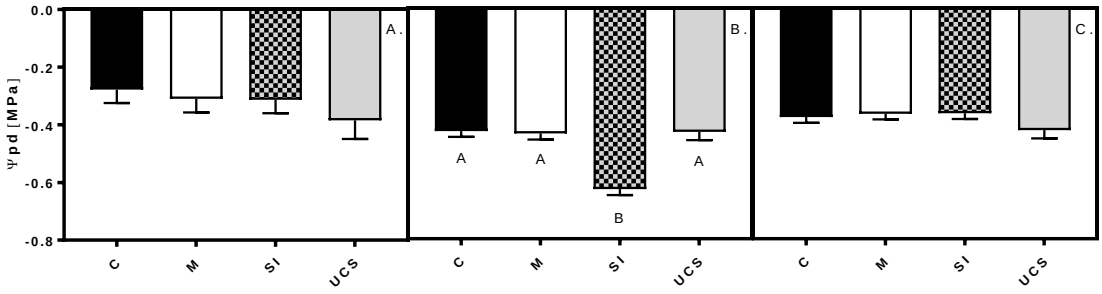


Figure 31: Predawn water potential (Ψ_{pd}) in Cabernet Sauvignon vines at ALV, 2017-18. **A**. Pre-heatwave Ψ_{pd} on 24/1/2018, **B**. Mid-heatwave Ψ_{pd} on 27/1/2018, **C.** Post-heatwave Ψ_{pd} on 31/1/2018.

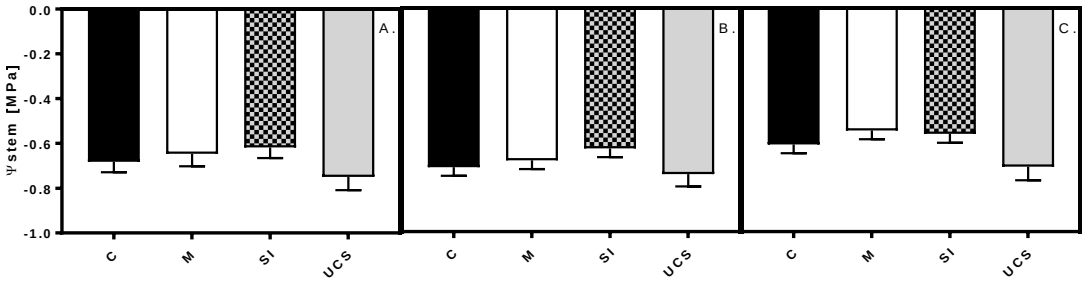


Figure 32: Mid-day stem water potential (Ψ_{stem}) in Cabernet Sauvignon vines at ALV, 2017-18. **A**. Pre-heatwave Ψ_{stem} on 24/1/2018, **B.** Mid-heatwave Ψ_{stem} on 27/1/2018, **C**. Post-heatwave Ψ_{stem} on 31/1/2018.

At Alverstoke vineyard in Season 2, soil moisture was similar between treatments leading up to a heatwave in January 2018 (Figure 31A) but during the heatwave, SI vines experienced a significant depletion of soil moisture compared to the control vines as well as other cooling treatments. Following the heatwave, all vines had the same soil moisture availability. Vine water status (Ψ_{stem}) was similar across all the treatments, therefore, misting or undercanopy water applications did not influence or affect vine water status in this block (Figure 32). Values of Ψ_{stem} were above -0.8 MPa indicating that the vines were not experiencing water stress even in the midst of the heatwave. This could possibly be due to partial stomatal closure that would raise the water potential above values when the stomata were completely open.

5.3.2 Leaf gas exchange

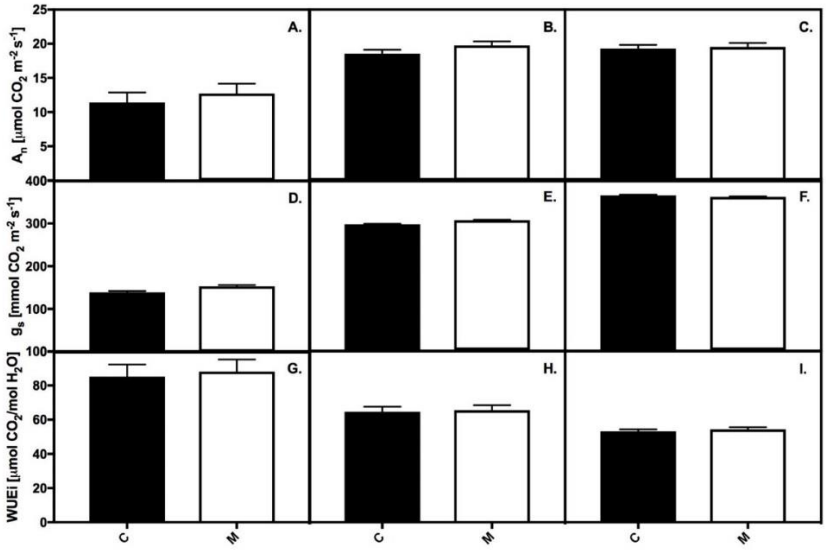


Figure 33: ICC trial at OLE, 2016-17. **A**. Pre-heatwave photosynthesis on 02/01/2017, **B**. Mid-heatwave photosynthesis on 08/01/2017, **C**. Post-heatwave photosynthesis on 11/01/2017, **D**. Pre-heatwave stomatal conductance on 02/01/2017, **E**. Mid-heatwave stomatal conductance on 08/01/2017, **F**. Post-heatwave stomatal conductance on 11/01/2017, **G.** Pre-heatwave water use efficiency on 02/01/2017, **H**. Mid-heatwave water use efficiency on 08/01/2017, **I.** Post-heatwave water use efficiency on 11/01/2017.

Leaf gas exchange in the Sauvignon Blanc vines in Season 1 at OLE showed small very few differences between misting and control vines (Figure 33). Net photosynthesis, stomatal conductance, and intrinsic water use efficiency were not affected by misting despite the resulting cooler canopy temperatures (Fig. 7c). Photosynthesis, shown in the first row of Figure 33 (A-C), appeared to be slightly but non-significantly higher in the misted vines but in both cases were higher than 15 µmol $CO_2/m^2/s$ during and after the heatwave. The increase in photosynthesis rates compared to pre-heatwave period could reflect the increased demand for transpirational cooling during the heatwave and more open stomata (Greer and Weedon 2013), as seen in the higher stomatal conductance values (Figure 33, D-F). This was supported by the lack of water stress in those vines (Figure 25). Intrinsic water use efficiency decreased under these warmer conditions (G-I) and can be explained by higher stomatal conductance relative to net photosynthesis as the efficiency of carbon assimilation decreases at higher temperatures (Greer and Weedon 2012), and there are known differences to temperature responses between different grapevine cultivars (Greer 2018).

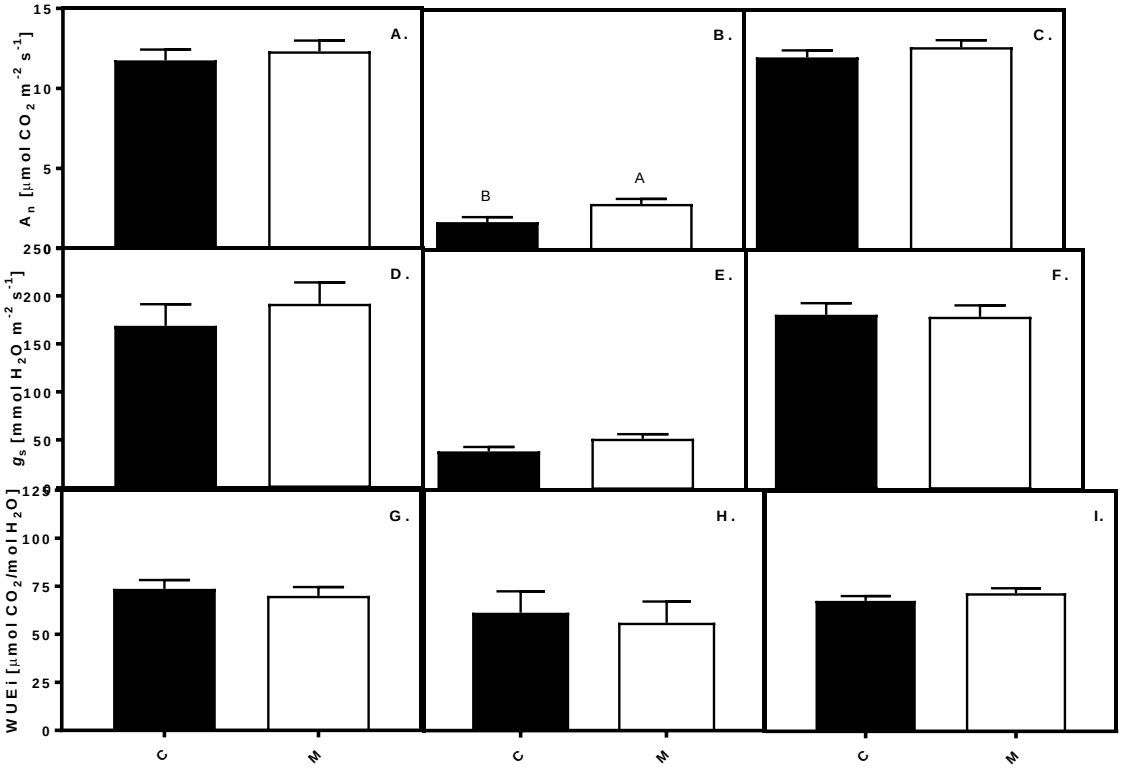


Figure 34: Leaf gas exchange data from the ICC trial at OLE in 2017-18. Photosynthesis (A_n), stomatal conductance (g_s), and intrinsic water use efficiency (WUEi). **A.** Pre-heatwave A_n on 9/12/17, **B**. Mid-heatwave A_n on 13/12/17, **C**. Post heatwave A_n on 16/12/17, **D**. Pre-heatwave g_s on 9/12/17, **E**. Mid-heatwave g_s on 13/12/17, **F**. Post-heatwave g_s on 16/12/17, **G.** Pre-heatwave WUEi on 9/12/17, **H**. Mid-heatwave WUEi on 13/12/17, **I.** Post-heatwave WUEi on 16/12/17.

In the second season at OLE (Riverland), no differences were observed in the gas exchange profiles of the misted vs uncooled Sauvignon Blanc canopies (Figure 34). Explanation for this lack of response to the misting treatment, which clearly had a cooling effect on the vines (Figure 13) include inadequate cooling due to long intervals (5 min) between misting events and also the possibility that a temperature reduction of 2-3 °C was insufficient to influence vine physiology. The misting temperatures inside the canopy exceeded the 35 °C threshold, rising above 40 °C in some cases, which could explain the lack of response to the misting.

Both pre- and post-heatwave in early December 2017, the vines had relatively high values of photosynthesis, albeit lower than in the previous season. Net photosynthesis and stomatal conductance values dropped dramatically during the heatwave by as much as 90% of the maximum values (Figure 34, B, E). The drastic reduction in leaf gas exchange was reflective of the early arrival of these heatwaves during the season with a smaller canopy and younger leaves that had not acclimated to the high temperatures. Although mature leaves were always measured in the study, the leaves may not have had an opportunity to adjust to extreme events such as this heatwave.

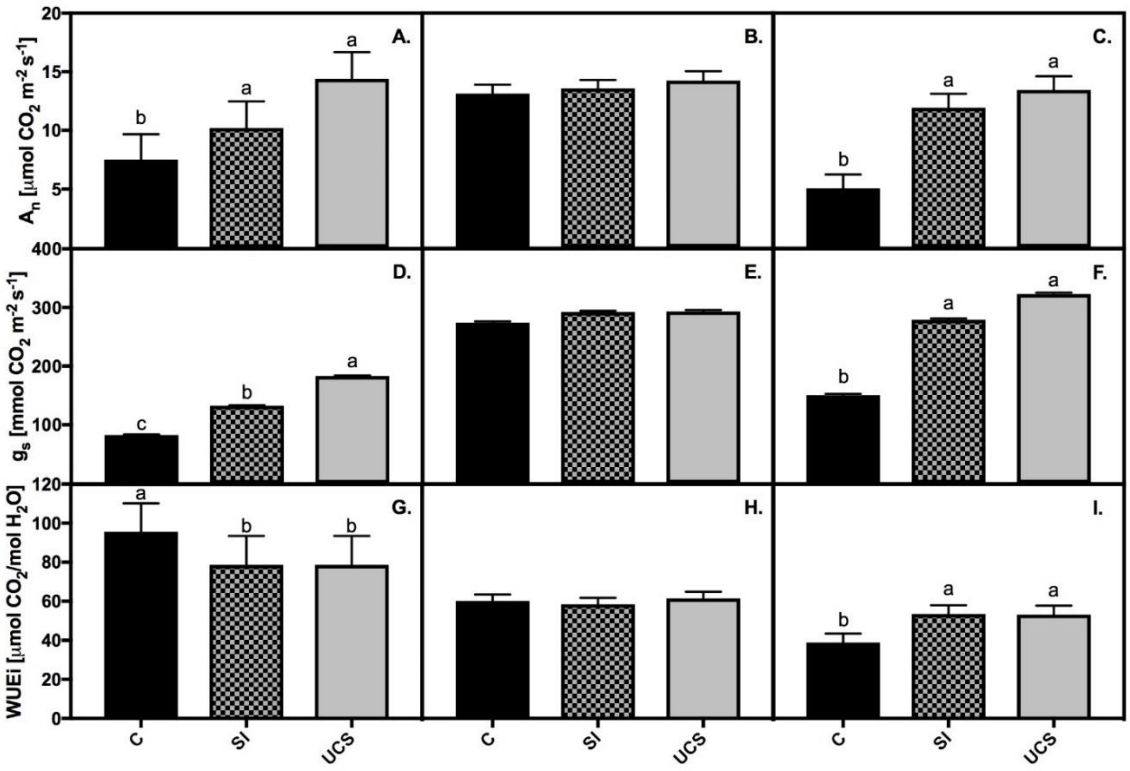


Figure 35: UCC trial at OLE, 2016-17. **A**. Mid-heatwave photosynthesis on 07/01/2017, **B.** Post-heatwave photosynthesis on 11/01/2017, **C**. Mid-heatwave photosynthesis on 10/02/2017, **D**. Mid-heatwave stomatal conductance on 07/01/2017, **E**. Post-heatwave stomatal conductance on 11/01/2017, **F**. Mid-heatwave stomatal conductance on 10/02/2017, **G**. Mid-heatwave water use efficiency on 07/01/2017, **H**. Post-heatwave water use efficiency on 11/01/2017, **I**. Mid-heatwave water use efficiency on 10/02/2017.

Significant differences in leaf gas exchange were observed between treatments in the Sauvignon Blanc vines that received the undercanopy cooling treatments at OLE in the first season. Highest net photosynthesis rates (A_n) were observed in the undercanopy cooled treatments pre- and post-heatwaves. Uncooled vines had A_n values under 7 µmol $CO_2/m^2/s$ while cooled vines had value 1.5× to 2× higher (Figure 35). Cooled vines also appeared not to suffer the adverse effects of the heatwave compared to control vines, with the latter having decreased their A_n rates (Figure 35C). Stomatal conductance (g_s) of these vines were also correspondingly higher: cooled vines had higher g_s values between 100-150 mmol $H_2O/m^2/s$ prior to the heatwave (Figure 35D), but during the heatwave, all vines seemed to show higher values (Figure 35E), while post-heatwave, the cooled vines again had higher values (Figure 35F). WUE_i values were higher in the control (un-cooled vines) prior to the heatwave but similar at later timepoints.

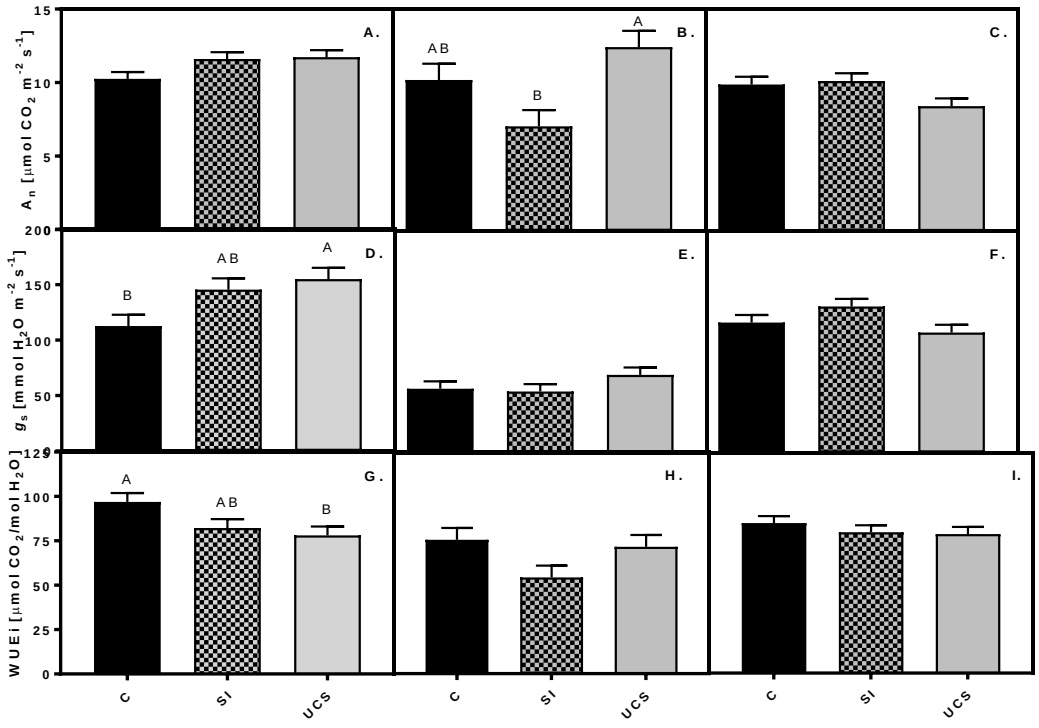


Figure 36: UCC trial at OLE, 2017-18. Stomatal conductance (g_s), and intrinsic water use efficiency (WUEi). **A**. Pre-heatwave A_n on 9/12/17, **B**. Mid-heatwave A_n on 13/12/17, **C**. Post heatwave A_n on 16/12/17, **D**. Pre-heatwave g_s on 9/12/17, **E**. Mid-heatwave g_s on 13/12/17, **F**. Post-heatwave g_s on 16/12/17, **G**. Pre-heatwave WUEi on 9/12/17, **H**. Mid-heatwave WUEi on 13/12/17, **I**. Post-heatwave WUE_i on 16/12/17.

In the second season at OLE, the Sauvignon Blanc vines showed few differences in A_n prior to the heatwave event in December 2017 although g_s values were significantly higher in the SI and UCS vines (Figure 36D) and WUE_i values lower (G). During the midst of the heatwave, A_n values were highest in the UCS vines compared to either of the other two treatments, control and SI (B). This could be the result of the more favourable canopy microclimatic conditions, specifically lower VPD levels in those vines (Figure 13). g_s levels were highest in the cooled vines, especially going into the heatwave (Figure 36D) but dropped significantly mid-heatwave to less than half their maximum values to around 50 mmol $H_2O/m^2/s$ indicating partial to near-complete stomatal closure. This indicated a considerable level of water stress on the vines even though there was adequate soil moisture (Ψ_{pd} > -0.3 MPa) and good vine water status (Ψ_{stem} > -0.8 MPa). No differences in WUE_i were observed except initially preceding the heatwave (G). All the vines returned to comparable levels of gas exchange following the heatwave (Figure 36, C,F,I).

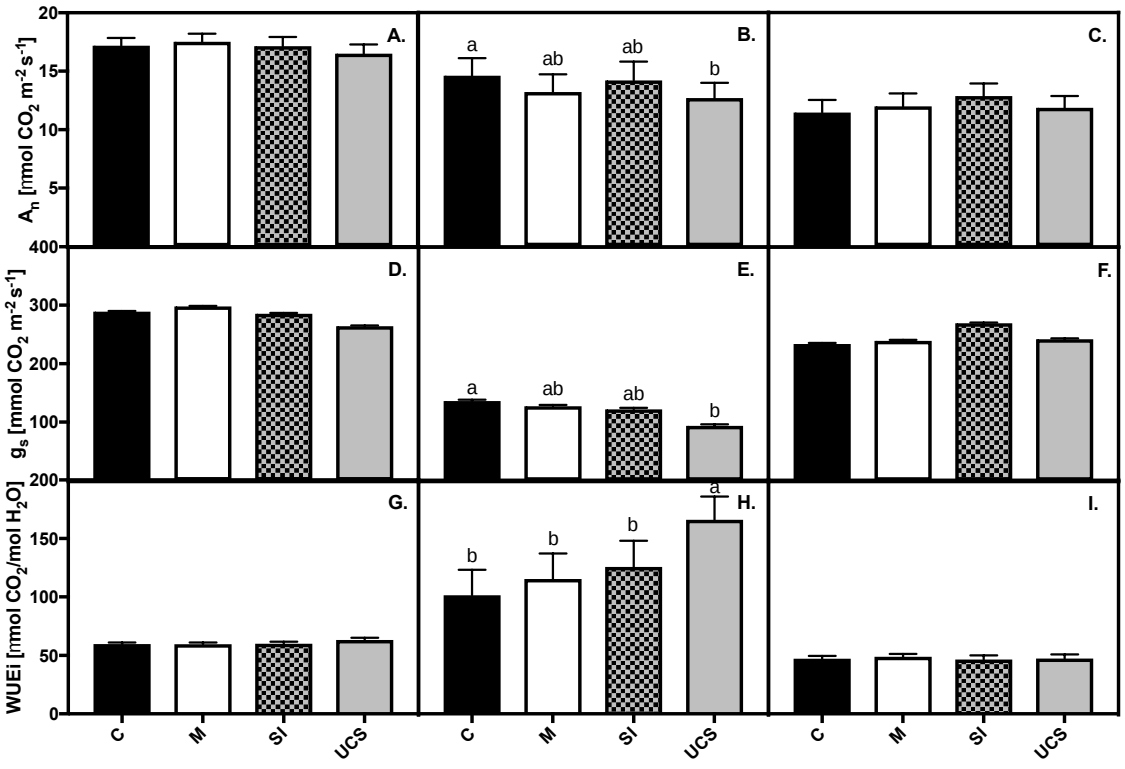


Figure 37: Gas exchange results from the Cabernet Sauvignon canopy cooling trial gas at ALV in 2016-17. **A**. Pre-heatwave photosynthesis on 15/01/2017, **B**. Mid-heatwave photosynthesis on 17/ 01/2017, **C**. Post-heatwave photosynthesis on 21/01/2017, **D**. Pre-heatwave stomatal conductance on 15/01/2017, **E**. Mid-heatwave stomatal conductance on 17/ 01/2017, **F**. Post-heatwave stomatal conductance on 21/01/2017 **G**. Pre-heatwave water use efficiency on 15/01/2017, **H**. Mid-heatwave water use efficiency on 17/ 01/2017, **I.** Post-heatwave water use efficiency on 21/01/2017.

At the ALV vineyard, in Season 1, the Cabernet Sauvignon vines appeared to be very similar in terms of leaf gas exchange parameters prior to the heatwave (Figure 37, A,D,G). In the midst of the heatwave in mid-January 2017, A_n rates were considerably higher in the control vines compared to the UCS vines, and similar to the SI and ICM vines (Figure 37B). g_s was concomitantly lower in the UCS vines compared to control vines (Figure 37E) while WUE_i was significantly higher in the UCS vines compared to the other treatments. Following the heatwave, all vines were observed to have very similar gas exchange levels (Figure 37, C,F,I).

These results were in contrast to those observed in the OLE vineyard during the same season indicating either cultivar-specific responses to heat, differences in the vineyard environment, or an unknown factor influencing vine physiological performance in the two vineyards. Cabernet Sauvignon is known to be relatively more sensitive to heat and water stress compared to, for example Shiraz, but not much is known about Sauvignon Blanc in this context as it tends to be grown in cooler viticultural regions around the world. The "tougher" OLE site with higher temperatures and deeper draining sandy soils may have conditioned the OLE vines to perform better under more adverse conditions compared to the Cabernet vines at Waite. The ALV vineyard is organically managed and relatively shaded with large Eucalyptus trees and other vegetation surrounding the block providing cooler and more favourable conditions during heatwaves. The presence of interrow fescues in this block might also result in higher organic matter and therefore better nutritional status of the vines that could have influenced the result (the OLE site had no vineyard floor vegetation). Finally, these grasses and other

cover crops could have lowered soil temperatures due to higher moisture retention (Celette et al. 2008) and subsequent evaporative cooling; these lower temperatures in turn may be positively influencing vine physiological performance, which is something that needs to be investigated in more detail in future studies of these undercanopy cooling strategies.

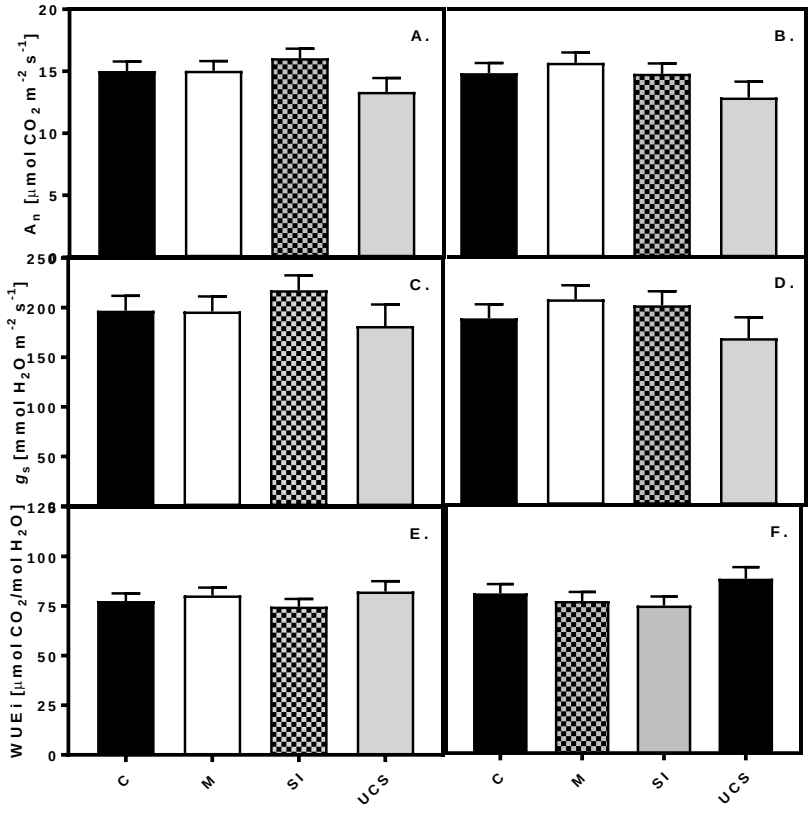


Figure 38: Gas exchange results from the Cabernet Sauvignon canopy cooling trial gas at ALV in the 2017-18 season. Photosynthesis (A_n), stomatal conductance (g_s), and intrinsic water use efficiency (WUE_i). **A**. Pre-heatwave A_n on 24/1/18, **B**. Mid-heatwave A_n on 27/1/18, **C**. Pre-heatwave g_s on 24/1/18, **D**. Mid-heatwave g_s on 27/1/18, **E**. Pre-heatwave WUE_i on 24/1/18, **F.** Mid-heatwave WUE_i on 27/1/18.

During the second season at ALV, the Cabernet Sauvignon vines appeared to have very similar leaf gas exchange values prior to the heatwave (Figure 38-A,C,E). A_n values were around 15 µmol $CO_2/m^2/s$ while g_s values were around 200 mmol $H_2O/m^2/s$, indicating no stress ion the vines. In the midst of the heatwave in late January 2018, similar levels of photosynthesis and stomatal conductance were observed in all vines, and these levels did not indicate any level of physiological stress (Figure 38-B,D,F). The lack of responses in gas exchange with the various canopy cooling treatments could reflect the buffering capacity of this organic vineyard due to some of the factors mentioned in the previous paragraph. Organic management of vineyards should be therefore considered in the context of future climate change, especially mitigation against heatwaves, and certainly more research needs to focus on this space in the Australian context.

5.3.3 Xylem ABA

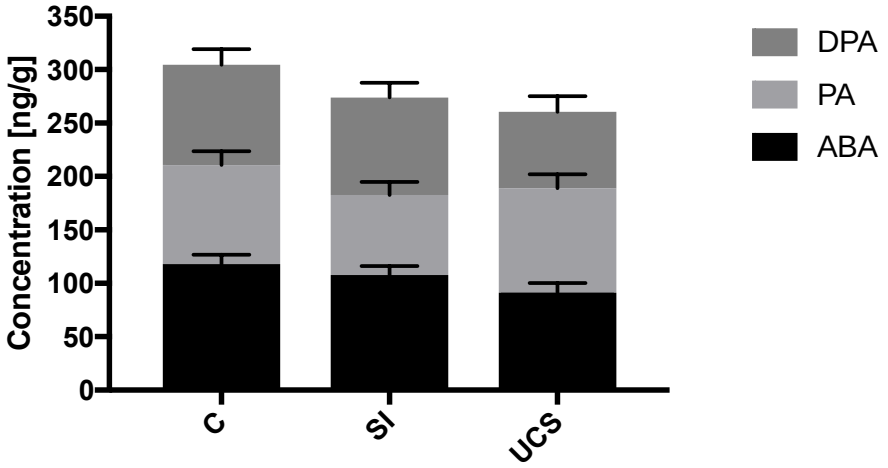


Figure 39: ABA, PA, and DPA concentrations extracted from Sauvignon Blanc xylem sap during a heatwave on January 30th, 2017 at OLE (Riverland).

Abscisic acid (ABA) concentrations were analysed in xylem sap obtained from leaves of the Sauvignon Blanc vines in the OLE vineyard in 2016-17. No significant differences were observed in ABA levels, or its catabolites phaseic acid (PA) and diphaseic acid (DPA) (Figure 39). This was not an unexpected result as soil moisture levels were the same across all treatments (Figure 25-A,B). However, observable trends indicate that the control vines had the highest absolute value of ABA, indicating current stress at the time of measurement relative to the treatments. This was reflected in the slightly lower Ψ_{stem} values in the control vines indicating a level of water stress (Figure 25-C,D).

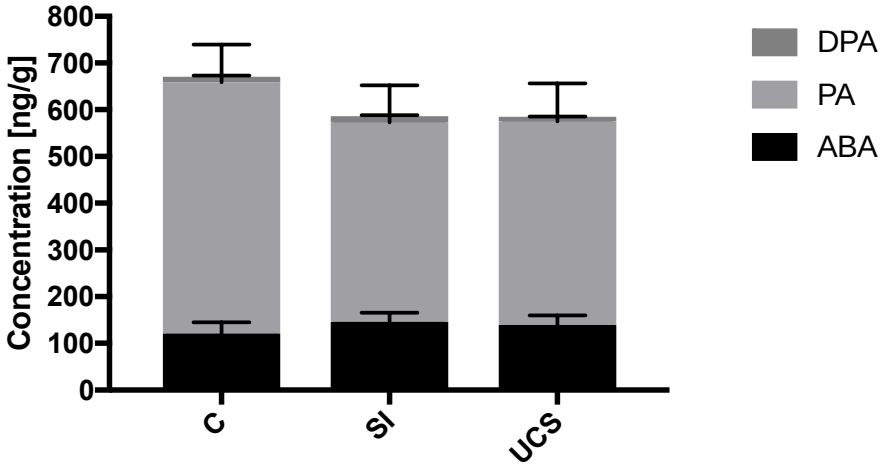


Figure 40: ABA, PA, and DPA concentrations extracted from Sauvignon Blanc xylem sap during a heatwave on January 29th, 2017 at ALV (Waite).

In the ALV vineyard (Waite) in the 2016-17 season, no significant differences were observed in abscisic acid (ABA) levels, or its catabolites phaseic acid (PA) and diphaseic acid (DPA) between the different

undercanopy cooling treatments (Figure 40). Soil moisture, as indicated by Ψ_{pd}, were similar across treatments (Figure 30-A,B,C). However, observable trends indicate that the control vines had the highest absolute values of PA, indicating past or accumulated water stress relative to the cooling treatments.

Xylem sap was collected in the second season but analysis of ABA was not completed by the time of submission of this report.

5.4 Berry development

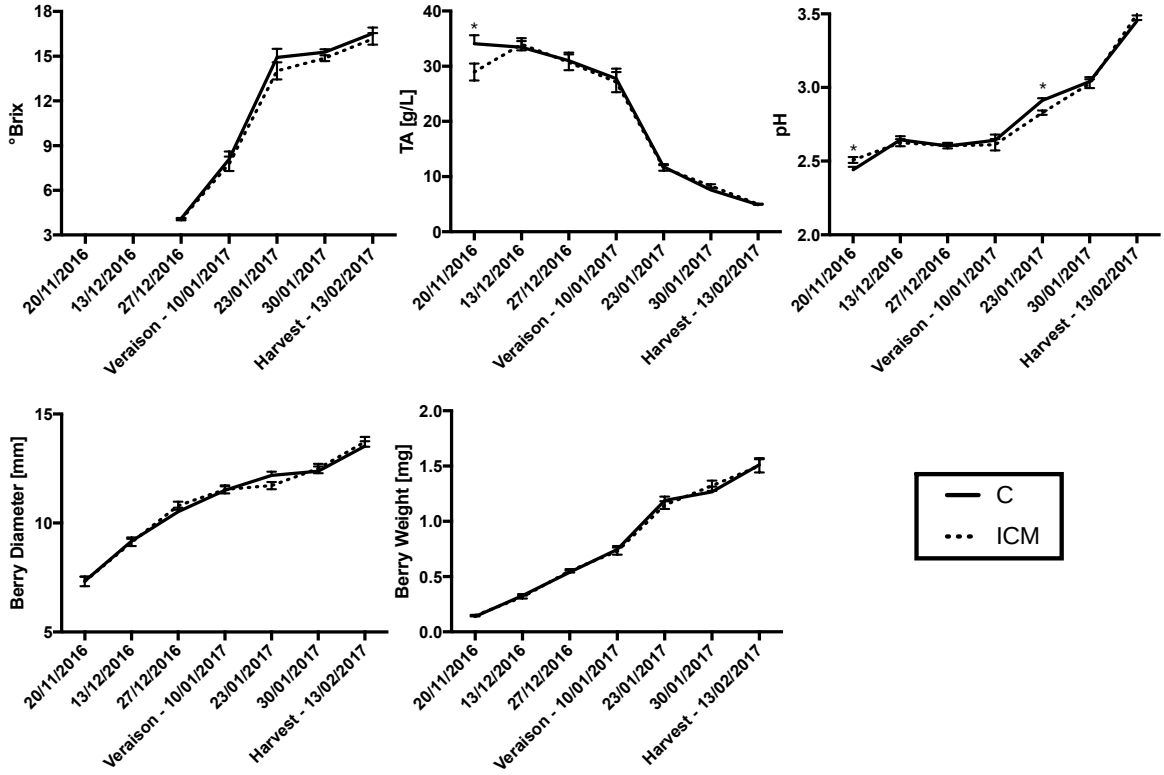


Figure 41: Bi-weekly Sauvignon Blanc berry development from the ICC trial in the 2016-17 season at OLE (Riverland). Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate and vine as nested random effects. Stars indicate significance determined at $P < 0.05$.

The ICC trial showed significant differences in pH and TA at fruit set early in the first season, 2016-17. The control fruit had significantly higher TA and lower pH than the ICM treatment at this stage (Figure 41). At two weeks post veraison, ICM was significantly lower in pH. However, these differences did not carry through to the must or the wines. Misting treatment had no discernible affect on berry size.

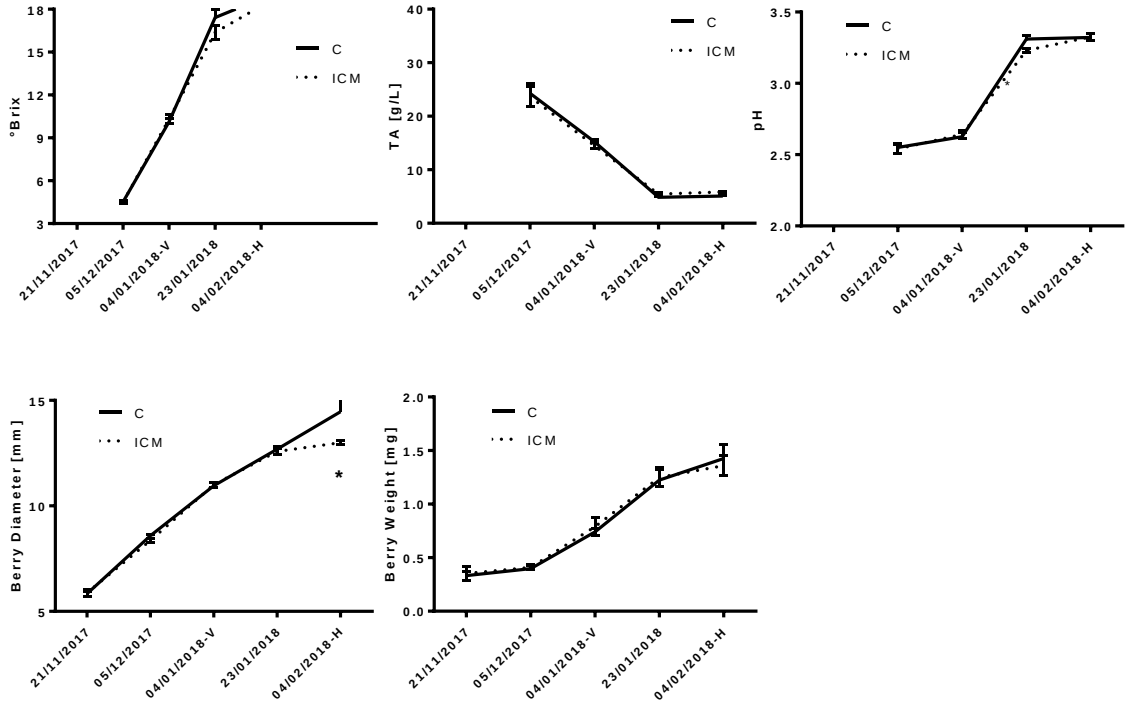


Figure 42: Bi-weekly Sauvignon Blanc berry development from the ICC trial in the 2017-18 season at OLE (Riverland).

In the second season, 2017-18, the Sauvignon Blanc berries developed similarly during the early stages of growth (Figure 42). However, post-veraison development was altered in the misting treatment compared to control, with soluble solids, berry diameter and weight being lower in the misted fruit. Water absorption through the berry cuticle cannot explain these results as the misted berries would be expected to be larger from the increased water content. It is therefore plausible that misting retarded the development of berries from both a sugar accumulation standpoint and also a growth standpoint.

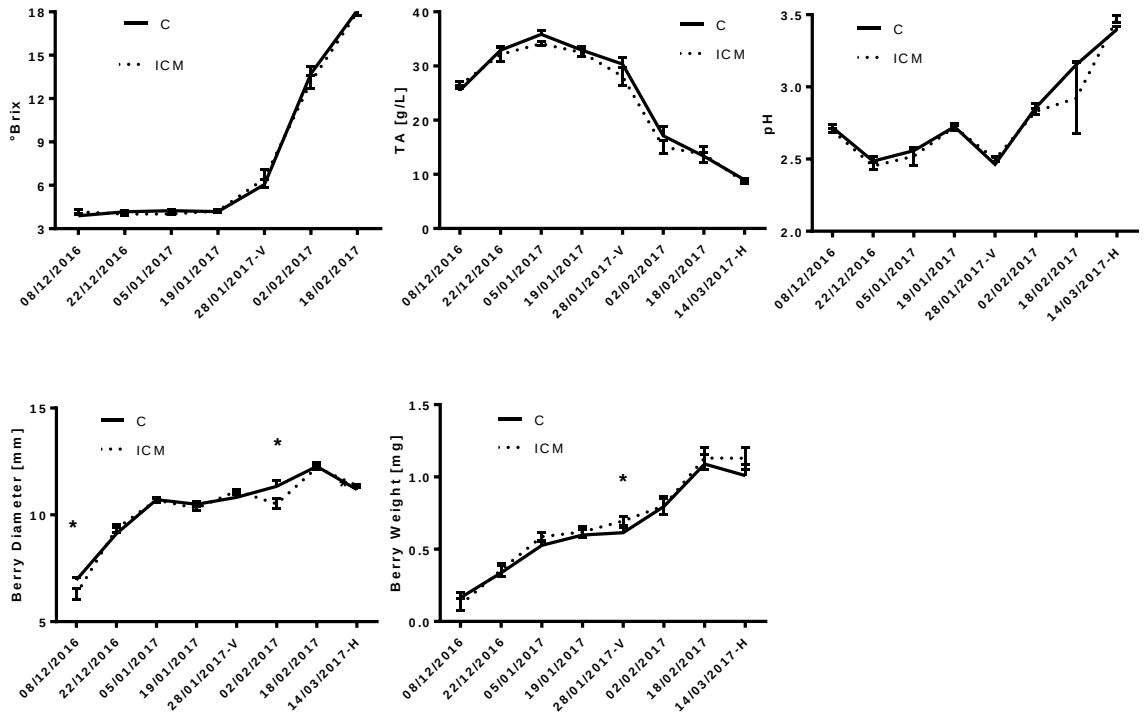


Figure 43: Bi-weekly Cabernet Sauvignon berry development from the ICC trial in the 2016-17 season at ALV (Waite).

Cabernet Sauvignon berries developed similarly in the two treatments at ALV vineyard in the first season, 2016-17. Post-veraison, there were differences observed between treatments, with the misting treatment having larger berries by harvest (Figure 43). There were no significant differences in fruit composition between the two treatments.

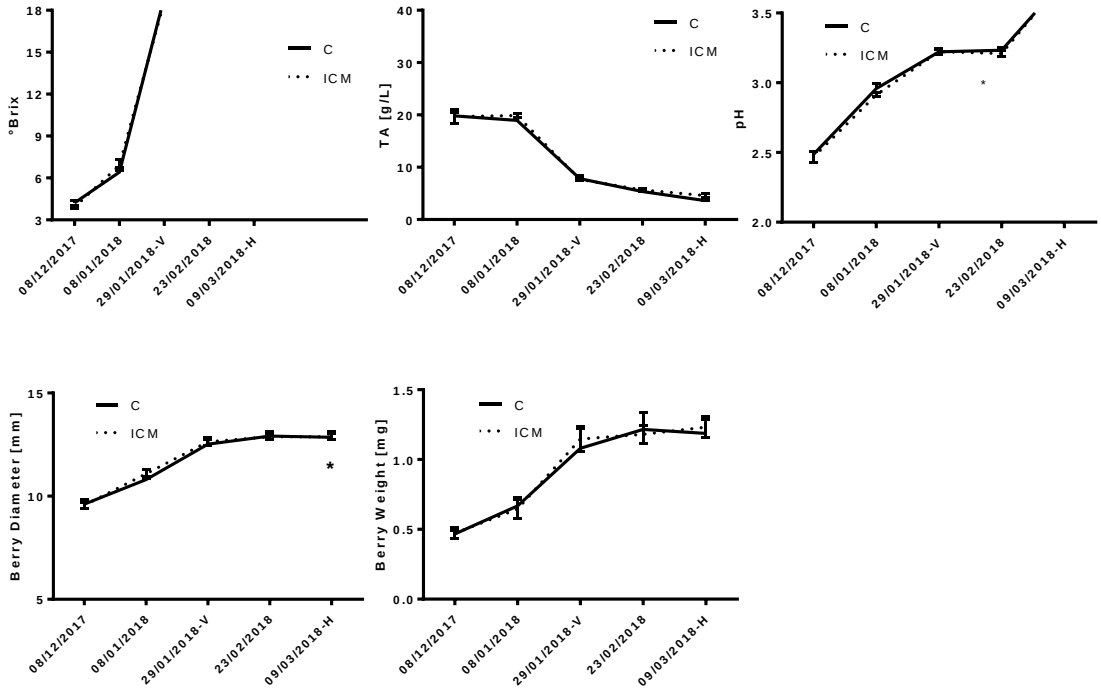


Figure 44: Bi-weekly Cabernet Sauvignon berry development from the ICC trial in the 2017-18 season at ALV (Waite).

In the second season, 2017-18, Cabernet Sauvignon berries at ALV also developed similarly in the two treatments (Figure 44). There were no significant differences in either fruit size or fruit composition between the two treatments. These results are consistent with the physiological measurements of the vines in this vineyard – the lack of treatment effect indicates that the misting treatments were not having any positive effect or rather the heat stress was not detrimental to the vines in this block, which could be due to several reasons as outlined in Sec. 5.3.2.

5.5 Berry cell vitality



Figure 45: An example of a FDA-stained Sauvignon Blanc berry with 84% live cells from the control treatment in the ICC trial at OLE (Riverland), 2016-17 season.

Table 4: Percentage of living cells in ICC trial Sauvignon Blanc berries at harvest, 2016-17, OLE (Riverland). Means ± SEM. Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate and vine as nested random effects. Stars indicate significance determined at $P < 0.05$.

Treatment	% Living Cells
C	70.2 ± 3.4
ICM	71.9 ± 3.4
P value	0.6815

There were no differences in berry cell vitality at harvest in the ICM treatment as determined by fluorescent microscopy (Table 4, Figure 45). Berry cell death has been shown to result in dehydration of the fruit and shrivelling in Shiraz and Cabernet Sauvignon in South Australia. Cell death results from depletions in oxygen in the berry, or hypoxic conditions, which are exacerbated by water stress and possibly heat/light stresses (see Wine Australia Incubator Project UA 1702). The approx. 30% incidence of non-vital cells in the berries suggests that cell death occurred in all the fruit grown in the Riverland, which may contribute to changes in fruit composition and wine quality and is a topic for future study.

Table 5: Percentage of living cells in ICC trial Sauvignon Blanc berries in 2017 at ALV. Means ± SEM. Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at $P < 0.05$.

Treatment	% Living Cells on 08/02/2017	% Living Cells at Harvest 15/03/2017
C	77.9 ± 4.3	81.1 ± 3.5
ICM	80.8 ± 4.3	84.1 ± 3.5
P value	0.389	0.5773

In the ALV vineyard in 2016-17, there were no differences in Cabernet Sauvignon berry cell vitality in the ICM treatment as determined by fluorescent microscopy (Table 5). Approx. 81% of cells were viable in the control vines while approx. 84% of cells in the misted treatment were viable. These differences are statistically non-significant. The approx. 15-20% cell death incidence in the fruit indicates that there could be a level of berry shrivel in this block of Cabernet under seasonal conditions that could be considered similar to historical averages (Figure 6). Preliminary results from the Wine Australia UA 1702 project on Cabernet berry shrivel suggest that 20-25% shrivelled fruit in the crush can lead to lower wine quality. It is therefore imperative that heatwaves and seasonal stresses from either temperature or water be mitigated in order to maintain wine quality.

5.6 Yield components and pruning weights

5.6.1 Sauvignon Blanc (OLE – Riverland)

Table 6: Yield components, pruning weights and crop load (Ravaz Index) of ICC trial Sauvignon Blanc at OLE (Riverland) in 2017. Means ± SEM. Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at $P < 0.05$.

Treatment	**Bunches per Vine**	**Bunch Weight (g)**	**Berry Weight (g)**	**Berries per Bunch**
C	158 ± 18.2 a	153.4 ± 8.88	1.51 ± 0.06	101 ± 5.81
ICM	222 ± 18.2 b	151.9 ± 8.88	1.50 ± 0.06	102 ± 5.81
P - Value	0.0330	0.9064	0.8688	0.9311
Treatment	**Yield per Vine (kg)**	**Pruning Weight (kg)**	**Ravaz Index (kg/kg)**	
C	19.9 ± 1.33	1.54 ± 0.09	12.4 ± 1.2	
ICM	22.9 ± 1.33	1.57 ± 0.09	14.7 ± 1.2	
P - Value	0.1514	0.8005	0.1779	

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at $P < 0.05$.

Yield components were measured at harvest in the OLE vineyard (Riverland). In the first season, yield per vine was slightly higher in the misting treatment, by approx. 3 kg/vine (Table 6). The higher yields can be explained by the greater number of bunches (higher bud fruitfulness) in the misting vines that had similar bunch weights, berry weights and berries per bunch. Simulated mechanical box hedge pruning of the vines was used to determine pruning weights. Both treatments had similar vine pruning weights. The crop load, indicated by the Ravaz Index, of the misted vines was slightly higher than controls due to the higher yields. This higher Ravaz Index indicates a degree of overcropping. Balanced vines are generally cropped lower to yield Ravaz Index values between 5-10 for higher fruit quality.

Table 7: Yield components of ICC trial Sauvignon Blanc at OLE (Riverland) in 2018. Means ± SEM. Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Treatment	Bunches per Vine	Bunch Weight (g)	Berry Weight (g)
C	248.4 ± 1.5	106.0 ± 4.6	1.50 ± 0.02
ICM	247.7 ± 1.9	99.4 ± 4.3	1.49 ± 0.02
P value	0.9770	0.3166	0.8117
Treatment	**Berries per Bunch**	**Yield per Vine (kg)**	
C	70.6 ± 3.0	26.1 ± 1.6	
ICM	66.5 ± 2.4	24.1 ± 1.1	
p value	0.3021	0.3136	

During the second season in the same OLE vineyard, yields per vine were higher in the control than in the misted vines by approx. 2 kg/vine (Table 7). This difference can be explained by the higher bunch weights in the control vines and the higher berries per bunch, an indication of superior fruit set. Berry weights in both treatments were virtually the same as were the number of bunches per vine. It can therefore be concluded from two seasons of this trial that yield components were not affected by misting.

Table 8: Yield components, pruning weights and crop load (Ravaz Index) of UCC trial Sauvignon Blanc at OLE (Riverland) in 2017. Means ± SEM. Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Treatment	Bunches per Vine	Bunch Weight (g)	Berry Weight (g)
C	256 ± 20.1	90.3 ± 8.29	1.50 ± 0.09
SI	206 ± 20.1	115.0 ± 8.29	1.57 ± 0.09
UCS	214 ± 20.1	104.5 ± 8.29	1.42 ± 0.09
p value	0.2156	0.2453	0.2466
Treatment	**Yield per Vine (kg)**	**Pruning Weight (kg)**	**Ravaz Index (kg/kg)**
C	22.5 ± 1.89	1.40 ± 0.17	16.1 ± 2.5
SI	23.2 ± 1.89	1.14 ± 0.17	20.0 ± 2.5
UCS	22.0 ± 1.89	1.37 ± 0.17	17.9 ± 2.5
p value	0.8795	0.5808	0.4431

In the UCC trial at OLE vineyard in the first season, yields were highest in the SI treatment followed by the control and UCS treatments; these values were non-significantly different (Table 8). The higher bunch weights observed in the cooled treatments can be explained by the higher berry weights in the SI but not the UCS. Interestingly, these treatments had the lowest fruitfulness as indicated by the lower bunches per vine. Vine vigour as indicated by the pruning weight was found to be highest in the control and UCS vines, and lowest in the SI vines. Ravaz Index values were highest in the SI treatment,

likely due to the relatively lower vine size or pruning weights. The UCS vines were similar to control vines.

Table 9: Yield components of UCC trial Sauvignon Blanc at OLE (Riverland) in 2018. Means ± SEM. Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Treatment	Bunches per Vine	Bunch Weight (g)	Berry Weight (g)
C	223.4 ± 19.6	91.1 ± 6.9	1.52 ± 0.03
SI	211.6 ± 25.9	133.7 ± 32.2	1.58 ± 0.04
UCS	227.3 ± 43.8	104.1 ± 8.1	1.57 ± 0.03
p value	0.8065	0.4985	0.7403
Treatment	**Berries per Bunch**	**Yield per Vine (kg)**	
C	59.9 ± 4.4	19.5 ± 1.3	
SI	83.1 ± 17.5	23.0 ± 1.0	
UCS	66.3 ± 5.0	21.2 ± 1.7	
p value	0.3098	0.6886	

In the second season at OLE, yields of the two cooling treatments were higher than the control vines (Table 9). SI vines had approx. 23 kg of fruit per vine while UCS had over 21 kg/vine. These higher yields can be explained by the higher bunch weights likely due to better fruit set indicated by the higher berries per bunch in those treatments. Compared to the first season, the yields were relatively higher in all treatments. The control vines had over 3 kg/vine higher yields while the SI and the UCS vines had 3 kg/vine and 4 kg/vine higher yields, respectively. The higher yields of these vines in the second season could be attributed to improved conditions at flowering and fruit set; warmer conditions in October during the second season were favourable for fruit set (Table 1).

Table 10: Pruning (vine vigour) metrics of ICC and UCC trials in Sauvignon Blanc at OLE (Riverland) in 2018. Sections were of the basal three internodes of the cane. Means ± SEM of three vines per treatment in each of three blocks. Different letters following means indicate significant differences in means as per Tukey's HSD test at P<0.05.

Treatment	Cane section length (cm)	Basal cane diameter (mm)	Cane section weight (g)	Cane Density (g/m)
C	23.4 ± 1.2	8.3 ± 0.3	8.9 ± 0.9	37.7 ± 2.7
ICM	23.0 ± 1.4	8.5 ± 0.2	9.1 ± 0.9	39.6 ± 2.6
p value	>0.9	>0.9	>0.9	>0.9
C	22.2 ± 1.0 b	7.7 ± 0.3 b	7.8 ± 0.7 b	35.1 ± 2.5 b
SI	20.5 ± 0.7 b	7.9 ± 0.3 b	7.0 ± 0.5 b	34.5 ± 2.4 b
UCS	23.8 ± 0.7 a	9.3 ± 0.3 a	11.9 ± 0.7 a	50.3 ± 2.9 a
p value	0.0239	< 0.0001	< 0.0001	< 0.0001

In the second season at OLE, due to trimming of the vine shoots prior to pruning, complete vine pruning weights could not be obtained. Therefore, sections of canes comprising of the basal three internodes were obtained and characterised for their vigour as an indication of vine vigour. In the misting trial, no significant differences were found between treatments for any vigour parameter (**Table *10***). However, in the undercanopy cooling trial, UCS vines had the highest vigour of any treatment. These vines had significantly longer cane sections, larger cane diameters, and heavier canes with higher cane density. SI vines had similar pruning metrics to the control vines. The higher vigour in the UCS treatment is consistent with their higher photosynthesis rates over the growing season, especially during heatwaves (Figure 35).

5.6.2 Cabernet Sauvignon (ALV – Waite)

Table 11: Yield components, pruning weights and crop load (Ravaz Index) of Cabernet Sauvignon grapevines at ALV in 2017. Means ± SEM.

Treatment	**Bunches per Vine**	**Bunch Weight (g)**	**Berry Weight (g)**	**Berries Per Bunch**
C	32 ± 7.12	125 ± 11.08	1.09 ± 0.04	116 ± 13.70
ICM	39 ± 7.12	138 ± 11.08	1.14 ± 0.04	124 ± 13.70
SI	46 ± 7.12	126 ± 11.08	1.17 ± 0.04	110 ± 13.70
UCS	36 ± 7.12	127 ± 11.08	1.06 ± 0.04	121 ± 10.14
p value	0.5456	0.258	0.258	0.5826
Treatment	**Yield (kg/m)**	**Pruning Weight (kg/m)**	**Ravaz Index (kg/kg)**	
C	2.12 ± 0.82	1.88 ± 0.2	1.2 ± 0.2	
ICM	2.63 ± 0.82	1.81 ± 0.2	1.4 ± 0.2	
SI	2.58 ± 0.82	2.20 ± 0.2	1.5 ± 0.2	
UCS	2.40 ± 0.82	2.35 ± 0.2	1.1 ± 0.2	
p value	0.8291	0.2093	0.5367	

In the first season at ALV vineyard (Waite), yield was found to be higher in the cooled vines compared to the control vines (Table 11). In particular, the misting and SI treatments had the highest yields at over 2.5 kg/vine, which can be explained by the higher bunch weights and berries per bunch, as well as, in the SI treatment, bunches per vine. These results point to higher bud fruitfulness in the ICM and SI vines, and also improved fruit set in the misting vines, none of which would be affected by the current season's treatments.

Vine vigour was higher in the two undercanopy cooling treatments, UCS and SI, as indicated by their pruning weights. UCS and SI vines had 2.2 kg/m or higher of prunings. Vine balance as indicated by the Ravaz Index was between 1-2 kg/kg indicating undercropped vines. Cabernet Sauvignon in California has been reported to be balanced if the Ravaz Index value is around 5. Lower values indicated that the vines had low productivity due to either low fruitfulness or poor fruit set. The Cabernet Sauvignon vines in this block are highly shaded and this might contribute to the low bud fruitfulness observed in general. Applications of additional water under the canopy during heatwaves appears to have

stimulated vegetative growth as indicated by the higher vine vigour; this growth would have further contributed to the shading of buds.

Table 12: Yield components, pruning weights and crop load (Ravaz Index = Yield/Pruning Weight) of Cabernet Sauvignon grapevines at ALV in 2018. Means ± SEM.

Treatment	Bunches per m	Bunch Weight (g)	Berry Weight (g)	Berries Per Bunch
C	26.7 ± 3.4	119.1 ± 9.0	1.18 ± 0.04	100.3 ± 5.9
ICM	32.3 ± 3.6	113.8 ± 5.5	1.23 ± 0.03	92.9 ± 6.3
SI	34.9 ± 2.9	117.6 ± 8.4	1.19 ± 0.02	98.6 ± 6.0
UCS	35.9 ± 2.4	117.9 ± 9.7	1.10 ± 0.06	108.4 ± 9.0
p value	0.1902	0.9718	0.1796	0.4756
Treatment	**Yield (kg/m)**	**Pruning Weight (kg/m)**	**Ravaz Index (kg/kg)**	
C	3.1 ± 0.3	1.14 ± 0.09	2.71	
ICM	3.6 ± 0.4	1.21 ± 0.07	2.98	
SI	4.1 ± 0.5	0.98 ± 0.11	4.18	
UCS	4.2 ± 0.2	0.89 ± 0.09	4.72	
p value	0.1243	0.0771	--	

In the second season at ALV, yield of the Cabernet Sauvignon vines was also higher in the two undercanopy cooled vines – UCS and SI – compared to the control and ICM vines. Both these undercanopy treatments resulted in vine yield over 4 kg/m compared to 3.1 and 3.6 kg/m for the control and misting treatment, respectively (Table 12). The higher yields in the UCC treatments can be explained by the higher berry numbers per bunch and greater bunch numbers. This indicates better fruit set and higher bud fruitfulness in those vines. This improvement in fruitfulness may be the cumulative effect of two seasons of favourable conditions for the vines from the application of undercanopy cooling.

Vine vigour, as indicated by pruning weight, was higher in the control and misted vines, and lower in the two undercanopy cooling treatments. The higher pruning weights in the control and misted vines cannot be explained by higher photosynthesis rates during the growing season as they were similar to the other treatments. Pruning weights of the control and misted vines were over 1.1 kg/m resulting in lower Ravaz Index values of between 2.7 and 3.0. In comparison, the two undercanopy treatments had Ravaz Indices of over 4.0 indicating good vine balance.

5.7 Juice and wine composition

5.7.1 Sauvignon Blanc (OLE – Riverland)

Table 13: Juice analysis of ICC trial Sauvignon Blanc post-harvest from OLE (Riverland) in 2017. Means ± SEM.

Treatment	Soluble Solids (Brix°)	Titratable Acidity (g/L)	pH	Yeast Assimilable Nitrogen (mg/L)
C	16.5 ± 0.38	4.91 ± 0.12	3.45 ± 0.38	260 ± 17.29
ICM	16.2 ± 0.38	5.03 ± 0.12	3.50 ± 0.38	253 ± 17.29
P value	0.6703	0.3159	0.5354	0.7619

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Table 14: Juice analysis of UCC trial Sauvignon Blanc post-harvest from OLE (Riverland) in 2017. Means ± SEM.

Treatment	Soluble Solids (Brix°)	Titratable Acidity (g/L)	pH	Yeast Assimilable Nitrogen (mg/L)
C	15.9 ± 0.42	4.79 ± 0.16 b	3.42 ± 0.03	216.9 ± 21.9
SI	15.6 ± 0.42	5.71 ± 0.16 a	3.30 ± 0.03	208.9 ± 21.9
UCS	15.8 ± 0.42	5.64 ± 0.16 a	3.33 ± 0.03	212.5 ± 21.9
P value	0.8936	0.0225	0.1832	0.9741

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and block as a random effect. Values are an average of three field replications. Significance determined at P < 0.05

In the Sauvignon Blanc juice samples from OLE (Riverland) in 2016-17, there were no significant differences in the juice composition between control and misted vines (Table 13). The low soluble solids in the must resulted from the early harvest and a high rain event shortly before this that resulted in dilution of the sugars. TA and pH values were similar as well, around 5 g/L of TA and pH of 3.50. YAN levels were adequate for a healthy fermentation with concentrations over 250 mg/L.

In the UCC trial in the same season, all treatments were similar in terms of sugar ripeness with soluble solids slightly below 16 °Brix, again reflecting the early harvest and rain event as stated above (Table 14). Both undercanopy cooling treatments had significantly higher titratable acidity values in the must compared to the control vines, an encouraging result that indicates that canopy cooling effectively preserved acids despite heatwaves. Additionally, pH was lower in the SI wine compared to the control. YAN levels in the must were again adequate for healthy fermentations, with all treatments above 200 ppm.

Table 15: Wine chemistry of ICC trial Sauvignon Blanc from OLE (Riverland) in 2017. Means ± SEM.

Treatment	Titratable Acidity (g/L)	pH	Alcohol (% v/v)
C	6.51 ± 0.23	3.33 ± 0.04	9.17 ± 0.278
ICM	6.59 ± 0.19	3.31 ± 0.03	9.02 ± 0.228
P value	0.8041	0.7844	0.7044

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Table 16: Wine chemistry of UCC trial Sauvignon Blanc from OLE (Riverland) in 2017. Means ± SEM.

Treatment	Titratable Acidity (g/L)	pH	Alcohol (% v/v)
C	6.44 ± 0.13 b	3.28 ± 0.02 a	8.90 ± 0.25
SI	7.37 ± 0.13 a	3.14 ± 0.02 b	8.63 ± 0.25
UCS	7.53 ± 0.13 a	3.22 ± 0.02 ab	9.12 ± 0.25
P value	0.0019	0.0083	0.4187

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and block as a random effect. Values are an average of three field replications. Significance determined at P < 0.05

Looking into the chemical composition of the wines made from the Sauvignon Blanc musts described above from OLE, both misting and control treatments were very similar (Table 15). The TA and pH values were around 6.5 g/L and 3.30, respectively, and the wine alcohol concentrations were just over 9% (v/v) reflecting the low soluble solids in the fruit/juice.

In the UCC part of the trial, both canopy cooling treatments had significantly higher TA compared to wine of control vines, by as much as 1 g/L (Table 16). The pH of these wines was lower, around 3.14-3.22, compared to 3.28 for the control. Alcohol concentrations in the wine were highest in the UCS wines followed by the C and SI wines, but these differences were non-significant.

Table 17: Juice analysis of ICC trial Sauvignon Blanc post-harvest from OLE (Riverland) in 2018. Means ± SEM.

Treatment	Soluble Solids (Brix°)	Titratable Acidity (g/L)	pH	Yeast Assimilable Nitrogen (mg/L)
C	20.1 ± 0.3	4.8 ± 0.1	3.42 ± 0.01	103.3 ± 25.4
ICM	20.0 ± 0.6	5.2 ± 0.1	3.40 ± 0.01	97.2 ± 17.2
P value	0.9228	0.0662	0.3739	0.8502

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Table 18: Juice analysis of UCC trial Sauvignon Blanc post-harvest from OLE (Riverland) in 2018. Means ± SEM.

Treatment	Soluble Solids (Brix°)	Titratable Acidity (g/L)	pH	Yeast Assimilable Nitrogen (mg/L)
C	20.3 ± 1.1	5.3 ± 0.0a	3.52 ± 0.04	113.7 ± 6.7
UCS	19.3 ± 1.0	5.4 ± 0.0a	3.40 ± 0.07	133.6 ± 19.3
SI	18.5 ± 0.6	4.5 ± 0.0b	3.35 ± 0.04	72.9 ± 27.2
P value	0.4178	<0.0001	0.1165	0.1626

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and block as a random effect. Values are an average of three field replications. Significance determined at P < 0.05.

In the second season at OLE, 2017-18, the juice composition of the misting treatment compared to control showed few differences. Soluble solids were similar, around 20 °Brix, while TA was slightly higher in the misted vines' fruit at around 5.2 g/L compared to 4.8 g/L in the control vines' fruit (Table 17). pH was very similar between treatments, around 3.40, while YAN was considerably lower than the first season, around 100 mg/L, which indicates inadequate nitrogen for healthy fermentation. In

winemaking, a supplemental nitrogen source, diammonium phosphate (DAP), was added at approx. 100 mg/L to ensure a successful and complete fermentation.

In the second season, 2017-18, the two undercanopy cooling treatments had slightly lower soluble solids. The SI treatment in particular was over 1.5 °Brix lower than the control and also had lower TA (Table 18). pH values ranged from 3.40 to 3.52 but these were not significantly different between treatments. SI juices also had considerably lower YAN levels compared to the UCS and control vines. While the UCS vines had YAN levels that were barely adequate for a successful and healthy fermentation, the other two treatments – SI and Control – did not.

Table 19: Wine chemistry of ICC trial Sauvignon Blanc from OLE (Riverland) in 2018. Means ± SEM.

Treatment	Titratable Acidity (g/L)	pH	Alcohol (% v/v)
C	4.6 ± 0.1	3.41 ± 0.02	13.0±0.0
ICM	4.9 ± 0.1	3.37 ± 0.06	13.3±0.2
P value	0.2069	0.4933	0.3049

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

Table 20: Wine chemistry of UCC trial Sauvignon Blanc from OLE (Riverland) in 2018. Means ± SEM.

Treatment	Titratable Acidity (g/L)	pH	Alcohol (% v/v)
C	4.5 ± 0.1	3.44 ± 0.03	13.1±0.2
UCS	5.5 ± 0.5	3.38 ± 0.06	12.9±0.1
SI	5.3 ± 0.1	3.24 ± 0.52	12.9±0.1
P value	0.1270	0.0714	0.3608

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and block as a random effect. Values are an average of three field replications. Significance determined at P < 0.05

In assessing the wine chemical composition from the OLE misting trial, the misted vines produced wines with slightly higher TA and lower pH (Table 19). This likely was the results of the cooler conditions in the canopy and less degradation of malic acid due to higher temperatures. In the case of the UCC trials, the highest TA values were found in the two undervine cooled treatments, UCS and SI, which had TA values of around 5.3-5.5 g/L and pH values around 3.24-3.38 (Table 20). In comparison, the control vines had lower TA values around 4.5 g/L with a higher pH of 3.44. The higher acidity in the cooled vines could reflect their cooler canopies as seen in the vine microclimate data (Figure 15). No differences in final wine alcohols levels were observed; the alcohols ranged from 12.9-13.3% v/v that were in the range of table wines.

5.7.1.1 Wine volatiles

Table 21: Select Sauvignon Blanc wine volatile compounds from OLE (Riverland), 2018.

Class/Compound	Control	ICM	Control	SI	UCS
Esters	(all concentrations in mg/L except where mentioned)				
Ethyl hexanoate	7.1±0.9	6.9±0.2	3.73±1.99	6.70±1.33	7.16±0.67
Ethyl octanoate	1.1±0.2	1.1±0.1	0.63±0.39	0.94±0.27	1.09±0.14
Isoamyl acetate	11.8±1.4	14.8±2.2	26.27±1.95a	11.61±3.50b	13.85±3.52ab
(Z)-3-Hexenyl acetate	0.06±0.01	0.05±0.01	0.04±0.01	0.05±0.01	0.07±0.00
C6 Alcohols					
1-hexanol	1.68±0.03	1.65±0.25	1.42±0.32	1.43±0.14	1.74±0.12
2-heptanol (µg/L)	15.05±0.4	16.34±8.3	18.9±1.4	15.3±2.6	16.9±4.1
(Z)-3-hexen-1-ol (µg/L)	33.19±2.9	29.44±7.3	19.6±6.4	22.3±2.6	38.8±5.9
Monoterpenes					
Limonene (µg/L)	0.98±0.3	0.69±0.3	1.74±0.13b	2.93±0.22a	2.31±0.15ab
Linalool (µg/L)	8.90±0.36	8.56±1.77	11.95±1.51	12.38±0.83	12.9±1.27
Nerol (µg/L)	0.36±0.00	0.35±0.01	0.46±0.00	1.18±0.27	0.71±0.28
Geraniol (µg/L)	0.92±0.00	0.95±0.16	1.37±0.18	1.05±0.18	1.25±0.17
C13 Norisoprenoids					
β-damascenone (µg/L)	8.10±0.92	9.28±4.77	11.88±1.11	12.83±4.85	13.35±3.25
TDN (µg/L)	2.09±0.11	1.75±0.83	3.33±0.51	5.94±0.66	3.55±0.90

Analysis of select volatile compounds in Sauvignon Blanc wines by HS-SPME-GC-MS provided an indication of the range and diversity of key aroma compounds that contribute to wine quality. Some of the key compounds that contribute to the varietal character of Sauvignon Blanc are shown in Table 21.

Esters contribute to fruity flavours in fruit including grape and in winegrape, they tend to be present in white varieties such as Riesling, Sauvignon Blanc and Muscat. Esters are synthesized via the LOX-HPL pathway with fatty acids as the initial substrates. High ester concentrations were found in cooled canopies, particularly in the UCS and SI treatments, with the exception of isoamyl acetate, which was higher in the control wines. The misting treatment also resulted in higher isoamyl acetate levels compared to control wines.

C6 alcohols are precursors to esters and can form in the berry both pre- and post-veraison depending on the alcohol. Hexan-1-ol tends to accumulate rapidly starting at véraison whereas hexen-1-ol accumulates early during berry development, decreases slightly at véraison, then remains stable (Kalua and Boss, 2010). In the misting trial, 1-hexanol was unaltered while hexen-1-ol was lower in the misted fruit. In the UCC trial, the UCS treatment had generally higher levels of alcohols compared to both the control and SI treatments. It has been suggested previously that environmental factors do not greatly influence C6 alcohol synthesis in the grape berry, however we found certain compounds such as hexen-1-ol to be greatly affected by the cooler canopies under the UCS treatment.

Monoterpenes are aroma compounds with fruity aromas and tend to be produced in many white winegrape cultivars. In this study, it was found that Sauvignon Blanc wines had linalool as the

dominant monoterpene with the highest concentrations found in the SI and UCS treatments. It has often been observed that cooler climates produce wines with greater aroma and higher terpene concentrations; our results concur with this observation. It was interesting to find that misting did not influence monoterpene concentrations in the wine compared to control or uncooled treatment. This could have been due to the inadequate misting level required to have a positive influence on fruit composition.

Surprisingly, methoxypyrazines were below the detection limit. Isobutyl methoxypyrazine, the dominant methoxypyrazine in Sauvignon Blanc wines, was not at noticeable concentrations.

Finally, beta-damascenone, a C13 norisoprenoid with fruity characters, was higher in the misted fruit as well as UCS and SI treatments. TDN (trimethyl dihydronapthaline), which is an undesirable compound in wine, was lower in misted fruit but higher in the SI fruit.

Overall, it can be concluded that canopy cooling treatments had a generally positive effect on wine aromatics, with many key compounds contributing to Sauvignon Blanc varietal character increasing in the cooled canopies.

5.7.2 Cabernet Sauvignon (ALV – Waite)

Table 22: Must analysis of Cabernet Sauvignon post-harvest from ALV in 2017. Means ± SEM. Different letters indicate significantly different means based on Tukey's HSD test at P<0.05.

Treatment	Soluble Solids (Brix°)	Titratable Acidity (g/L)	pH
C	22.2 ± 0.26	7.98 ± 0.18	3.42 ± 0.01 b
ICM	22.3 ± 0.29	7.76 ± 0.21	3.46 ± 0.01 a
SI	22.6 ± 0.29	7.97 ± 0.21	3.40 ± 0.01 b
UCS	22.5 ± 0.29	7.83 ± 0.21	3.47 ± 0.01 a
P - Value	0.3516	0.3516	0.0001

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

In the Cabernet Sauvignon musts from the first season, 2016-17, all treatments reached the same levels of fruit ripeness as indicated by the similar soluble solids concentrations of between 22-23 °Brix (Table 22). Similarly, the differences in acidity and pH were insignificant between treatments this year. TA values were approx. 8 g/L while pH values were between 3.40-3.50.

Table 23: Wine chemistry of Cabernet Sauvignon from ALV in 2017. Means ± SEM.

Treatment	Titratable Acidity (g/L)	pH	Alcohol (%V/V)
C	7.9 ± 0.34	3.64 ± 0.04	10.9 ± 0.16
ICM	7.4 ± 0.34	3.71 ± 0.04	10.7 ± 0.16
SI	8.0 ± 0.34	3.62 ± 0.04	11.0 ± 0.16
UCS	7.9 ± 0.34	3.72 ± 0.04	10.8 ± 0.16
P - Value	0.6512	0.1803	0.5246

Data was analyzed using a mixed model ANOVA, with treatment as a fixed variable and replicate as a random effect. Significance determined at P < 0.05.

In the first season at ALV, there were no differences in wine chemistry (Table 23). Likewise, no effects of cooling were observed on wine acidity and pH, as well as alcohol levels, which were between 10-

11% (v/v). These results indicate that canopy cooling treatments during heatwaves did not translate to differences in juice and wine composition presumably due to the unseasonable precipitation (higher than average) and fewer heatwaves in the first season.

Table 24: Fruit (berry) composition of Cabernet Sauvignon from ALV in 2018: total anthocyanins and polyphenols. Means ± SEM. Different letters indicate significantly different means based on Tukey's HSD test at *P*<0.05.

Treatment	Total Anthocyanins (mg/berry)	Total Anthocyanins (mg/g berry weight)	Total Phenolics (au/berry)	Total Phenolics (au/g berry weight)
Control	1.3 ± 0.1	1.1±0.1a	0.30 ± 0.01ab	0.24 ± 0.01
Mist	1.4 ± 0.1	1.1±0.0a	0.30 ± 0.00ab	0.24 ± 0.01
UCS	1.0 ± 0.1	0.8±0.1b	0.23 ± 0.03b	0.18 ± 0.03
SI	1.3 ± 0.1	1.0±0.1ab	0.32 ± 0.02a	0.24 ± 0.01
P Value	0.0492	0.0202	0.0413	0.0597

Table 25: Fruit (berry) composition of Cabernet Sauvignon from ALV in 2018: skin and seed tannins (in epicatechin equivalents). Means ± SEM.

Treatment	Total Skin Tannin (mg/berry)	Total Seed Tannin (mg/berry)
Control	0.93 ± 0.49	1.68 ± 0.06
Mist	0.59 ± 0.22	1.34 ± 0.26
UCS	0.94 ± 0.13	1.23 ± 0.16
SI	1.15 ± 0.51	1.43 ± 0.66
P Value	0.7936	0.8360

Fruit was assessed for the phenolic composition, specifically anthocyanins and polyphenols. The misted vines had the highest anthocyanins per berry, similar to the control vines' fruit, and these were also highest on a fresh weight basis (Table 24). The UCS fruit consistently had the lowest values of anthocyanins. Total polyphenols were the highest in the SI fruit, similar to the control and misted vines' fruit, but UCS fruit had significantly lower values (although not on a fresh weight basis).

Detailed polyphenol (tannin) analysis of the components of the berry including skins and seeds revealed that seeds had higher tannins than skins, which is well-known (Table 25). Seeds had over 1 mg of tannin per berry while skins had under 1 mg, with the exception of berries from SI-treated vines. However, no differences in skin tannins were observed between treatments.

Table 26: Must analysis of Cabernet Sauvignon from ALV in 2018. Means ± SEM. Different letters indicate significantly different means based on Tukey's HSD test at *P*<0.05.

Treatment	Soluble Solids (°Brix)	pH	Titratable Acidity (g/L)	Colour Density	Hue/Tint	Total Phenolics (mg/L)	Total Anthocyanins (mg/L)	YAN (mg/L)
Control	25.1±0.2	3.77±0.03	3.7±0.1b	0.93±0.10	1.28±0.04	286±36	34±11	52±15
Mist	25.3±0.1	3.69±0.03	3.9±0.0b	0.75±0.26	1.18±0.08	245±37	19±8	40±6
UCS	24.3±0.5	3.65±0.06	4.5±0.1a	0.92±0.25	1.30±0.08	174±22	17±8	64±11
SI	24.6±0.3	3.64±0.03	3.9±0.1b	0.46±0.06	1.09±0.03	271±38	22±9	59±9
P Value	0.2268	0.1849	0.0005	0.3403	0.1199	0.1662	0.5932	0.4756

Detailed chemical composition was carried out of musts from the Cabernet Sauvignon trial at ALV in the 2017-18 season. While the pH of all treatments was very similar, the UCS vines had significantly higher TA levels at around 4.5 g/L, with the other treatments consistently below 4 g/L (Table 26). Soluble solids also did not differ significantly between treatments. Colour density or intensity reflects the brightness of the juice; no differences were observed between treatments. Hue or tint of the juice is its shading; no differences were found between treatments with hue values ranging from 1.1-1.3. Total polyphenols in the juice were highest in the control fruit and lowest in the UCS fruit. Total anthocyanin concentrations were also very low in the musts as there was little skin contact during crushing. Control fruit had the highest anthocyanin concentrations while all the cooling treatments had lower values. YAN levels were relatively low across the treatments with values between 40-64 mg/L, indicating that the nitrogen availability to the yeasts were likely inadequate for a healthy fermentation. There were no significant differences in must YAN levels between treatments.

Wine chemical composition was assessed in the Cabernet Sauvignon from ALV in season 2; results are presented in Table 27. Significant differences in pH were observed between the treatments: the lowest pH was in the control vines while the cooled treatments had significantly higher pH values. There were no differences in the TA values between treatments. Alcohol levels were the same between treatments, between 12.5% and 13% v/v. Colour density or intensity were similar between the treatments. Hue or tint were also similar. Total wine phenolics were higher in the control vines compared to the mist and other treatments. This was possibly due to increased light exposure (not specifically measured in this trial) that would have enhanced polyphenol synthesis (Bergqvist et al. 2001). Total anthocyanins were highest in the UCS wines and lowest in the misted vines. The differences between treatments was statistically non-significant.

Table 27. Wine chemistry of Cabernet Sauvignon from ALV in 2018. Means ± SEM. Different letters indicate significantly different means based on Tukey's HSD test at *P*<0.05.

Treatment	pH	Titratable Acidity (g/L)	Alcohol (%)	Colour Density	Hue/Tint	Total Phenolics (g/L)	Total Anthocyanins (mg/L)
Control	3.44±0.03b	5.8±0.1	13.0±0.0	10.2±0.7	0.8±0.0	2.40±0.21	105±14
Mist	4.08±0.02a	5.7±0.0	12.9±0.0	8.1±0.3	0.8±0.0	1.93±0.53	83±15
UCS	4.11±0.032a	5.7±0.1	12.5±0.0	8.9±1.5	0.8±0.0	2.29±0.13	125±15
SI	4.04±0.02a	5.8±0.0	12.6±0.0	8.8±0.6	0.8±0.0	2.08±0.21	98±7
P Value	<0.0001	0.2192	0.7551	0.4324	0.8475	0.2747	0.2489

5.7.2.1 Wine anthocyanins and polyphenols

Wine polyphenols were analysed by HPLC-DAD of the Cabernet Sauvignon wines made from the ALV (Waite) canopy cooling trial in 2018. Five major non-acylated anthocyanin glucosides were analysed in the wine samples in triplicate. The dominant anthocyanin in red wine, malvidin-3-O-glucoside, was the most abundant in these Cabernet Sauvignon wines as well, as expected, with concentrations ranging from 63-73 mg/L (Table 28). These concentrations are relatively low compared with many Cabernet Sauvignon wines analysed elsewhere around the world (Mazza et al. 1999, Pagay et al. 2013). Petunidin and peonidin were the next most abundant but less than malvidin by an order of magnitude. Dephinidin and cyanidin were by far the lowest in concentration in all wine samples.

Table 28: Individual anthocyanins and polyphenols in the Cabernet Sauvignon wines from ALV in 2018. Means ± SEM.

Compound Name	Control (mg/L)	Mist (mg/L)	UCS (mg/L)	SI (mg/L)	*P*-Value
Anthocyanins					
Delphinindin-3-O-glucoside	0.80±0.25	0.82±0.25	0.79±0.18	0.55±0.12	0.7375
Cyanidin-3-O-glucoside	0.20±0.03	0.30±0.07	0.23±0.09	0.22 *	0.7856
Petunidin-3-O-glucoside	2.62±1.10	2.58±0.37	2.21±0.81	1.91±0.30	0.9058
Peonidin-3-O-glucoside	1.08±0.28	1.29±0.39	0.73±0.15	0.58±0.13	0.2289
Malvidin-3-O-glucoside	71.90±23.07	64.23±5.12	73.49±22.39	63.34±7.32	0.9738
Non-pigmented Polyphenols					
Gallic acid	7.52±0.29	6.07±0.55	6.98±0.61	6.463±0.23	0.1961
Caffeic acid	0.27±0.06	0.28±0.03	0.25±0.05	0.30±0.02	0.8932
Quercetin	3.87±0.25	4.40±0.70	3.30±0.53	2.60±0.33	0.1339
Epicatechin	55.14±3.83	49.74±12.67	53.93±8.57	46.60±6.38	0.8849

* Only single value available

In comparing treatments, there were no significant differences in the anthocyanin profiles or concentrations. The highest malvidin concentrations were found in the control and UCS wines; these were approx. 10 ppm higher than the SI and misted wines. These trends correlated with the total anthocyanins measured in the wines using UV-Vis spectrophotometry (Table 24), consistent with other studies (Spayd et al. 2002). The two B-ring anthocyanins, petunidin and peonidin, had the highest concentrations in the misted fruit compared to other treatments. Dephinidin tended to be similar across treatments with the exception of the SI treatment, which was much lower than the other three treatments. Finally, cyanidin, the lowest in abundance of all anthocyanins analysed in this study, was comparable across all treatments. Anthocyanin biosynthesis occurs primarily after véraison and is affected by environmental conditions including temperature and light (Spayd et al. 2002). It is therefore plausible that increased incidence of heatwaves would be detrimental to anthocyanin biosynthesis and/or degradation.

Non-pigmented polyphenols were analysed in the wine samples also by HPLC; the results are presented in Table 28. Gallic acid and caffeic acid, non-flavonoid compounds, had similar concentrations across all treatments. Gallic acid concentrations were around 6-7.5 ppm while caffeic acid samples were between 0.25-0.3 ppm. The trend of gallic acid concentrations closely matched total phenolics based on spectrophotometry (Table 24).

Quercetin, a powerful antioxidant and UV-protectant found in the skins of grapes (Cheynier and Rigaud 1986), was highest in the misted fruit and lowest in the SI fruit. Misted fruit had approx. 4.4 ppm of quercetin, indicting perhaps increased light exposure to the fruit compared to the other treatment. However, the light environment of these canopies was not specifically measured in this study. Quercetin biosynthesis occurs early during berry development (Downey et al. 2003) and our results indicate that higher quercetin levels in the misted fruit might be resulting from more favourable conditions (higher light exposure) during the early part of the season. Although it is known that high light exposure increases quercetin biosynthesis (Price et al. 1995) and that shading negatively affects it (Downey et al. 2004), it is still unknown whether high temperatures are detrimental to quercetin biosynthesis or degradation; this could be considered for future work.

Finally, epicatechin concentrations were found to be slightly higher in the control wines compared to any of the cooled treatments. In particular, differences between the control and SI wines were the greatest suggesting that higher temperature may be favourable for tannin biosynthesis and accumulation. Tannin concentration in the fruit is generally related to light exposure with higher light levels resulting in greater tannin biosynthesis (Downey et al. 2004). However, the role of temperature is still unclear and is an opportunity for future work.

Overall, it can be concluded that the various canopy cooling treatments had a marginal effect on wine anthocyanins and polyphenols in 2018. In some cases, higher levels of these polyphenols were observed in the non-cooled (control) fruit due to the higher temperatures and possibly light levels in the canopy due to increased exposure. The latter needs to be addressed in an extended study in the future.

Young Cabernet Sauvignon wines were analysed for their volatile composition using HS-SPME-GC-MS; the results are presented in Table 29. Esters are formed early in berry development and have a fruity character. Wine esters were dominated by methyl butanoate and ethyl methyl butanoate, which were very similar across treatments. Higher alcohols are derived via the lipoxygenase (LOX) pathway from aldehydes and were found to be similar between the misting treatment and control. Fruity and floral volatiles such as the monoterpenes were also similar between the misted and control fruit (for linalool and geraniol). Similar to Sauvignon Blanc, no methoxypyrazines were detected in the wines. C13 norisoprenoids are important flavour compounds in Cabernet Sauvignon (Lee et al. 2007), and it was found that ß-damascenone concentration was highest in the misted wine and lowest in the UCS wine. It is likely that cooler canopy temperatures contribute positively to increased synthesis or reduced breakdown of this compound (Lee et al. 2007). Benzene derivatives tend to dominate the post-veraison period of berry development (Kalua and Boss 2009), and in the wines, benzaldehyde was found to be higher in warmer fruit, i.e. control wines. Benzyl alcohol and phenylethanol concentrations were found to be similar between treatments.

Table 29: Select volatile compounds in Cabernet Sauvignon wines from ALV, 2017-18.

Class/Compound	Control	ICM	SI	UCS
	(concentrations in mg/L except where indicated)			
Esters				
Methyl hexanoate (µg/L)	20.3±3.6	20.7±5.6	19.0±9.7	19.2±6.1
Ethyl-2-methyl butanoate	0.14±0.02	0.16±0.02	0.14±0.03	0.14±0.00
C6 Alcohols				
1-hexanol	1.93±0.04	2.16±0.08	1.89±0.06	1.96±0.09
2-heptanol	0.15±0.00	0.13±0.01	0.13±0.01	0.17±0.02
(Z)-3-hexen-1-ol (µg/L)	44.4±1.2	52.1±2.9	43.3±3.4	44.1±6.4
(E)-3-hexen-1-ol (µg/L)	32.3±1.9	34.1±0.9	30.8±0.5	29.7±2.3
Monoterpenes				
Linalool (µg/L)	7.0±1.5	6.8±0.6	6.0±0.7	5.5±0.2
Nerol (µg/L)	0.5±0.1	0.5±0.1	0.4±0.0	0.5±0.1
Geraniol (µg/L)	4.1±0.4	3.6±0.1	3.5±0.4	4.0±0.2
1,8 Cineole / Eucalyptol (µg/L)	0.4±0.0	0.4±0.1	0.4±0.1	0.5±0.2
C13 Norisoprenoids				
β-damascenone (µg/L)	17.9±2.0	18.5±0.6	17.4±1.5	15.7±1.1
TDN (µg/L)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Benzene derivatives				
Benzaldehyde	65.0±28.9	39.7±3.1	38.4±3.7	35.7±3.0
Benzyl alcohol	0.34±0.02	0.28±0.03	0.30±0.06	0.35±0.01
Phenylethyl alcohol	23.0±1.6	24.5±0.2	27.3±3.5	22.7±0.2

5.8 Modelling of Canopy Misting

A simulation model was developed to predict canopy, leaf and bunch temperatures in response to various misting programs. Model results based on a 60 µm droplet size, ca. 1 L/h/vine mist application, 35 °C temperature and 15% RH predicted an air temperature drop of between 10-15 °C from ambient during the misting operation when using the same spray regime as used in field trials (20 s every 300 s) are presented in Figure 46.

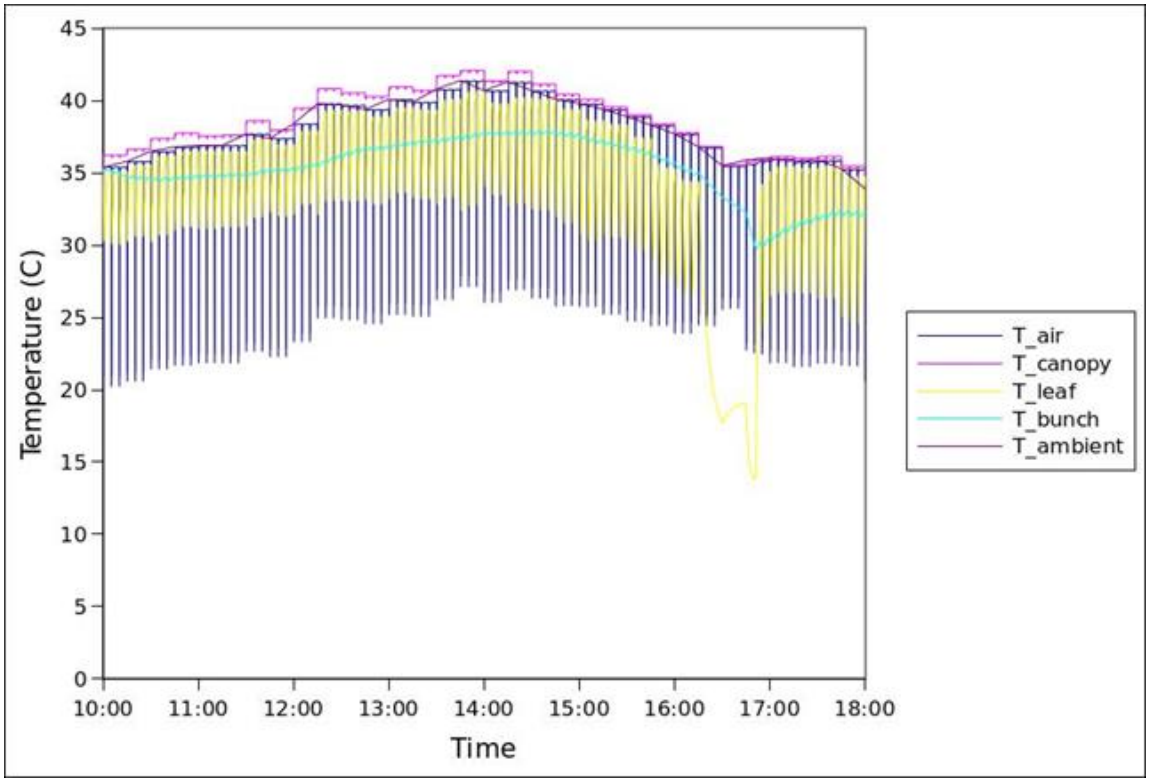


Figure 46: Canopy cooling simulation results based on misting for 20 s every five minutes on a canopy structure and dimension similar to OLE Sauvignon Blanc vines. Ambient temperature was set at 35 °C and relative humidity was 15%.

When used to simulate daytime temperature trends during a heatwave event, the model was found to accurately predict the temperature behaviour experienced by leaves and bunches during canopy mist cooling. Simulated leaf temperatures were found to drop rapidly during misting, then quickly recover to near canopy air temperature, with similar results obtained from field observations. Simulated bunch temperatures were maintained at approximately 5 °C below ambient. Bunches did not experience the same temperature oscillations as leaves due to their higher thermal mass, which resulted in more stable temperatures throughout. When comparing simulated bunch temperatures with field data, it should be noted that field temperatures were surface bunch temperatures, whereas the model simulation determines an *averaged* bunch temperature across all bunches and accounts for berries within the bunch (not exposed directly to mist) as well as those on the bunch surface. Consequently, the predicted bunch cooling (when averaged across all berries/bunches within the canopy) is not as extreme as that observed with bunch surface temperature measured in the field.

From the simulation results, cooling of bunches and leaves was dramatically increased during the period of 16:00-17:00 (Figure 47).

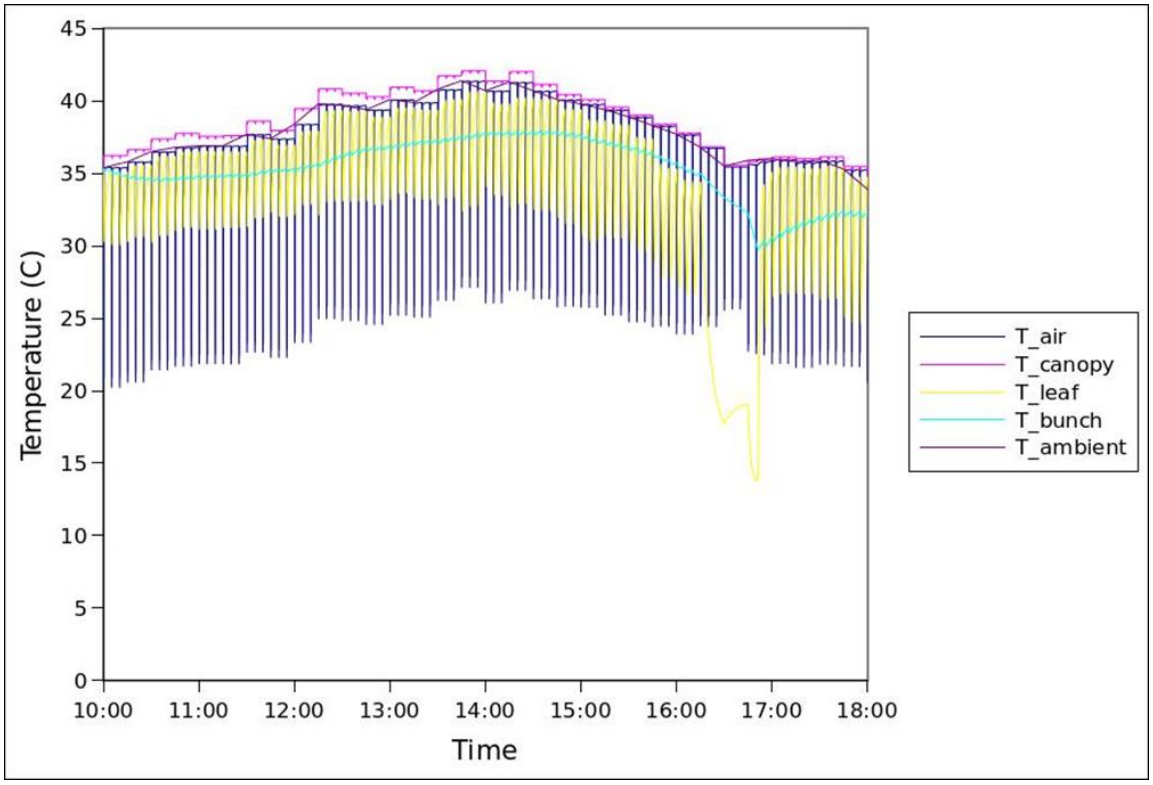


Figure 47: Canopy cooling field observations (from the thermocouple data) based on misting for 20 s every five minutes at OLE Sauvignon Blanc vines. Ambient temperature was approx. 35 °C and relative humidity was approx. 15%.

Comparison with weather station observations showed that this corresponded to a brief period of higher ambient humidity, reduced solar radiation and still air (i.e. no wind). During this time, evaporation of droplets in flight was reduced, resulting in significantly more liquid water accumulating on leaves and bunches. Subsequent evaporation of this water (in addition to heat exchange with cooled moist air) results in greater cooling than that otherwise achieved from heat exchange with moist air alone. This suggests that greater cooling may be achieved if misting is controlled to ensure leaves and bunches are fully wetted, rather than relying on air cooling alone.

Simulation of alternative spray regimes (20 s every 150 s, and 20 s every 900 s) support these observations. Not surprisingly, the cooling effect is more pronounced when the frequency of misting is increased. A greater proportion of liquid water accumulates on leaves and bunches in the “20 s every 150 s” scenario, leading to enhanced cooling. In practice, achieving a cooling effect in this manner would need to be balanced against any increased risk of fungal diseases from high humidity conditions.

The model is now fully customisable, allowing for what-if analysis and prediction of cooling performance based on changes to any of the parameters mentioned. Simulation code has also been optimised to allow operation on a low-cost (< $50) Raspberry Pi microprocessor (Figure 48), which will permit the future development of low cost misting control systems suitable for field deployment.



Figure 48: Raspberry Pi 3 microprocessor that can be used to automatically trigger and control the misters in the vineyard.

6 Conclusion

The canopy cooling trials conducted over the two seasons at OLE and ALV provided an opportunity to investigate novel strategies for mitigation of heatwaves in vineyards based on evaporative cooling of water. These strategies were considered based on their feasibility of implementation, in particular the infrastructure that was in place at OLE, and also on convenience for most growers to install relatively inexpensively in the future. The misting infrastructure was not as convenient because, in some cases, extra posts had to be installed over the canopies to support an overhead trellis wire that would in turn support the misters at OLE. In addition, manual removal of the misters was required prior to harvest, which increased the labour input.

In-canopy misting resulted in improved canopy microclimate and, at least initially, was effective in maintaining the canopy temperature around 35 °C. Leaf and bunch temperatures were also lowered by 3-12 °C as a result. Misting did not affect soil and vine water status, which was an expected outcome due to the small amounts of water applied directly to the canopy, but this was adequate to positively influence Sauvignon Blanc vine photosynthesis rates during a heatwave. The more moderate ALV site did not show such differences, which could be attributed to several factors including extensive cover crop, shading, and heavier soils that would have buffered adverse temperature effects such as heatwaves. Misting resulted in higher yields and pruning weights in the first season at OLE and ALV but not in the second season at either site. Misting did not alter Sauvignon Blanc juice and wine chemical composition. Misting lowered the polyphenol concentrations in the Cabernet Sauvignon wines, perhaps due to increased shading and lower temperatures.

The misting system had the advantage of utilising the least amount of water compared to either of the other undercanopy-based cooling systems, however, given the lack of large differences to fruit composition, it is questionable whether the amount of water applied was adequate. Providing larger amounts of water could be detrimental from a disease incidence standpoint, as well as increasing risk of leaf scorching should the water be even slightly saline, which is a risk in inland irrigated regions. By contrast, the amount of water used by the undercanopy systems were approx. 0.5 ML/ha in the second season, similar to the additional irrigation applied by the grower in advance of each heatwave.

Undercanopy cooling treatments applied for only two hours at night preceding a heatwave were also effective in lowering canopy temperatures during the day by as much as 5 °C. The lower vapour pressure deficit (VPD) resulting from cooling the vineyard floor provided a favourable environment for vine physiological function. Thermal images of the vineyard floor revealed dramatic decreases in floor temperatures as a result of sprinkler use or undervine irrigation. UCS-based cooling resulted in the greatest cooling of the floor and generally best performing canopies during a heatwave. Despite similar soil moisture in the two undercanopy water applications and control, the cooled vines had the best water status and higher net photosynthesis rates, particularly in the second season. ABA concentrations were highest in the control vines indicating a higher level of water stress. The ALV results were mixed; no significant effects of undercanopy cooling were observed on vine physiology. Yields and pruning weights were generally higher in the cooled Cabernet canopies compared to control. However, must composition was relatively unaffected by the cooling treatments except for slightly higher TA in the cooled fruit.

The simulation model of canopy cooling accurately predicted the actual degree of cooling based on in-canopy misting. Air temperatures were found to drop by as much as 15 °C with the mist application resulting in leaf and bunch temperature drops of 10 °C and 5 °C, respectively. The model is a powerful tool for future optimisation and control of misting applications in vineyards as it can be deployed on

a low-cost microcontroller in the vineyard. Future development of misting controllers will allow automatic solenoid operation for canopy misting based on near real-time feedback of canopy microclimate.

7 Recommendations

The two-year study of canopy cooling systems for heatwave mitigation in South Australian vineyard resulted in useful and practical information to manage vineyards under high temperatures events and also revealed promising avenues for further investigation.

All methods of cooling tested in this trial were effective in moderating canopy microclimate, however, the practicality of setting up and running in-canopy (mister-based) systems in a field setting is more challenging compared to the undercanopy (UCS or SI) approaches. Furthermore, the mister-based approach relies on high quality water as saline water may result in leaf scorching under high temperatures. The effects of various cooling approaches on vine performance were variable based on season, site, and cultivar making generalised recommendations difficult. In inland vineyards such as OLE in Riverland, a warmer and "tougher" site than ALV at the Waite, any cooling approach has positive effects and in the case of undercanopy treatments, cooling of the vineyard floor (soil and roots) resulted in positive effects to the vine. This needs to be explored in future studies of root growth and function with undercanopy cooling.

At the ALV site, the organic management of the block along with permanent cover crop (fescue) moderated the effects of extreme weather events such as heatwaves, therefore, effects of the treatments were less marked. Future work in this situation should consider the specific effects of vineyard floor cover on vine performance under adverse conditions.

Specific recommendations for growers to mitigate against heatwaves include:

- Apply drip irrigation on the day preceding the heatwave event and maintain higher rates throughout the heatwave.
- Install undervine sprinklers to increase the water distribution to the vineyard floor to include both the undervine and midrow regions for enhanced vine performance.
- Maintain shading of fruit in warm inland regions.
- Where undervine sprinklers are not available, use overheard sprinklers to lower the VPD around canopies. Sprinklers should be operated at night to minimise evaporative losses.
- Maintain a green vineyard floor using cover crops for improved water retention and lower soil/root temperatures.
- Long term, use of more heat-tolerant cultivars, e.g. southern Mediterranean varieties.

Future work in the area of heatwave mitigation strategies in vineyards should investigate the role of broadcast water applications on the vineyard floor on root physiology and vine performance, as well as automation of sprinkler or mister control using microcontrollers and software that have been developed in this study.

Appendix 1: Communication

The information from this trial has been featured or presented at the following sites and locations:

- Article on Wine Australia website (08/12/2017)
  https://www.wineaustralia.com/au/news/articles/a-little-water-can-go-a-long-way

- 2017 Crush Wine Symposium, Waite Campus, University of Adelaide – oral presentation by Raquel Kallas in November 2017

- Cornell University (USA) - Department of Horticulture Seminar – oral presentation by Raquel Kallas in December 2017

- Elders Viticulture Grower Information Day, Tanunda, SA (26/06/2018) – oral presentation by Vinay Pagay

- Upcoming industry presentations:
  - Landmark Viticulture Information Evening, Belvidere, SA (Langhorne Creek) (15/08/2018)
  - Adelaide Hills Wine Seminar, Woodside, SA (25/09/2018)

- Two peer-reviewed manuscripts currently being prepared for publication and one in an industry journal (*Wine and Viticulture Journal*)

Appendix 2: Intellectual Property

There is potential IP associated with the canopy cooling model developed to predict temperatures as a function of environmental conditions, canopy architecture and size, and mister settings.

Appendix 3: Additional Canopy Cooling Simulation Results

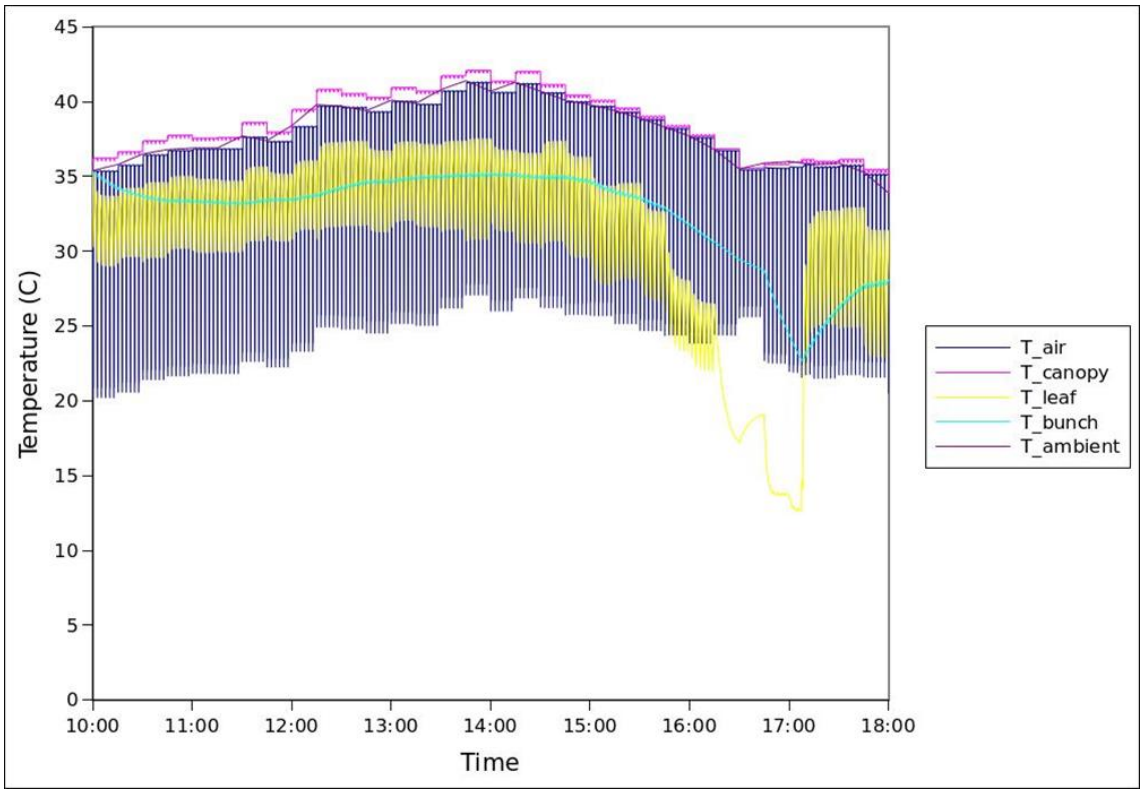


Figure A1. Canopy cooling simulation results based on misting for 20 s every 2.5 min on a canopy structure and dimension similar to OLE Sauvignon Blanc vines. Ambient temperature was set at 35 °C and relative humidity was 15%.

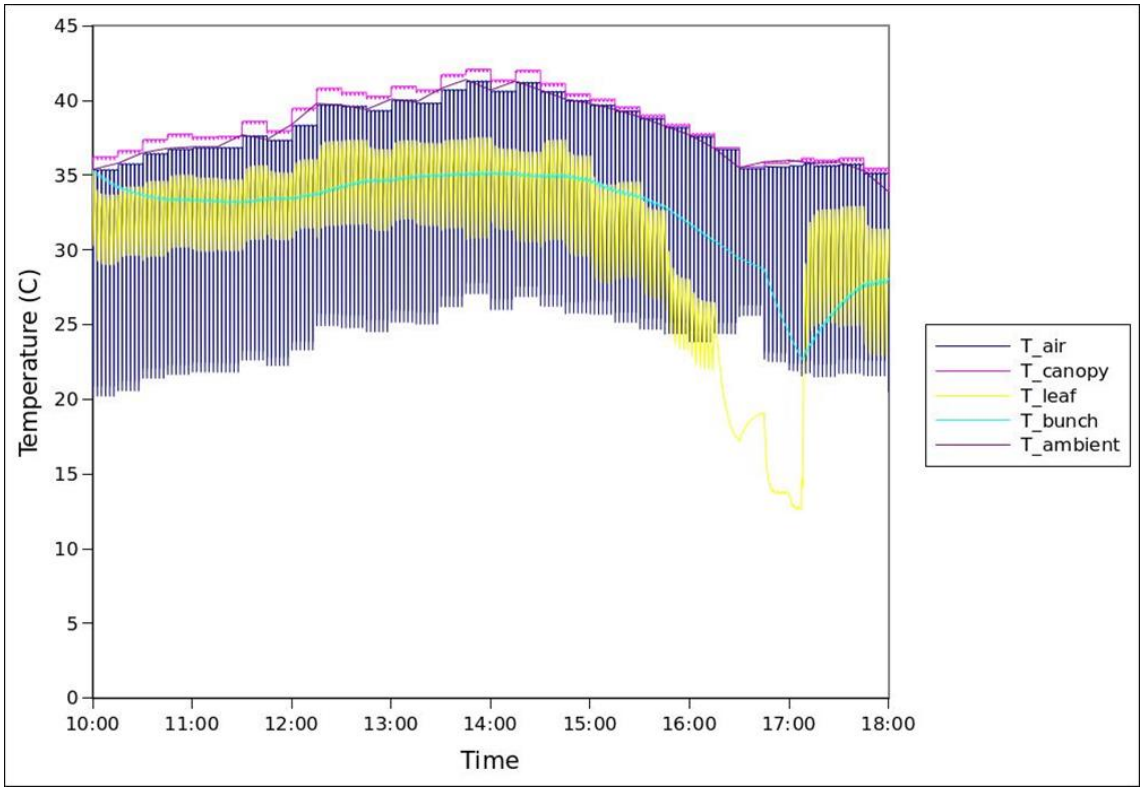


Figure A2. Canopy cooling simulation results based on misting for 20 s every 15 min on a canopy structure and dimension similar to OLE Sauvignon Blanc vines. Ambient temperature was set at 35 °C and relative humidity was 15%.

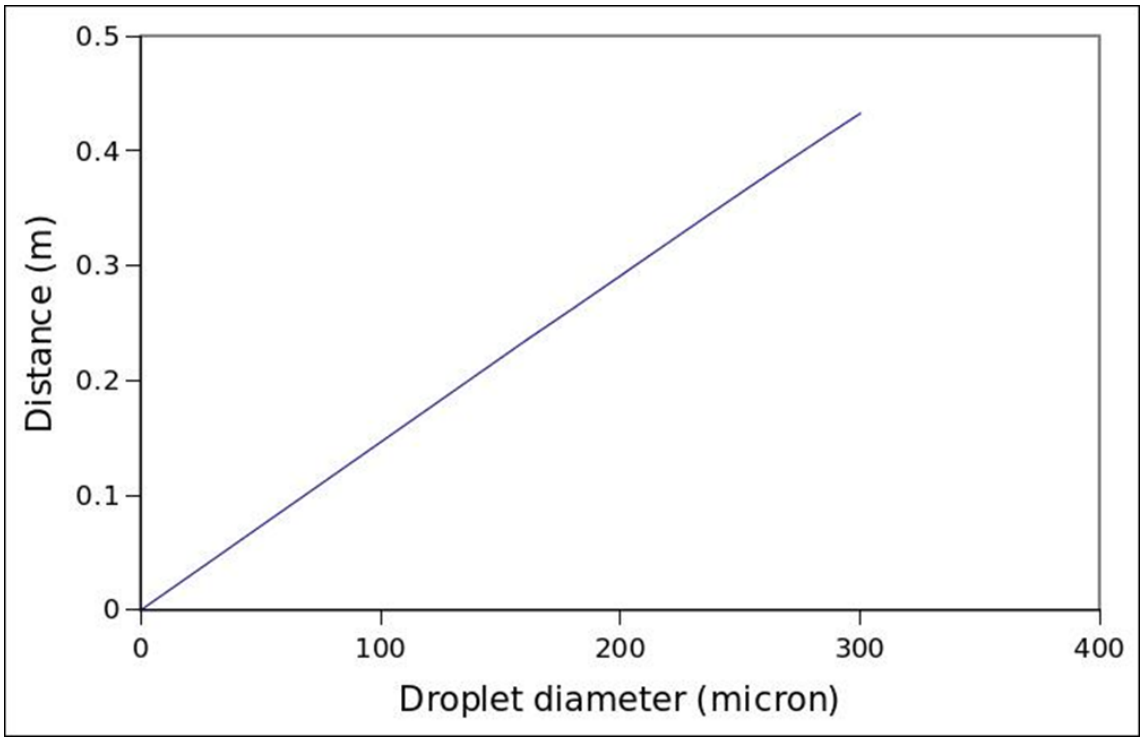


Figure A3. Total distance travelled by droplet before evaporation (T_ambient = 35 °C, RH = 15%).

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