

Evaluating Viticulture Manipulations Effects on Glycoside Abundance and Diversity in  
Vancouver Island Pinot Gris

By

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B.Sc., Lincoln University 2009

A Thesis Submitted in Partial Fulfillment

Of the Requirements for a Degree of

MASTER OF SCIENCE

In the School of Environmental Studies

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University of Victoria

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## Abstract

Common techniques to modify growing conditions of wine grapes such as leaf removal, kaolin application and cluster thinning are assumed to improve grape quality. Abundance and diversity of appropriate aroma compounds are key markers of wine grape quality. Grape varietal and location specific responses to these common vineyard management techniques have not been explored on Vancouver Island. To evaluate the response of Pinot gris to common vineyard management techniques a stratified random block design encompassing three management strategies in two Vancouver Island vineyards, both growing Pinot gris over the 2018 and 2019 growing seasons was conducted. Vines were manipulated with seven treatment combinations that included reference, heavy leaf removal, kaolin application on fruit and cluster thinning. Vine physiology metrics were monitored during the growing season, while mature grapes were evaluated at harvest for total soluble solids (TSS), titratable acidity (TA), and (pH). Further, gas chromatography and mass spectrometry were used to quantify glycoside aroma compounds abundance and diversity across the treatments and vineyards.

Results show heavy leaf removal decreased incidence of botrytis bunch rot and affected aroma compound abundance over the two growing seasons. Cluster thinning yielded consistent increased TSS and decreased TA at both vineyards. Kaolin did not significantly affect grape quality metrics. These results suggest heavy leaf removal and/or cluster thinning may yield significant benefit in the form of reduced botrytis pressure and improved grape quality in Vancouver Island grown Pinot Gris.

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## Acronyms

BC	British Columbia
Anova	Analysis of Variance
LR	Basal Leaf Removal
CT	Cluster Thinning
R	Reference or Regular Leaf Removal
H	Heavy Leaf Removal
HK	Heavy Leaf Removal + Kaolin
RK	Reference Leaf Removal + Kaolin
RC	Reference Leaf Removal + Cluster Thinning
HC	Heavy Leaf Removal + Cluster Thinning
HCK	Heavy Leaf Removal + Cluster Thinning + Kaolin Application
GC/MS	Gas Chromatography Mass Spectrometry
LAI	Leaf Area Index
PW	Pruning Weight
VOC	Volatile Organic Compound
TSS	Total Soluble Solids
TA	Titrateable Acidity
GDD	Growing Degree Days
V1	Vineyard 1
V2	Vineyard 2
NAFTA	North American Free Trade Agreement
Sub-GI	Sub geographic Indication Region
MTBE	Methyl Tert Butyl Ether
LOX	Lipid Oxidation Pathway
MEP	2-C-Methylerythritol-4-Phosphate Pathway

## Dedication

I would like to acknowledge all of the people who helped make this research possible. Firstly I would like to thank the folks in the University System, my supervisor Dr John Volpe and commity member Dr Simone Castellarin. Without their help this project would would not have been started or finished repectivley. Mitch MacFarlane, it was great to have someone to share the work load with. I would like to mention Dr Joana Pico Carbajo, who helped with all of the GCMS work. With out her help I would not have been able to complete this work. Secondly I would like to thank all of the family and friends who provided inspiration, and constant support during the length of this project. My Father Tim Watts, for being a constant sounding board and inspiration during this Project. Madeleine Greig and Dan Ting, for housing me for so long during the chemistry portion of this work. Most of all I would like to thank my wife Adra Greig for your constant support and encouragement. It has taken a long time for me to finish this thesis, without her this would not have been possible to complete.

# 1. Introduction

## 1.1 Wine Production in British Columbia

The British Columbia (BC) wine industry has changed dramatically over the past 30 years. Local wine production, consumption and regional tourism have all increased in wine regions across BC during this period (Cartier 2014, Hira and Bwenge 2011). The North American Free Trade Agreement (NAFTA) enacted in 1989 has significantly shaped the Canadian wine market, catalysing the rise of the BC wine industry by allowing competition into the Canadian market and driving an increase in domestic quality wine production.

Hybrid or non-*vinifera* grape varieties were most common in BC wine production prior to NAFTA, as growers during this period had little competition and quantity was the focus over quality (Hira and Bwenge 2011). Upon wines of objectively higher quality entering the Canadian market from the United States, wine producers in BC began replacing hybrid varieties with more recognisable European *Vitis vinifera* cultivars such as Merlot, Pinot noir, and Chardonnay (Hira and Bwenge 2011). With the shift to European *Vitis vinifera* cultivars came a shift in focus to quality over quantity to remain viable in the now much more competitive wine market. Some 30 years after NAFTA, BC's wine industry is valued at approximately C\$3 billion (Co.LLP 2017) and growing, as smaller wine regions in the province such as Vancouver Island follow the trend to planting European *Vitis vinifera* cultivars and favouring quality over quantity of wine.

Wine production on Vancouver Island and the Southern Gulf Islands has developed in parallel with the more widely recognized Okanagan Valley wine region. Vancouver Island is classified as a cool climate wine region and is in the coastal Douglas-fir moist maritime biogeoclimatic zone. A typical year is characterized by warm, dry summers and mild, wet winters

(Canada 2011). The region experiences an average of 900 – 1200 growing degree days with a base of 10°C (GDD), a mean maximum summer temperature of 25°C and fewer than 10 days above 30°C during the growing season (Smith et al. 2019). Vancouver Island's Cowichan Valley, a sub-geographic indication region (sub-GI), is located on the southeast coast of Vancouver Island and boasts the largest number of wine producers in the region due to the favourable growing conditions. Popular examples of European *Vitis vinifera* cultivars grown on Vancouver Island are early ripening Ortega and Pinot Gris, both early maturing at ~1000 GDD (Jones 2015, Smith et al. 2019). By comparison, the Okanagan Valley, which experiences 1200 to 1500 GDD in a growing season, produces a much larger range of varieties and wine styles (Rayne et al. 2011).

The challenging cooler climate of the Cowichan Valley delayed the shift away from cold-hardy hybrid varieties, however, plantings of European *Vitis vinifera* cultivars have recently increased significantly (Council 2018). As of 2018, Pinot gris is the most planted white varietal in British Columbia, and the most planted varietal on Vancouver Island (Council 2018). It is a versatile grape and is a popular choice for use in several wine styles produced on Vancouver Island, from dry and crisp Pinot gris/grigio produced in stainless steel tanks, barrel aged whites, and sparkling wine. Despite its popularity, research into the aroma compounds that comprise Pinot gris' varietal identity is extremely limited. This could be due to Pinot gris' wine aroma being dominated by esters produced during the fermentation process (Francis et al. 2005). A study conducted in Oregon profiled the monoterpene isomers in Pinot gris wines and showed increasing concentrations of monoterpenes in the wine had positive outcomes on perceived wine aroma (Tomasino et al. 2020). This shows a need to better understand varietal aromas in Vancouver Island's most planted grape, Pinot gris.

## 1.2 Glycoside Aroma Compounds

Varietal aromas or volatile organic compounds (VOC) in grapes are classified into a number of groups including: C13-norisoprenoids, alcohols, carbonyls, phenylpropanoids, methoxypyrazines, thiols and terpenes (Robinson et al. 2014). VOC's accumulate in the grape in a free molecular form (volatile), or bonded to other molecules such as sugars (Robinson et al. 2014). These bound volatiles are called glycosides, and are referred to as bound or potential volatiles, because they are non-volatile in this form. Glycosides represent the largest pool of aroma compounds despite being non-volatile (Kennison et al. 2009, Robinson et al. 2014). An experiment that involved grafting clusters of different varieties onto different host vines confirms that the glycosides are not transported from the vines to the fruit but instead are synthesized in the berries themselves (Günata et al. 2001). The volatile component of the glycoside can be liberated from its sugar molecule by enzymatic or acid hydrolysis during the winemaking process. Despite the possibility of release, it is common to have many glycosides present at harvest remaining in similar concentrations after winemaking (Parker et al. 2018) meaning the potentially volatile compounds were not liberated from their sugar molecules. In some cases, like the cultivar Riesling, glycosides play an important role in varietal flavour as they are released during the aging process (Simpson 1979). Understanding what compounds are present in the largest pool of bound volatile compounds in the berry and how they vary between vineyards, vintages and under different management strategies is fundamental to improving wine quality.

## 1.3 Viticultural Techniques

Many vineyard techniques have been subject to varietal and regional specific research as benefits are often unique to varietal, region and growing season (Bledsoe et al. 1988, Jackson et al. 1993, Poni et al. 2009, Reynolds and Wardle 1989). The hallmark of a maturing wine region

is investigation of best practices based on the dominant varieties and wine styles of the region. This research is the first rigorous evaluation of viticultural techniques commonly used on Vancouver Island in the production of Pinot gris, the most abundant variety planted in the region and province of BC, and how these techniques may affect glycoside abundance.

Common techniques used to improve fruit quality during the growing season are focused on improving the rate of ripening in terms of sugar accumulation and acid degradation, maximizing aroma compounds, and reducing disease pressure. Adjusting vine balance (optimized vegetative vs. reproductive growth) through cluster thinning (CT), improving cluster microclimate through leaf removal (LR) and the protection of fruit with the application of kaolin (K) clay are common practices in grape production.

The importance of viticultural techniques is clear when you consider how environmental and climate factors such as temperature, light exposure, and rainfall affect grape and wine quality. Temperature is positively correlated with better wines (Jones et al, 2005). Warmer temperatures have also been shown to accelerate plant developmental stages during the growing season (Jones and Davis 2000, Sadras and Moran 2013, Webb and Whetton 1989). Exposure of grapes to light during development increases the concentration of glycosides present in fruit at harvest (Reynolds and Wardle 1989b, Young et al 2016). ‘Climate’ in viticulture can be thought of at three levels. The macro-climate, or the region the vineyard is located in. The meso-climate, the specific location in the region the vineyard is located. The micro-climate, the specific conditions the cluster experiences on the vine. Since both macro- and meso-climate are fixed, the vineyard manager focusses attention on adjusting the microclimate to facilitate the production of the highest quality fruit possible. An extremely common practice is basal leaf removal (LR); the removal of leaves surrounding the clusters allowing them to be exposed to increased light and

thus increasing the micro-climate temperature. LR is the easiest way to modify the micro-climate of the grapes.

### 1.3.1 Basal Leaf Removal

LR is typically pre-formed during the early stages of berry development shortly after fruit set, but before lag phase (Ferrari et al. 2017, Kemp 2010). Lag phase is the stage during berry development where berry growth slows and the vines energy is focused on maturing the seed inside the berry. LR is done by removing the leaves at the bottom of the shoots surrounding clusters. Generally speaking, LR improves cluster exposure to sunlight, which has been shown to increase total soluble solids (TSS) and improve acidity in fruit at harvest (Bledsoe et al. 1988). LR also increases air flow around the cluster and reduces relative humidity in the cluster zone (Percival and Fisher 1994) which, in combination with the increased sunlight exposure, reduces disease pressure from *Uncinula necator* or grapevine powdery mildew, and *Botrytis cinerea*.

. Powdery mildew is very susceptible to ultraviolet light exposure (Willoquet et al. 1996), while *Botrytis* is reduced as a result of faster drying times after rainfall events (English 1989, Zoecklein et al. 1992). The amount and timing of LR can be varietal and region specific.

LR application on Vancouver Island typically takes the form of removing leaves only on the east side of the vine row, as that is common practice in larger wine regions that are typically warmer. East side leaf removal allows for the benefits of increased airflow and sun exposure while protecting the fruit from high afternoon temperatures. As Vancouver Island typically receives only around 1000GDD (Brooks 2022), it is considered amongst the coolest regions where grape production is viable (Jones et al. 2010). Cool and wet weather conditions are typical during the ripening period so performing LR on both sides of the canopy (heavy LR) or (H) may improve micro-climate conditions and ripening in addition to more rapid post-rain drying,

improving grape quality. Research on the effects of different types of leaf removal on grape aroma and berry chemistry have not been undertaken on Vancouver Island. However, grower concerns with the application of heavy LR include the possibility of excess light exposure of the clusters leading to sunburn, which causes negative flavours in the end product (Ferrari et al. 2017, Kolb et al. 2003).

### 1.3.2 Kaolin Clay Application

Sunburning of grapes typically occurs with the sudden exposure of previously shaded berries to sunlight and temperatures exceeding 35°C. Leaf removal on a warm day could produce these conditions. Sunburn symptoms include browning of skin, glossy berries, and eventually shriveling (Bondada and Keller 2012). Sunburn is typically worse in the case of post-veraison LR, as fruit that has developed in the shade has not produced the necessary components to protect itself against excess sunlight exposure. This is why LR is best pre-formed pre-veraison, early in the season when fruit is still green. Pre-veraison berries are better able to modulate temperature relative to post-veraison berries because they can transpire water like a leaf, creating an evaporative cooling effect. Further, pre-veraison berries exposed to sun will compensate by producing protective compounds such as norisoprenoids that diminish sunburn risk (DÜring and DAVtyan 2002). The risk of sunburn due to LR can be further mitigated by the use of physical barriers such as kaolin clay. Kaolin is an inert compound used in agriculture to protect sensitive crops like grapevines and cherries from the effects of excess heat, ultraviolet radiation, and insect pressure. Used to cool the vines, kaolin can be applied as a spray to the entire canopy (Frioni et al. 2017, Shellie and King 2013), or just to the fruit clusters. Application of kaolin to the fruit zone typically takes place after LR has been performed (Ferrari et al. 2017).

### 1.3.3 Cluster Thinning

The concept of vine balance or crop load describes the grape vine's energy source to sink ratio (Bravdo et al. 1984, Jackson et al. 1993). Sources such as plant's overwintering material, especially roots, and photosynthetically efficient organs such as fully developed leaves are balanced against sinks of carbohydrates such as young shoots, new root biomass and fruit (Kliewer and Dokoozlian 2005, Kliewer and Weaver 1971) . Optimal vine balance is determined by region and varietal (Kliewer and Dokoozlian 2005, Skinkis 2013, Skinkis and Schreiner 2011) and is normally calculated by comparing vegetative growth to reproductive growth on a single vine. Consequences of improper vine balance vary from over-cropping (metabolic demand of fruit exceeds the capacity of the vine's green tissue to support it) leading to underripe fruit and delayed ripening (Kliewer and Dokoozlian 2005); to under-cropping (an excess of green tissue relative to fruit metabolic demand) resulting in excessive shading and increased disease pressure (Bravdo et al. 1984, Smart 1985). Achieving optimal vine balance is important to fruit quality.

Decisions regarding vine balance are made at multiple points in a vineyard's life. During the planning and planting stage, vine and row spacing, and rootstock choices determine the general vine balance characteristics at a given site (Sala et al. 2005, Winkler 1969). Annual vine balance decisions are made during winter pruning via the number of buds left intact on the vine, and at pre-veraison with the number of clusters the vine will be permitted to carry through to harvest. Measuring and comparing the vegetative (green shoots) plant growth through either leaf area measurement or the ratio of pruning weight to the reproduction growth (vine yield) on a given vine allows a vineyard manager to assess the vine balance and make informed decisions regarding whether applying strategies to optimize the vine balance is warranted (Kliewer and Dokoozlian 2005, Skinkis 2013, Skinkis and Schreiner 2011).

## 1.4 Grape Maturity

Grape maturity can be assessed in a variety of ways. Botanically, the grape is mature when it has produced a viable seed, this occurs when the berry has completed veraison. Oenologically, the grape is considered mature when it reaches its peak phenological ripeness or flavour maximum. Important components of the grapes in relation to wine quality include total soluble solids (TSS), acidity, colour, and aroma compounds. Wine quality is linked to varietal characteristics expressed in the wine as a function of the specific suite of aroma compounds (and relative concentration) intrinsic to the grape variety (Francis and Williams 1993, Sefton et al. 1994a, 1994b).

Generally speaking TSS is a combination of a number of types of compounds including Sugars, Acids, as well as flavour and aroma compounds found in solution extracted from grapes. for winemaking TSS represents the sugar content of the grape. TSS is typically measured with a refractometer which reads as °brix or by measuring the specific gravity of the grape juice is. The sugar content of the grapes at harvest is an important factor in ripeness, it directly determines the alcohol content of the wine being made.

Titrateable acidity measured in (g/L) is the primary measure of acid concentration of the grape during ripening. The two acids produced in the highest quantities are tartaric acid and malic acid. During the ripening phase, malic acid is consumed during respiration of the berry. This leads to a decrease in TA as the grape matures. Typically a wine maker would target a °brix and TA level to produce the best balance for a given wine style (Coombe, BG. 1980).

As with determining exactly what effect each volatile compound plays in a varietal's aroma composition, determining maturity in a grape is a difficult task. Sugar and acid are relatively easy to taste and test but the presence and abundance of aroma compounds are difficult to perceive and measure. The difficulty arises because most volatile (aroma) compounds are bound to sugar molecules (Kennison et al. 2009). In this form the compounds are referred to as a glycoside or a bound volatile. Human saliva lacks glycosidase enzymes and thus cannot detect volatile compounds in their bound form. However, the sugar-volatile link can be cleaved during fermentation, releasing the glycosides (Gambetta et al. 2014, Jackson 2000) and thus rendering the volatile compounds perceptible to human senses. As many of the wine quality-related compounds are synthesized in the berry during the later stages of ripening (Luan and Wu 2002), measuring TSS in the berry has been the traditional proxy measure of physiological ripeness (Fang and Qian 2006, Hardie et al. 1996, Park et al. 1991).

TSS is an important tool for monitoring ripeness as sugar content and acidity are key components of specific wine styles. Variation in aroma compounds is large from season to season. Sugar content often rises in parallel with aroma compounds in the berry. So, in each individual season, maximizing sugar could also be thought of as maximizing aroma as well though the same TSS measurements in different seasons will not guarantee the same volatile compound quantities.

## 1.5 Objectives

This research explores the effects of heavy leaf removal, kaolin clay application and cluster thinning as a means of altering the basic berry chemistry and abundance and diversity of glycoside volatile compounds in Pinot gris grown on Vancouver Island.

- 1) Do the viticultural strategies of heavy leaf removal, kaolin clay application and/or cluster thinning significantly alter basic berry chemistry or glycoside compounds abundance at harvest? Are these techniques viable options to consistently improve grape quality?
- 2) Of the basic berry chemistry measurements (TSS, Ta, pH) what is the best method for a grower of Pinot gris on Vancouver Island to determine when their grapes have reach their peak phenological ripeness.

## 2. Materials and Methods

### 2.1 Study Sites and Experimental Design

This study took place over the 2018 and 2019 growing seasons, in two commercial *Vitis Vinifera* L. cv. Pinot gris vineyards in the Cowichan Valley sub-geographic indicator (sub-GI) on Vancouver Island, British Columbia Canada. Vineyard one (V1) is Pinot gris clone 93, planted on the rootstock 101-14. Vineyard two (V2) is Pinot gris of unknown clone, planted on unknown rootstock. Utilizing two vineyards over two growing seasons permits the effects of vineyard and year to be controlled for in assessing the treatment effects on variation in vine performance and bound volatile compound (VC) composition. Both vineyards had north/south row orientation, regulated deficit drip irrigation, and were planted at a 2.13m X 1.2m row and vine spacing. Vines at (V1) were 10 years old whereas the vines of (V2) were ~25 years. The two vineyards are both on a similar silt loam soil ~10 km apart. V1 is roughly 2.5 km from the ocean, whereas V2 is 3.5 km.

Three treatments were applied: heavy vs. industry standard leaf removal, kaolin application vs. no kaolin, and cluster removal vs. no cluster removal. The reference treatment (R) was industry standard leaf removal in the east-facing fruit zone only, while the heavy treatment (H) completely removed all six basal leaves on both sides of the canopy. All leaf removal treatments were performed at pea sized berries, stage 29 – 31 on the Modified EL system (Coombe 1995) .

Kaolin treatment also had two levels, reference panels with no kaolin applied (R) and treated panels (K) that received applications of kaolin clay and water mixture (50 kg kaolin / 1000L) to only the fruit zone via backpack sprayer, at a rate of (50g/L). The kaolin mixture was

applied to all sides of the treated vines at veraison and was re-applied after major rainfall events which necessitated three applications in each year.

In 2018 there were a total of four treatments: regular leaf removal (R), heavy leaf removal (H), regular leaf removal with kaolin (RK), and heavy leaf removal and kaolin (HK). Five replicates per treatment at both vineyards yielded a total of 20 experimental panels defined as all vines between line posts in the same row (a “panel”). (Table 1).

In 2019, the previous year’s treatments were repeated with an additional cluster thinning treatment. Treated panels (C) had fruit removed during lag phase (stage 33) leaving only one cluster per shoot. This treatment was combined with the reference treatment (RC), the heavy leaf removal (HC), and a treatment that combined all three (HCK). These treatments therefore yielded 35 experimental units, in both vineyards for the 2019 season (Table 1).

A semi-structured randomized block design was used, where each experimental unit was a panel. Vines per panel varied per vineyard, six at V1, and five at V2. In total 20 panels were selected at each vineyard for their even growth and a treatment was randomly assigned to each.

*Table 1* Treatment name and code, number of replicates in 2018 and 2019, and description of the treatment.

<b>Treatment name (code)</b>	<b>2018</b>	<b>2019</b>	<b>Description</b>
<b>Regular Leaf Removal (R)</b>	5	5	Standard leaf removal
<b>Heavy Leaf Removal (H)</b>	5	5	Heavy leaf removal
<b>Regular LR + Kaolin (RK)</b>	5	5	Standard leaf removal with kaolin
<b>Heavy LR + Kaolin (HK)</b>	5	5	Heavy leaf removal with kaolin
<b>Regular LR + Cluster thinning (RC)</b>	0	5	Regular leaf removal with cluster removal
<b>Heavy LR + Cluster thinning (HC)</b>	0	5	Heavy leaf removal with cluster removal
<b>Heavy LR + Cluster thinning + Kaolin (HCK)</b>	0	5	Heavy leaf removal, cluster removal and kaolin
<b>Total Experimental Units</b>	20	35	

**Table 2** Vine-related variables assessed in the experimental vineyards in 2018 and 2019, including their units and description of data collected.

<b>Variable</b>	<b>Units</b>	<b>Description</b>
Leaf area (LA)	m <sup>2</sup>	Measured after Veraison, mean per panel
Pruning Weight (PW)	kg	Vine vegetative growth, mean per panel
Yield (Y)	kg	Mean per vine yield of each panel
Ravaz index	Y/PW	Fruit yield divided by the pruning weight, mean per panel
Crop load	LA/Y	Leaf area divided by yield, mean for the panel
Cluster number	#	Clusters per vine, mean per panel
Botrytis incidence %	#	Percent of clusters on vine with visible botrytis infection
Leaf Area index	Na	Proxy measure of canopy density
Cluster Weight	g	Per Panel from sub sample of 7 clusters
Berry Number	#	Mean per panel from sub sample
Average berry weight	g	Berry number/ (cluster weight – Rachis) from sub sample
Brix	#	Soluble solids measured at Panel level
Titrateable acidity (TA)	g/L	Titrateable acidity measured at panel level
pH	#	Measured at panel level
Leaf water potential	Bar	The (–) pressure the vine is using to hold onto water
Berry External temperature	°C	Temps taken over the course of a day, mean per panel

## 2.2 Environmental Variables

Vineyard soil temperature (°C) was measured using Hydrochron iButtons® (d1923-f5). Two data loggers were placed in the root zone at a depth of 30cm at both vineyards recording every 30 min, from bloom to harvest in both growing seasons. Growing season data was collected from the North Cowichan weather station. Average, Daily Minimum and Max air temperature as well as and rainfall by month from April to October in both 2018 and 2019.

## 2.3 Vine Morphology

Leaf area was measured soon after veraison when canopy growth was completed by classifying all leaves on two shoots per vine into six size categories as described in (Table 3) and in more detail in the extension paper by. (Skinkis and Schreiner 2011), with the number of leaves in each category summed to yield a non-destructive leaf area for each vine and treatment (Feng et al. 2014). Canopy density or the number of leaf layers in the vine canopy is referred to as the leaf area index (LAI); a proxy measure of vine vigour and light penetration. LAI was measured using Viticanopy (De Bei et al. 2016) which analyzes images captured by a cell phone from beneath the canopy looking up. Two images were taken per vine, one on each side of the main trunk with the final LAI calculated as a composite of the two images. Images were take at a distance of 70 to 80 cm below the canopy as recommended by the app developer. Pruning weight (PW) was recorded for each individual vine during the winter following harvest by weighing all one-year-old wood removed during pruning (Skinkis 2013). Fruit yield (kg) and pruning weights (kg) were used to calculate the Ravaz index for each vine's reproductive growth to vegetative growth ratio each season (Ravaz, 1911).

Table 3 Leaf area template categories converted to metric from the article by (Skinkis and Shriener, 2011)

<b>CATEGORY</b>	<b>CIRCLE DIAMETER (CM)</b>	<b>MEAN LEAF SIZE FOR EACH CATEGORY IN WITH PINOT GRIS. (CM ^2)</b>
<b>1</b>	17.78	194.2
<b>2</b>	15.87	157.4
<b>3</b>	13.34	127.1
<b>4</b>	11.43	83.2
<b>5</b>	7.62	40.6
<b>6</b>	5.72	18.1

**Equation 1**

$$Ravaz\ Index = \frac{(Fruit\ Yield\ [KG])}{(Pruning\ weight\ [KG])}$$

The leaf area to yield ratio is an additional measure of the relationship between vegetative and reproductive growth of the vine and is computed as leaf area (m<sup>2</sup>) divided by fruit yield (kg) for each vine.

**2.4 Harvest**

At harvest, fruit from individual vines within experimental panels was collected and weighed separately to one decimal place with a portable electronic scale (Washdown Stainless Scale, Western Scale Co Ltd.). Clusters from each vine were counted, and visible *Botrytis*

infection was recorded as % of clusters per vine with *Botrytis*. Seven randomly selected clusters from each panel were stored on ice for a maximum of five hours prior to undergoing berry physiology assessments (see below). After berry physiological assessment a 100-berry sample was haphazardly selected from the seven-cluster sub-sample and frozen at -23°C prior to VOC composition analysis (GC/MS). The remaining berries were used in basic chemical analysis.

#### 2.4.1 Berry Morphology

Berry and cluster size were assessed by recording whole cluster weights and rachis weights (Ohaus Scout Pro, Ohaus Corporation). Berries from the seven-cluster sample taken in the field were removed from the rachis and counted. The average berry weight was calculated by:

*Equation 2*

$$(\text{Average Berry Weight}) = \frac{\text{Whole Cluster weight} - \text{Rachis weight}}{\text{Berry Number}}$$

#### 2.4.2 Berry Chemical Analysis

The berries remaining from the seven-cluster sample for each experimental replicate were crushed and screened with a household sieve prior to measuring total soluble solids (TSS, reported as °Brix), titratable acidity (TA) and pH. The °Brix was measured with a hand-held refractometer. Titratable acidity g/L and pH was measured with 0.1M NaOH to an endpoint of 8.2 using an Orion Star A111 pH meter (Iland et al. 2013).

### 2.5 Extraction, Hydrolysis, and Determination of Glycosidically Bound Volatiles

This analysis generally follows methods from (Kovalenko et al. 2022). 100 Frozen grapes were crushed gently with mortar and pestle using liquid nitrogen, and seeds were removed with forceps. Remaining grape tissue was powdered with an analytical mill (IKA, NC, USA) under

liquid nitrogen, with the aim of avoiding enzymatic activity and preserving the volatile composition. Frozen grape powder ( $1\text{g} \pm 0.01$ ) was extracted with 8 mL of deionized water and 100  $\mu\text{l}$  of ascorbic acid (200g/L) at room temperature using the Vortex Genie 2 (Scientific Industries, Bohemia, NY, USA) at 3200 rpm for 10 min. Afterwards, samples were centrifuged for 20 min at 3500g at 4°C, the pellet was discarded, and the supernatant was purified by solid-phase extraction (SPE).

Six ml of sample supernatant were loaded into Oasis HLB SPE cartridges (6cc volume with 150mg sorbent, Waters, MA, USA) previously equilibrated with 10 ml of methanol followed by 10 ml of deionized water. Polar matrix compounds (e.g., sugars, acids) were eluted with 10 ml of deionized water, and free volatiles were subsequently eluted with 18 ml of pentane. These solutions were captured and discarded. Glycosidically bound volatiles were eluted with 10 ml of methanol and dried using a Labconco nitrogen evaporator (RapidVap Vertex Evaporator) for 15h at room temperature.

Dried samples were reconstituted with 5 ml of citrate-phosphate buffer (0.05 M: 0.1M, pH 5.0), vortexed for 30 seconds, and sonicated for 1 min. Samples were then transferred to 20 ml glass GC vials and 1 ml of the AR2000 (DSM, Netherlands) hydrolytic enzyme solution was added in a concentration of 416 mg/mL in citrate phosphate buffer containing deuterated ethyl butyrate (12ppm) and 3-octanol (14ppm) as internal standards. After 20 seconds of mixing by vortex, 1 mL of methyl tert-butyl ether (MTBE) was gently overlaid on the sample surface. Samples were capped and sealed, and they were incubated in a water bath for 14 hrs at 40 °C. Samples were then vortexed for 20 seconds, centrifuged for 10 min at 3500 rpm and 4 °C, and frozen for 45 min at – 80 °C. The aqueous phase containing the sugar moieties was frozen, and

the MTBE liquid layer was transferred to a 2 ml vial and capped. Samples were stored at -80 °C until further injection within 7 days.

### 2.5.1 Gas Chromatography and Mass Spectrometry Analysis

Volatile compounds were determined using a 7890A gas chromatograph coupled to a 5975C single quadrupole mass spectrometer detector, both from Agilent Technologies (Santa Clara, CA, USA). Volatiles were separated using a polar DB-Wax Column (30 m length x 0.25 mm inner diameter x .25 um film) from J&W Scientific (Folsom, CA, USA).

The GC was operated under programmed temperature conditions: from 35 °C (4.36 min) to 65 °C (3 min) at 1.5 °C/min, subsequently up to 138 °C (0 min) at a rate of 3 °C/Min and finally up to 230 °C (2 min) at 60 °C/min. This gave a total run time of 55 min. Helium was used as the carrier gas with a flow rate of 0.9 ml/min. For each sample, 1 µL of hydrolyzed sample was injected, with the injector temperature working at 250°C and in pulsed splitless mode. The interface, ion source and quadrupole temperatures were 250, 230, and 150 °C, respectively. Samples were simultaneously injected in SCAN mode (included a mass range of 35 – 500 m/z) and selected ion monitoring (SIM) mode, operating in electron ionization mode with energy of 70 eV. All volatile compounds were identified by comparing their retention times and accurate mass spectra with standards and the Mass Spectra Library (NIST MS Search 2.2 and MS interpreter). The identified and quantified terpenes are shown in Table 4.

Quantification of volatiles was performed using an external calibration curve built with six points (2 – 0.32 mg/L) prepared in methyl tert-butyl ether (MTBE) containing deuterated ethyl butyrate (12ppm) and 3-octanol (14ppm) as internal standards. The concentration of each volatile was calculated using the corresponding external calibration curve with a subsequent correction to the recovery %, which was calculated using the following.

*Equation 3*

$$\% \text{ Recovery} = \left( \frac{\text{area spiked sample} - \text{area non-spiked sample}}{\text{area standard in solution}} - 1 \right) \times 100$$

As identifications can only be claimed for compounds with concentrations above their limits of detection (LOD) and quantifications can only be performed in volatile compounds with concentrations above their limits of quantification (LOQ), the sensitivity of each volatile was assessed for LODs and LOQs as three and ten times the signal to noise ratio (S/N), respectively. For linearity, the coefficient of determination ( $R^2$ ) was subsequently calculated, and the linear range was also established.

Table 4 List of compounds measured.

<b>Quantified volatile compounds with sensory importance for wine aroma</b>	<b>Other volatiles quantified in grapes</b>	<b>Identified volatile compounds found &lt; LOQ</b>
2-Methyl-1-propanol	$\alpha$ -(+)-Pinene	Ethyl isovalerate
3-Methyl-1-butanol	1-Butanol	Hexanal
3-(Z)-Hexen-1-ol	2-(E)-Hexenal	1-Penten-3-ol
2-(E)-Hexen-1-ol	2-Hexanol	2-Heptanone
Linalool Oxide (isomer 1)	Furfural	Heptanal
Linalool Oxide (isomer 2)	1-Heptanol	Eucalyptol
$\alpha$ -Terpineol	Phenylacetaldehyde	Cymene
Nerol	Hexanoic acid	Nonanal
Geraniol	Nonanoic acid	2-(Z)-Hexen-1-ol
Benzyl alcohol	Decanoic acid	Benzaldehyde
Phenylethyl alcohol	Farnesol	2-(E)-Nonenal
		5-Methylfurfural
		1-Octanol
		Furfuryl alcohol
		Citral (isomer 1)
		1-Nonanol
		Eugenol

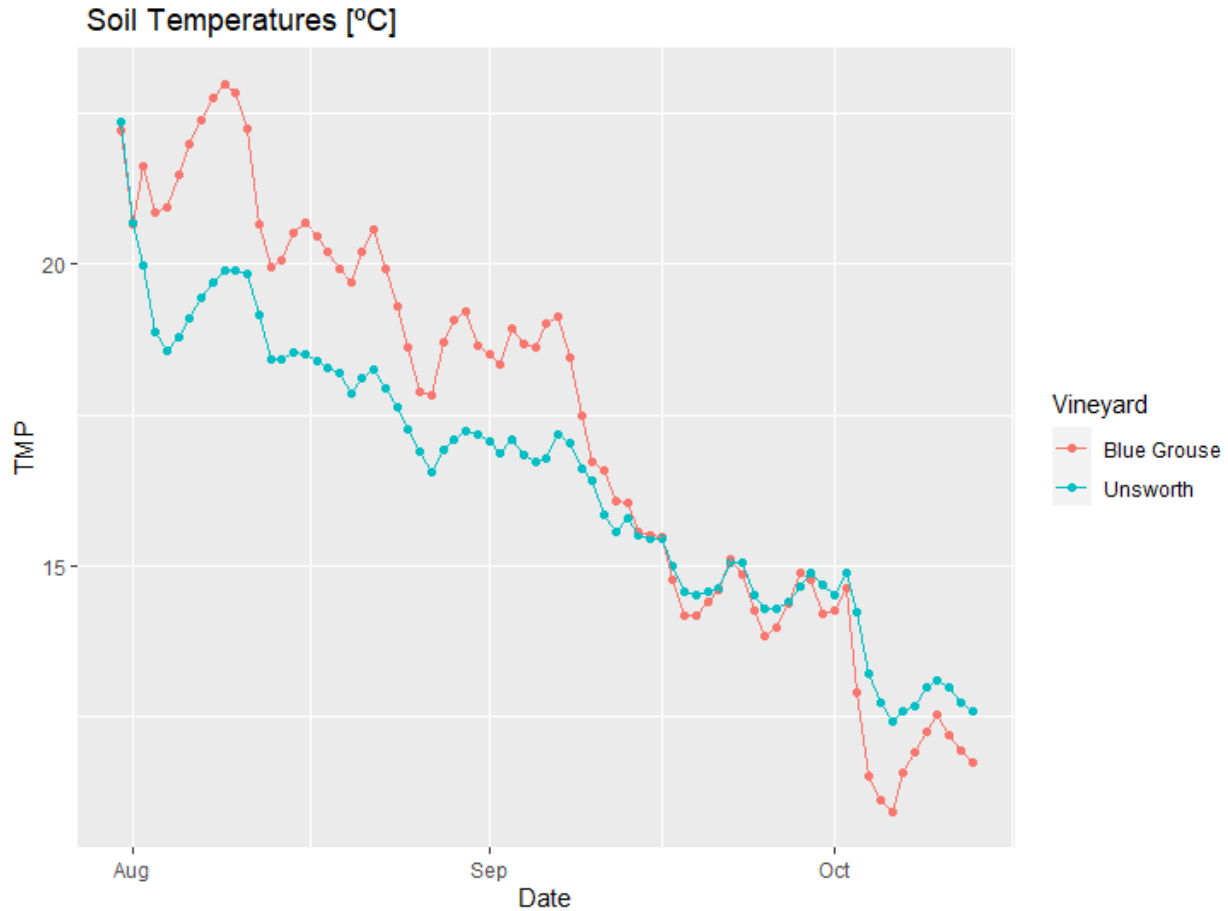
## 2.6 Statistical analysis

Data analysis was completed in R 4.1.1 (R Core Team, 2021), using the agricolae package (Mendiburu, Felipe, 2021). Analysis of Variance (ANOVA) was used to assess the differences between treatments. Fishers LSD was used to determine differences between groups. Differences between the independent variables Vineyard and Year were assessed using the whole dataframe. While test for interaction effects were also performed. Ultimately each variable was assessed individually for each vineyard and year.

*Variable ~ Leaf Removal + Kaolin + Cluster Removal*

### 3. Results

#### 3.1 Environmental context of the vineyards



**Figure 1** Vineyard soil temperature recorded in 2018 growing season, TMP refers to average Temperature per day °C of the soil.

During the warmer months, specifically August, the south facing slopes of V2 labeled Blue Grouse in (Fig.1) are assumed to expose the vines to more sun than those located on flatter ground at V1 labeled Unsworth in (Fig. 1) leading to a differential soil temperature of up to 5°C. To generalise, the temperatures at the two sites are similar when the weather is cool. During warmer periods V2 experiences more heat likely due to the southern facing slope and more

inland location resulting in warmer temperatures and more heat units available to the vines and greater potential fruit ripening.

*Table 5 Surface temperature mean, range and rainfall by month during the 2018 and 2019 growing seasons (North Cowichan weather station).*

Month	2018.0					2019.0				
	Air Temperature °C				Rainfall (mm)	Air Temperature °C				Rainfall (mm)
	Min	Avg	Max	GDD		Min	Avg	Max	GDD	
April	4.5	9.2	14.0	20	7.5	4.4	9.5	14.6	10	55.0
May	8.4	15.1	21.9	157	11.2	8.0	14.8	21.5	148	11.3
June	9.6	15.5	21.4	166	23.0	9.8	16.3	22.8	190	20.1
July	12.2	20.1	28.1	314	0.2	12.2	18.2	24.2	255	21.5
August	11.7	19.0	26.4	280	2.1	12.6	19.1	25.5	281	8.7
September	9.2	14.4	19.7	133	81.3	10.4	15.3	20.2	160	89.2
October	3.4	9.8	15.6	19	82.4	3.2	8.3	13.4	10	85.0
Overall	8.4	14.7	21.0	1089	207.7	8.7	14.5	20.3	1054	290.8

Weather conditions were similar between the two seasons (Table 5). The average air temperature by month shows 2018 being slightly warmer overall, but similar to 2019. However, the 2018 season was among the driest summers on record. Rainfall was higher in the spring and early summer of 2019 compared to 2018. Growing degree days between the two seasons were similar overall. It is important to note that 2018 had a much warmer July, while 2019 had more

GDD during September. During the latter half of the growing seasons rainfall was very similar in each year.

## 3.2 Harvest

For all the following results, interaction effects between treatments leaf removal, kaolin application and cluster thinning were assessed using (Anova). No interaction effects were found between the treatments. Consistent significant differences between vineyard and year were found with the majority of results. The results following were tested within their year and vineyard.

### 3.2.1 Yield

Neither leaf removal nor kaolin application significantly affected fruit yield in 2018 or 2019 at both vineyards. Cluster removal significantly reduced yield in 2019 at both vineyards, V1 experienced a 32% reduction in yield or roughly 1 kg per vine and V2 a 22% reduction or .5 kg per vine. Yield per vine was also significantly different between vineyards in both years. Yield increased in 2019 at V1 compared to 2018 whereas decreased at V2 compared to 2018 (Table 5).

Table 6 Anova results of vine physiology. Letters a, b denotes groupings from Fishers LSD. \* Denotes significance at p .05.

Vineyard	Treatment	Yield (kg/vine)	Pruning Weight (Kg)	Leaf area (m2)	Ravaz	Leaf area to Yeild Ratio (m2/kg)	LAIE	Cluster number/ vine	Berry Number	Rachis Weight (g)	Cluster (g)	Berry Weight (g)	Botrytis (%) cluster infected per vine		
2018	1	Leaf Removal Heavy	2.61 ±.50 a	.25 ±.08 a	1.85 ±.61 a	11.09 ± 2.3 a	.70 ±.15 a	.374 ± .07a	21.82± 3.0 a	140.62 ±16.7a	7.26± .6a	148.53 ± 14.1a	0.76 ±.1 a	1.80 ±1.2b *	
		Leaf Removal Regular	2.61 ±.59 a	.26 ±.07 a	2.14± .34 a	10.77 ± 2.0 a	.87 ± .24 a	.419 ± .08a	22.29 ± 2.7a	126.24 ±22a	6.5 ±1.0a	136.37 ±136.4a	0.72 ±.1 a	5.72 ±4.7a	
	Kaolin	Yes	2.59 ±.65 a	.23 ±.07 a	1.99 ±.56 a	11.55 ± 2.5 a	.77 ± .16 a	.409 ±.09a	22.14 ± 3.2a	137.14 ±18.6a	6.87 ±.7a	141.02 ± 19.6a	0.72 ±.1a	5.17 ±5.0a	
		No	2.63 ±.42 a	.28 ±.08 a	2.0 ±.46 a	10.31 ± 1.56 a	.80 ±.26 a	.384 ±.06a	21.97 ± 2.5a	129.73 ±22.3a	6.88 ± 1.1a	143.89 ± 22a	0.76 ±.1a	2.35 ±1.6b	
	2	Leaf Removal	Heavy	2.13 ±.45 a	.36 ±.1 a	2.93 ±.55 b *	7.16 ± 2.2 a	1.45 ±.46a	.55 ±.084 a *	22.94 ± 3.8b *	94.8 ± 15.8a	4.77 ±.7a	100.97 ± 10.2a	0.51 ±.1a	2.91 ±2.3a
			Regular	2.30 ±.35 a	.39 ±.1 a	3.43 ±.56 a	7.32 ± 2.4 a	1.53 ±.28a	.68 ±.18 a	26.56 ± 2.4 a	92.98 ± 12.8a	5.34 ±2.4a	101.19 ± 15.5a	0.54 ±0.5a	3.16 ±1.9a
		Kaolin	Yes	2.20 ±.41 a	.39 ±.1 a	3.16 ±.72 a	7.22 ± 2.6 a	1.48 ±.37a	.615 ± .19a	24.8 ± 4.0a	94.19 ±17.1a	5.33 ±2.4a	102.35 ± 14.4a	0.54 ±0.8a	2.66 ±1.9a
			No	2.23 ±.41 a	.36 ±.1 a	3.2 ±.48 a	7.26 ± 2.0 a	1.50 ±.39a	.615 ±.11 a	24.7 ± 3.4a	93.57 ± 11a	4.76 ±.7 a	99.81 ±11.5a	0.51 ±0.7a	3.41 ±2.41a
	2019	1	Leaf Removal Heavy	2.89 ±.86 b	.44 ±.2 a	3.30 ±.70 b *	8.35 ± 3.0 a	1.28 ±.35a	.48 ±.09b **	20.67 ± 5.8b	148.92 ± 38.2a	7.37 ± 1.6a	138.87 0±32.2a	0.91 ±.1a	11.1 ± 6.7b ***
			Leaf Removal Regular	3.33 ±.69 a	.47 ±.5 a	3.89 ±.52 a	8.09 ± 1.7 a	1.28 ±.31a	.56 ±.07 a	23.37 ± 4.7a	142.52 ± 34.5a	6.92 ± 1.4a	139.16 ±26.8a	0.97 ±0.7b	19.4 ±8.4a
Kaolin		Yes	3.21 ±.64 a	.42 ±.2 a	3.47 ±.57 a	9.2 ± 2.1 a	1.35 ±.28a	.50 ±.1 a	22.96 ± 5.3a	165.43 ± 32.6a **	7.67 ± 1.4a	151.31 ± 22.6a	0.90 ±.1b *	11.2 ±7.5b	
		No	2.98 ±.92 a	.48 ±.1 a	3.63 ±.76 a	7.5 ± 2.5 a	1.18 ±.35a	.53 ±.09a	20.98 ± 5.5a	131.733 ± 32.7b	6.81 ± 1.5a	129.76 ±31.4a	0.96 ±.08a	17.3 ±8.4a	
2		Cluster	Yes	2.43 ±.35 b ***	.40 ±.1 a *	3.45 ±.60 a	7.65 ± 2.6 a	1.5 ±.31a ***	.51 ±.1 a	17.4 ± 1.7b ***	155.76 ±43.5a	7.49 ±1.6a	144.91 ±31.6a	0.90 ±.1a *	15.6 ±9a
			No	3.57 ±.072 a	.49 ±.2 a	3.65 ±.75 a	8.68 ± 2.3 a	1.11 ±.24b	.53 ±.09 a	25.34 ± 4.6a	138.99 ± 29.0a	6.94 ± 1.3a	134.56 ±28a	0.96 ±.08a	13.9 ±8a
		Leaf Removal	Heavy	1.69 ±.41 a	.54 ±.2 a	3.29 ±.70 b **	4.04 ± 1.4 a	2.18 ±.58a *	.60 ±.12 a	18.49 ± 4.7a	94.18 ± 15.6a	5.27 ±1.1 a	93.61 ±16.6a	0.93 ±.05b	4.1 ±4.0a
			Regular	1.83 ±.49 a	.57 ±.2 a	3.97 ±.40 a	4.21 ± 1.9 a	2.46 ±.71a	.64 ±.13 a	19.06 ± 3.5a	100.48 ± 15.8a	5.73 ±1.0 a	103.18 ±16.4a	0.97 ±0.5a	6.3 ±4.1a
2		Kaolin	Yes	1.67 ±.43 a	.54 ±.2 a	3.36 ±.71 a	3.98 ± 1.5 a	2.24 ±.51a	.622 ±.14 a	17.91 ± 4.2a	92.76 ±15.9a	5.20 ± 1.0a	92.35 ±16a	0.94 ±.05a	3.3 ±2.9b
			No	1.81 ±.46 a	.56 ±.2 a	3.73 ±.62 a	4.21 ± 1.8 a	2.35 ±.74a	.618 ±.1 a	19.34 ± 4.20a	99.86 ±15.5a	5.69 ± 1.1 a	101.56 ±17.0a	0.95 ±0.6a	6.3 ±4.5a
	Cluster	Yes	1.52 ±.43b **	.56 ±.2 a	3.58 ±.77 a	3.44 ± 1.2 b **	2.61 ±.64a **	.60 ±.12 a	16.2 ± 3.5b ***	97.73 ±15.1a	5.75 ±.9 a	97.33 ± 16a	0.93 ±.05b	5.6 ±4.4a	
		No	1.97 ±.33 a	.54 ±.2 a	3.57 ±.59 a	4.74 ± 1.7 a	2.01 ±.51 b	.64 ±.13 a	21.12 ± 3.4a	96.02 ±16.8a	5.23 ± 1.2 a	98.00 ± 18.4a	0.97 ±0.5a	4.5 ±3.9b	

### **3.2.2 Cluster Number**

H and kaolin application did not affect cluster number. Cluster number was similar between vineyards in 2018, but V1 had a higher cluster number in 2019. Cluster number was significantly reduced by cluster thinning in 2019, as expected (Table 5).

### **3.2.3 Cluster Weight**

Cluster weight was not significantly altered by any of the treatments though cluster weights were significantly different between the two vineyards. Cluster weights in V1 were similar in both years and larger compared to V2 (Table 5).

### **3.2.4 Berry number**

Berry number was not altered by any of the treatments. The one significant difference in berry number was due to poor fertilization prior to treatment application in 2019. This resulted in numerous small berries with no seeds. V1 consistently produced significantly more berries in both years (Table 5).

### **3.2.5 Berry Weight**

Treatments did not affect berry size consistently. V1 had significantly larger, heavier berries in 2018 compared to V2, whereas in 2019 both vineyards had significantly larger berries relative to 2018 and berry size was similar between vineyards. Only at V1 in 2019 was berry size significantly decreased, likely the result of poor fertilization in some experimental panels.

## **3.3 Vine Morphology**

Pruning weights were not affected by any of the treatments. Leaf area was significantly reduced by (H) in most cases. Kaolin and cluster thinning treatments did not affect leaf area.

Leaf area index or the density of the canopy was reduced by H in both years and at both vineyards as expected, but statistically significantly so only in 2019 at V1. Cluster thinning also had a trend of reducing canopy density but was not significant (Table 5).

### **3.4 Combined Metrics**

Ravaz index in V1 was significantly lower than V2 in both years. Ravaz index was also significantly lower in both vineyards in 2019 compared to 2018. H and kaolin treatment did not significantly alter the Ravaz index. Cluster thinning lowered the Ravaz index in both years and vineyards (Table 5).

Leaf area to yield ratio followed a similar trend to the Ravaz index. V2 had larger leaf area to yield ratio in both years. Leaf area to yield ratio increased at both vineyards in 2019. H consistently lowered leaf area to yield ratio but not significantly. Kaolin application showed no effect on the leaf area to yield ratio. Cluster thinning significantly increased leaf area to yield ratio at both vineyards in 2019.

### **3.5 Berry Chemistry**

Total soluble solids were significantly higher at V2 in both years. Both vineyards had higher TSS on average in 2019 compared to 2018. H and kaolin application did not produce consistent trends in TSS. The 2019 cluster thinning treatment significantly increased TSS content in both vineyards (Table 7).

V2 had significantly lower TA than V1 in both years. In 2019 both vineyards had lower TA than in 2018. H and kaolin application did not affect TA. Cluster thinning lowered TA in both vineyards (Table 7).

Table 7 Anova results of basic berry chemistry. Letters a, b denotes groupings from Fishers LSD. \* Denotes significance at p

.05

Year	Vineyard	Treatment		Brix	Ta	PH
2018	1	Leaf Removal	Heavy	15.57 ± 1.7 a	10.30 ± 1.4 a	3.00 ± .06 a
			Regular	16.4 ± .9 a	9.83 ± 1.4 a	3.014 ± .08 a
		Kaolin	Yes	15.3 ± 1.4b	10.07 ± 1.4a	2.99 ± .07a
			No	16.67 ± 1.12a	10.06 ± 1.4 a	3.02 ± .07 a
	2	Leaf Removal	Heavy	19.3 ± .58 a	9.08 ± .5a	3.12 ± .06b *
			Regular	19.9 ± .74 a	9.18 ± 1.3 a	3.20 ± 0.8a
		Kaolin	Yes	19.8 ± .77a	9.03 ± .6 a	3.18 ± .1a
			No	19.37 ± .6 a	9.24 ± 1.3 a	3.14 ± .07a
2019	1	Leaf Removal	Heavy	16.9 ± .85 a	7.97 ± 1.1 a	3.03 ± .05a
			Regular	17.1 ± .63 a	8.46 ± 1.2 a	3.02 ± .05a
		Kaolin	Yes	16.7 ± .9 a	8.32 ± 1.0 a	3.03 ± 0.5a
			No	17.2 ± .6 a	8.00 ± 1.3 a	3.03 ± .05a
		Cluster	Yes	17.32 ± .9 a *	7.72 ± 1.1 b *	3.05 ± .05 a **
			No	16.73 ± .6 b	8.53 ± 1.1 a	3.02 ± .04 b
	2	Leaf Removal	Heavy	21.3 ± .7 a	7.03 ± .3 a	3.41 ± .07a
			Regular	21.3 ± .8 a	7.08 ± .4 a	3.39 ± .06 a
		Kaolin	Yes	21.3 ± .5 a	7.02 ± .4 a	3.41 ± .07a
			No	21.3 ± .9 a	7.07 ± .3 a	3.39 ± .07 a
		Cluster	Yes	21.6 ± .7 a *	6.92 ± .3 b *	3.41 ± .06 a
			No	21.0 ± .6 b	7.17 ± .4 a	3.39 ± .07 a

V2 fruit consistently had higher pH than V1 fruit and overall, with 2019 showing an increase in pH on average compared to 2018. H generally did not produce significant changes in pH with the exception of a significant increase in V2 in 2018. Kaolin had no effect on pH. Cluster thinning consistently raised pH in both vineyards, significantly so at V1 (Table 7).

### 3.6 Botrytis Incidence

Both vineyards saw a higher *Botrytis* incidence in 2019 than in 2018. H consistently reduced *botrytis* incidence in both years and vineyards, significantly so at V1 in both years.

Kaolin application increased *Botrytis* incidence at both vineyards in 2018, but this trend reversed in 2019. In neither year did kaolin treatment result in statistically significant effects. Cluster removal did not significantly affect *Botrytis* incidence (Table 6).

### 3.7 Volatile Organic Compounds

Bound volatile compounds were higher in 2019 than in 2018 for most compounds. Differences in compounds varied between the two vineyard sites depending on the compound. The 2018 season gave a similar concentration in compounds between the two sites, while in 2019 V1 had more of many compounds, than V2. Significant differences in compound abundance were observed at V1 in both seasons between treatments. Kaolin application did not alter compound abundance significantly. Cluster thinning was not a major driver of differences. H produced significant changes in a variety of compounds during both growing seasons, but only at V1.

Kaolin application did not produce any significant changes to compound abundance at either site, in both years of the study (Table 8).

Cluster thinning applied in 2019 had a trend of producing slightly higher abundances of compounds at both vineyards. Only Benzyl alcohol was significantly increased by cluster thinning at V1 in 2019 (Table 8).

H yielded significant differences in compounds abundance at V1 in both seasons. In 2018 H at V1 shows a trend of increased compound abundance with H. The compounds 3-Hexen-1-ol, linalool oxide (isomer 1), and linalool oxide (isomer 2) were significantly increased by H (Table 7). In the 2019 season H produced the opposite trend of decreasing compound abundance with

H. The compounds 2-Methyl-1-propanol, 3-methyl-1-butanol, 3-(Z)-hexen-1-ol, terpineol, nerol, geraniol, benzyl alcohol, and phenylethylalcohol were decreased significantly by H (Table 8).

**Table 8** Anova results of glycoside aroma compounds. Letters a, b denotes groupings from Fishers LSD. \* Denotes significance at p .05. All values are ug/kg

Year	Vineyard	Treatment	2 - Methyl - 1 - Propanol	3 - Methyl - 1 - Butanol	3 - (Z)- Hexen-1-ol	X2..E..Hexen.1.ol	Linalool oxide (Isomer 1)	Linalool Oxide (Isomer 2)	Terpineol	Nerol	Geraniol	Benzyl alcohol	Phenylethyl alcohol	
2018	1	Leaf Heavy	284.0 ± 43.6a	2975.0 ± 472.1a	39.0 ± 16.9a **	564.4 ± 108.9a	218.0 ± 41.8a *	430.9 ± 105.5a *	167.5 ± 127.3a	162.0 ± 28.8 a	181.3 ± 42.2a	1787.5 ± 368a	982.1 ± 208.5a	
		Removal Regular	297.9 ± 55.7a	2842.1 ± 244a	17.5 ± 6.0a	537.0 ± 28a	173.6 ± 21.2b	345.8 ± 46.7b	116.8 ± 40a	119.2 ± 63.6 a	163.8 ± 10.6 a	1491.3 ± 230.1a	870.6 ± 149.4a	
		Kaolin	Yes	288.2 ± 46.9a	2864.8 ± 248.2a	29.0 ± 13.6a	541.8 ± 54.2a	197.0 ± 36.2a	370.7 ± 49.5a	125.6 ± 39a	135.2 ± 48.2a	162.8 ± 10.0a	1600.9 ± 246.6a	901.9 ± 153.5a
			No	293.7 ± 53.8a	a	27.5 ± 19.7a	559.7 ± 99.7a	194.6 ± 44.4a	406.0 ± 119.1a	158.7 ± 130.8a	146.0 ± 59.2 a	182.3 ± 41.8 a	1677.9 ± 415.9a	950.8 ± 218.6a
	2	Leaf Heavy	297.3 ± 35.0a	3135.6 ± 262.6a	22.7 ± 10.2a	566.1 ± 54.1a	203.1 ± 43.9a	350.2 ± 76.8a	348.1 ± 84.9a	<LOQ	203 ± 17.2a	1974.0 ± 177.6a	875.4 ± 73.1a	
		Removal Regular	274 ± 99.9a	3181.7 ± 345.6a	20.2 ± 29.5a	589.5 ± 55.2a	191.3 ± 51.1a	318.5 ± 81.9a	340.6 ± 76a	<LOQ	167.8 ± 61.1a	2017.9 ± 327.3a	853.4 ± 104.8a	
		Kaolin	Yes	293.4 ± 30a	3107.8 ± 319.3a	15.9 ± 9.3a	588.0 ± 55.3a	202.4 ± 42.3a	327.3 ± 73.4a	323.4 ± 78.8a	<LOQ	192.3 ± 15.7 a	1950.0 ± 246.2a	838.6 ± 89.4a
			No	278.5 ± 102.5a	3209.5 ± 286.2a	27.0 ± 28.7a	567.5 ± 54.7a	192.0 ± 52.5a	341.4 ± 87.6a	365.3 ± 76.3a	<LOQ	178.5 ± 66.1 a	2041.9 ± 272.8a	890.2 ± 84.4a
2019	1	Leaf Heavy	264.3 ± 67.6b ***	4140.7 ± 1466.2b	47.4 ± 39.7b *	653.3 ± 62.3a	345.5 ± 119.6a	683.1 ± 272.4a	263.5 ± 120.6b *	172.8 ± 20.2b *	218.2 ± 52.5 b *	**	1239 ± 504.3b ***	
		Removal Regular	341.4 ± 14.2a	5878.4 ± 318.1a	73.7 ± 16.7a	597.0 ± 92.3b	387.2 ± 41.4a	781.2 ± 85.6 a	341.2 ± 49.6 a	188.4 ± 11.2 a	279.3 ± 79.8a	3839.8 ± 512.7a	1766.5 ± 144.5a	
		Kaolin	Yes	271.6 ± 65.9b	4381.1 ± 1512.6b	48.2 ± 38.3a	652.5 ± 76.4a	336.8 ± 90.6a	689.7 ± 251.4a	282 ± 133.9a	173.8 ± 20.3 a	239.0 ± 101.7a	2860 ± 1086.6a	1289.9 ± 492.7b
			No	316.7 ± 57.3a	5263.7 ± 1244.7a	66.6 ± 29.3a	611.7 ± 80.9a	383.3 ± 96.5a	751.7 ± 188.8 a	307.9 ± 74.6 a	183.7 ± 16.1 a	248.5 ± 38.3 a	3527.1 ± 971.8 a	1596.5 ± 417.1a
	Cluster	Yes	310.7 ± 61a	5123.8 ± 1321.4a	67.9 ± 31.7a	629.1 ± 74.6a	397.0 ± 99.8a	784.1 ± 209.8 a	293.0 ± 114.2 a	183.3 ± 18.3a	239.9 ± 53.3 a	3565.9 ± 987.8a *	1553.7 ± 449.9a	
		No	287.4 ± 66.4a	4706.7 ± 1490.4a	51.8 ± 35.1a	629.2 ± 86.5a	338.1 ± 86.2a	680.9 ± 216.1 a	299.6 ± 97.2a	176.6 ± 18.5 a	247.8 ± 83.7 a	2997.5 ± 1072.7a	1398.6 ± 485.0a	
	2	Leaf Heavy	219.5 ± 55a	3780.7 ± 1109.4a	22.7 ± 12.8a	676.8 ± 182.6a	266.2 ± 81.3a	518.5 ± 190.6 a	438.8 ± 153.6 a	158.6 ± 39.3 a	207.5 ± 59.6 a	2503.1 ± 570.0a	1032 ± 195.4a	
		Removal Regular	231.7 ± 18.1a	3530.3 ± 983.7a	21.9 ± 17.7a	712.2 ± 201.4a	270.1 ± 61.8a	495.9 ± 160.5 a	465.9 ± 181.5 a	165.1 ± 11.4 a	211.2 ± 35.1a	2597.2 ± 1104.6a	1052 ± 351.6a	
		Kaolin	Yes	208.9 ± 60.9a	3215.6 ± 896.9b	26.4 ± 8.5a	674.7 ± 177.7a	242.3 ± 81.0a	441.1 ± 153.2 a	415.8 ± 156.3a	151.9 ± 44.2 a	191.4 ± 62.3 a	2277.5 ± 476.4a	953.5 ± 169.3a
			No	236.2 ± 17.1a	3946.8 ± 1031.6a	16.9 ± 17.3a	704.5 ± 200a	286.8 ± 61.2a	558.8 ± 178.9 a	475.7 ± 168.7 a	168.3 ± 11.2 a	222.0 ± 34.8 a	2738.6 ± 975.1a	1105.6 ± 310.7a
Cluster	Yes	237.6 ± 17.5a	3827.7 ± 1017.1a	25.0 ± 16.0a	663.8 ± 156.1a	282.9 ± 62.9a	560.1 ± 189.2a	457.7 ± 162.2 a	169.3 ± 11.5 a	226.1 ± 39.7 a	2727.5 ± 992.9a	1063.7 ± 325.5a		
	No	212.5 ± 55.7a	3456.7 ± 1038.9a	19.9 ± 13.7a	718.3 ± 216.2a	253.8 ± 80.0a	460.7 ± 153a	443.4 ± 170.0 a	153.8 ± 40.2 a	193.0 ± 54.4 a	2369.3 ± 609.1a	1019.7 ± 208a		

**Table 9** Anova results of glycoside aroma compounds by individual treatment code. Letters a, b denotes groupings from Fishers LSD. \* Denote s significance at p .05. All values are ug/kg.

Standard deviations for this table can be found in appendix one, in Table 10.

Year	Vineyard	Treatment	2 - Methyl - 1 - Propanol	3 - Methyl - 1 - Butanol	3 - (Z)- Hexen-1-ol	X2..E..Hexen.1.ol	Linalool oxide (Isomer 1)	Linalool Oxide (Isomer 2)	Terpineol	Nerol	Geraniol	Benzyl alcohol	Phenylethylalcohol	
2018	1	Regular	302.0 a	2790.6 a	14.5 c	535.8 a	168.7 b	334.7 b	116.6 a	173.3 a	166.4 a	1443 b	840.6 a	
		Heavy	285.5 a	3114.2 a	40.4 a	583.6 a	220.5 a	477.0 a	200.8 a	118.8 a	198.3 a	1912.9 a	1060.9 a	
		Heavy + K	282.6 a	2836 a	37.5 ab	545.2 a	215.6 a	384.5 ab	134.2 a	150.7 a	164.3 a	1662.2 ab	903.3 a	
		Regular +K	293.8 a	2893.7 a	20.5 bc	538.4 a	178.5 ab	356.9 b	116.9 a	119.6 a	161.3 a	1539.7 ab	900.6 a	
		p	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2	Regular	251 a	3187.9 a	30.3 a	570.8 a	166.7 a	303.1 a	374.4 a	< LOQ	149.5 a	2054.1 a	863.8 a	
		Heavy	306.0 a	3231.2 a	23.7 a	564.3 a	217.4 a	379.6 a	356.1 a	< LOQ	207.6 a	2029.7 a	916.5 a	
		Heavy + K	289.9 a	3040.0 a	21.8 a	567.9 a	188.8 a	320.8 a	340.1 a	< LOQ	198.5 a	1918.4 a	834.2 a	
		Regular +K	297 a	3175.6 a	10.012 a	608.2 a	216 a	333.9 a	306.8 a	< LOQ	186.0 a	1981.7 a	842.9 a	
		p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2019	1	Regular	339.4 a	5815.6 a	66.6 abc	600.7 a	390.6 ab	744.6 abc	365 a	185.6 ab	268.3 ab	3515.2 a	1742.6 a
			Heavy	236.8 b	3496.2 b	37.3 cd	639 a	273.7 c	549.6 c	220.8 c	166.5 b	209.2 b	2356.3 b	1044.4 b
Heavy +K			236 b	3589.8 b	26.6 d	686.1 a	303.9 bc	610.2 bc	260.2 abc	166 b	204.0 b	2229.9 b	1044.0 b	
Regular +K			337.5 a	5925.1 a	76.8 ab	591.1 a	384.3 ab	819.4 ab	352.3 ab	188.5 a	309.6 a	3888.8 a	1763.4 a	
Heavy +Cluster			343.3 a	5848.6 a	84.6 a	607.7 a	482.1 a	933.2 a	339.4 abc	191.8 a	256.4 ab	4121.4 a	1805.3 a	
Regular + Cluster			347.5 a	5894.4 a	77.8 ab	599.3 a	386.7 ab	779.7 abc	306.2 abc	191.1 a	260.1 ab	4115.6 a	1793.6 a	
Heavy + Cluster + K			241.2 b	3628.2 b	41.2 bcd	680.3 a	322.1 bc	639.5 bc	233.4 bc	166.9 b	203.4 b	2460.7 b	1062.1 b	
p		***	***	*	ns	**	ns	ns	ns	*	ns	***	***	
2		Regular	221.8 a	3893.8 a	19.8 a	852.3 a	270.1 a	472.5 ab	488.1 a	162.1 a	208.0 a	2548.1 a	1107.5 a	
		Heavy	234.1 a	3885.8 a	28.6 a	754.9 ab	292.4 a	582.2 ab	481.9 a	171.1 a	224.6 a	2675.4 a	1073 a	
		Heavy + K	164.1 b	2666.0 a	15.7 a	586.0 b	206.8 a	348.6 b	354.6 a	119.9 b	139.7 b	2079.4 a	1005.2 a	
		Regular + K	227.6 a	3271.9 a	15.7 a	646.4 ab	241.7 a	436.4 ab	438 a	160.1 ab	196.0 ab	2129.7 a	871.2 a	
	Heavy +Cluster	243.8 a	3920.6 a	28.7 a	596 b	292.8 a	618.5 a	468.1 a	168.5 a	229.6 a	2698.2 a	1094.2 a		
	Regular + Cluster	244.9 a	4074.8 a	28.9 a	624.9 ab	292.9 a	566.8 ab	466.2 a	172.1 a	226.5 a	3020.3 a	1141.1 a		
p	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		

## 4. Discussion

### 4.1 Intro

With roughly 1000 GDD per season, which is lower than most other wine regions (Jones et al. 2010) and the threat of heavy rain in the spring and fall, mean Vancouver Island growing conditions are currently on the margin of where it is possible to consistently grow high-quality wine grapes. Within the Cowichan Valley, growing conditions can vary widely, with some vineyards accumulating up to 200 GDD (Base 10 °C) more than cooler properties such as V1 in this study. However, growing seasons have been trending towards warmer and drier recently, with 2018 being one of the warmest, driest summers on record.

Variation in volatile compounds between growing seasons and between vineyards are major contributors to grape quality at harvest (Oliveira et al. 2004; Reynolds et al. 2007; Reynolds et al. 2013). Variation in volatile compounds is evident even in seasons with similar numbers of GDD, as the timing of when the GDD accumulate in the season can affect aroma and grape quality. In the case of this experiment, the 2019 growing season had 30 more GDD than 2018, which may contribute to increase aroma compounds abundance at harvest. Other environmental factors undoubtedly also play a role. The significant increases in leaf area at both vineyards in 2019 compared to 2018 may be attributed to more spring rainfall in the month of April, reducing water stress and increasing vigour during the summer. Larger leaf area in 2019 could have been responsible for the increased grape sugar content at harvest compared to 2018 for both vineyards despite V1 having larger yields (Kliewer and Dokoozlian 2005, Kliewer and Weaver 1971). Greater sugar content at V2 compared to V1 may be attributed to its warmer growing conditions (Buttrose 1969, Rebutti et al. 1997). Warmer growing conditions advance ripening and allow more berries to reach a uniform ripeness (Rankine et al. 1962). This in turn results in diminished effects of treatments on berry chemistry and bound volatile composition. V1 is a cooler site with greater yield and lower leaf area to yield ratio and therefore showed greater responses in regards to

TSS, TA and pH in treatments H, and CT. This suggests that, due to the relative short application time needed to implement the techniques of LR or CT, decisions about application can be made during the growing season depending on vintage and site characteristic.

Glycoside bound aroma precursors are the largest pool of volatile organic compounds found in the berry. This study was able to detect and quantify a total of 11 aroma compounds from Pinot gris grown on Vancouver Island (Table 7). Those compounds are derived from three different developmental pathways in the berry; four C6 compounds produced in the lipid oxidation pathway (LOX), five terpenes produced in the MEP pathway, two alcohols produced in the Shikimate pathway (SA) pathway. Of these compounds, 3-Methyl-1-Propanol (LOX) and Benzyl alcohol (SA) are present at the highest concentrations. When considering human odour threshold, the most important compounds from this list on wine quality are the terpenes as they are perceptible at very low concentrations and even small differences yield significant effects on perceived aroma (Larsen and Poll 1990). Recent research from (Tomasino et al. 2020) showed linalool oxide, and terpineol were present in many Pinot gris wines.

## **4.2 Kaolin**

The use of kaolin applied at veraison as a particle film protecting clusters from adverse effects of increased light exposure showed no effects on vine growth or berry chemical composition. Sun burn was not observed in the two years of this study in any of the treatments. This is most likely due to the cool climate of Vancouver Island and the timing of LR application being early in the season. Early season application of LR regardless of severity reduces risk of sunburn by allowing berries to acclimatize to sun exposure (Kolb et al. 2003). Increased *Botrytis* infection could be seen with the use of kaolin after veraison (Broome et al. 1995, Gubler et al. 1987) as was observed in this study. Kaolin needs to be reapplied following rain events, this re- application requires more water to be applied to clusters and therefore increases the free standing water which is advantageous to *Botrytis* infection. If kaolin is applied for sun protection, after the rainy season starts re-application of kaolin might not be warranted. The

application of kaolin did not have negative effects on fruit quality and can be considered as a tool for protection from sunburn if timing of LR and changes in climate warrant its use.

### **4.3 Leaf removal**

The treatment H consistently reduced leaf area across both vineyards and seasons but did not translate into a reduction in the leaf area to yield ratio. This is important, if H was significantly lowering the source to sink ratio, grape quality could be reduced however, no negative effects on berry chemistry were associated with H. Some studies ( Bledsoe et al. 1988) have found increased sugar accumulation and reduced acidity, but other research report no measurable change in berry chemistry associated with leaf removal (Feng et al. 2014). The absence of an effect here could be due to the reference treatment, east side leaf removal, which already provides the benefit of increased sun exposure and air flow. One interpretation may be that heavy leaf removal can be employed for the benefits of reduced *Botrytis* infection and increased berry temperature without fear of reduced berry quality. A clear benefit of leaf removal, especially in cooler, wetter regions like Vancouver Island is reduced *Botrytis* infection due to reduced moisture on clusters during the ripening phase.

Heavy leaf removal was the only treatment which significantly altered VOC abundance in both years . In 2018 H produced increases in some terpene compounds, while in 2019 H reduced the abundance of many VOC compounds. Concentrations of bound terpenes have been shown to vary by season and intensity of LR. Season-to-season variation makes it difficult to conclude that leaf removal will yield consistent effect on compound abundance in every season (Reynolds et al. 1996). It should be noted that 2019 fruit had higher abundance of almost all measured compounds across all treatments. Different compounds in the vine responded differently to the seasonal variation in combination with the effects of H at V1. Overall, the benefit of consistently reduced *Botrytis* through leaf removal outweighs the variability in volatile compounds seen.

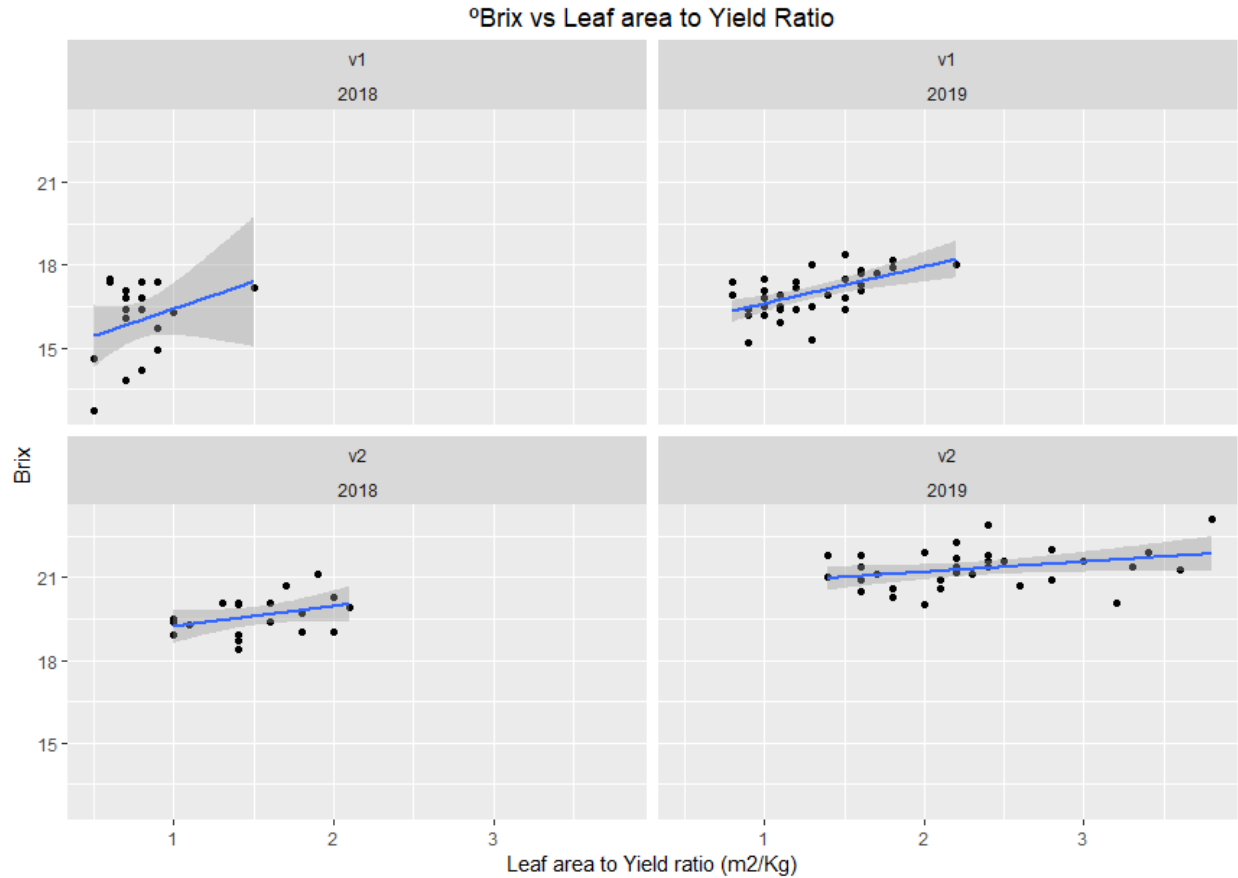
Compounds produced in the LOX pathway such as 2-methyl-1-propanol, and 3-methyl-1butanol showed some of the largest variation attributable to H. Production of these compounds is not fully understood in plants (Robinson et al. 2014, Schwab et al. 2008). Other studies have linked increases in LOX compounds to increases in vine maturity (Mendez-Costabel et al. 2014, Pedneault et al. 2013, Song et al. 2012). The increases seen between seasons in this study make sense in this context as vines varied in vine size between seasons and maturity at harvest was greater in 2019. But this does not explain why these compounds decreased due to H as maturity was similar between LR treatments as was the leaf area to yield ratio.

The low odour threshold for human perception of terpenes compared to other compounds (Larsen and Poll 1990) means effect size of changes in terpenes is particularly important to grape composition and perceived wine quality. Berry respiration increases with temperature which in turn increases bound terpene production (Kruse et al. 2008). Canopy management decisions significantly affect berry temperature. Both the R and H treatments result in berry temperature varying throughout the day, with both treatments permitting warming early in the day and staying relatively warm through the day while the H treatment facilitates greater warmth in the afternoon. It could be that in the case of the reference R treatment the clusters are warming early in the day, and this gives sufficient warming to upregulate the MEP pathway and terpene production to a similar amount to the H (Reynolds and Wardle 1989, Young et al. 2016). Terpene production was higher at both vineyards in 2019, and this is despite the slightly cooler growing season overall. Though 2019 had a much 30 more growing degree days when most of the ripening happened. It is possible that the increase leaf area at both sites in 2019 is responsible for the increase in volatile compounds, as the improved vine balance advanced maturity of the clusters allowing for increased production of volatile compounds. Which in turn allowed the vines to take advantage of 2019, a season with warmer temperatures during the ripening period. Due to the variable effect of leaf removal on volatile compounds season-to-season, vineyard managers should employ leaf removal more for the reduction in *Botrytis* than for any effect on volatile compounds.

## 4.4 Cluster thinning

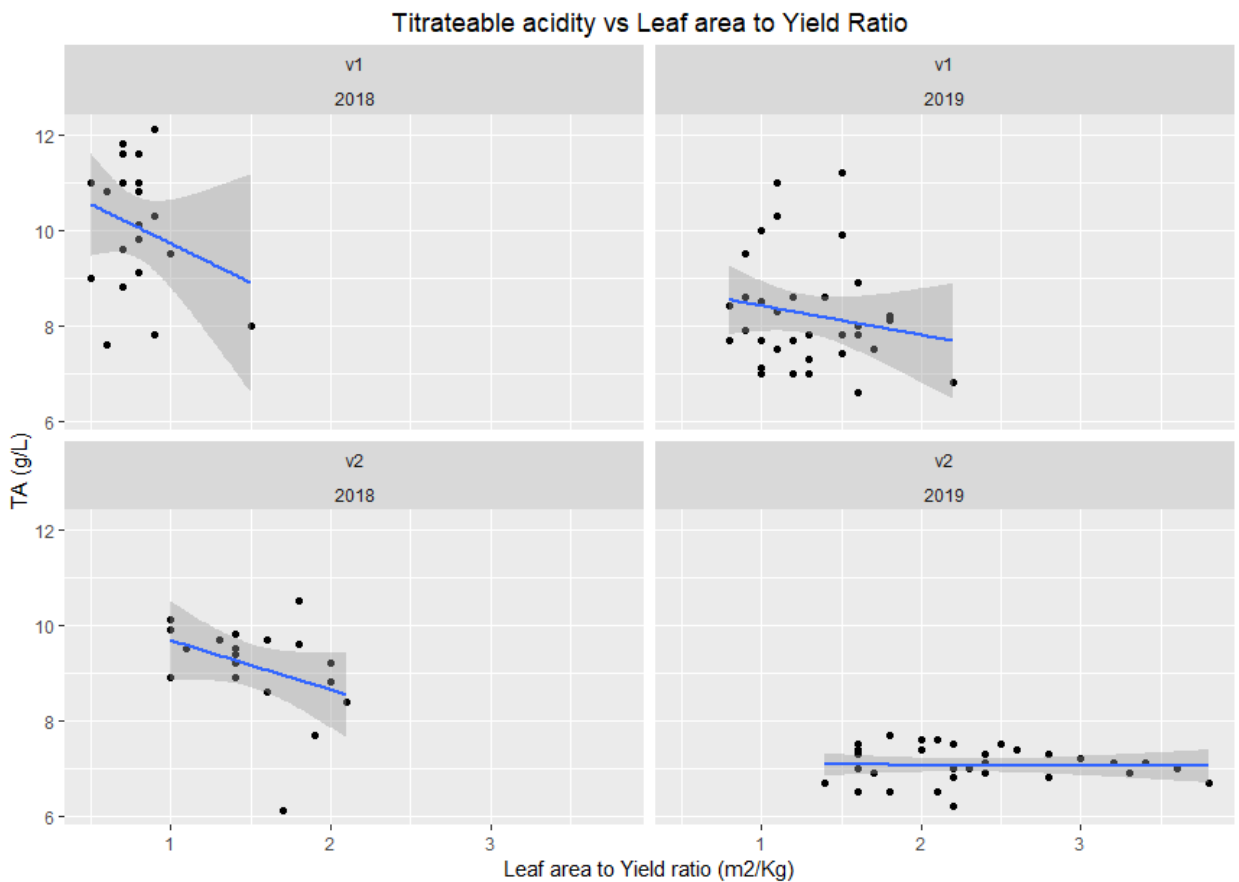
Cluster thinning was responsible for significant changes in yield, leaf area to yield ratio, TSS and titratable acidity. All these significant responses can be explained by significantly altered leaf area to yield ratio.

The impact of improving vine balance through cluster thinning was seen in improved berry chemistry at both sites. Cluster thinning increased TSS at both sites, but more so at V1 where the leaf area to yield ratio was lower to begin with and the site accumulates less heat units. The relationship between TSS and leaf area to yield ratio is shown in Fig2.



**Figure 2** Linear regression of Total soluble solids as °Brix vs Leaf area to Yield ratio by vineyard and year. Positive relationship with increased Brix appears stronger in vineyards with lower leaf area to yield ratio and slower ripening conditions.

Cluster thinning also reduced titratable acidity at both sites. The mechanism here is thought to be larger leaf area to yield ratios allow vines to mature fruit earlier or advance ripening (Kliewer and Dokoozlian 2005). By advancing the ripening process, cluster thinning treatments have increased respiration in the warm temperatures experienced, increasing the break down rate of malic acid, greatly reducing acidity at harvest (Martinez-Esteso et al. 2011, Ruffner 1982). The relationship between titratable acidity and crop load can be seen in Figure 3. Most importantly there is a larger benefit of cluster thinning at V1, the cooler site, as the increase in respiration which reduces malic acid may become more pronounced compared to the warmer site V2.



**Figure 3** Linear regression of Titratable acidity vs Leaf area to Yield Ratio by vineyard and year. Shows increased speed of acid metabolism occurring with higher leaf area to yield ratios

Cluster thinning alone did not appear to have a direct effect on the abundance of volatile compounds but, when observing the individual treatments seen in Table 8, cluster thinning elevated compound abundance. Treatments heavy leaf removal plus cluster thinning and regular leaf removal plus cluster thinning produced significantly higher abundances of most compounds than other treatments at V1 in 2019 (Table 8). V1 had a much higher yield and difference in leaf area to yield ratio on vines receiving cluster thinning compared to V2 where these differences in compound abundance were not seen. Up to 1kg of fruit was removed per vine at V1 equating to a 32% reduction in yield. The advancing of ripening compared to the reference is particularly important as many terpene compounds are synthesised in the berry late in the ripening stages. Research on the effect of cluster thinning in Gewürztraminer conducted in Okanagan Valley found that leaf area to yield ratio was correlated with increased TSS and *cis*-rose oxide concentration (Kovalenko et al. 2022). Cluster thinning advances ripening which in turn allows for more time in the growing season to accumulate volatile compounds. In other words, more compounds are synthesized due to increased time and speed of respiration (Kruse et al. 2008). At V2, a much warmer site with higher leaf area to yield ratio, an increase in volatile compounds due to cluster thinning, as observed at V1, was not observed, consistent with the expectation that warmer sites with better leaf area to yield ratios are better able to ripen fruit and therefore reduce variation in ripeness between clusters that would otherwise be present at cooler sites. In cool climate regions, with larger yields, cluster thinning can be profitably employed to ensure an economical quantity of fruit is grown but with higher quality.

#### **4.5 Determining Ripeness**

Typically in cool regions, wine grapes are left on the vine as long as possible with the goal of gathering as much potential of the season for aroma and sugar production (Kalua and Boss 2009, Ryona et al. 2008). This research confirms this assumption for Vancouver Island's cool climate with TSS being significantly positively correlated with increases in volatile compounds at V1 (Fig. 4). This trend was seen at Vineyard 2 as well, but the results were not significantly correlated. This could be due to increased GDD at V2, combined with a higher leaf

area to yield ratio. This combination allows for much advanced ripening and reduction in variability of TSS, TA and volatile organic compounds between clusters and berries. Regardless of vineyard meso-climate, increased ° brix may be related to increased aroma compounds in Vancouver Island vineyards.

## 5. Conclusion

The use of kaolin is typically not warranted in the Vancouver island's cool climate, though it can be used to protect clusters from sun if using heavy leaf removal without risk of increasing *Botrytis* or reducing chemical composition of the fruit at harvest. H reduced *Botrytis* infection without negative effects to basic berry chemistry, which is a novel finding. Heavy leaf removal produced varied effects on bound volatile composition across the two seasons of study. Heavy leaf removal applied early, after fruit set is an important tool for quality grape production on Vancouver Island but more research is needed to refine this tool. Cluster removal had the largest effect on vine size, yield and basic berry composition. In cool vineyard sites with large yields, applying cluster thinning can be an economic solution to increasing quality. Instead of targeting a specific yield, vineyard managers should target the largest yield possible to ripen in each season by making cluster thinning decisions based on leaf area to yield measurements.

Volatile compound abundance has positive relationship with TSS in the berry, and at any given vineyard the berries with the highest sugar have the highest volatiles. Targeting a specific leaf area to yield ratio  $>2 \text{ m}^2/\text{kg}$  through cluster thinning, can allow growers to get the most potential ripeness out of any given season consistently.

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on Yield, Fruit Composition, and Fruit Rot Incidence of Chardonnay and White Riesling (*Vitis vinifera* L.) Grapes. *Am J Enol Vitic* 43:139–148.

# Appendix 1

Table 10 Standard Deviations corresponding to means in Table 9

Year	Vineyard	Treatment	2-Methyl-1-propanol	3-Methyl-1-butanol	3-(Z)-Hexen-1-ol	2-(E)-Hexen-1-ol	Linalool Oxide (isomer 1)	Linalool Oxide (isomer 2)	?-Terpineol	Nerol	Geraniol	Benzyl alcohol	Phenylethyl alcohol
	1	Regular	± 62.0	± 126	± 5.53	± 32.3	± 17.6	± 34.3	± 33.2	± 67.2	± 10.8	± 154	± 73
		Heavy	± 50.0	± 654	± 20.6	± 141	± 49.5	± 134	± 181	± 38.8	± 56.4	± 477	± 268
		Heavy + Kaolin	± 42.1	± 158	± 14.5	± 76.7	± 38.1	± 40.3	± 27.3	± 6.16	± 10.0	± 195	± 102
		Regular + Kaolin	± 55.6	± 334	± 5.34	± 26.6	± 25.3	± 58.4	± 49.8	± 67.7	± 10.9	± 299	± 207
2018	2	Regular	± 143	± 343	± 40.8	± 39.7	± 59.4	± 101	± 92.2	0	± 84.9	± 363	± 111
		Heavy	± 34.2	± 256	± 12.9	± 71.6	± 32.7	± 58.7	± 66.2	0	± 23.0	± 188	± 45.4
		Heavy + Kaolin	± 37.8	± 258	± 8.15	± 38.0	± 52.5	± 87.6	± 108	0	± 9.11	± 167	± 75.8
		Regular + Kaolin	± 23.9	± 389	± 6.26	± 66.4	± 28.7	± 66	± 40.1	0	± 19.4	± 326	± 110
	1	Regular	± 13.2	± 492	± 22.7	± 119	± 42.8	± 140	± 52.7	± 11.9	± 25.8	± 646	± 219
		Heavy	± 63.6	± 1354	± 39.1	± 70.3	± 93.2	± 207	± 79.5	± 20.8	± 42.8	± 1165	± 482
		Heavy + Kaolin	± 59.3	± 1283	± 38.7	± 37.4	± 107	± 318	± 121	± 18.1	± 46.3	± 939	± 449
		Regular + Kaolin	± 14.4	± 215	± 14.5	± 89.2	± 26.0	± 38.9	± 51.2	± 13.3	± 138	± 479	± 89.1
		Heavy + Cluster	± 20.7	± 118	± 16.0	± 54.7	± 54.7	± 111	± 40.9	± 5.87	± 32.5	± 282	± 142
		Regular + Cluster	± 15.9	± 240	± 12.5	± 86.9	± 59.0	± 35.8	± 29.0	± 9.96	± 27.2	± 208	± 125
		Heavy + Cluster + K	± 57.3	± 1358	± 41.8	± 63.0	± 111	± 294	± 189	± 23.2	± 75.0	± 1001	± 468
2019	2	Regular	± 5.79	± 978	± 17.3	± 210	± 63.3	± 125	± 218	± 5.52	± 26.3	± 839	± 273
		Heavy	± 10.7	± 696	± 13.7	± 170	± 32.7	± 84.5	± 72.2	± 6.46	± 20.5	± 545	± 141
		Heavy + Kaolin	± 110	± 1480	± 14.6	± 300	± 146	± 238	± 247	± 80.0	± 93.3	± 549	± 162
		Regular + Kaolin	± 14.7	± 605	± 6.67	± 99.4	± 29.8	± 51.8	± 92.3	± 6.86	± 14.5	± 277	± 196
		Heavy + Cluster	± 15.4	± 1027	± 15.6	± 141	± 72.8	± 230	± 156	± 13.1	± 38.5	± 666	± 301
		Regular + Cluster	± 22.8	± 1545	± 24.1	± 205	± 79.3	± 236	± 228	± 16.1	± 51.1	± 1661	± 502
		Heavy + Cluster + K	± 8.07	± 430	± 5.26	± 90.2	± 45.4	± 116	± 132	± 6.44	± 37.8	± 501	± 166

