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Research Article

Manipulating Nitrogen and Water Resources for Improved Cool Climate Vine to Wine Quality

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Abstract: Low yeast assimilable nitrogen (YAN) concentrations (<140 mg/N/L) can result in wines of inferior aroma and flavor, regardless of supplemental nitrogen (N) additions in the winery. The impact of doubling commercial field N and irrigation rates was explored in Vitis vinifera L. cv. Chardonnay and Pinot noir over three growing seasons (2016 -2019) in Southern Tasmania, Australia, with the aim of improving YAN concentrations and observing the concurrent influence on vine canopy, yield, and grape and wine composition. Six combinations of irrigation and N rates were applied to 20 vines for each treatment combination and replicated across both cultivars. The treatments included, the standard irrigation rate (~530 L/vine/year) / control N (0 kg/N/ha/year) rate, standard irrigation / standard commercial N rate (~18 kg/N/ha/year), standard irrigation / double commercial N rate (~36 kg/N/ha/year), double irrigation rate (~1060

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L/vine/year) / control N, double irrigation / standard N and double irrigation / double N. ANOVA was used to analyze main treatment effects and treatment interactions on the measured variables for a sub-set of the vine population in each growing season. Increasing N rate improved YAN concentrations across both cultivars in 2 out of 3 growing seasons, with the double N rate associated with increasing YAN to acceptable (>140 mg/N/L) levels. Irrigation had no impact on YAN concentrations. Treatment influences on vine vegetative growth, yield, and grape and wine composition were marginal and inconsistent, and were largely influenced by climatic conditions. Cool-climate grape growers would benefit from applying more N in the vineyard around veraison to improve YAN, without stimulating vigor and negatively impacting chemical grape and wine composition. Increasing irrigation rates may be advantageous in seasons of high crop load, however current commercial irrigation rates are considered adequate.

Key words: irrigation, nitrogen fertilization, vineyard management, wine composition, yeast assimilable nitrogen (YAN), yield

Introduction

In vineyards, nitrogen (N) is required to achieve sustainable yields in a manner that maintains overall vine balance and produces grapes that meet winery specifications. In cool climate regions, N fertilization is recommended at modest rates (20 kg/N/ha) (AWRI 2010) to avoid excess vine vigor (Spayd et al. 1993, Neilsen et al. 2010), which can disrupt source-sink relationships (Vasconcelos et al. 2009), alter canopy microclimate (Bell and Henschke 2005), increase disease pressure (Thomidis et al. 2016), and negatively impact berry composition (Kliewer 1977, Hilbert et al. 2015). Achieving N balance is important as inadequate N can result

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in low yields, poor fruit ripening and a reduction in yeast assimilable nitrogen (YAN) (Bell and Henschke 2005), whereas excess N can result in adverse environmental impacts, such as waterway contamination via leaching (Nováková and Nágel 2009) and the release of potent greenhouse gases (Swarts et al. 2016).

YAN is comprised of ammonium ions and free alpha-amino acids, which serve as key nutrient sources to yeast and dictate the speed and efficiency of fermentation (Hannam et al. 2013). Suboptimal YAN concentrations can lead to stuck or sluggish ferments, the production of undesirables metabolites, such as hydrogen sulfide (Jiranek et al. 1995), and other wine faults (Bell and Henschke 2005). The YAN threshold to ferment grape juice to dryness under moderate initial sugar concentration is 140 mg/N/L (Bell and Henschke 2005, Hannam et al. 2013), however the interplay of other fermentation impacts, such as yeast strain, can influence YAN requirements (Bell and Henschke 2005). As growers limit their N application to manage vine vigor and fruit quality, low YAN concentrations remain a key issue in cool-climate winemaking regions, such as Tasmania.

In the winery, low juice and must YAN concentrations are often supplemented through the addition of ammonium salts, such as diammonium phosphate (DAP). This can prevent problems associated with fermentation kinetics, yet it increases ammonium only and thereby creates an unbalanced must (Bell and Henschke 2005). As amino acid composition is an integral part of dictating wine flavor and aroma, it is preferred to optimize grape N concentration in the vineyard (Holzapfel et al. 2015).

Several studies have demonstrated that N application in the vineyard increases berry amino N and YAN (Bell et al. 1979, Linsenmeier et al. 2008, Hannam et al. 2013, Hilbert et al. 2015),

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yet the impact on vine vegetative variables, and grape and wine composition is less clear. In a long-term, 15-year trial, Linsenmeier et al. (2008) observed a reduction in Riesling pruning weights, leaf size, yield and must amino acids in a zero N control compared to N fertilized treatments (30, 60 and 90 kg/N/ha). A decrease in grape must titratable acidity (TA) and an increase in must pH was reported in the 90 kg/N/ha treatment, yet no other notable impacts of N rate on grape quality were observed. Similarly, in a 5-year trial in British Columbia, Hannam et al. (2013) reported an increase in Merlot juice pH with increasing N rate (0, 16.6, 32.2, and 64.4 kg/N/ha) in 3 out of the 5 trial years, yet juice total soluble solids (Brix), juice TA and yield were largely unaffected. An increase in grape juice YAN concentration with N rate was observed, but only the highest N application rate (64.4 kg/N/ha) consistently increased YAN over the trial period, and in 2 out of the 5 trial years, YAN concentrations did not reach the desired 140 mg/N/L minimum threshold. In another British Columbian trial, Neilsen et al. (2010) observed no impact of N rates (40 and 80 kg/N/ha) on Merlot yield (per vine) and no consistent impact of N rate on yield components, juice pH or juice TA, whereas juice Brix tended to be lower at the higher N rate. Neilsen et al. (2010) observed adequate (>140 mg/N/L) YAN concentrations in the 80 kg/N/ha treatment, whereas standard commercial rates (40 kg/N/ha) resulted in frequently low YAN concentrations (<140 mg/N/L). In Washington State, Spayd et al. (1994) reported an increase in juice and wine pH in Riesling with increasing N rates (0, 56, 112 and 224 kg/N/ha), whereas no impact on juice and wine TA were reported. Juice total amino N, including free amino N, increased linearly with N rate, but a decrease in wine total phenolics was also observed and attributed to dense canopy development.

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The high variability of the impact of N fertilization, the limited research conducted in cool climate wine regions, and the lack of studies exploring the impact of N on YAN in tandem with vine vegetative growth, yield, and grape and wine composition, has led to uncertainty within industry, resulting in the use of modest N applications and low YAN concentrations that require winery N additions to prevent fermentation issues. A benchmark N rate of application in the vineyard to improve YAN concentrations, without negatively impacting vine vigor and grape and wine composition is yet to be determined.

As nutrient uptake by grapevines is reliant on water flow through the soil-root-shoot pathway (Keller 2005), water availability and N fertilization collectively impact grapevine physiology and grape and wine composition. The impact of irrigation level under low N supply on vine vigor, yield and grape and wine composition is yet to be fully observed and the influence of water availability on YAN has been varied (Wade et al. 2002, Keller 2005, Hannam et al. 2013, Holzapfel et al. 2015).

This current study was undertaken to investigate the impact of doubling annual N rates (~18 kg/N/ha and 36 kg/N/ha) and irrigation rates (~530 and 1060 L/vine) on vine vigor, vegetative growth, yield and grape and wine composition of Vitis vinifera Chardonnay and Pinot noir vines in a cool-climate vineyard. We tested the hypothesis that increasing N application rates under additional irrigation will increase YAN concentration in the grape juice must. We also investigated the influence of additional N and irrigation inputs on vegetative growth, yield and grape and wine composition and explored the influence of climatic conditions on these variables over three growing seasons.

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Materials and Methods

Trial site and experimental set-up. The trial was established in winter 2016, at a commercially managed vineyard (Coal River Valley, Southern Tasmania, 42°45'09.7"S 147°29'27.9"E) in Australia using Vitis vinifera L. cv. Pinot noir (clone 115) and Chardonnay (clone 95) 3-year-old vines on Paulsen 1103 rootstock. Vines were vertically shoot positioned and bilaterally cane pruned to 16 nodes per vine plus two 2-node spurs. Vine spacing was 1.25 m with a row spacing of 2.5 m. The Chardonnay block was planted with a South-East row orientation on a shallow black soil on dolerite, comprised of a black clay loam A1 horizon to 50 mm depth, on top of a heavy black clay A1-2 horizon (50 - 300 mm), with underlying B1 and B2 soil horizons characterized as brown heavy clays (200 – 1200 mm). The Pinot noir block was planted with a North-West row orientation on a lateritic sandy loam with a grey sandy loam A1 horizon to 100mm depth, with clay content gradually increasing down the soil profile to a sandy clay loam B1 horizon (100 – 200 mm), and sandy clay B2 horizon (200 – 700 mm) on top of a mudstone C horizon.

Prior to setting up the experiment, the vineyard was scouted for appropriate cultivar blocks with relatively flat landscapes and uniform soil type within the treatment rows. Nitrogen and irrigation treatments were integrated into the commercial vineyard irrigation infrastructure and controlled by the grower to simulate true commercial conditions over the three-vintage period. As such, treatments were established in two rows, one reflecting the standard growers practice irrigation regime (SI) and the other the double standard irrigation rate (DI). This was achieved through the addition of an extra irrigation dripper line (Netafim, Hatzerim, Israel) in the DI row at the same rate (L/hr) as the original line. All the dripper lines had pre-existing holes set at 0.3 m

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apart and the second dripper line was off-set resulting in only a 0.15 m gap between holes. Within each row, vines received no nitrogen (N) for the control rate (0N), the "standard" grower's annual N practice rate (ST) or "double" the grower's standard N practice (DBL) rate (Table 1). The three nitrogen treatments were applied to 20 neighboring vines down the length of each row in treatment blocks to allow ease of management for the grower to accurately maintain the treatment protocol. Within each treatment, eight monitor vines of similar vigor and health were tagged to collect measurements and samples from. The treatments were controlled through a series of taps, maintained at the start of each row to supply either fertigation or irrigation at the desired times. This resulted in a total of six treatment combinations, including SI/0N, SI/ST, SI/DBL, DI/0N, DI/ST and DI/DBL.

The standard N treatment was determined by the grower and considered dry ash analysis (N removal from fruit from previous harvest), previous season's yield, historical site data, vine number/ha, petiole and nitrate and soil analysis. The double N treatment delivered double the annual standard N rate for each site through a second, newly installed fertigation dripper line.

Nitrogen fertilizer (type, timing and delivery) varied across blocks and vintages at the discretion of the vineyard manager to reflect standard commercial vineyard practice (Table 2, 3). Application of other macro and micro nutrients were managed by the grower and were uniform across the treatment blocks and rows, and chosen in accordance with fertilizer budgets and additional nutrient requirements (e.g. calcium, boron, phosphorous). In 2016/17, N fertilizer was first applied mid-February, whereas it was applied mid-January in 2017/18, and late January in 2018/19 for both cultivars.

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Irrigation scheduling and duration of application were controlled by the vineyard manager, subject to cultivar requirements and seasonal conditions (Table 1). Irrigation volume (iNTELLiTROL®, MAIT industries, Victoria, Australia) and soil moisture (AquaCheck®, MAIT industries, Victoria, Australia) were monitored at each site for each growing season and the timing of application was monitored in the Pinot noir block via the installation of flow meters (GSD8, BMeters, Udine, Italy) in both the standard and double irrigation lines to ensure treatments were being managed as desired. Irrigation was generally applied at a lower limit of 50% readily available water and terminated when soil moisture reached field capacity. Evapotranspiration was also monitored by the vineyard manager throughout the season to track water requirements and help determine irrigation frequency via an onsite weather station (Vaisala weather transmitter WXT520, Helsinki, Finland).

Vines were shoot thinned in spring at EL 15 and trimmed in summer at 40 cm above top canopy wire height (≈ 130 cm shoot length). Additionally, the Chardonnay vines were leaf-plucked after bloom. Leaf plucking was targeted at the fruiting zone just above the cordon to remove enough leaves to expose fruit to dappled light. Both blocks were netted just prior to veraison (EL 35). The trial was held over three consecutive growing seasons (2016/17, 2017/18, 2018/19).

Climatic conditions. Tasmania has a cool, temperate climate, with an annual 30-year mean rainfall of ≈ 600 mm as described on the Australian Bureau of Meteorology website (www.bom.gov.au). Southern Tasmania has mild summer temperatures (average 16.8 °C), relatively cool winter temperatures (average 8.8 °C), and an overall maritime influence. The wine grape growing season extends from September (budbreak) through to April/May (leaf fall). Weather data was obtained from the on-site weather station (Vaisala weather transmitter WXT520

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Helsinki, Finland) located approximately 500 m from each trial site, and historic climate data was sourced from the closest Bureau of Meteorology site (Hobart Airport, Tasmania) located ≈ 10 km away from the field site. Annual growing season weather data was recorded from May to April. For each production season, climatic conditions were split into four phases as dictated by important phenological stages for further analysis: dormancy to budbreak (1st May – 31st August), budbreak to bloom (1st September – 31st November), bloom to veraison (1st December – 31st January) and veraison to harvest (1st February – 30th April) (Supplementary Table 1).

Field measurements and lab analysis. *Canopy analysis.* Vine canopy size was determined each growing season in January on three randomly selected monitor vines per treatment using the modified Point Quadrat method for estimating canopy structure of grapevines as described by Poni et al. (1996). A 1 m long, firm, thin (~1 m x 2 mm) length of wire was inserted into the canopy fruiting zone at pre-determined randomly generated heights between 80 and 115 cm. Contact with leaves, clusters and gaps were recorded to determine gaps (%), leaf layer number (LLN), and the number of leaves and clusters of fruit per vine (Smart 1985). Any contact with the cordon, stems, petioles and peduncles were ignored as per the modifications of the original method presented by Poni et al. (1996). To ensure an unbiased random sample, measurements were repeated 40 times per vine at randomly generated points marked on a drop sheet of 1.25 m in length to represent the projected canopy length.

Vine vigor. Vines were bi-lateral cane pruned by hand each season, according to the previous practice at the site. Prunings, excluding the old wood (>1 year old), were weighed in four vines per treatment, using a set of digital hanging scales (model WS603, Wedderburn, Sydney,

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199 Australia). The same vine was measured each year to determine the cumulative impact of
200 treatments on vine vigor.

201 *Leaf nitrogen analysis.* Leaves were sampled for N analysis at harvest in the 2017/18 and
202 2018/19 growing seasons. 30 leaves were collected at random and pooled across five neighboring
203 vines to make one measurement sample. This was repeated another three times in each treatment
204 block (n = 4). The leaves within each sample were dried at 60 °C, hand-crushed and mixed to
205 obtain a homogenous sample. Samples were ground to a fine powder using a Mixer Mill MM 200
206 (Retsch, Haan, Germany) and total N analysis was performed using a Thermo Finigan EA 1112
207 Series Flash Elemental Analyzer (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA).
208 The total N analysis occurred according to the method described in detail in Walker et al. (2021).

209 *Yield analysis.* Grapes were hand harvested at target commercial ripeness of 21 Brix.
210 Before picking, the total number of clusters per vine was recorded for each tagged monitor vine (n
211 = 8). Five random clusters were then sub-sampled per monitor vine, and a sample was considered
212 as the sum of the two neighboring monitor vines (n = 4). The clusters were kept cool and frozen
213 later that day for analysis. For four of the 10 clusters, cluster weight (g), cluster density (OIV scale)
214 and number of berries per cluster were recorded. Berry size was evaluated by sieving the berries
215 into small (<2 mm), medium (2-10 mm) and large (>10 mm) size categories. The number of small
216 berries was generally very low, therefore the small to medium berry size category was combined
217 as <10 mm.

218 *Microvinification.* Small-scale winemaking, otherwise known as microvinification, was
219 undertaken on the day of harvest or the following day, with clusters refrigerated at 4 °C overnight
220 in this instance and then warmed to ambient temperature prior to processing. For the red

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winemaking process, ≈ 1 kg of the harvested fruit per sample (one sample = grape collection from 5 neighboring vines) was weighed out as whole clusters before being destemmed and crushed by hand. The remaining clusters were frozen for future grape homogenate analysis of yeast assimilable nitrogen (YAN) and grape composition (phenolics, tannins, anthocyanins). The must generated from the 1 kg of fruit was decanted into 1.5 L BodumTM 'Kenya' coffee vessels and ≈ 100 mL of juice was sampled and frozen for chemical analysis, including Brix, pH and titratable acidity (TA; g/L). 50 mg/L of potassium metabisulfite (PMS) was added to each Bodum. After a minimum of 10 minutes, RC212 (Lallemand, France) yeast was rehydrated and added at 0.4 g/L. Grapes were fermented on skins for seven days at 27 °C (± 1 °C) using a modified version of the "Bodum French Press" method (Dambergs and Sparrow 2011) as outlined by Carew et al. (2014). The ferments were monitored and weighed daily, with daily weight loss indicating ongoing fermentation through the release of carbon dioxide (Carew et al. 2014). On day 3, 200 mg/L of diammonium phosphate (DAP) was added to each ferment as a yeast nutrient source to ensure fermentation progression and completion, in reflection of standard winery practice when YAN is considered low (<140 mg/N/L). The end of fermentation was confirmed using Clinitest® reagent tablets for sugar testing (Bayer, Barmen, Germany) via the 5-drop method and considered complete when sugar was below 0.25 g/L. The wines were decanted into 375 mL green glass sparkling bottles, covered with parafilm and left at ambient temperature for 24 hours to ensure complete alcoholic fermentation. The bottles were crown sealed and stored at 4 °C for 2 weeks to promote settling. The settled wine was racked under CO2 cover and stabilized by the addition of 60 mg/L of PMS, then left for a further two weeks at ambient temperature before final racking

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under CO2 cover into three 50 mL amber glass bottles for each replicate. The wines were stored in a dark room at ambient temperature (~20 - 23°C) until analysis.

For the white winemaking process, ≈ 2 kg of grape clusters from the four 5-vine samples were basket pressed (1.3 L, Ferrari, Italy) to extract 700 mL of juice (35% extraction). 100 mL of pressed juice was collected and frozen for chemical analysis [Brix, pH, TA, Yeast Assimilable Nitrogen (YAN), total phenolics] and ≈ 600ml of filtered juice was transferred for microvinification into 500ml Schott bottles, leaving no headspace and fitted with an airlock. To break down pectin and prevent protein haze formation, 50 mg/L of PMS and 1 mL/L of VinoClear® classic enzyme (Winequip, Australia) was added to each replicate. The juice was cold settled at 4 °C for 7 days, after which the clear juice was racked and warmed to 12-14 °C, before addition of 200 mg/L of DAP and inoculation with EC1118 (Lallemand, France) yeast at 0.3 g/L. The juice was fermented at 14 °C for 2 weeks and finished at 20 °C for ≈ 1 week. Wines were fermented to dryness (< 2.5 g/L), and settled at 4 °C for one week, then racked under CO2 cover and stabilized by the addition of 80 mg/L of PMS. The wines were left for a further two weeks at ambient temperature (~20 - 23°C) before further racking into 3, 50 mL amber glass bottles, leaving no headroom, and stored at ambient.

Juice analysis. Juice samples were defrosted overnight at 4 °C and centrifuged (Hettich Universal 320R, Hettich, Germany) at 4000 rpm for 10 minutes to clarify. Brix was measured using a hand-held digital refractometer (Pocket Refractometer Pal-1, Tokyo, Japan) and the pH and TA were measured using an auto-titrator (702 Metrohm SM Titrino, Metrohm, Switzerland). Measurement consistency of each method was tested by duplicating measurements of every fourth (refractometer) and tenth (auto-titrator) sample. Total phenolics was measured in clarified

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Chardonnay juice samples using UV-vis spectrometry as described in Kerslake et al. (2018). Total phenolics were measured in absorbance units (AU) and calculated as the absorbance at 280 nm (Iland et al. 2004).

Grape analysis. The 100 g sub-samples of frozen Pinot noir grapes from each sample were defrosted at 4 °C overnight. An Ultra Turrax T25 (Ika, China) was used at 4,186 rcf for two blocks of 30 seconds. For yeast assimilable nitrogen (YAN) analysis, 50 g of grapes were homogenized. The homogenate for YAN analysis used only raw material and did not include ethanol extraction. The samples were centrifuged at 1,450 rcf for 10 minutes to clarify before analysis.

The other 50 g of grapes was used as an homogenate for anthocyanin extraction as described in Iland et al. (1996). UV-visible spectroscopy was used to measure total phenolics, anthocyanins and tannins, which were calculated using the Australian Wine Research Institute WineCloudTM (AWRI).

Yeast assimilable nitrogen (YAN). Chardonnay juice samples and Pinot noir homogenate samples (no ethanol extraction) were used to measure YAN using Vintessential Laboratories (Melbourne, Australia) Ammonia Nitrogen and Primary Amino Acid Nitrogen test kits according to the manufacturer's instructions.

Wine analysis. In each trial season, Chardonnay wine samples were clarified by centrifugation (Hettich Universal 320R), diluted 1:5 in 1M HCL, and dark incubated at ambient temperature (20 – 22 °C) for one hour, before scanning at 280 nm with a spectrophotometer (Genesys 10S, Thermo, USA). Total phenolics was calculated using absorbance at 280 nm (Iland et al. 2004). Total phenolics, total anthocyanins, total tannins, total pigment and non-bleachable

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pigment were quantified using UV-visible spectroscopy for the Pinot noir wine samples in the 2017/18 and 2018/19 growing seasons using the modified Somers method (Mercurio et al. 2007).

Data analysis. All data was analyzed using IBM SPSS Statistics V22. The data was subjected to normality tests prior to analysis. ANOVA analysis was used to test for significant differences of the dependent variables due to the fixed effects of N and irrigation treatments, and the treatment interaction term. Each ANOVA model was limited to a single year. Tukeys post hoc test was used to determine significant differences among sample means at $\alpha = 0.05$.

Results

Weather. Mean daily temperatures for the trial period were below the historical average, yet higher mean maximum and minimum temperatures and an increase in solar radiation compared to historical climate data was reported. Each growing season was warmer and drier than the previous over the three-year trial period. The 2017/18 season had the highest growing degree days (Table 4).

Canopy architecture and vine vigor. In Chardonnay, a reduction in cluster contacts was observed with double irrigation in 2017-18, but only in the standard N treatment (Table 5). No other treatment interactions were observed in the Chardonnay block within the measured canopy variables. There were no interactions between N and irrigation treatments on vine canopy (effective insertions (%), gaps (%), leaf contacts and leaf layer number (LLN)) in the Pinot noir block over the three trial seasons.

N treatments did not have an impact on leaf contacts and LLN for either Chardonnay or Pinot noir over the three trial seasons (Table 5). Effective insertions and gaps varied between 90.2

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306 – 98.4 % and 1.6 – 9.8 % for Chardonnay and 89.9 – 98.7 % and 1.3 – 10.2 % for Pinot noir
307 respectively, but no N effects were detected.

308 The influence of irrigation rate on vine canopy was season dependent (Table 5). Effective
309 insertions (%) and gaps (%) were unaffected by irrigation rate across both cultivars and seasons,
310 ranging from 90.4 – 98.6 % and 1.4 – 9.6 % for Chardonnay and 89.9 – 97.7 % and 2.3 – 10.1 %
311 for Pinot noir. Leaf contacts, LLN and cluster contacts responded inconsistently to the irrigation
312 treatments (Table 5). In 2017/18, vine canopy was less dense in Pinot noir but denser in
313 Chardonnay under the double irrigation treatment as demonstrated by the number of leaf contacts
314 and LLN. In Chardonnay in 2016/17 and Pinot noir in 2018/19, double irrigation increased cluster
315 contacts compared to standard irrigation.

316 In Chardonnay, pruning weights were lower in the control (0N) treatment in 2018/19
317 (Table 5). In Pinot noir, pruning weights were increased in the double N treatment in 2018/19, but
318 only in comparison to the standard N treatment. The double irrigation treatment increased pruning
319 weights in both Pinot noir and Chardonnay in 2017/18 and for Pinot noir only in 2018/19 (Table
320 5). No other irrigation treatment effects were observed.

321 **Leaf nitrogen.** In 2017-18, both cultivars exhibited a decrease in leaf N (%) as a result of
322 the double irrigation treatment at harvest (Table 6). Similar results were observed for leaves
323 collected at harvest in the 2018-19 growing season, but the means in Table 4 are representative of
324 pooled leaves from each of the four treatment replicates and statistical analysis could not be
325 undertaken. In 2017-18, lower leaf N was observed in the control N treatment for Chardonnay, yet
326 no response was observed in Pinot noir (Table 6). In 2018-19, a trend for increased leaf N with
327 increasing N rate was observed across both cultivars.

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328 **Yield analysis.** The interaction between N and irrigation treatments on yield variables
329 varied among seasons for both cultivars. For Chardonnay in 2016/17, both the number of berries
330 per cluster and cluster density were higher under double irrigation, yet only in the double N
331 treatment (Table 7). Cluster weight was also increased with double irrigation under both control
332 and double N rates (Table 7). Interactions between N and irrigation were also observed in Pinot
333 noir in 2016/17 for cluster weights and the ratio of berry size (>10 mm : <10 mm) (Table 8). In
334 2016/17, the number of big berries (>10 mm) in Pinot noir was lower with double irrigation in the
335 control N treatment but increased by double irrigation in the double N treatment. In the same
336 season, the number of small to medium-sized (<10 mm) berries was increased with double
337 irrigation, but only in the standard N treatment (Table 8).
338 For Chardonnay in 2017/18, the number of small to medium sized berries (<10 mm) was
339 lower with double irrigation at both standard N and double N rates, whereas no treatment effect
340 was observed in the control N treatment (Table 7). For Pinot noir in 2017/18, double irrigation
341 reduced the number of small to medium sized berries (< 10mm), but only in the control N treatment
342 (Table 8).
343 Main effects of the N treatments on yield variables were again inconsistent across cultivars
344 and seasons. In Chardonnay in 2018/19, yield was decreased in the control N treatment compared
345 to the double N treatment (Table 9). This was driven by a decrease in cluster number, larger berries
346 (>10 mm), the ratio of berry size (>10 mm : <10 mm) and cluster weight under the control N
347 treatment (Table 9, 10). In Pinot noir in 2016/17, an increase in average cluster count was observed
348 in the double N treatment in comparison to the standard N treatment, whereas a decrease in berries
349 per cluster was apparent under the standard N treatment compared to the double N treatment (Table

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11). No differences between double N or standard N with control N were observed in the above variables. N treatments had no significant impact on berry size or berry size ratio in Pinot noir (data not shown).

Irrigation treatments influenced cluster weight, berry number and berry size, although treatment effects were inconsistent across cultivars and seasons. For Chardonnay, the average cluster count was decreased at the double irrigation rate in 2016/17, but this effect was not observed in the following seasons (Table 9). In 2017/18, cluster weight was higher in the double irrigation treatment, whereas in 2018/19, it was decreased with double irrigation (Table 9). The decrease in cluster weight in 2018/19 was coupled was a decrease in the number of big berries (>10 mm) (Table 10). In Pinot noir, the mean (μ) berry size ratio was higher with double irrigation in 2017/18 ($\mu = 3.2$, SE = 0.43) than with standard irrigation ($\mu = 2.0$, SE = 0.24). In 2018/19, there was a lower number of berries per cluster (Table 9) and small berries (<10 mm) at the double irrigation rate ($\mu = 64.6$, SE = 6.87) compared to the standard irrigation treatment ($\mu = 88.8$, SE = 7.87).

Grape and wine composition. *Yeast assimilable nitrogen (YAN).* Irrigation treatments had no impact on YAN in either cultivar over the three trial seasons (Tables 12 and 13). However, N treatments did influence YAN for both Pinot noir and Chardonnay. In Pinot noir, YAN was highest in response to applying double N in 2017/18. In 2018/19, response differences among N treatments became more distinct with an increase in YAN observed with increasing N rate (Table 12). A similar positive increase was observed in Chardonnay in both 2016/17 and 2017/18, but not the 2018/19 season (Table 13). Overall, YAN concentrations were very low in 2017/18 across both cultivars (Table 12, 13).

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372 *Juice Brix, pH, TA, and phenolics.* No interactions between irrigation and N treatments
373 were observed for juice Brix, pH or TA for either cultivar over the three seasons. N treatments also
374 had no influence on juice Brix, pH or TA over the trial period (Tables 12, 13). As the TA analysis
375 was not performed on fresh juice samples, the TA values observed are not considered entirely
376 accurate as freezing and defrosting samples may result in tartrate precipitation. However, relative
377 differences between treatments are still considered valid as the samples were handled and treated
378 in the same manner.

379 The impact of irrigation rate on juice Brix, pH and TA was generally inconsistent and
380 minor across seasons (Tables 12, 13). In Chardonnay, juice TA was higher under double irrigation
381 in 2016/17 only (Table 12). In Pinot noir, juice Brix was higher in response to double irrigation
382 only in 2017/18(Table 13). In Chardonnay in 2016/17, juice phenolics were higher in the control
383 N treatment, but only under standard irrigation (Table 7). In Pinot noir in 2018/19, grape phenolics
384 and tannins were decreased under double irrigation and in the double N treatment (Table 13).

385 *Wine pH and TA.* Wine pH and TA were inconsistently affected by N and irrigation
386 treatments across cultivars and seasons (Table 14, 15). In general, no interaction between
387 treatments were observed, except for Chardonnay in 2018/19, where grapevines receiving double
388 irrigation produced wine with higher pH levels than standard irrigation, but only under the control
389 N treatment (Table 7). In Chardonnay in 2017/18, wine TA was increased in the double N
390 treatment in comparison to the control N treatment yet remained unaffected in the other trial
391 seasons (Table 14). In Pinot noir in 2018/19, wine pH was higher in the standard N treatment than
392 in the control N treatment, despite a lack of effect on juice pH in the same year (Table 15). The
393 imposed irrigation treatments had a minimal influence on wine TA or pH. Wine TA was increased

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with double irrigation in Chardonnay in 2016/17 and 2017/18 (Table 14), whereas wine pH and TA were unaffected by irrigation rate in Pinot noir (Table 15).

Wine phenolics. In the first season of the Chardonnay trial (2017/18), double irrigation led to a reduction in total phenolics in wine under the control N treatment, whereas irrigation had no impact under the other N treatments (Table 7). In 2018/19, this same pattern was observed, and lower wine phenolics were observed with double irrigation but only in the standard N treatment. In Pinot noir in 2018/19, a decrease in wine phenolics and tannins was observed with double irrigation under the standard N treatment (Table 9). Irrigation had no impact on wine phenolics at the other N rates, however conversely, double irrigation increased wine total tannins under the double N treatment.

The impact of N treatments on wine phenolics was limited. In Chardonnay in 2016/17, the wine phenolics response to N followed an inverted bell curve-shape, with standard N treatments producing wine with lower phenolics than in double N (Table 14). However, no differences were observed among treatments in the following trial seasons. In Pinot noir in 2018/19, total wine pigment was decreased under the double N treatment (Table 15). Non-bleachable pigment was likewise lower under the double N treatment, but only in comparison to standard N rates (Table 15).

Irrigation effects on wine phenolics was inconsistent among seasons. In 2016/17 in Chardonnay, wine phenolics were lower under double irrigation (Table 14). In Pinot noir, in 2017/18, wine phenolics and tannins were higher with double irrigation (Table 15).

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Discussion

This study on two cool-climate grapevine cultivars showed that the most discernable influence of N treatments was on YAN concentrations, whereas marginal and inconsistent treatment impacts were observed on vine canopy, vigor, yield, and grape and wine composition. These findings align closely with observations made by Bell and Henschke (2005) in their review of the impact of N nutrition on grapes, fermentation and wine, demonstrating that the only consistent effect of increased N application on grape berry quality was an increase in berry nitrogenous compounds, often leading to an increase in juice and must YAN. The vines used in our study were relatively young at trial commencement (3 years-old), and as a result, vine reserves were likely low and the root systems small, resulting in higher N requirements relative to mature vines (Verdenal et al. 2021). Despite this, the additional N supplied by the double N treatment had minimal impact on the measured variables apart from YAN, suggesting that under lower N rates, YAN is not prioritized by the vine. In the final year of the trial (2018/19), Chardonnay vine vigor and yield were lower in the control (0N) treatment, suggesting that over time, low N rates in young vines reduce productivity as N reserves are depleted. Due to the limited amount of research on grapevine N fertilization rate, the following discussion is not limited to cool-climate wine regions.

Yeast assimilable nitrogen (YAN). In 2 out of the 3 trial years, Pinot noir homogenate extract and Chardonnay juice YAN concentrations showed an increase with the additional N applied compared to the 0N control. A large proportion of N is located in grape skins and seeds, and for skin-contact wine-making cultivars such as Pinot noir, it is likely that this contact during pressing, maceration and fermentation may provide yeast with increased accessibility to N than

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436 reported from juice YAN measurements (Stines et al. 2000, Bell and Henschke 2005, Ribéreau-
437 Gayon et al. 2006). Stines et al. (2000) observed the amino acid contribution of grape seeds, skins
438 and pulp in Riesling and Cabernet Sauvignon and reported 19 – 29 % of yeast assimilable amino
439 acids were present in the skins and 10 – 15 % in the seeds, whereas Miele et al. (2000) reported
440 50 % of final yeast assimilable amino acids were present in Cabernet Sauvignon skins. Although
441 the Pinot noir YAN measurements in this study are not directly comparable to others due to the
442 homogenization procedure undertaken, the differences observed between N treatments are
443 legitimate as the samples were treated homogenously. It was expected that we would observe
444 higher YANs than reported in other studies, given our homogenization procedure also took in to
445 account the skin and seeds. Regardless of the YAN measurement procedure, no minimum YAN
446 concentration for high solid red wine ferments has been published (Bell and Henschke 2005).
447 Using the levels considered for clarified juice samples, the Pinot noir YAN concentrations in this
448 study could be considered deficient or low across all N treatments relative to the conventionally
449 used 140 mg/N/L YAN threshold, under the assumption that juice extractions are considered to
450 fall 29 – 50 % lower than actual YAN concentrations due to the contribution of N in grape skins
451 (Miele et al. 2000, Stines et al. 2000). Due to the nature of this study, DAP was added to all
452 ferments to ensure fermentation completion and therefore, the direct impact of N rates on
453 fermentation kinetics and actual YAN thresholds could not be evaluated. This is however a realistic
454 scenario, as DAP addition in low YAN (<140 mg/N/L) grape juices and musts is a common winery
455 intervention, particularly in cool climate wine regions. Nonetheless the relative differences in wine
456 composition between N treatments are comparable. YAN concentrations in Chardonnay also
457 tended to be deficient (< 140 mg/N/L) or at the lower acceptable range of suggested concentrations.

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458 Yet in this case, increasing N from the standard N rate to the double N rate did increase deficient
459 (< 140 mg/N/L) YAN concentrations to close to acceptable YAN levels (> 140 mg/N/L) in
460 2017/18. Nevertheless, it should be noted that the increase in YAN was not proportional to the
461 amount of N applied in the double N treatment, suggesting that N use efficiency may be reduced
462 with higher N application rates. There was however a trend for increased N in the double rate
463 treatment canopies in each of the three seasons (Table 6), suggesting that the additional N may
464 have increase N storage in the perennial organs. Yet as the N was applied in a mobile nitrate form,
465 there is the potential that surplus N could have been lost to the environment as either N2O gasses
466 (Swarts et al. 2016) or leached below the root zone (Nováková and Nágel 2009). It should be noted
467 that in another Tasmanian texture contrast soil, the infiltration of fertigation nitrate in an apple
468 orchard was largely restricted to A1 soil horizons under standard fertigation and irrigation
469 practices, despite infiltrating water penetrating further below in to the A2 and upper B2 horizons
470 (Hardie et al. 2018). This demonstrates that nitrate losses through leaching can be minimized with
471 good fertigation management and suggests that nitrate leaching in this study should have been
472 minimal given the careful monitoring of crop evapotranspiration and soil moisture undertaken by
473 the grower.

474 The amount of N required to achieve suggested YAN concentrations (140 mg/N/L) varied
475 between growing seasons. YAN concentrations across both Pinot noir and Chardonnay blocks
476 were substantially lower in the 2017/18 season, likely in response to the high yields experienced
477 in that season. This suggests that in high crop load years, N application needs to be monitored
478 closely and increased to meet YAN requirements.
479

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The relatively high rainfall during the bloom to veraison (113.6 mm) and veraison to harvest (117.4 mm) growing periods (Supplementary Table 1) when N fertilization was applied may have also contributed to nitrate leaching in the soil and decreased N uptake, reducing N translocation to grape bunches and therefore YAN. On the other hand, increasing irrigation had no influence on YAN concentrations across both cultivars over the trial period. Given most of the research undertaken on the impact of irrigation rate on YAN is based on decreasing standard irrigation rates (Wade et al. 2002, Hannam et al. 2013, Holzapfel et al. 2015), the impact of additional irrigation on YAN is largely unknown. Yet, in reduced irrigation studies, YAN improvements appear to be mainly driven by reductions in yield and/or berry size (Hannam et al. 2013, Holzapfel et al. 2015). Based on this principle, it is not surprising that YAN was unaffected by the double irrigation rate in this study given the lack of impact of irrigation rate on yield and the inconsistent influence of irrigation rate and treatment interactions (N x irrigation) on berry size. There were no interactive effects of N and irrigation on YAN, indicating that the double irrigation rate did not influence the amount of N translocated into grape clusters.

Vine canopy and vigor. Vine canopy characteristics, as measured by the Point Quadrat method (PQ), were not influenced by N and irrigation treatment interactions or N treatments alone. This suggests that the double N rate, which averaged ~36 kg/N/ha per year across both blocks, may have been too low to have had an impact on vegetative growth or that the timing of N application, which in most cases occurred just prior to veraison (Table 2, 3), may have limited the influence of N supply on vine vegetative response. Whilst other studies on grape cultivars suited to cooler climates have applied upwards of 60 kg/N/ha for a moderate to high N rate (Keller et al. 1999, Linsenmeier et al. 2008), the current study was designed as a grower managed manipulation

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of current vineyard practices, where the double N rate was set as a comparison to their standard practice. Despite the modest N rates utilized in this study, vine canopy response to N application in cool climate wine regions appears to be small and inconsistent even at higher N rates.

No changes to vine canopy variables measured for the control (0N) treatment over the three-year period suggests sufficient vine N reserves were available and the supply of N from the soil was enough to sustain adequate vegetative growth. Bell and Robson (1999) reported a decrease in LLN measured at veraison in unfertilized Cabernet Sauvignon vine canopies (0 kg/N/ha) compared to fertilized canopies (92.6, 185.2, 370.4 and 740.8 kg/N/ha) in each season of a three-year trial. However, comparatively, much higher N treatment rates were applied, and the trial was conducted on a site characterized by low soil fertility, suggesting that N reserves may have been initially low. Löhnertz (1991) suggested that fertilizer studies that only span a few years are not representative of how N rate impacts vegetative growth due to the nature of how N is stored in wood. Yet, despite the lack of impact of N treatments on vine canopies, pruning weights were lower in the Chardonnay control treatment by the conclusion of the trial in 2018/19, indicating that N was becoming deficient. In Pinot noir, an increase in pruning weights in the double N treatment in the final year of the trial was also observed, again demonstrating an impact of N rate on vine vigor, although no differences in canopy architecture were observed.

Irrigation rate only impacted vine canopy and vine vigor in the 2017/18 growing season, with denser vine canopies observed for Chardonnay under double irrigation and the opposite effect observed for Pinot noir. This response is likely a result of soil type differences between the two blocks. The Chardonnay grapevines were grown in a shallow black clay loam soil, with a heavy clay subsoil characterized by a high-water holding capacity, whereas the Pinot noir grapevines

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were grown in a lateritic sandy loam. It is possible that increased rainfall in 2016/17 helped to recharge soil water reserves in the Chardonnay block, meaning more water was available in the following season. This increase in water availability in 2017/18, particularly earlier in the season, coupled with double irrigation, may have stimulated vegetative growth as shown by the increase in leaf contacts and LLN in Chardonnay. Alternatively, the decrease observed in leaf contacts and LLN in Pinot noir in 2017/18 may reflect nutrient leaching as a result of additional water supply in the high draining sandy loam soil, resulting in less dense canopies. Indeed, leaf N concentrations measured at harvest in the 2017/18 growing season showed that double irrigation decreased leaf N compared to standard irrigation. This same pattern was also observed with Chardonnay however, the additional water held by the soil may have been sufficient to stimulate vegetative growth regardless of nutrient availability.

Despite the inconsistent irrigation treatment effect on canopies, double irrigation increased vine vigor in both cultivars in 2017/18 as shown by an increase in pruning weights. Paranychianakis et al. (2004) reported a strong correlation between shoot growth and soil moisture in Sultanina grapevines and observed a strong positive association between shoot length and irrigation level (0.50, 0.75, 1.00 of evapotranspiration) over a three-year period. As the dormancy period in this study was uncharacteristically dry in 2017/18, a greater amount of irrigation was applied earlier in the season (budbreak – bloom) than in other trial years, and therefore the earlier application of water may be responsible for the differences observed in pruning weights, as this may have stimulated early shoot growth in this season.

Yield and yield variables. Nitrogen fertilization and irrigation in the vineyard aims to drive higher yields through an overall increased capacity of the vine. Interactions between N and

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546 irrigation treatments existed mainly for cluster weight and berry size variables, although these
547 were inconsistent across trial seasons and cultivars. In 2016/17 only, applying double irrigation to
548 vines receiving the double N treatment resulted in increases in cluster weight in both Chardonnay
549 and Pinot noir. In Chardonnay, the cluster weight increase was driven by an increase in berries per
550 cluster, which is determined by conditions influencing inflorescence initiation and differentiation
551 in the prior season, as well as fruit-set in the existing season (Guilpart et al. 2014, Li-Mallet et al.
552 2016). Keller et al., (1998) reported that inadequate N around fruit-set can lead to flower
553 abscission. In this study, a low annual growing season rainfall of 256 mm in the prior season
554 (2015/16), may have led to some degree of vine water stress, which may have influenced the
555 response of vines to the additional N through the double N treatment in 2016/17. The additional N
556 may have improved fruit-set and thereby increased berries per cluster, but only when irrigation
557 was sufficient to allow effective N uptake. In Pinot noir, the observed increase in cluster weight
558 was driven by more larger berries as represented by the berry size ratio. In agreement with Triolo
559 et al. (2018), our results suggest that berry weight (size) increased when N and water supply are
560 not limited, most likely due to their influence on cell division and/or cell expansion.

561 The main effects of N and irrigation treatments on yield variables were minimal and
562 inconsistent. In Chardonnay, yield increases with N fertilizer application in 2018/19 reflected
563 more, heavier clusters and larger berries, yet no influence of N treatments were observed for Pinot
564 noir. Several studies have found that cluster number accounts for ≈ 60 % of seasonal yield variation
565 (Dunn and Martin 2007, Guilpart et al. 2014, Li-Mallet et al. 2016), and although it is primarily
566 determined by pruning approach, secondary influences on bud fruitfulness and differentiation,
567 through climate, irrigation, and mineral nutrition, can play a significant role. Whilst the influence

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of N supply on cluster number remains poorly understood (Li-Mallet et al. 2016), N stress has been associated with a decrease in potential cluster number in response to a decrease in bud fruitfulness (Guilpart et al. 2014). Indeed, sufficient N is necessary for optimal inflorescence primordium formation and flower differentiation (Vasconcelos et al. 2009). Keller et al. (1998) reported reduced fruit set as a result of inflorescence necrosis due to low N fertilization at bloom, whilst Linsenmeier et al. (2008) reported a 16 % reduction in yield for zero N treatments in Riesling when averaging yields over a fifteen year trial period. In alignment with these findings, it is therefore possible that in Chardonnay, the lack of N provided in the 0N treatment disrupted yield formation, suggesting that the reproductive capabilities of the vine were impacted more by reduced N supply than that of vegetative production, as suggested by Keller et al. (2001).

Grape and wine composition. Reduced berry size, such as through low N supply and water deficit, has been linked to an increase in juice and grape phenolics (Bell and Henschke 2005). In 2016/17, grape juice phenolics were higher under standard irrigation in the Chardonnay 0N treatment, which may be a response of the lower cluster weights and reduced berry size in this treatment. In agreement with Triolo et al. (2018), our findings suggests that water availability is a greater driver of berry size than N, as low N supply does not appear to limit berry size or increase juice phenolics when water supply is increased. In Chardonnay, wine phenolic content also tended to be lower with double irrigation with different N rates and between seasons, which could again be attributed to changes in berry size, suggesting that the standard irrigation rates applied by the grower are generally adequate for juice and wine quality.

Juice composition (Brix, TA, pH) was unaffected by N treatments in both cultivars, whereas wine composition (TA, pH) was influenced inconsistently. A similar result on juice Brix,

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590 TA and pH was found by Conradie and Saayman (1989) in a 11-year study on Chenin blanc vines,
591 finding no influence of N rate (16, 56, 96 kg N ha year-1). The impact of N fertilization on juice
592 Brix, TA and pH was generally inconclusive, due to the high variability both within and between
593 studies, where differences appear to be more influenced by external factors including climate,
594 environment, cultural practices and genetics (Bell and Henschke 2005).

595 Microvinification allows adequate replication and control of the winemaking process,
596 which is often difficult to achieve on an industrial scale due to production logistics, risks and cost
597 (Dambergs and Sparrow 2011). Pinot noir phenolic composition has been found to be independent
598 of fermentation vessel and must size for volumes ranging from 0.2 to 10 kg, and similar phenolic
599 profiles have been obtained using fruit from the same source in an 330 kg fermenter (Sparrow and
600 Smart 2015). The Bodum french press method, as used in the red winemaking of this current trial,
601 is a simple, submerged cap method that can be non-invasively monitored and results in good
602 extraction with reproducible ferments (Dambergs and Sparrow 2011, Dambergs et al. 2012). There
603 were few effects of N treatments on phenolics, tannin and anthocyanin content of grape
604 homogenates over the period of the trial. Nevertheless, in the final season (2018/19), Pinot noir
605 grape phenolics and tannins were lower in the double N rate treatment, likely as a result of
606 compounding seasonal additions. As the double N rate did not influence canopy and yield
607 components in the same season, it is unlikely this treatment response is a consequence of
608 differences in berry size and phenolic dilution. Rather higher phenolic contents in the control and
609 standard N treatments may have resulted from altered phenolic metabolism, in which the N rates
610 imposed may have been low enough to impose a scenario of vine stress, leading to the upregulation
611 of secondary metabolite production (Keller, 2005). The total phenolics in wine from the same

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612 vintage also correspond with this finding, with the double N rate treatment having lower phenolics
613 than the other N treatments, although a treatment interaction with irrigation was also observed.
614 Thomidis et al. (2016) also found a reduction in Xinomavro berry phenolics with increasing N
615 rates (0, 60, 150 kg N ha-1), yet the standard N rate applied in that study was much greater than
616 the double N treatment in this trial, highlighting that there may be thresholds in which N rate starts
617 to impact secondary metabolism and berry phenolics.

618 The only main effect of increased irrigation on Chardonnay juice and wine quality was for
619 TA, although seasonal variation was apparent. As found in the current study, an increase in TA in
620 response to additional irrigation is a common finding (Williams and Matthews 1990) and has been
621 linked to an accelerated decrease in malic acid during berry ripening when water supply is limited
622 (Esteban et al. 1999). Seasonal variations in response to irrigation on juice TA have been
623 previously reported in cool-climate growing systems (Hannam et al. 2013, Balint and Reynolds
624 2014).

625 In Pinot noir, wine phenolics and tannins were increased by double irrigation in 2017/18,
626 which contrasts with other research that showed an increase in wine phenolics with water deficit
627 (Roby et al. 2004). However, in this study, 2017/18 was a high cropping year and double irrigation
628 also increased vine canopy and vine vigor, suggesting that vines with double irrigation benefited
629 from more source capacity which may have enabled better phenolic development. As for N supply,
630 additional water during high yielding years is necessary for maintaining consistent wine
631 composition. Although beyond the scope of this study, sensory analysis may have provided further
632 insight into the influence of irrigation and N treatments on vine vigor and YAN and its impact on
633 wine composition and should be a focus of further research.

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Conclusions

Excessive N rates and surplus water availability are generally associated with increased vine vigor and vegetative growth, increased yields, and negative impacts on grape and wine composition. Similar to Neilsen et al. (1989), the influence of N and irrigation treatments in this study were marginal and seasonally variable.

Given the minimal effect of the double N treatment on canopy, yield, grape and wine composition, cool climate growers would benefit from applying more N in the vineyard around veraison to increase YAN levels without driving excessive vegetative vigor. For a more conservative approach, foliar urea application at veraison has shown promise to improve YAN without resulting in substantial changes to vine N retention, vine growth, and no to little change to juice quality (Hannam et al. 2014, Mataffo et al. 2020). The results also suggest that the standard irrigation rates used in this trial (430 – 600 L vine-1) were adequate and there is no benefit from adding additional irrigation in a standard season. Our results showed that additional irrigation may be beneficial in high cropping years when carbohydrate and nutrient demands are higher.

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Table 1 Nitrogen (control, standard, double) and irrigation (standard, double) treatment rates for Chardonnay and Pinot Noir cultivars for 2016/17, 2017/18 and 2018/19 production seasons (May – April).

		Chardonnay			Pinot Noir		
		2016/17	2017/18	2018/19	2016/17	2017/18	2018/19
Nitrogen (kg/N/ha)	Control	0	0	0	0	0	0
	Standard	15.6	18.1	21.2	16.1	15.0	21.2
	Double	31.2	36.2	42.2	32.3	30.0	42.2
Irrigation (L/vine)	Standard	432.4	578.6	561.2	443.5	598.6	568.1
	Double	864.8	1157.2	1122.4	887.0	1197.2	1136.2

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Table 2 Nitrogen (N) fertilizer source, rate and timing of application across trial period (2016-2019) for Chardonnay.

Chardonnay	2016/17		2017/18		2018/19	
Fertilizer	Application date	Rate (kg/N/ha)	Application date	Rate (kg/N/ha)	Application date	Rate (kg/N/ha)
Liquid N (32.3%) plus humic acid (Nitro Humus 323)	Feb-17	4.29	-	-	-	-
N (42%)[a] plus fulvic and seaweed extract (VitalGold 42)	Mar-17	8.35	Jan-18	6.30	-	-
Calcium Nitrate (13.1% N, Campbells Aqua-Fert)	Feb-17	1.12	Feb-18	2.62	-	-
Calcium Nitrate plus Boron (15.5% N, Aqua-Fert Calcium Nitrate + Boron)	-	-	Feb-18	3.10	-	-
Ammonium (15.8% N, Campbells Nitro-P)	Feb-17	1.41	Feb-18	2.37	Feb-19	4.66
	-	-	Mar-18	3.16	Feb-19	4.66
Fish emulsion (2.5% N, Sustainable Farming Solutions)	May-17	0.42	Apr-18	0.50	-	-
N (42.5%, Easy N)[b]	-	-	-	-	Jan-19	8.49
	-	-	-	-	Jan-19	6.37
Total N/season		15.6		18.1		21.2

[a]Nitrogen as ammonium (10.5%), nitrate (10.5%) and urea (21%).
[b]Nitrogen as nitrate (25%), ammonium (25%) and urea (50%).

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Table 3 Nitrogen (N) fertilizer source, rate and timing of application across trial period (2016-2019) for Pinot Noir.

Pinot Noir	2016/17		2017/18		2018/19	
Fertilizer	Application date	Rate (kg/N/ha)	Application date	Rate (kg/N/ha)	Application date	Rate (kg/N/ha)
Liquid N (32.3%) plus humic acid (Nitro Humus 323)	Feb-17	5.98	-	-	-	-
N (42%)[a] plus fulvic and seaweed extract (VitalGold 42)	Mar-17	8.03	Jan-18	6.30	-	-
Calcium Nitrate (13.1% N, Campbells Aqua-Fert)	Feb-17	1.18	Feb-18	2.62	-	-
Ammonium (15.8% N, Campbells Nitro-P)	Feb-17	0.49	Feb-18	2.37	Feb-19	4.66
	-	-	Mar-18	3.16	Feb-19	4.66
Fish emulsion (2.5% N, Sustainable Farming Solutions)	May-17	0.48	Apr-18	0.50	-	-
N (42.5%, Easy N)[b]	-	-	-	-	Jan-19	8.49
	-	-	-	-	Jan-19	6.37
Total N/season		16.6		15.0		21.2

[a]Nitrogen as ammonium (10.5%), nitrate (10.5%) and urea (21%).
[b]Nitrogen as nitrate (25%), ammonium (25%) and urea (50%.)

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Table 4 Climate data from on-site weather station at trial site for 2016/17, 2017/18 and 2018/19 production seasons (May – April) with historical climate data (1991 – 2020) obtained from Hobart Airport weather station.

	2016/17	2017/18	2018/19	Long term average
Mean average growing season temperature (°C)	13.2	13.3	13.6	13.8
Mean maximum temperature (°C)	17.7	18.1	18.5	17.9
Mean minimum temperature (°C)	9.3	9.4	9.6	8.4
GDD (Oct – Apr)[1]	1178.6	1373.7	1200.6	-
Solar radiation (MJ m^{-2})[2]	4861.3	4919.6	4923.8	4845.2
Total rainfall (mm)	499.8	400.0	317.5	461.2

[1]GDD represents growing degree days, calculated using a base of 10°C.
[2]Online database for solar radiation only available from 2009 onwards.

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Table 5 Dormant pruning weights and vegetative growth variables, as measured by the modified Point Quadrat method, for *Vitis vinifera* Chardonnay and Pinot Noir grapevines in response to nitrogen and irrigation treatments across the trial period (2016 – 2019).

	Leaf contacts			LLN			Pruning weights (kg)		
	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19
Chardonnay									
Nitrogen									
Control (0N)	60.5	84.0	89.7	1.51	2.10	2.24	1.13	1.00	0.71 a
Standard	63.8	87.0	97.0	1.60	2.18	2.43	1.32	1.27	1.45 b
Double	54.5	83.0	97.3	1.36	2.08	2.43	1.25	1.39	1.52 b
Significance	ns	ns	ns	ns	ns	ns	ns	ns	***
Irrigation									
Standard	55.4	77.1	95.7	1.39	1.93	2.39	1.14	1.00	1.34
Double	63.8	92.2	93.7	1.59	2.31	2.34	1.33	1.44	1.17
Significance	ns	**	ns	ns	*	ns	ns	**	ns
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns
Pinot Noir									
Nitrogen									
Control (0N)	52.2	57.7	90.8	1.3	1.4	2.3	0.93	0.92	1.06 ab
Standard	50.0	63.0	88.0	1.3	1.6	2.2	0.75	0.86	0.94 a
Double	48.5	63.0	95.0	1.2	1.6	2.4	0.84	1.22	1.57 b
Significance	ns	ns	ns	ns	ns	ns	ns	ns	*
Irrigation									
Standard	53.7	66.0	94.1	1.34	1.65	2.35	0.86	0.75	0.99
Double	46.8	56.4	88.4	1.17	1.41	2.21	0.82	1.25	1.39
Significance	ns	*	ns	ns	*	ns	ns	**	ns
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 6 Mean total leaf N (%) recorded at harvest in the 2017-18 and 2018-19 growing seasons.

	Chardonnay		Pinot Noir	
	2017/18	2018/19[a]	2017/18	2018/19
Nitrogen				
Control (0N)	1.42 a	1.53	1.54	1.92
Standard (ST)	1.65 b	1.71	1.50	2.06
Double (DBL)	1.77 b	1.85	1.68	2.30
Sig.	***		ns	
Irrigation				
Standard	1.66	1.74	1.68	2.20
Double	1.56	1.65	1.47	1.83
Sig.	*		**	

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.
[a]In 2018/19, samples were pooled; no statistical analysis could be performed.

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Table 7 Interactive effects of N and irrigation treatments on yield characteristics, and fruit and wine composition of Chardonnay in three growing seasons (2016-2019).

		2016-17				2017-18			2018-19		
		Berries per cluster	Cluster weight	Cluster density	Juice phenolics (AU)	Cluster density	Berries per cluster < 10 mm	Wine phenolics (AU)	Berries per cluster < 10 mm	Wine pH	Wine phenolics (AU)
Irrigation	**Nitrogen**										
Standard	Control	157.4	112.7	4.1	2.01	6.8	10.0	1.01	33.8	3.09	1.11
	Standard	168.1	136.3	5.6	1	8.6	22.7	0.99	35.3	3.09	1.11
	Double	123.4	107.9	4.4	1.06	7.0 a	11.4	1.03	23.9	3.14	1.1
Double	Control	167.1	155.5	6.5	1.23	6.9	10.9	0.91	101.6	3.14	1.12
	Standard	138.0	148.3	6.1	0.98	8.0	8.8	1.03	43.1	3.12	1.03
	Double	163.7	184	6.0	1.01	8.1 b	6.1	1.03	27.1	3.09	1.09
Interaction		*	**	*	*	*	**	*	**	*	*

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 8 Interactive effects of N and irrigation treatments on yield characteristics, and fruit and wine composition of Pinot Noir in three growing seasons (2016-2019).

		2016-17				2017-18	2018-19	
		Cluster weight (g)	Berries per cluster >10 mm	Berries per cluster <10 mm	Berry size ratio (>10 : <10mm)	Berries per cluster <10 mm	Wine Phenolics (AU)	Wine Tannins (g/L)
Irrigation	**Nitrogen**							
Standard	Control	115.6	81.3	87.8	1.2	68.1	28.1	0.3
	Standard	93.3	45.5	54.6	0.9	77.1	37.1	0.7
	Double	118.1	53.6	62.8	0.9	64.1	22.6	0.2
Double	Control	103.2	43.2	81.2	0.6	45.4	30.4	0.5
	Standard	105.8	44.8	83.0	0.7	60.4	28.5	0.4
	Double	149.4	82.6	46.6	1.8	57.9	23.7	0.3
Interaction		*	***	*	***	*	*	**

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 9 Yield characteristics of Chardonnay in response to N and irrigation rates in three growing seasons (2016 – 2019).

	Yield (kg/vine)			Cluster count			Berries per cluster		Cluster weight (g)		Cluster density (OIV)
	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2018-19
Nitrogen											
Control (0N)	3.0	6.9	3.2 a	23.1	31.6	22.3 a	141.4	126.6	217.5	141.7 a	4.3 a
Standard (ST)	3.0	6.9	4.6 b	21.5	30.3	24.8 b	152.8	140.8	225.9	185.5 b	4.6 a
Double (DBL)	3.5	6.5	5.3 b	24.2	29.4	28.6 b	134.7	133.8	211.6	187.2 b	5.4 b
Significance	ns	ns	**	ns	ns	*	ns	ns	ns	***	**
Irrigation											
Standard	3.0	6.1	4.5	25.6	29.7	24.1	138.7	135.5	200.1	184.7	4.9
Double	3.3	7.4	4.2	20.2	31.2	26.4	147.3	132.0	236.6	158.2	4.6
Significance	ns	ns	ns	**	ns	ns	ns	ns	*	**	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 10 Berry size of Chardonnay in response to N and irrigation rates in three growing seasons (2016 – 2019).

	Berries >10 mm			Berries <10 mm	Berry size ratio (>10:<10mm)		
	2016-17	2017-18	2018-19	2016-17	2016-17	2017-18	2018-19
Nitrogen							
Control (0N)	66.6	131.0	74.6 a	119.6	0.9	20.9	2.4 a
Standard (ST)	68.6	137.1	112.0 b	79.9	1.6	17.9	3.4 a
Double (DBL)	74.8	125.9	114.6 b	62.0	2.1	19.6	7.3 b
Significance	ns	ns	***	ns	ns	ns	**
Irrigation							
Standard	62.0	124.0	116.1	91.5	1.4	18.0	5.5
Double	78.0	138.7	84.7	82.8	1.7	20.9	3.3
Significance	ns	ns	***	ns	ns	ns	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 11 Yield characteristics of Pinot Noir in response to N and irrigation rates in three growing seasons (2016 – 2019).

	Yield (kg/vine)			Cluster count			Berries per cluster			Cluster weight (g)		Cluster density (OIV)		
	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2017-18	2018-19	2016-17	2017-18	2018-19
Nitrogen														
Control (0N)	3.5	6.0	4.1	30.7 ab	30.3	32.1	132.0 a	178.5	123.1	199.2	128.3	6.8	8.6	5.0
Standard (ST)	2.9	6.2	3.5	27.8 a	31.4	32.8	109.0 b	180.5	118.9	196.7	107.9	6.4	8.8	4.4
Double (DBL)	4.3	6.1	3.3	31.5 b	31.4	28.8	130.4 a	168.3	114.9	191.5	112.3	7.1	8.8	4.3
Significance	ns	ns	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
Irrigation														
Standard	3.5	6.6	3.5	30.8	32.5	29.6	117.7	185.6	127.9	201.0	118.2	6.7	8.9	4.8
Double	3.7	5.6	3.8	29.2	29.5	32.6	129.6	165.9	111.5	190.6	115.0	6.8	8.6	4.4
Significance	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 12 Berry composition of Chardonnay in response to N and irrigation rates in three growing seasons (2016-2019).

	Juice TSS (°Brix)			Juice TA (g/L)			Juice pH			Juice Phenolics (AU)		YAN (mg/N/L)		
	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2017-18	2018-19	2016-17	2017-18	2018-19
Nitrogen														
Control (0N)	20.63	20.89	20.24	9.50	7.42	8.28	2.61	2.71	3.06	1.15	0.74	130.0 a	42.0 a	119.9
Standard	21.65	20.99	19.38	9.26	8.76	9.46	3.04	2.97	3.02	1.06	0.76	154.3 ab	94.9 b	118.9
Double	20.89	21.03	18.68	9.13	9.22	8.13	3.08	3.00	3.02	0.96	0.79	174.6 b	135.5 c	148.7
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	***	ns
Irrigation														
Standard	21.43	21.47	19.44	8.70	8.39	8.09	3.04	3.00	3.02	1.13	0.86	159.9	95.5	129.4
Double	20.68	20.47	19.42	9.89	8.54	9.16	2.77	2.79	3.04	0.98	0.66	146.1	86.1	128.9
Significance	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 13 Berry composition of Pinot Noir in response to N and irrigation rates in three growing seasons (2016-2019).

	Juice TSS (°Brix)			Juice TA (g/L)			Juice pH			Grape Phenolics (AU)		Grape Tannins (g/L)		Grape Anthocyanins (AU)		YAN (mg/N/L)		
	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19	2016-17	2017-18	2018-19
Nitrogen																		
Control (0N)	20.4	21.6	20.1	8.0	8.0	8.0	3.2	3.0	3.2	112.7	116.4 a	1.8	1.7 a	88.1 a	244.8 a	283.7	88.1 a	244.8 a
Standard	22.5	19.6	19.4	7.4	7.4	7.4	3.2	3.1	3.3	111.7	108.9 a	1.7	1.5 a	103.4 a	298.5 b	308.7	103.4 a	298.5 b
Double	21.2	21.6	19.0	7.8	7.8	7.8	3.2	3.1	3.3	116.7	90.6 b	1.9	1.1 b	166.5 b	378.2 c	330.6	166.5 b	378.2 c
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	**	**	***	ns	***	***
Irrigation																		
Standard	20.7	19.4	19.7	7.7	7.7	7.7	3.2	3.0	3.3	112.7	113.2	1.8	1.6	127.0	304.8	316.3	127.0	304.8
Double	22.0	22.4	19.3	7.8	7.8	7.8	3.2	3.1	3.2	114.7	97.5	1.8	1.3	112.4	309.5	299.0	112.4	309.5
Significance	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	*	ns	ns	ns	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 14 Wine composition of Chardonnay in response to N and irrigation rates in three growing seasons (2016-2019).

		Wine TA (g/L)			Wine pH		Wine Phenolics (AU)
		2016-17	2017-18	2018-19	2016-17	2017-18	2016-17
Nitrogen							
	Control (0N)	9.12	9.67 a	9.14	3.12	3.00	1.20 ab
	Standard	8.91	9.95 ab	8.93	3.30	3.00	1.12 a
	Double	8.78	10.61 b	10.48	3.31	3.05	1.25 b
Significance		ns	*	ns	ns	ns	*
Irrigation							
	Standard	8.52	9.79	9.04	3.24	3.02	1.23
	Double	9.35	10.37	10.00	3.24	3.01	1.15
Significance		***	*	ns	ns	ns	*

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatments as determined by Tukeys post-hoc test.

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Table 15 Wine composition of Pinot noir in response to N and irrigation rates in three growing seasons (2016-2019).

	Wine TA (g/L)			Wine pH			Wine Phenolics (AU)	Wine Tannins (g/L)	Wine Anthocyanins (AU)		Wine Total Pigment (AU)		Non-bleachable Pigment (AU)	
	2016-17	2017-18	2018-19	2016-17	2017-18	2018-19	2017-18	2017-18	2017-18	2018-19	2017-18	2018-19	2017-18	2018-19
Nitrogen														
Control (0N)	7.4	7.7	6.7	3.6	3.4	3.8 a	29.7	0.7	32.5	35.8	4.4	4.7 a	1.6	1.9 ab
Standard	7.2	8.1	5.9	3.7	3.0	3.9 b	28.8	0.4	29.6	25.1	4.2	5.3 a	1.6	2.6 b
Double	7.2	9.0	6.2	3.7	3.3	3.8 ab	30.9	0.6	33.1	22.0	4.2	3.1 b	1.5	1.3 a
Significance	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	**	ns	**
Irrigation														
Standard	7.3	9.1	6.3	3.7	3.0	3.8	25.1	0.4	21.7	26.0	3.4	4.5	1.4	2.1
Double	7.3	7.3	6.2	3.6	3.4	3.8	33.7	0.7	39.9	29.5	4.9	4.2	1.7	1.8
Significance	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns

*, **, ***Means significantly different at P = 0.05, 0.01, 0.001, respectively, or non-significantly (ns) different.
Lowercase letters denote significant differences between treatment means as determined by Tukeys post-hoc test.

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Supplemental Table 1 Climate data grouped by phenological stages (dormancy to budbreak (1st May – 31st August), budbreak to bloom (1st September – 31st November), bloom to veraison (1st December – 31st January) and veraison to harvest (1st February – 30th April)) from on-site weather station at trial site for 2016/17, 2017/18 and 2018/19 growing seasons (May – April) with historical average climate data (1991 – 2020) obtained from Hobart Airport weather station.

		Average daily temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Solar radiation (MJ/m^2)	Rainfall (mm)	Rainfall (L/vine)	Irrigation (L/vine)	
								Chardonnay	Pinot Noir
Dormancy to budbreak	16/17	10.2	13.6	7.2	990.8	227.6	711.3	0.0	0.0
	17/18	9.5	13.3	6.3	1037.9	82.3	257.2	6.0	8.8
	18/19	10.2	13.9	7.2	844.0	139.6	436.3	7.2	12.1
	Historical	9.6	13.9	5.3	761.8	149.0	465.6	-	-
Budbreak to bloom	16/17	12.3	16.7	8.3	1677.8	144.8	452.5	14.4	14.4
	17/18	13.5	18.9	9.2	1818.8	86.7	270.9	94.6	100.6
	18/19	12.7	17.6	8.4	1659.9	94.3	294.7	50.3	50.3
	Historical	12.8	17.6	8.0	1453.7	121.8	380.6	-	-
Bloom to veraison	16/17	16.8	22.4	12.1	1544.5	88.3	275.9	160.5	165.8
	17/18	17.7	23.2	12.8	1513.5	113.6	355.0	226.0	231.6
	18/19	18.1	24.2	13.2	1606.6	36.8	115.0	261.5	260.4
	Historical	17.1	22.3	11.9	1432.0	86.2	269.4	-	-
Veraison to harvest	16/17	15.7	21.2	11.4	1504.3	39.1	122.2	257.5	263.4
	17/18	15.3	20.3	11.3	1443.4	117.4	366.9	252.0	257.6
	18/19	16.7	22.4	12.2	1087.4	46.8	146.3	242.3	245.3
	Historical	15.7	20.6	10.7	1197.7	104.7	327.2	-	-