THE EFFECTS OF CLUSTER THINNING ON VINE PERFORMANCE, FRUIT, AND WINE COMPOSITION OF PINOT NOIR (CLONE 115) IN THE EDNA VALLEY OF CALIFORNIA

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ABSTRACT

The effects of cluster thinning on vine performance, fruit and wine composition of Pinot noir (clone 115) in the Edna Valley of California

Paul Frederick Wolf Mawdsley

A three-year study was conducted at a commercial vineyard site in California's Edna Valley AVA to evaluate the physiological and agronomical effects of the timing of cluster thinning on Pinot noir (clone 115) grapevines. Vines were thinned to one cluster per shoot at three selected time-points during the growing season (bloom, bloom + 4 weeks, bloom + 8 weeks), and fruit from each treatment was harvested and made into wine. Across all growing seasons, yield decreased 43% in thinned vines relative to un-thinned control vines. No effect of cluster thinning or interaction with growing season was found in vine shoot diameter, internode length, fruit zone light level, or cluster weight. Growing season significantly affected more fruit and wine parameters than did cluster thinning treatment, with interactions between treatment and growing season found in fruit Brix, titratable acidity, and anthocyanins, as well as wine anthocyanins and wine b* (yellow component). For example, bloom + 8 and bloom + 12 thinning treatments advanced Brix in 2017 but had no effect in 2018. Cluster thinning treatments increased berry anthocyanins by 43% in 2017 and by 103% in 2018 relative to the control. Similarly, cluster thinning increased berry total phenolics by 87% in 2017 and by 140% in 2018 relative to the control, with no significant differences found between the different thinning treatments. However, the levels of anthocyanins and total phenolics were generally not affected by cluster thinning treatment in the resulting wines. The fact that different cluster thinning treatments resulted in nil or minor effects on fruit and wine suggests that the vines tested were at or below a balanced crop load prior to the application of cluster thinning. Edna Valley AVA could likely support higher crop loads than 3.2 on the Ravaz index without negatively impacting fruit or wine composition and reducing crop load below that level is unlikely to increase fruit or wine quality.

Keywords: Cluster thinning, Yield manipulation, Vine balance, Crop load, Pinot noir, Central Coast of California

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

1. LITERATURE REVIEW

1.1. Grapevine Physiology and Agricultural Practices

Pinot noir (*Vitis vinifera* L.) is a challenging grape cultivar from both a viticultural and winemaking perspective. Viticulturally, Pinot noir is genetically diverse and complex with many phenotypes displaying plasticity in cluster architecture (Blaich, Konradi, Rühl, & Forneck, 2007; Whiting & Hardie, 1990), berry chemistry at maturity (Anderson, Smith, Williams, & Wolpert, 2008; Blaich et al., 2007; Mercado-Martín, Wolpert, & Smith, 2006), and shoot growth (Anderson et al., 2008; Blaich et al., 2007). The cause of Pinot noir's clonal prolificacy appears to be two-fold: Pinot noir has been cultivated and propagated since at least the late 14th century (Anderson et al., 2008) and Pinot noir is chimeric at several loci (Blaich et al., 2007), creating ample opportunities for mutations. Pinot noir grapevines are grown primarily in 'cool' to 'moderately warm' growing regions (Robinson, 2006). Vines grown in cool regions are photosynthetically limited due to reduced solar radiation (if at higher latitudes) and lower temperatures that reduce photosynthesis rates and slow vine phenological development relative to vines grown in warm climates (Reeve et al., 2018). Pinot noir grapes and wines are also inherently low in phenols (Dimitrovska, Bocevska, Dimitrovski, & Murkovic, 2011; Harbertson et al., 2008), which results in wines that are perceived as light in color and astringency (Cliff, King, & Schlosser, 2007; Harbertson, Kennedy, & Adams, 2002). As color is one of the main drivers of perceived wine quality (Somers & Evans, 1974), viticultural practices such as cluster thinning are often applied to Pinot noir grapes in an attempt to influence fruit polyphenol composition by lowering vine crop load (Cañón, González, Alcalde, & Bordeu, 2014; Reynolds, Price, Wardle, & Watson, 1994; Uzes & Skinkis, 2016).

1.1.1. Crop Load

The traditionally accepted yield paradigm in wine grapes (*Vitis vinifera* L.) is that of a negative linear relationship between yield and fruit quality (Gamero et al., 2014; Palliotti & Cartechini, 2000; Reynolds et al., 2007; Uzes & Skinkis, 2016). This relationship has been shown to be an oversimplification and may be

more accurately represented by crop load (Bravdo, Hepner, Loinger, Cohen, & Tabacman, 1985; Jackson & Lombard, 1993). Crop load, expressed as the unitless ratio of fruit yield to dormant pruning weight in the Ravaz Index (Ravaz, 1903; Smart, Dick, Gravett, & Fisher, 1990), relates fruit yield, the primary vine photosynthetic sink during ripening, to the photosynthetic capacity of the vine represented by the weight of canopy growth removed during dormant season pruning (Kliewer & Dokoozlian, 2005). Crop load can also be expressed as the relationship between leaf area and fruit yield. Measuring leaf area directly may offer a more accurate representation of vine photosynthetic capacity than pruning weight, and thus crop load, especially when comparing between trellis systems (Kliewer & Dokoozlian, 2005). As pruning weights are simpler to measure than leaf area, most extension agencies recommend that growers utilize pruning weights to evaluate vine capacity and the Ravaz Index to evaluate crop load. Despite the Ravaz Index being a popular measurement in industry, the measurement has been widely criticized as overly simplistic or reductive. The Ravaz Index, while a useful tool to assess general vine balance after the fact, cannot account for many factors that affect vine photosynthate production and fruit ripening. For example, differences in solar radiation intensity and season length that occur at different latitudes will change the amount of vegetation required to ripen a set amount of fruit (Howell, 2001). Additionally, vegetative differences in leaf number, layers, and positioning that are not accounted for in dormant vine pruning weight will change the vine microclimate, fruit sun exposure and berry temperature which will in turn impact fruit composition and maturation (Price, Breen, Valladao, & Watson, 1995; Rienth et al., 2016; Spayd, Tarara, Mee, & Ferguson, 2002).

Vine photosynthate production capacity is affected by exposed leaf area (Kliewer & Dokoozlian, 2005), climate (Reeve et al., 2018), growing season (Frioni et al., 2017; Gamero et al., 2014; Reeve et al., 2018; Zhuang et al., 2014) site characteristics (Uriarte et al., 2015), rootstock (Santarosa, Dutra de Souza, de Araujo Mariath, & Lourosa, 2016), and cultural practices (Reeve et al., 2018). Given that the ability to ripen a set amount of fruit is dependent upon a sufficient vine capacity, and vine capacity is affected by a myriad of factors, there is no single optimal yield for wine grapes across regions and cultivars (Kliewer & Dokoozlian, 2005; Reeve et al., 2018), and crop load metrics must be utilized. Optimal crop load is considered to be between 5 and 10 on the Ravaz Index for most cultivars and regions (Bravdo et al., 1985). However, it has been proposed that optimal crop load for small-clustered cultivars, such as Pinot noir, grown in cool climates

should be between 3 and 6 on the Ravaz Index (Kliewer & Dokoozlian, 2005), although this range has not been substantiated by published research.

Crop load is affected by climate, with warmer regions supporting higher crop loads than cooler regions (Kliewer & Dokoozlian, 2005; Reeve et al., 2018). Lower daytime temperatures in cooler regions result in lower leaf photosynthetic capacity and berry carbon assimilation (Howell, 2001; Jackson & Lombard, 1993; Kliewer & Dokoozlian, 2005), necessitating lower crop loads to achieve fruit maturity than would otherwise be required in warmer climates (Howell, 2001; Kliewer & Dokoozlian, 2005; Reeve et al., 2018). Additionally, these crop loads are intrinsically tied to seasonal limitations and meteorological variations in mean temperature and heat accumulation (Frioni et al., 2017; Gamero et al., 2014; Reeve et al., 2018; Zhuang et al., 2014). As such, the crop load that can be supported in a region, while generally restricted by the climate, will ultimately be determined by the meteorological conditions of any given growing season.

1.1.2. Climate and Meteorology

Wine grape growing climates are classified by growing degree day (GDD) accumulation during the typical growing season, April 1 – October 31 in the Northern Hemisphere. Regions are classified from the coolest region (I) to the warmest region (V) following the Winkler scale (Jones, Duff, Hall, & Myers, 2010; Winkler, Cook, Kliewer, & Lider, 1974). While many meteorological factors such as rainfall, humidity, and frost-free days determine the viticultural suitability of a site or region, temperature indices, specifically the Winkler scale, are the most common tools used to compare wine region climates (Jones et al., 2010). Many areas of economic importance for Pinot noir production are classified as region I, II, and, less commonly, III (Robinson, 2006), which are considered cool climate (I and II) or moderately warm climate (III) areas according to the Winkler scale.

Ambient temperature, along with sunlight level and exposure, is directly related to grape composition and canopy growth. It is well documented that vine capacity and the ability to achieve ripeness by soluble solids accumulation in cool regions is dependent upon climatic conditions such as temperature (Kliewer, 1973; Reeve, Skinkis, Vance, Lee, & Tarara, 2016; Winkler et al., 1974), with a base level of temperature required to adequately ripen fruit. Increased temperature was associated with increased Brix in Merlot fruit (Spayd et al., 2002), increased skin and seed tannins in Pinot noir (Pastor del Rio & Kennedy, 2006), and increased pruning weights in Shiraz (Moran, Petrie, & Sadras, 2019). Similarly, increased sun exposure was

associated with increased fruit anthocyanin content in Merlot (Spayd et al., 2002) and quercetin content in Pinot noir (Feng, Yuan, Skinkis, & Qian, 2015; Price et al., 1995), although the latter study found no effect on Pinot noir berry anthocyanins. Warmer temperatures during bloom have been shown to decrease berry size but increase berry set (Kliewer, 1977). Fruit organic acid levels are affected by berry temperature and light exposure post-véraison, with increasing temperatures and light exposure corresponding to increased respiration and lower malate levels (Price et al., 1995; Rienth et al., 2016; Ruffner, 1982).

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1.1.3. Yield Control

Grape yield, expressed as the mass of grapes produced per unit farmed land, is primarily determined by pruning practices: vines are pruned to leave a certain number of buds which will grow into an equal number of shoots that, in turn, will theoretically develop two clusters per shoot. However, variation in bud fruitfulness affects both cluster number per shoot and cluster weight (Dry, 2000). Bud fruitfulness can be determined prior to pruning by counting cluster primordia present in a sample of dissected compound buds, and that number, combined with bud counts, can be used to estimate cluster number per vine. Variation in cluster number per vine, directly affected by pruning practices and bud fertility, has been shown to represent 60% of variation in wine grape yields (Martin, 2002).

Grape yield is also affected by berry size and berry set percent during bloom. Several factors influence berry size, including berry temperature during various phenological growth stages, light incidence, water and nutrient availability, and seed number per berry (Coombe, 1976; Gillaspy, Ben-David, & Gruissem, 1993; W. M. Kliewer, 1977; Van Volkenburgh, 1999). Berry size can be reduced by increased heat and resultant increased berry temperature prior to Stage II: Lag Phase (Kliewer, 1977). Typical berry set in winegrapes ranges from 20% to 50% (Mullins, Bouquet, & Williams, 1992), and can be reduced by temperatures during bloom below 15 °C (Kliewer, 1977). Because of these uncontrollable factors that can reduce berry set and size, viticulturists may be inclined to prune to a higher bud count than that which would achieve their target crop load if berry set and size were maximized. If cluster counts and sizes at lag phase predict a higher-than-desired yield, yield-reduction practices such as cluster thinning may be performed.

Cluster thinning is a common viticultural practice in Pinot noir production to reduce vine crop load. Cluster thinning is performed with the aim of either improving fruit quality or conforming with commercially

expected crop loads (Uzes & Skinkis, 2016). Color is a primary component of the consumer perception of wine quality (Somers & Evans, 1974). As Pinot noir wines are typically lighter in color and astringency, the practice of cluster thinning is often used to lower yields with the intention of increasing phenolic content. (Gamero et al., 2014; Guidoni, Allara, & Schubert, 2002; Keller, Mills, Wample, & Spayd, 2005; Reynolds et al., 1994; Santesteban, Miranda, & Royo, 2011; Uzes & Skinkis, 2016; Valdés et al., 2009). This thinning is performed, at times, regardless of the crop load of the vineyard (Uzes & Skinkis, 2016).

Cluster thinning is purported to have a multitude of beneficial effects on berry composition, although experimentation has shown mixed results. Several studies have shown that cluster thinning increased the concentration of fruit anthocyanins in Pinot noir (Cañón et al., 2014), Cabernet franc (Frioni et al., 2017), Cabernet Sauvignon (Cañón et al., 2014; Palliotti & Cartechini, 2000), Sangiovese, and Merlot (Palliotti & Cartechini, 2000). However, when the effects of cluster thinning have been evaluated over multiple years, results have generally indicated no effect of cluster thinning on anthocyanin concentration in Cabernet Sauvignon (Keller et al., 2005) and inconsistent effects in Pinot noir, with thinning increasing berry anthocyanins in one season and no effect in two other seasons (Reynolds et al., 1994). Half-crop thinning in Pinot noir has increased pH (Reeve et al., 2016; Reynolds et al., 1994) in both fruit and juice, but with mixed effects on TA, showing either no impact (Reynolds et al., 1994) or a reduction in TA with cluster thinning (Reeve et al., 2016). Compositional effects as a result of cluster thinning were found in Chardonnay Musqué but did not change the finished wines correspondingly (Reynolds et al., 2007).

Many studies on cluster thinning have been conducted in warm, arid climates and on cultivars such as Cabernet Sauvignon (Bergqvist, Dokoozlian, & Ebisuda, 2001) and Tempranillo (Gamero et al., 2014; Gil-Muñoz et al., 2009; Valdés et al., 2009). Pinot noir grown in cool climates may be more suited to benefit from cluster thinning due to the inherently low polyphenol content of the fruit and reduced carbon assimilation capacity of vines in cool climates (Howell, 2001; Jackson & Lombard, 1993; Reeve et al., 2018). However, in cool climate regions where cluster thinning effectively advanced ripening in one season, it has been observed that performing this practice during a subsequent warmer season can have no impact on fruit maturation (Frioni et al., 2017). These results suggest that cluster thinning is a practice that should be evaluated on not only a cultivar basis but on a season to season basis as well, as source limitation will vary with the meteorological conditions of each growing season. Regardless of site-specific factors that affect

vine capacity, growers may perform cluster thinning to reduce yields below what they believe is necessary to achieve commercially acceptable fruit ripeness in order to conform with the expectations of the winemaker (Uzes & Skinkis, 2016). As there is considerable economic cost to the practice of cluster thinning associated with both yield reduction and labor, it would be beneficial for viticulturists to understand when thinning can be applied to positive effect on the Central Coast of California, where Pinot noir represents over 7,000 hectares of the planted vineyards (nass.usda.gov).

Additionally, the timing of cluster thinning implementation is also subject to debate. For example, cluster thinning performed during the lag phase of berry development has been suggested as the most appropriate time to perform this task, due to the popularity of using lag phase cluster weights to estimate yields and the purported ability of pre-véraison thinning to affect fruit maturity (Jackson & Lombard, 1993). However, an experiment conducted in Cabernet Sauvignon reported that cluster thinning performed during lag phase of berry development had little to no impact on canopy growth, cluster yield components, and fruit maturity (Keller, Smithyman, & Mills, 2008). It has also been hypothesized that removing crop earlier in the season, *e.g.* at bloom, may lead to lower leaf transpiration rates and therefore lower leaf photosynthesis rates (Naor, Gal, & Bravdo, 1997), which could negate the potential benefits of reduced crop load by reducing the photosynthetic capacity of the vine. For example, a temporary photosynthetic reduction was observed after thinning at lag phase in Cabernet Sauvignon grapes (Wang et al., 2018), but photosynthetic capacity was not affected after véraison indicating a compensation effect of increased vegetative growth in response to cluster thinning. Cluster thinning performed during bloom also increased berry size in Pinot noir (Reynolds et al., 1994) as well as Cabernet Sauvignon, Chenin blanc, and Riesling grapes (Keller et al., 2005). In the latter study, this effect was only seen in one season and only thinning at bloom resulted in increased berry size, while véraison thinning resulted in berry size indistinguishable from the non-thinned control. Thinning at véraison has also been shown to have no effect on berry size in Tempranillo (Valdés et al., 2009).

Berry size reduction has been traditionally considered desirable from a winemaking perspective based on the empirical assumption that comparatively smaller berries have higher berry surface area to volume ratio than larger ones. However, the relationship between berry size and phenolic composition is not a simple linear relationship, and larger berries may not be necessarily undesirable as once thought (Matthews & Nuzzo, 2007). Although berry flesh and juice volume does increase as berry size increases, berry skin and

seed mass grow along with berry size (Matthews & Nuzzo, 2007). While larger berries have been found to have lower anthocyanin and tannin content than smaller barriers (Roby, Harbertson, Adams, & Matthews, 2004; Roby & Matthews, 2008; Romero-Cascales, Ortega-Regules, López-Roca, Fernández-Fernández, & Gómez-Plaza, 2005), the extractability does not appear to scale with berry size, and phenolic differences present in fruit do not always transfer to wine at the same magnitude, if at all (Roby et al., 2004; Roby & Matthews, 2008). It has also been observed that smaller-magnitude berry size differences caused by seasonal variation had a more substantial effect on wine quality than larger-magnitude berry size differences caused by viticultural treatments, suggesting that the relationship between berry size and wine characteristics is not simple, and that seasonal meteorological conditions may be more important than the simple size of berries (Holt, Francis, Field, Herderich, & Iland, 2008).

In much of the previous research performed on cluster thinning, external factors independent of (although at times in combination with) vine crop load impacted berry and wine chemical composition to a greater degree than crop load. Factors such as climatic variation in growing season (Keller et al., 2005) and viticultural practices such as cover cropping practices (Reeve et al., 2018), deficit irrigation (Keller et al., 2005; Valdés et al., 2009), and leaf thinning (Burbola, Sivilotti, Janjanin, & Poni, 2017) have all been found to have a greater effect on fruit composition than cluster thinning. Additionally, in many studies the effect of cluster thinning was dependent upon the meteorological conditions of any single growing season (Cañón et al., 2014; Gamero et al., 2014; Guidoni et al., 2002; Keller et al., 2005).

1.2. Fruit Composition

V. vinifera fruit composition at the time of harvest, along with winemaking practices, is largely responsible for the composition and quality of the wine produced from that fruit. Quantitative winemaking and harvest-timing decisions are based largely on fruit or juice total soluble solids concentration (TSS) (Conde et al., 2007), which is expressed as Brix and used as an estimate of sugar concentration, pH, acidity, measured as titratable acidity or enzymatically as tartaric or malic acid, and polyphenol content. The ability to ripen fruit and the relative content of each of these components at harvest is affected by temperature and growing season heat accumulation (Holt et al., 2008; Keller et al., 2005; Rienth et al., 2016), berry light exposure during ripening (Price et al., 1995) and canopy microclimate (Burbola et al., 2017), crop load (Frioni et al., 2017; Gamero et al., 2014; Keller et al., 2005; Naor, Gal, & Bravdo, 2002), irrigation regime (Keller

et al., 2005; Valdés et al., 2009), soil type (Poni et al., 2018; van Leeuwen et al., 2004), and the interactions of these factors.

1.2.1. Primary Metabolites

After water, which accounts for approximately 75-85% of juice volume (Conde et al., 2007), the most abundant compounds in *V. vinifera* fruit are hexose sugars. Maximum sugar concentration varies slightly by cultivar, but generally does not progress past 25 Brix, when sugar import to the berry is halted (Keller, 2010); any further increase in sugar concentration is the result of berry dehydration (Keller, 2010). Glucose and fructose are the predominate sugars found in grape berries, along with trace amounts of sucrose (Liu, Wu, Fan, Li, & Li, 2006). Wine alcohol concentration is fundamentally tied to fruit sugar content, as sugar is converted to ethanol by yeast during alcoholic fermentation. As such, the ability to ripen fruit, while the exact desired sugar content will vary with cultivar and wine style, is important to both viticulturists and winemakers. Ripening ability is affected largely by the climate and crop load of a vine, with warmer climates supporting larger crop loads, and cooler climates requiring smaller crop loads (Frioni et al., 2017; Kliewer & Dokoozlian, 2005; Reeve et al., 2018; Tozzini, Sabbatini, & Howell, 2013). When a vine is overcropped, the photosynthetic capacity of the vine is unable to produce and translocate the amount of sugars that would be required to achieve the desired TSS content in the entire crop (Keller, 2010; Winkler et al., 1974). Thus, crop load must be balanced with GDD accumulation to achieve maturity. Brix accumulation, however, is not driven solely by temperature. In fact, differences in berry temperature may not directly impact Brix accumulation at all (Kliewer, 1973). Other factors such as soil moisture content (McIntyre, Kliewer, & Lider, 1987; Triolo et al., 2018; van Leeuwen et al., 2009) and berry light exposure (Dokoozlian & Kliewer, 1996) will impact fruit ripening rate irrespective of atmospheric temperature and may even have a larger effect than GDD accumulation in cases where GDD accumulation is adequate for fruit ripening (McIntyre et al., 1987).

After sugar, the next most abundant metabolites in ripe *V. vinifera* fruit are organic acids. The primary acids found in *V. vinifera* fruit are tartaric acid and malic acid, representing 69 to 92% of fruit acid content (Kliewer, 1966). Tartaric acid is formed in berries from anthesis to véraison (DeBolt, Cook, & Ford, 2006), when fruit tartaric acid concentration peaks (Conde et al., 2007). After véraison, tartaric acid concentration in berries decreases via dilution as berries grow and water and sugar content increases, although the tartaric acid content remains relatively unchanged (Conde et al., 2007; Possner & Kliewer, 1985). Like tartaric acid,

malic acid reaches its maximum concentration just prior to véraison, but unlike tartaric acid, malic acid production does not halt at véraison, and the decreasing concentration of malic acid during fruit ripening is due to respiration and enzymatic degradation (Conde et al., 2007). Respiration, and the corresponding drop of malate concentration, increases during ripening with temperature and light exposure (Price et al., 1995; Rienth et al., 2016), resulting in reduced malate concentration in fruit grown in warmer climates (Conde et al., 2007). Fruit pH is, generally, inversely proportional to fruit organic acid concentration, however fruit pH has also been shown to increase with vine potassium level (Conradie & Saayman, 1989) due to the constant flow of potassium into the fruit with phloem influx (Keller, 2010). Vine potassium level is in turn affected by soil potassium level, cultivar, and viticultural practices (Davies, Shin, Liu, Thomas, & Schachtman, 2006; Mpelasoka, Schachtman, Treeby, & Thomas, 2003). Fruit pH, in the absence of winemaking adjuncts and ameliorations such as tartaric acid and potassium bicarbonate additions, is directly responsible for juice pH and eventual wine pH. Wine pH is tied intrinsically to a wine's microbial and oxidative stability, with lower pH values inhibiting (synergistically with ethanol) microbial growth (Boban et al., 2010) and increasing the ability of polyphenols to protect the wine from premature oxidation (Singleton, 1987).

1.2.2. Secondary Metabolites

Polyphenols are biomolecules present in the grapes (and subsequently extracted into wine) possessing a three-aromatic ring system defined by a C6−C3−C6 structure bearing diverse hydroxyl and non-hydroxyl substitutions (Waterhouse, 2002). Due to the high electron density of these phenol rings (each one characterized by a hydroxyl active group) polyphenols are very reactive compounds, readily participating in oxidation and electrophilic aromatic substitution reactions (Fulcrand, Dueñas, Salas, & Cheynier, 2006), as well as non-covalent interactions with many other components of the wine matrix (Casassa, 2017). Depending on pH and oxidation-reaction potential, polyphenols can act as both electrophiles and nucleophiles, contributing to their potential for activity. Different oxidation states and substitutions on the C ring define the different classes, ranging from the highest oxidation state, anthocyanins, to the most reduced state, flavan-3-ols (Casassa, 2017). In wines, polyphenols are responsible for color (Bimpilas, Panagopoulou, Tsimogiannis, & Oreopoulou, 2016; Boulton, 2001; Jordheim, Fossen, & Andersen, 2006), tactile sensations such as astringency (Casassa & Harbertson, 2014; Poncet-Legrand, Gautier, Cheynier, & Imberty, 2007), and taste sensations such as bitterness (Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón,

2014; Harbertson, Parpinello, Heymann, & Downey, 2012; Lesschaeve & Noble, 2005). In addition to sensory effects on astringency, taste, and aroma modulation (Villamor, Evans, Mattinson, & Ross, 2013), flavonoids also play a critical role in the chemical stability of the wine during aging as these molecules intervene in metal-catalyzed oxidation reactions (Danilewicz, 2003; Fulcrand et al., 2006). In *V. vinifera* fruit, polyphenol content is modulated by cultivar (Dimitrovska et al., 2011; Harbertson et al., 2002), climate (Mateus, Marques, Gonçalves, Machado, & De Freitas, 2001), growing season (Downey, Dokoozlian, & Krstic, 2006; Guidoni et al., 2002), berry sun exposure (Lenk, Buschmann, & Pfündel, 2007), canopy biomass (Cortell, Halbleib, Gallagher, Righetti, & Kennedy, 2005), vine water status (Mirás-Avalos et al., 2017), yield (Reynolds et al., 2018), and fruit maturity (Allegro et al., 2018).

Anthocyanins are the primary compounds responsible for the color of red grapes and wine. Anthocyanins are formed in the vacuoles of grape hypodermis tissue (Fontes et al. 2011) during fruit maturation where they contribute to skin pigmentation. Six anthocyanins are present in grape in a glycosylated (bonded to a glucose molecule) form: malvidin, cyanidin, petunidin, peonidin, delphinidin, and pelargonidin (Cheynier 2006). The maximum absorbances of these compounds range from 475-540 nm, resulting in red and blue hues. Within this range, individual anthocyanin absorbance properties are largely pH dependent (Heredia, Francia-Aricha, Rivas-Gonzalo, Vicario, & Santos-Buelga, 1998). As pH decreases, the equilibrium of anthocyanin form shifts to favor the flavylium cation, which increases red hue, and as pH increases the equilibrium shifts to favor the quinoidal hydrobase, which increases blue hue (Heredia et al., 1998). Acylated anthocyanins are also present in grapes as esters of coumaric and caffeic acid, and are abundant in some cultivars such as Cabernet Sauvignon or Syrah and may increase color stability (Brouillard, Chassaing, & Fougerousse, 2003; Van Buren, Bertino, & Robinson, 1968). Acylated anthocyanins are notably lacking from Pinot noir fruit (Dimitrovska et al., 2011), which contributes to the light color profile associated with Pinot noir wines.

Flavan-3-ols occur in grape seeds and skins as five monomers: catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin-3-O-gallate. These different isomeric configurations have an impact on sensory properties (Brossaud et al. 2001). Flavan-3-ols are formed primarily within the cells between the cuticle and lignified layers of grape seed tissue (Adams 2006), although some flavan-3-ols are found in skin tissue as well. This distribution heavily favors the seeds: in one study, Cabernet Sauvignon flavan-3-ol

content was 179 mg/kg fresh weight vs 4.8 mg/kg fresh weight in seeds and skins, respectively (Guerrero et al., 2009). Flavan-3-ols do not absorb light on the visible spectrum, but following oxidation the resulting quinones that are formed exhibit visible yellow hues (Waterhouse & Nikolantonaki, 2015) that contribute to the yellow-brown color of aged red wines. Flavan-3-ols are responsible for the bitter taste of wine (Rossi, Sing-leton, & Gallo Winery, 1966), with bitterness varying between isomer (Kallithraka, Bakker, & Clifford, 1997). Flavan-3-ols can interact with anthocyanins to form polymeric pigments.

Tannins are oligomeric (degree of polymerization 2 to 4) and polymeric (degree of polymerization of 5 units or higher) flavonoids, consisting of sub-units of monomeric flavan-3-ols (Foo & Porter, 1980). Tannins are responsible for the tactile sensation of astringency found in wine, occurring from the precipitation of salivary proteins by the tannins (Ma et al., 2014). In addition to the native ability to precipitate proteins, tannins also interact readily with polysaccharides, which can modulate astringency (Escot, Feuillat, Dulau, & Charpentier, 2001). Tannins in wine are characterized by the mean degree of polymerization (mDP), determined by HPLC phloroglucinolysis and referring to the average number of subunits in the oligomer or polymer (Kennedy & Jones, 2001). Tannins occur in both grape skins and seeds, although up to 80% of the total extractable tannin is located within the seeds (Haslam, 1998). Skin tannins exhibit higher mDP than seed tannins, ranging from 6-85 compared to 2-22 for seed tannins (Casassa 2017).

Nitrogen is found in ripe *V. vinifera* fruit in both mineral and organic forms. The nitrogen content of *V. vinifera* fruit destined for wine is represented as yeast assimilable nitrogen (YAN), which is measured as primary amino nitrogen (PAN), representing organic nitrogen sources, plus mineral nitrogen in the form of ammonium and ammonia. Organic nitrogen, in the form of, primarily, the amino acids proline, arginine, glutamine, alanine, glutamate, serine and threonine, represents 20-50% of nitrogen found in grape must (Conde et al., 2007). The relative proportion of these amino acids differs between cultivars, and to a lesser extent rootstock (Huang & Ough, 1989), region, and growing season (Huang & Ough, 1989, 1991). Wine yeast will readily utilize most amino acids present in *V. vinifera* fruit with the notable exception of proline, which is not included in PAN.

Mineral nitrogen found in ripe fruit consists of ammonium and ammonia, the concentrations of which vary widely with cultivar, vine nitrogen status, and fertilization practices (Bell & Henschke, 2005). In fertilized vineyards, ammonium has been found to represent as little as 9% (Conradie, 2001), and as much as

40% (Bell & Henschke, 2005) of YAN at harvest. Similarly, between cultivars at the same site ammonium has been found to range between 2-53% (Huang & Ough, 1989) of YAN at harvest and ammonia has been found to represent between 32-53% of YAN (Huang & Ough, 1989). These cultivar differences are driven by differences in preferential production of amino acids, particularly proline and arginine (Bell & Henschke, 2005). Nitrogen fertilization in the vineyard consistently increases inorganic nitrogen content of fruit, however the impact on ammonium as a percentage of YAN is not consistent (Bell & Henschke, 2005). Minimum YAN requirements for healthy fermentations in Pinot noir are in the range of 100 mg/L (Schreiner, Osborne, & Skinkis, 2018) to 140 mg/L (Bell & Henschke, 2005), although exact YAN requirements are dependent upon yeast strain (Bell & Henschke, 2005).

1.3. Wine Composition

Wine composition is determined by a combination of fruit physical and chemical composition and winemaking practices. Although there are many variations of practice that can be incorporated at every step, red winemaking proceeds, generally, as follows: fruit is harvested from winegrapes either by hand or mechanically. If fruit is harvested by hand, fruit is generally destemmed to remove berries from the rachis, and berries are crushed to create must, the slurry of juice and fruit solids. Some amount of fruit may be kept on the rachis, generally as a percentage by weight, in a practice known as whole cluster fermentation. Whole cluster fermentation is conducted with the objective of increasing phenolic extraction or wine aromatic complexity (Casassa et al., 2019; Suriano, Alba, Tarricone, & Di Gennaro, 2015). If fruit is harvest mechanically, only berries are collected and the rachises remain attached to the vine, which eliminated the step of destemming. After fruit has been crushed, if fermentation by commercial yeast cultures (*Saccharomyces cerevisiae*) is desired, the must will be treated with SO_2 to inhibit the growth of native yeast flora and commercial yeast will be inoculated. If native yeast fermentation is desired, no SO_2 will be added to the must. A pre-fermentation cold soak period may be undertaken prior to the addition of yeast with the objective of increasing phenolic extraction, although the efficacy of this practice is not clear and studies have shown mixed results in Pinot noir, reporting either nil (Casassa et al., 2019) or negative effects (Casassa, Bolcato, & Sari, 2015) on color and aromatic profile. Fermentation duration ranges widely, varying with initial must sugar content, yeast strain (Reynolds et al., 2007), and fermentation temperature (Du et al., 2012; Ough & Amerine, 1967). During fermentation, cap management practices such as punch-downs or pump-

overs are performed once or twice daily to wet the cap and homogenize temperature within the fermentation vessel. At the completion of fermentation, wine is removed from the remaining solids (skins, seeds, and rachis), and the solids may be pressed to extract more wine. Variations in winemaking practices at any of these steps can create differences in final wine chemical composition and sensory characteristics.

Wine composition affects consumer acceptance and commercial viability of wine (Jaeger, Danaher, & Brodie, 2009). Particularly, consumers have been found to prefer wines that have deeper color (Parpinello, Versari, Chinnici, & Galassi, 2009; Somers & Evans, 1974), and color has been shown to affect consumer perception of flavors and aromas in food and wine (Delwiche, 2004). Anthocyanins are notoriously low in Pinot noir wines (Dimitrovska et al., 2011), which has generated interest in practices that could increase polyphenol content in fruit or increase phenolic extraction into wine.

1.3.1. Phenolic Extraction

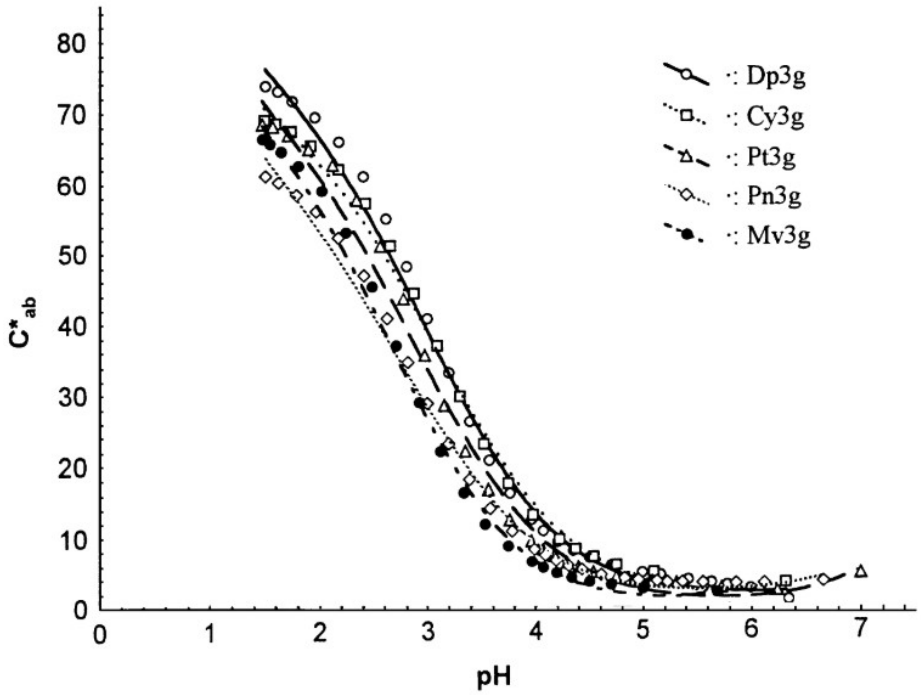
While viticultural practices may be undertaken with the objective of increasing fruit polyphenol content, changes in fruit polyphenol content are not always reflected in wine. In one study, it was observed that Pinot noir fruit tannin content increased with later pick dates while wine tannin content decreased (Pastor del Rio & Kennedy, 2006), suggesting the influence of other factors. The diffusion of polyphenols from grape tissue into the must requires both the breakdown of cell walls and vacuoles in grape skin and seed tissue and contact between the grape solids and the juice (Lerno et al., 2015). The extraction of polyphenols from fruit into wine is modulated by several factors secondary to fruit native phenolic content, including the relative skin, seed, and pulp content of fruit (Sparrow, Dambergs, Bindon, Smith, & Close, 2015), fermentation temperature (Lerno et al., 2015), wine ethanol concentration (Casassa, Beaver, Mireles, & Harbertson, 2013), and maceration technique (Busse-Valverde, Gómez-Plaza, López-Roca, Gil-Muñoz, & Bautista-Ortín, 2011).

Temperature affects both the rate and total extraction of polyphenols into grape must, with higher temperatures generally leading to higher extraction (Girard, Yuksel, Cliff, Delaquis, & Reynolds, 2001; Girard, Kopp, Reynolds, & Cliff, 1997; Lerno et al., 2015; Ough & Amerine, 1961; Sacchi, Bisson, & Adams, 2005). This effect of elevated temperatures on phenolic extraction has been attributed to heat-increased permeability of polyphenol containing grape hypodermal cells, which allows increased diffusion and solubility of polyphenols (Sacchi et al. 2005). Temperature affects extraction from seed tissue and skin

tissue differently, increasing the rate and total extraction of polyphenols from seed tissue over a set period linearly but increasing only the rate of extraction from skin tissue without increasing total polyphenol extraction (Lerno et al., 2015). Higher temperatures increase the initial rate of skin polyphenol extraction but do not increase the total amount of polyphenol extracted (Lerno et al., 2015). In other words, skin polyphenols exhibit a non-linear extraction pattern in relation to temperature whereas seed polyphenols exhibit a linear extraction model (Lerno et al., 2015). The mDP distribution of tannins in wine most closely resembles that of seed tannins (Casassa, 2017), implying that although skin tannins are extracted quickly and readily the relatively high portion of seed tannins present in fruit is reflected in the finished wine product.

1.3.3. Wine Color

Wine color is a function of anthocyanin content and composition, wine pH (Heredia et al., 1998), polymeric pigment content (Casassa & Harbertson, 2014; Somers, 1971; Somers & Evans, 1977), and anthocyanin copigmentation (Boulton, 2001). Wine color is described with CIE L*a*b* tri-stimulus colorimetry, measured by spectrophotometry. CIE L*a*b* color space describes wine color on three axes: L* represents light to dark, a* represents red to green, and b* represents blue to yellow. Hue angle and chroma represent perceived color and chromatic intensity, respectively.



1. Relationship of anthocyanin chroma and pH. Excerpted from Heredia et al., 1998.

The ratio of anthocyanins present as well as the total anthocyanin content of a wine affect wine color and differ between cultivars (Dimitrovska et al., 2011; González-Neves et al., 2007). Indeed, anthocyanin ratios have been proposed as varietal markers for identification of wines (Dimitrovska et al., 2011; González-Neves et al., 2007; Monagas, Núñez, Bartolomé, & Gómez-Cordovés, 2003). Anthocyanin color expression is dependent upon wine pH: as pH decreases, the equilibrium of anthocyanin forms shifts to favor the flavilium cation, which increases red hue, and as pH increases the equilibrium shifts to favor the quinoidal hydrobase, which increases blue hue (Heredia et al., 1998). Additionally, as pH increases there is a linear decrease in chroma value (Figure 1), indicating decreased chromatic intensity (Heredia et al., 1998).

Polymeric pigments, formed by the covalent polymerization of anthocyanins with flavan-3-ols or tannins (Casassa & Harbertson, 2014), provide protection for anthocyanins against oxidation (Jurd, 1969; Somers & Evans, 1977), which can be beneficial in wines made from cultivars that have relatively limited phenolics such as Pinot noir. However, as the formation of polymeric pigment may also lower wine saturation on accounts of their lower molar extinction coefficient relative to that of intact anthocyanins (Casassa, 2017; Weber, Greve, Durner, Fischer, & Winterhalter, 2013), increasing polymeric pigments may

result in comparative decreases in wine color saturation, although the full chromatic properties of polymeric pigments are not yet known. Saturation may be lowered through the transformation (and subsequent reduction) of anthocyanins, or through the modulation of the chromatic properties of the anthocyanin subunit following a reduction of the molar extinction coefficient relative to the native anthocyanin, although there is only indirect experimental evidence of this molar extinction coefficient reduction (Casassa, Larsen, et al., 2013; Weber et al., 2013). Notwithstanding, polymeric pigments generally provide desirable mouthfeel properties as they are less astringent than tannins of the same molecular weight (Casassa & Harbertson, 2014). Polymeric pigments are classified as large or small based on their ability to precipitate proteins during the Adams-Harbertson tannin assay, in which a sample of wine undergoes protein precipitations and bisulfite bleaching, which differentiates polymeric pigments from monomeric anthocyanins (Harbertson, Picciotto, & Adams, 2003). Within polymeric pigments, large polymeric pigments can precipitate proteins, creating astringency and altering wine mouthfeel (Weber et al., 2013), whereas small polymeric pigments cannot precipitate proteins.

Copigmentation entails the non-covalent bonding of anthocyanins to other, generally uncolored, phenolic compounds known as cofactors (Boulton, 2001). Flavonols and other cofactors engage in π - π interactions with anthocyanins in a reaction known as copigmentation which causes a hyperchromatic shift, resulting in enhanced color intensity (Bimpilas et al., 2016; Boulton, 2001; Jordheim et al., 2006). The ability of this interaction to produce positive results is described primarily anecdotally, although some studies have found that this copigmentation association accounts for almost half of the observed color in "young red wines" (Boulton, 2001). The role of cofactors and copigmentation in wine is threefold: first, binding free anthocyanins into a copigmented form enables more pigment to be retained, resulting in higher total anthocyanin content; second, the copigmented anthocyanins provide more color than they would in the free form (Boulton, 2001); third, copigmentation is considered a necessary step to color stabilization in wines via the formation of polymeric pigments (Boulton, 2001).

2. AGRONOMICAL AND CHEMICAL EFFECTS OF THE TIMING OF CLUSTER THINNING ON PINOT NOIR (CLONE 115) GRAPES AND WINES

The second chapter of this thesis consists of an article that was published in *Fermentations* on July 31, 2018, volume 4, issue 3, a special issue on wine fermentation. This article was written and published as a comparison of the cool 2016 growing season and the warm 2017 growing season, focusing on wine chemistry and color. Co-authors Dr. Jean C. Dodson Peterson and Dr. L. Federico Casassa. The article has been reformatted to integrate within this thesis.

2.1. Abstract and Keywords

A two-year study was performed to evaluate the effects of the timing of cluster thinning on Pinot noir grapes and wines in the central coast of California. Vines were thinned to one cluster per shoot at three selected time-points during the growing season, and fruit was harvested and made into wine. No consistent effect of cluster thinning was found in wine phenolic profile or color across a cool (2016) and a warm (2017) growing season. The growing season proved to have a more significant effect than the cluster thinning treatment for most parameters measured. There was no detectable overall sensory difference between the non-thinned control wines and any of the thinned treatment wines. Based on current results, Pinot noir vineyards on the central coast of California can support crop loads that result in Ravaz Index values from 3 to 6 without concern for impacting ripening potential or negatively affecting fruit composition.

Keywords: cluster thinning; yield manipulation; vine balance; crop load; Pinot noir; Central Coast of California

2.2. Introduction

Pinot noir (*Vitis vinifera* L.) is a challenging grape cultivar from both a viticultural and winemaking perspective. Viticulturally, Pinot noir grapevines produce compact clusters of thin-skinned berries, which increase susceptibility to fungal pathogens relative to other *V. vinifera* cultivars. Pinot noir grapes (and their wines) are also inherently low in phenols (Dimitrovska et al., 2011; Harbertson et al., 2008). Phenols are biomolecules originally present in the grapes (and subsequently extracted into wine). Phenols can be broadly classified as simple phenols having a C6–C1 or C6–C3 structure and a single aromatic ring containing one

or more hydroxyl groups; and polyphenols, which contain multiple phenol rings and are defined by a C6−C3−C6 structure bearing hydroxyl and non-hydroxyl substitutions (Waterhouse, 2002). In wines, polyphenols are responsible for color (Bimpilas et al., 2016; Boulton, 2001; Jordheim et al., 2006), tactile sensations such as astringency (Casassa & Harbertson, 2014; Poncet-Legrand et al., 2007), and taste sensations such as bitterness (Ferrer-Gallego et al., 2014; Harbertson et al., 2012; Lesschaeve & Noble, 2005). In addition to sensory effects on astringency, taste, and aroma modulation (Villamor et al., 2013), flavonoids also play a critical role in the chemical stability of the wine during aging as these molecules intervene in metal-catalyzed oxidation reactions (Danilewicz, 2003; Fulcrand et al., 2006). Because of the relatively lower phenolic content of Pinot noir, wines produced from it are lighter in color and astringency than wines made from other cultivars (Cliff et al., 2007). Pinot noir is also notable for lacking acylated anthocyanins (Dimitrovska et al., 2011), which are abundant in other cultivars such as Cabernet Sauvignon or Syrah and may in turn provide more stable color (Brouillard et al., 2003; Van Buren et al., 1968). As color is one of the main drivers of perceived wine quality (Somers & Evans, 1974), viticultural practices such as cluster thinning are often applied to Pinot noir grapes in an attempt to lower yields and influence fruit polyphenol composition by lowering vine crop load (Gamero et al., 2014; Guidoni et al., 2002; Keller et al., 2005; Reynolds et al., 1994; Santesteban et al., 2011; Uzes & Skinkis, 2016; Valdés et al., 2009).

Polyphenols such as anthocyanins and tannins, and their reaction products, known as polymeric pigments (Casassa & Harbertson, 2014), are positively associated with wine quality (Somers & Evans, 1974). In turn, and as mentioned above, vineyard crop load manipulation techniques such as cluster thinning are often applied to influence phenolic development (Gamero et al., 2014; Guidoni et al., 2002; Keller et al., 2005; Reynolds et al., 1994; Santesteban et al., 2011; Uzes & Skinkis, 2016; Valdés et al., 2009). The traditional yield to fruit quality paradigm of a linear relationship with quality increasing as yield decreases (Gamero et al., 2014; Palliotti & Cartechini, 2000; Reynolds et al., 2007; Uzes & Skinkis, 2016), has been shown to be an oversimplification, and yields are more accurately described as a function of vine balance (Bravdo et al., 1985; Jackson & Lombard, 1993). Vine balance, better described as the source/sink ratio, relates vine vegetative and reproductive growth, either through leaf area/yield (LA/Y) ratios (Kliewer & Dokoozlian, 2005) or through the ratio between dormant vine pruning weights and yields, the latter known as the Ravaz Index (Ravaz, 1903; Smart et al., 1990). For most cultivars, in warm climates, 0.8–1.2 m^2 of

leaf area is needed to ripen 1 kg of fruit, which generally results in a yield/pruning weight ratio of 5 to 10 (Kliewer & Dokoozlian, 2005).

Crop load metrics are dependent upon vine capacity, which is in turn influenced by regional and viticultural factors such as climate (Frioni et al., 2017), canopy training and trellising (Reeve et al., 2018), rootstock, and cultivar (Keller et al., 2005; Moreno Luna, Reynolds, & Di Profio, 2017). Grapes grown in cool climates require higher source/sink ratios than those grown in warmer regions because of lower daytime temperatures that restrict both leaf photosynthetic capacity and berry carbon assimilation (Howell, 2001; Jackson & Lombard, 1993; Reeve et al., 2018). As a result of this restricted photosynthetic capacity, the ability of grapes grown in cool climates to ripen fruit to commercially viable total soluble solids (TSS) levels is limited and is significantly affected by seasonal variations in weather (Frioni et al., 2017; Reeve et al., 2018).. As such, in cool climates, seasonal variations in weather may have a greater effect than the source/sink ratio on Pinot noir ripening (Frioni et al., 2017).

Despite the prevalence of cluster thinning in Pinot noir, few studies have been conducted to investigate the effects of this viticultural technique on this cultivar. Indeed, most studies on cluster thinning have been conducted in warm, arid climates and on cultivars such as Cabernet Sauvignon (Bergqvist et al., 2001; Cañón et al., 2014) and Tempranillo (Gamero et al., 2014; Gil-Muñoz et al., 2009; Valdés et al., 2009). The bulk of this research suggests conflicting results, whereby it has not been conclusively shown that manipulating yields by cluster thinning will uniformly affect fruit composition. For example, some research has indicated that cluster thinning can positively impact fruit composition (Ough & Nagaoka, 1984; Palliotti & Cartechini, 2000; Reynolds et al., 2007), while other studies have found no effect on fruit composition (Cañón et al., 2014; Dayer, Prieto, Galat, & Perez Peña, 2013; Freeman & Kliewer, 1983; Keller et al., 2005) or that the effect of cluster thinning was dependent upon the climate conditions of any single growing season (Gamero et al., 2014; Guidoni et al., 2002; Keller et al., 2005). In other instances, compositional effects as a result of cluster thinning have been found in grapes, but these have not translated into the finished wines (Reynolds et al., 2007). Pinot noir grown in cool climates may be more suited to benefit from cluster thinning because of the inherently low polyphenol content of the fruit and reduced carbon assimilation capacity of vines in cool climates (Howell, 2001; Jackson & Lombard, 1993; Reeve et al., 2018).

The timing of cluster thinning may also have an impact on vine physiology and fruit composition. For example, it has been hypothesized that removing crop at bloom may lead to lower leaf transpiration rates, and therefore lower leaf photosynthesis rates (Naor et al., 1997), which could negate the desired effect of enhanced berry ripeness (Keller et al., 2005). In addition, if photosynthesis rates remain unchanged but the source/sink ratio increases upon cluster thinning, the increased photo-assimilates may stimulate vegetative growth, counteracting or negating the benefits of the decreased crop load (Jackson & Lombard, 1993; Smart et al., 1990). In a study spanning five seasons, early thinning at bloom increased berry weight in Cabernet Sauvignon, Riesling, and Chenin blanc, while late thinning performed at véraison was intermediate to early thinning and non-thinned vines (Keller et al., 2005). However, this effect was not found in all years of the previous study. Cluster thinning applied at bloom to Pinot noir vines resulted in increased berry size (Reynolds et al., 1994), although there was no late thinning treatment included in the study. Other research has shown no effect of thinning on berry size in Tempranillo (Valdés et al., 2009), thereby suggesting that there is likely a cultivar-specific response of berry size as a result of cluster thinning. Berry size reduction has traditionally been considered desirable from a winemaking perspective based on the empirical assumption that comparatively smaller berries have higher berry surface area/volume than larger ones. However, the relationship between berry size and phenolic composition is not a simple linear relationship, as berry skin and seed mass grow along with berry size (Matthews & Nuzzo, 2007), and therefore larger berries may not be necessarily undesirable as once thought. Multiple studies conducted with a variety of cultivars investigating the relationship between cluster thinning and berry size have found conflicting results dependent upon cultivar and growing season (Keller et al., 2005; Reynolds et al., 1994; Valdés et al., 2009), and as such, there is no current conclusive understanding of the relationship between cluster thinning and berry size, and the subsequent effect of the latter on wine composition.

In the central coast of California (USA), consisting of Santa Barbara, San Luis Obispo, and Monterey counties, there are over 7000 hectares of Pinot noir being grown ("Grape acreage report," 2018), representing a substantial contribution to the wine industry of the region. Indeed, in 2016, wine grapes were the most valuable crop produced in San Luis Obispo county ("Annual crop report," 2017). Despite the economic importance of Pinot noir on the central coast of California, no research has been undertaken to understand the relationship between Pinot noir crop load and fruit quality in the cool climate of San Luis Obispo county

of the central coast. While research in cooler areas such as Oregon's Willamette Valley (USA) have indicated that grape ripeness increased in a curvilinear fashion with increasing LA/Y ratios up to 1.25–1.75 m^2/kg (Reeve et al., 2018), which is higher than the LA/Y ratios observed in warm climates (Kliewer & Dokoozlian, 2005), these source/sink ratios are intrinsically tied to the seasonal limitations of the region that result in inconsistent ripening and may not be translatable to more moderate cool climates such as California's Central Coast.

In the present study, the effect of crop load reduction by cluster thinning was explored for the first time on Pinot noir grapes (clone 115) and wines grown in the cool climate conditions of the Edna Valley of the Central Coast (San Luis Obispo county) of California (USA). The objectives of this study were to evaluate the effect of the timing of cluster thinning crop reduction on vine capacity, berry composition, and wine composition over two consecutive seasons. An additional objective was to identify appropriate crop loads in cool climate Pinot noir grown on the central coast of California.

2.3. Materials and Methods

2.3.1. Vineyard Site

This study was conducted at a commercial vineyard located in Edna Valley (35°11′58.3″ N 120°34′12.6″ W), San Luis Obispo county, California, during the 2016 and 2017 growing seasons. Treatments of cluster thinning were applied to Pinot noir grapevines (clone 115) planted in 1996 on 5C rootstock. Cluster thinning was applied to these Pinot noir grapevines at selected phenological growth stages. Cluster thinning was conducted by removing the second cluster from each fruiting shoot of the vine. Any third clusters (second crop) were also removed during the treatment, resulting in one cluster per fruiting shoot. No second-crop thinning or reduction was performed in the control vines. Vineyard treatments were applied as 100-vine sets replicated five times (n = 5), organized as a randomized complete block. Bloom was defined as stage 23 on the modified Eichhorn and Lorenz scale (Coombe, 1995), and occurred on 1 June in 2016 and 15 May in 2017. Cluster thinning was applied at four weeks post-bloom (bloom + 4) approximating fruit set, eight weeks post-bloom (bloom + 8) approximating véraison, and 12 weeks post bloom (bloom + 12) shortly before harvest. Vines were pruned to two ten bud canes trained in a vertical shoot positioning (VSP) system with two catch-wires. Canes removed during the 2018 winter pruning were collected and weighed on a per-vine basis to determine 2017 growing season vegetative growth. Vine spacing

was 2.75 × 1.52 m in north–south aligned rows planted in silty clay loam soil. Precipitation and daily minimum and maximum temperatures were recorded from California Irrigation Information Management System (CIMIS) weather station 52 (35°18′19.6″ N 120°39′42.4″ W), located 14.41 kilometers from the experimental site. Cumulative growing degree days (GDD) were calculated using a baseline temperature of 10 °C and the average daily temperature from 1 April to 31 October of each year (Winkler et al., 1974).

2.3.2. Winemaking

Fruit was harvested when a composite sample of all treatments (n = 25, 250 berries each) reached 22.5 Brix. Harvest dates were 6 September 2016 and 6 September 2017. Fruit was harvested manually from three independent vineyard replications of each treatment (n = 3). Approximately 80 kg of fruit per replicate was harvested both in 2016 and 2017, for a total of 960 kg of fruit harvested in each season. The three replicates of each of the five treatments were independently destemmed and crushed using a crusher–destemmer (Bucher Vaslin, Niederweningen, Switzerland), and placed separately in individual 60-L plastic containers (Speidel, Swabia, Germany), where fermentation took place. Musts were inoculated with a commercial wine yeast (*Saccharomyces cerevisiae*, EC-1118, Lallemand, Rexdale, ON, Canada) at a rate of 30 g/hL. Musts were inoculated with commercial malolactic bacteria 48 hours after crushing to ensure a standardized fermentation across treatments. In 2016, musts were inoculated with VP-41 (*Oenococcus oeni*, Scott Laboratories, CA, USA); in 2017, musts were inoculated with ML Prime (*Lactobacillus plantarum*, Lallemand, Rexdale, ON, Canada). Temperature and Brix were followed daily during alcoholic fermentation using a density meter (Anton Paar, Graz, Austria) with temperature and sugar consumption curves showing good reproducibility within replicates of the same and different treatments (data not shown). Following 10 days of maceration, wines were drained off from solids, with free run wines immediately transferred to 20-L glass carboys fitted with airlocks until the completion of malolactic fermentation. Following the completion of malolactic fermentation, wines were adjusted to 0.3 mg/L molecular SO_2, bottled using a DIAM 5 microagglomerated cork closure (G3 Enterprises, Modesto, CA, USA) and kept in cellar-like conditions until analysis.

2.3.3. Fruit Composition

Berry chemistry and physical properties were measured at harvest from random samples of 250 berries taken from each replication (n = 3). Brix was measured using a density meter (Anton Paar, Graz, Austria); titratable acidity (TA) was measured by titrating a known quantity of juice (5 mL) in a deionized water solution against 0.067 N NaOH (Fisher Scientific, Waltham, MA, USA) to a pH endpoint of 8.2 in accordance with an established procedure (Iland et al. 2004); pH was measured with a Benchtop pH meter (ThermoFisher Scientific, Waltham, MA, USA. Yeast assimilable nitrogen (YAN) was measured enzymatically from juice utilizing an analyzer and commercially available kits (Biosystems, Barcelona, Spain). Individual vine pruning weights were taken during vine dormancy and compared with individual vine fruit yields to calculate vine Ravaz Index (Ravaz, 1903; Smart et al., 1990).

2.3.4. Wine Composition

Wine titratable acidity (TA) and pH were measured in the same method as juice TA and pH. Wine ethanol was measured with an alcolyzer wine M/ME analysis system (Anton Paar, Graz, Austria); wine residual sugars, acetic acid, lactic acid, and malic acid were measured with a Y15 analyzer (Biosystems, Barcelona, Spain) using commercial enzymatic analysis kits (Biosystems, Barcelona, Spain). Wine phenolics and color were measured at pressing and following the completion of malolactic fermentation (malic acid < 0.4 g/L). Anthocyanins, non-tannin phenolics, small polymeric pigments (SPP), large polymeric pigments (LPP), and total polymeric pigments (herein reported as SPP + LPP), were measured as previously described (Harbertson et al., 2003). Tannins in the wines were analyzed by protein precipitation (Harbertson et al., 2002). Full-visible-spectrum absorbance scans were taken using a spectrophotometer (Cary UV-VIS60, Agilent Technologies, Santa Clara, CA, USA) and absorbance data is used to construct visible light absorbance curves and run through Cary WINUV Color module software (Agilent Technologies, Santa Clara, CA, USA) to extract CIE-L*a*b* tri-stimulus colorimetry values (D65 illuminant).

2.3.5. Duo-Trio Test

The 2016 wines were analyzed three months after bottling for overall sensory difference using a duo-trio test with constant reference as described (Meilgaard Civille, G. V., & Carr, B. T., 1999). Briefly, the test was administered to 21 enology students of the Wine and Viticulture Department, Cal Poly San Luis Obispo.

After a brief training session (1 h) to familiarize the subjects with the test, each subject received three consecutive flights, each containing three wine ISO glasses (Libbey, Toledo, OH, USA). One glass was labeled as R ("reference") and the other two glasses were labeled with three-digit random code numbers. All the treatment replicates were contrasted against all the replicates of the control treatment. Significance was established at $p < 0.05$ and for $n = 21$, 15 correct responses were needed to establish an overall sensory difference between any of the control wines and any of the cluster thinning treatments (Meilgaard Civille, G. V., & Carr, B. T., 1999). The wines were poured 30 min before the sessions and glasses were covered with plastic lids to trap volatiles, with each glass receiving exactly 25 mL of wine.

2.3.6. Statistical Analyses

Statistical analyses were carried out with JMP (SAS Institute, Cary, NC, USA) for analyses of variance (ANOVA). Fisher's least significant difference (LSD) test at the $\alpha = 0.05$ level was used for means separation. Two-way ANOVA models considering treatment and growing season were carried out for all parameters with data from both growing seasons. Statistical analysis on juice and wine pH was performed using the hydrogen ion concentration and presented as pH for clarity.

2.3. Results

2.3.1. Seasonal Climate

Weather data from the San Luis Obispo CIMIS weather station (Station 52, located 14.41 km from the vineyard site) was used to calculate climatological parameters during the study (Table 7). Growing degree days (GDD) were calculated for each season of the study. While GDD accumulation in 2016 placed the vineyard site in region II, considered to be a 'cool climate', there was sufficient heat in 2017 during the growing season to classify San Luis Obispo as region III, which corresponds with a 'moderately warm' climate (Winkler et al., 1974).

1. Growing degree days (GDD); Winkler region classification; and precipitation for San Luis Obispo, California (USA).

Growing Season	Growing Degree Days (GDD) [1]	Winkler Region	Annual Precipitation (mm) [2]	Seasonal Precipitation (mm) [3]
2016	1462.1	II	521.9	66.2
2017	1780.6	III	733.6	79.7

[1] Calculated from 1 April–31 October in Celsius units with a baseline of 10 °C; [2] Sum of precipitation from 1 January–31 December; [3] Sum of precipitation from 1 April–31 October.

2.3.2. Yield

As intended, all cluster thinning treatments resulted in a reduction of clusters per vine and vine fruit yield relative to the non-thinned control (Table 2). There was no significant difference in either cluster number or vine yield between thinning treatments, indicating that the thinning treatment was evenly applied. Cluster weight was lower in bloom + 8 relative to control fruit (Table 2). However, the growing season had a larger effect on cluster weight than the thinning treatments, with cluster weights being significantly higher in the warmer 2017 growing season than in the cooler 2016 growing season (Table 13). Berry weight was generally unaffected by the timing of cluster thinning, with the effect of the growing season having a significant impact on this parameter. Indeed, berry weight was generally higher in the cooler 2016 growing season (Table 13). No significant treatment × growing season interaction was found in any yield component, indicating that the effect of the timing of cluster thinning on yield components was equivalent in both the cooler 2016 and warmer 2017 growing seasons. In addition, in 2017, pruning weights were collected and the Ravaz index was calculated to assess vine balance. Non-thinned control vines had a Ravaz Index of 3.23 (Table 13). The Ravaz Index was lower in bloom + 4 vines relative to control vines; bloom + 8 and bloom + 12 vines were indistinguishable from control vines or one another (Table 13).

2. Two-way analysis of variance (ANOVA) with interaction showing mean values and *p-values* of vine yield components by cluster thinning treatment. Combined two-year averages followed by standard error of the mean. Also shown are *p-values* corresponding to main effects and the interaction between treatments and growing season. Different letters within a column indicate significant differences between treatment groups for Fisher's least significant difference (LSD) test at $p < 0.05$. p-values below 0.05 are shown in bold font.

Treatment	Clusters per Vine	Yield per Vine (kg)	Cluster Weight (g)	Berry Weight (g)
Control	32.13 ± 3.05 a	2.43 ± 0.34 a	74.80 ± 6.35 a	1.08 ± 0.06 a
Bloom + 4	21.06 ± 3.03 b	1.27 ± 0.14 b	64.42 ± 5.98 ab	1.05 ± 0.03 a
Bloom + 8	21.52 ± 0.99 b	1.34 ± 0.15 b	58.82 ± 5.78 b	0.96 ± 0.03 a
Bloom + 12	22.10 ± 1.59 b	1.44 ± 0.15 b	65.91 ± 6.35 ab	1.07 ± 0.07 a
Treatment (T)	**0.0228**	**0.0029**	0.2179	0.3189
Growing Season (S)	0.7436	0.0569	**0.0008**	**0.0489**
T × S Interaction	0.8149	0.6569	0.8883	0.5432

2.3.3. Fruit Composition

All treatments were harvested manually on a single day in both 2016 and 2017. Table 3 shows Brix, titratable acidity (TA), and pH, which were determined at harvest. No significant difference was found in Brix level at harvest between treatments or between growing seasons (Table 3), indicating no effect of cluster thinning at any point during the growing season on the ability of fruit to ripen at this site. Fruit pH was lower in bloom + 12 relative to all other treatments and the non-thinned control (Table 3). There was a significant effect of the growing season on fruit pH, indicating that the growing season had a larger effect than treatments on fruit pH, with the cooler season resulting in lower fruit pH (Table 13). There was no significant treatment × growing season interaction on fruit Brix or pH, suggesting that the effect of cluster thinning timing (or lack thereof) was the same across both a warm growing season (2017) and a cool growing season (2016). No consistent effect of treatment on fruit TA was seen across both growing seasons (Table 3). In 2016, which was the cooler growing season, bloom + 12 showed higher fruit TA relative to control fruit and in 2017, the warmer growing season, bloom + 12 had lower fruit TA relative to control fruit (Table 13). However, no difference was observed in 2017 (Table 13). There was a significant interaction of treatment and growing season, indicating that the effect of cluster thinning timing on fruit TA was dependent on environmental factors pertaining to the climate of the individual growing seasons.

3. Two-way analysis of variance (ANOVA) with interaction showing mean values and p-values of fruit composition parameters at harvest by cluster thinning treatment. Combined two-year averages followed by standard error of the mean. Also shown are *p-values* corresponding to main effects and the interaction between treatments and growing season (n = 3). Different letters within a column indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Treatment	Brix	pH	Titratable Acidity (g/L)
Control	22.28 ± 0.30 a	3.54 ± 0.03 a	6.35 ± 0.17 a
Bloom + 4	22.42 ± 0.24 a	3.53 ± 0.04 ab	6.37 ± 0.12 a
Bloom + 8	22.72 ± 0.14 a	3.55 ± 0.04 a	6.35 ± 0.14 a
Bloom + 12	22.22 ± 0.19 a	3.48 ± 0.05 b	6.59 ± 0.50 a
Treatment (T)	0.3237	0.1246	0.725
Growing Season (S)	0.4125	**<0.0001**	**0.0115**
T × S Interaction	0.0585	0.3309	**0.001**

2.3.4. Wine Composition

2.3.4.1. Wine Basic Chemistry

Basic wine chemistry was analyzed on the finished wines at the time of bottling. Bloom + 4 wine had higher pH relative to the control and bloom + 12 wines (Table 4). The growing season also significantly affected wine pH (Table 4), with pH being higher in the warmer growing 2017 season. However, there was not a significant treatment × growing season interaction, indicating that while seasonal variation in environment did influence wine pH, the effect of cluster thinning on wine pH was not affected by seasonal variation. No effect of thinning treatment was found on wine TA (Table 4). Wine TA levels were significantly higher in 2016 than 2017, indicating an effect of the growing season (Table 15). No effect of thinning treatment was found on ethanol (Table 4). However, wine ethanol levels were higher in 2016 than in 2017 (Table 15), indicating, once again, a significant effect of the growing season over the cluster thinning treatments on the basic chemistry of the resulting wines. Bloom + 4 and bloom + 8 wines had significantly higher acetic acid levels relative to control and bloom + 12 wines (Table 4), which were statistically indistinguishable from one another. Bloom + 4 and bloom + 8 thinning treatments resulted in higher wine acetic acid levels relative to bloom + 12 and control wines (Table 4), which were statistically indistinguishable from one another. Wine acetic acid was lower in 2017 relative to 2016 (Table 16), and a significant interaction of treatment and growing season was observed (Table 4), indicating that seasonal variation in climate affected the impact of cluster thinning treatments on wine acetic acid. Although there

were differences observed in wine acetic acid, because of the low nature of the acetic acid levels in the wines, these differences are unlikely to be of sensory relevance.

2.3.4.2. Wine Phenolics

Wine phenolics, including anthocyanins, tannins, polymeric pigments, and total phenolics, were measured as previously described (Harbertson et al., 2003). There was no effect of cluster thinning treatment on wine anthocyanins or polymeric pigments in either growing season (Table 5). Total phenolics, the summation of total tannins and non-tannin phenolics, was significantly higher in bloom + 4 relative to other cluster thinning treatments, although none of the treatments were statistically distinguishable from the control (Table 5). Polymeric pigments, tannins, and non-tannin phenolics exhibited greater differences due to seasonal variation than to the cluster thinning treatment (Table 5), with polymeric pigments and tannins being lower in the warmer season than in the cooler season and non-tannin phenolics being higher in the warmer season (Table 18).

2.3.4.3. Wine Color

Wine CIE L*a*b* color space values were determined in the finished wines at bottling. Bloom + 12 wines exhibited higher a* and chroma than bloom + 4 and bloom + 8 wines, but all were statistically indistinguishable from control wines (Table 6). No effect of cluster thinning treatment was found in wine L*, b*, or hue angle. The growing season had a comparatively higher impact than cluster thinning treatment in every chromatic parameter (Table 6), with 2016 wines having lower L*, b*, and hue angle than 2017 wines, and higher a* and chroma than 2017 wines (Table 17). No treatment × growing season interaction was found in any chromatic parameter.

4. Two-way analysis of variance (ANOVA) with interaction showing mean values and *p-values* of wine composition parameters post-malolactic fermentation by cluster thinning treatment. Combined two-year averages followed by standard error of the mean. Also shown are *p-values* corresponding to main effects and the interaction between treatments and growing season ($n = 3$). Different letters within a column indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Treatment	pH	Ethanol (% v/v)	Titratable Acidity (g/L)	Acetic Acid (g/L)	Lactic Acid (g/L)	Malic Acid (g/L)	Residual Sugar (g/L)
Control	3.81 ± 0.03 b	13.03 ± 0.16 a	5.36 ± 0.29 a	0.25 ± 0.04 b	1.23 ± 0.06 b	0.09 ± 0.03 a	0.45 ± 0.03 ab
Bloom + 4	3.86 ± 0.03 a	13.05 ± 0.16 a	4.91 ± 0.25 a	0.31 ± 0.03 a	1.32 ± 0.06 a	0.09 ± 0.02 a	0.43 ± 0.02 ab
Bloom + 8	3.84 ± 0.03 ab	13.17 ± 0.16 a	5.21 ± 0.24 a	0.31 ± 0.02 a	1.25 ± 0.07 b	0.04 ± 0.02 a	0.41 ± 0.02 b
Bloom + 12	3.77 ± 0.02 c	13.24 ± 0.12 a	5.25 ± 0.16 a	0.24 ± 0.04 b	1.32 ± 0.05 a	0.06 ± 0.03 a	0.47 ± 0.01 a
Treatment (T)	**0.0011**	0.4711	0.2637	**<0.0001**	**0.0046**	0.4302	0.1378
Growing Season (S)	**<0.0001**	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**	0.6511	0.0024
T × S Interaction	0.6207	0.7141	0.6896	**0.0031**	0.2296	0.5036	0.8459

5. Two-way analysis of variance (ANOVA) with interaction showing mean values and *p-values* of wine phenolic parameters post-malolactic fermentation by cluster thinning treatment. Combined two-year averages followed by standard error of the mean. Also shown are *p-values* corresponding to main effects and the interaction between treatments and growing season ($n = 3$). Different letters within a column indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Treatment	Anthocyanins (mg/L Malvidin Equivalents)	Polymeric Pigments (Absorbance at 520 nm)	Tannins (mg/L CE [1])	Non-Tannin Phenolics (mg/L CE [1])	Total Phenolics (mg/L CE [1])
Control	193.29 ± 2.80 a	0.86 ± 0.14 a	22.85 ± 7.43 ab	520.11 ± 20.64 ab	542.96 ± 22.86 ab
Bloom + 4	197.43 ± 4.00 a	0.95 ± 0.11 a	26.99 ± 4.35 a	553.86 ± 11.40 a	580.85 ± 8.85 a
Bloom + 8	197.56 ± 3.96 a	0.82 ± 0.10 a	16.64 ± 1.42 ab	516.66 ± 16.87 b	533.30 ± 16.29 b
Bloom + 12	197.22 ± 5.43 a	0.85 ± 0.10 a	14.66 ± 0.94 b	522.81 ± 10.91 ab	537.46 ± 10.92 b
Treatment (T)	0.8413	0.4305	0.1078	0.1629	0.1175
Growing Season (S)	0.2201	**<0.0001**	**0.0304**	**0.0183**	0.1208
T × S Interaction	0.2255	0.8451	0.1186	0.0672	0.2156

[1] CE: Catechin equivalents.

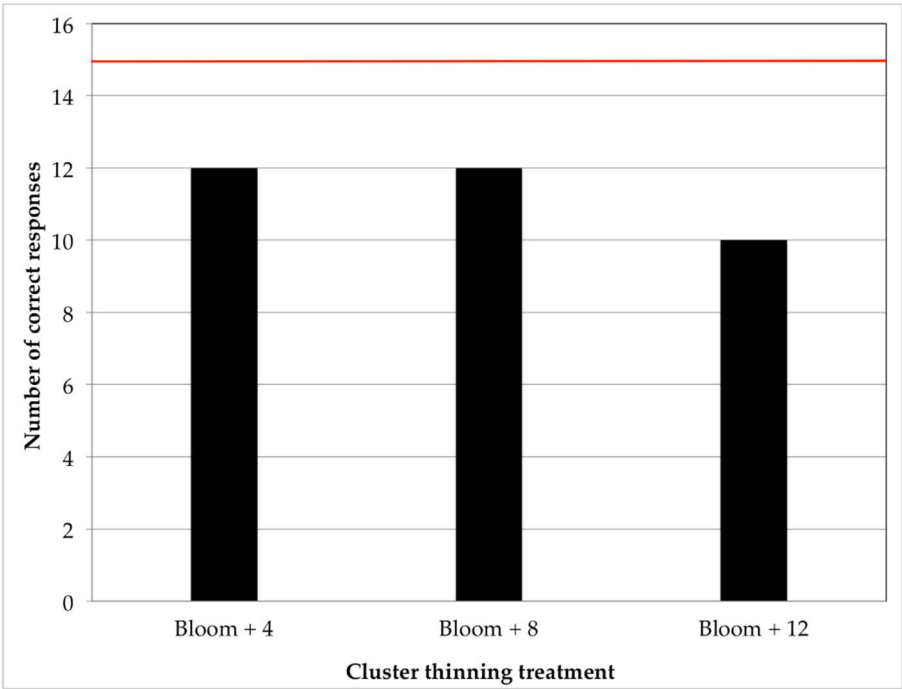
6. Two-way analysis of variance (ANOVA) with interaction showing mean values and *p-values* of wine CIE L*a*b* chromatic parameters post-malolactic fermentation by cluster thinning treatment. Combined two-year averages followed by standard error of the mean. Also shown are *p-values* corresponding to main effects and the interaction between treatments and growing season (n = 3). Different letters within a column indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Treatment	L*	a*	b*	Hue Angle	Chroma
Control	85.34 ± 2.14 a	18.37 ± 2.60 ab	2.51 ± 1.53 a	−0.86 ± 0.60 a	19.03 ± 2.31 ab
Bloom + 4	85.67 ± 2.32 a	17.67 ± 2.79 b	2.55 ± 1.38 a	−0.34 ± 0.54 a	18.31 ± 2.51 b
Bloom+8	85.85 ± 2.24 a	17.71 ± 2.61 b	2.65 ± 1.39 a	−0.28 ± 0.49 a	18.36 ± 2.32 b
Bloom+12	84.37 ± 1.80 a	19.57 ± 2.46 a	2.71 ± 1.62 a	−0.77 ± 0.54 a	20.24 ± 2.17 a
Treatment (T)	0.1982	0.1149	0.8018	0.4819	0.1072
Growing Season (S)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
T × S Interaction	0.3599	0.8472	0.067	0.5101	0.8306

2.3.5. Duo-Trio Test

Figure 2 shows a duo-trio test performed in the 2016 wines three months after bottling. Under the "constant reference" variant of this test, each wine treatment is contrasted against the control (Meilgaard Civille, G. V., & Carr, B. T., 1999). The results of this test indicated that none of the cluster thinning treatments produced wines that had overall sensory differences relative to the control wine. The panel (n = 22) failed to find an overall sensory difference between the control and any of the cluster thinning wines.

Based on these results and considering that a panel of four experience industry professionals deemed the differences in the 2017 wines to be even less evident than those in 2016, no sensory analysis was performed in the 2017 wines.



2. Results of the duo-trio test with constant reference performed in the 2016 wines. Each cluster thinning treatment was contrasted against the control treatment. A total of 15 correct responses were needed for a statistically distinguishable sensory difference ($p < 0.05$). The horizontal red line indicates the number of correct responses required to attain statistical significance.

2.4. Discussion

A study was conducted over two growing seasons (2016 and 2017) to determine the agronomical effects (on grapes) and chemical effects (on the resulting wines) of cluster thinning timing in Pinot noir grown in the Edna Valley of California's central coast. A secondary objective of the study was to identify appropriate

crop loads for Pinot noir on the moderately cool climate of California's central coast. Cluster thinning treatments were applied at 4, 8, and 12 weeks post-bloom, approximating the timing of the phenological growth events of fruit set and véraison and including a pre-harvest "red drop". Thinning consisted of removing any second or third cluster from fruit bearing shoots, reducing cluster number by an average of 34.3% across all treatments (Table 2), and reducing yield by an average of 44.4% across all treatments (Table 2). Previous studies conducted on cluster thinning practices have applied variable yield reduction rates depending on cultivar. However, most research in Pinot noir has applied "half crop" treatments such as the one performed in the current study, removing all but the basal cluster on each fruiting shoot (Reeve et al., 2018) or reducing cluster number by 50% (Reynolds et al., 1994).

Growing degree day (GDD) accumulation varied between 2016 and 2017 enough to place each growing season in a separate Winkler Index region, with 2017 being warmer by 318.5 GDD than 2016 and qualifying as a region III (Table 1). An increase in average temperature by 1 °C increases GDD by 214 over the course of a growing season. The GDD variation observed at this site between 2016 and 2017 corresponds to approximately 1.5 °C higher average temperatures in 2017. It is well documented that vine capacity and the ability to achieve ripeness by Brix accumulation in cool regions is dependent upon climatic conditions such as temperature (Kliewer, 1973; Reeve et al., 2016; Winkler et al., 1974), with a base level of temperature required to adequately ripen fruit. However, Brix accumulation is not driven solely by temperature and, by extension, by GDD accumulation. In fact, differences in berry temperature may not directly impact Brix accumulation at all (Kliewer, 1973). Indeed, other factors such as soil moisture content (McIntyre et al., 1987) and berry light exposure (Dokoozlian & Kliewer, 1996) may also impact fruit ripening rate irrespective of atmospheric temperature and may even have a larger effect than GDD accumulation in cases where GDD accumulation is adequate for fruit ripening (McIntyre et al., 1987). Consequently, in the present study, there was no significant effect of the growing season on Brix at harvest to indicate an impact of the increased GDD accumulation in 2017 (Table 3), despite the fruit being picked on the same date in both growing seasons.

While in the present study there was no impact of the thinning treatments on Brix accumulation, the climatic conditions of the growing season did have a clear impact on fruit pH and TA (Table 3), resulting in lower TA and higher pH in 2017 fruit (Table 14). While bloom + 12 fruit did have significantly lower pH than control fruit (Table 3), after accounting for growing season in the model, the effect of cluster thinning

treatment was not significant, indicating that the climatic conditions prevalent during the growing season had a greater effect on fruit pH and TA than cluster thinning timing. The decrease of malate concentration in grapes post-véraison through respiration increases with temperature and light exposure (Price et al., 1995; Rienth et al., 2016); as such, the decrease in TA and the corresponding increase in pH observed in 2017, which was the warmer season (Table 14), is likely a function of the increased temperature. There was a significant treatment × growing season interaction found in TA (Table 3). In both growing seasons, bloom + 12 fruit had significantly different TA from the control treatment. However, the direction of the difference varied, with bloom + 12 having higher TA in 2016 and lower TA in 2017 relative to the control treatment. In both growing seasons, bloom + 4 was indistinguishable from the control treatment. Bloom + 8 was indistinguishable from the control or any treatment in 2016 and significantly lower than the control in 2017. Overall, there was no consistent effect of cluster thinning on fruit TA across both growing seasons (Table 3). Previous studies on Pinot noir, in which half of the crop was thinned, have found increased pH (Reeve et al., 2016; Reynolds et al., 1994) in both fruit and juice, but mixed effects on TA, showing either no impact (Reynolds et al., 1994) or a reduction in TA with cluster thinning (Reeve et al., 2016). While no significant treatment effect of pH was found in the present study after considering the growing season, the inconsistent results observed in bloom + 12 fruit is similar to what has been found in previous research, suggesting the influence of some external factor on malate degradation in late-thinned fruit. It is possible that late thinning resulted in more convective heat exchange between clusters and air within the canopy, which, given the substantially warmer air temperatures in August of 2017 (Table 19), resulted in an increase in berry temperature and therefore malate degradation in the 2017 bloom + 12 fruit that was not seen in 2016. Indeed, cooler air temperatures in 2016 during the same period (Table 19) may have resulted in decreased berry temperatures and the observed increase in fruit TA relative to the non-thinned control.

Wine pH is tied intrinsically to a wine's microbial and oxidative stability, with lower pH values inhibiting (synergistically with ethanol) microbial growth (Boban et al., 2010) and increasing the ability of phenolics to protect the wine from premature oxidation (Singleton, 1987). There was a significant effect of thinning timing on wine pH, with bloom + 12 having lower wine pH relative to the control in accordance with the observed lowered fruit pH. Conversely, the bloom + 4 wines showed higher wine pH relative to the control wines despite indistinguishable fruit pH (Tables 3 and 4). The growing season also significantly

affected wine pH (Table 4) with the warmer 2017 growing season resulting in higher wine pH than 2016, much like the effect seen on fruit pH (Table 14). However, the interaction for treatment and growing season was not significant, suggesting that while seasonal variances in environment do influence wine pH, environmental variance did not affect the response of wine pH to thinning timing. Interestingly, differences in fruit pH did not correspond linearly with differences in wine pH. Both growing season and treatment affected wine lactic acid content; fruit malic acid was measured in 2017 and no significant difference was found between treatments ($p = 0.38$, df = 4,7; data not shown), indicating that a difference in fruit malic acid content was not responsible for the differences observed in lactic acid content in 2017. Different strains of malolactic bacteria were used in 2016 and 2017; VP-41 (*Oenococcus oeni*) in 2016 and ML-Prime (*Lactobacillus Plantarum*) in 2017. Unrelated to malic acid content, *Lactobacillus* and *Oenococcus* fermentation activity in wine is affected by wine temperature, ethanol level, pH, and acetic acid levels (Guerzoni, Sinigaglia, Gardini, Ferruzzi, & Torriani, 1995), each of which exhibited some degree of variation within the wines that could be responsible for the differences observed between treatment groups in wine lactic acid and pH levels, irrespective of differences in fruit composition. Lower average and maximum fermentation temperatures in 2017 wines corresponded with higher wine pH and higher acetic acid levels (Tables A3 and A4), indicating that fermentation temperature was a likely contributor to differences in wine pH and acetic acid levels. Indeed, a two-way ANOVA utilizing treatment, average temperature, maximum temperature, and treatment × average temperature and treatment × maximum temperature interactions, found average temperature to be a significant predictor of wine pH ($p = 0.0136$) and acetic acid ($p = 0.0136$).

Despite no differences being found in fruit Brix (Table 3), wine ethanol was significantly higher in 2016 than 2017 (Table 16). As yeast and fermentation practices were constant between growing seasons, observed differences in ethanol content are most likely the result of variations of the alcohol conversion ratio of the yeast. Average must temperature during the 10-day maceration period in 2016 was 21.3 °C and 24.9 °C in 2017, with peak fermentation temperatures of 26.7 °C and 29.9 °C, respectively (Table 15). Similar to *Lactobacillus* and *Oenococcus* fermentation activity, temperature is one of the most influential factors on *Saccharomyces cerevisiae* fermentation activity and ethanol biosynthesis (Du et al., 2012; Ough & Amerine, 1967), with ethanol formation decreasing as fermentation temperature increases (Ough & Amerine, 1967). In addition to decreasing ethanol biosynthesis in wine yeast, increased fermentation temperatures also

increase the rate of ethanol volatilization, further lowering already diminished wine ethanol levels (Ough & Amerine, 1967).

The growing season also affected cluster and berry weight (Table 2), with 2017 resulting in fruit having 34% higher cluster weight and 9% lower berry weight relative to 2016 (Table 13). Several factors influence berry size, including berry temperature during various phenological growth stages, light incidence, water and nutrient supply, and seed number per berry (Coombe, 1976; Gillaspy et al., 1993; Kliewer, 1977; Van Volkenburgh, 1999). Berry size can be reduced by increased heat and resultant increased berry temperature prior to lag phase (Kliewer, 1977). Ambient temperature was on average 1.25 °C warmer in May 2017 than May 2016 (Table 19), which may explain current results. Cluster weight is a function of berry size and berry number, so as berry weight decreased while cluster weight increased, berry number must have increased in 2017. Typical berry set in wine grapes ranges from 20% to 50 (Mullins et al., 1992), and can be reduced by temperatures during bloom below 15 °C (Kliewer, 1977). From 15 April to 1 June, there were 29 days with an average air temperature below 15 °C in 2016 and 14 days in 2017 (Table 19). It is likely that warmer temperatures during bloom in 2017 resulted in a higher fruit set and therefore higher cluster weight than 2016.

Pruning weights were collected in January 2018 and Ravaz Index was calculated for the 2017 growing season. The non-thinned control vines had a Ravaz Index of 3.23, and Ravaz Index was not significantly different between treatments (Table 13). Within the control vines, one replication had a substantially lower cluster number than other replications, which inflated the deviation of cluster number, vine yield, and Ravaz Index of the sampled population (Table 13). This was confirmed by conducting outlier analysis of control treatment repetition 1, which, for the Ravaz Index model, had a Cook's Distance value of 16 (data not shown), indicating high influence on the model. It is possible that the low cluster number on the vines within this repetition is due to natural site variation (e.g., block to block variations in soil composition or water holding capacity), affecting vine capacity. While the abnormally low cluster number affected vine yield, cluster number, and Ravaz Index, little influence of this repetition was found in models of fruit or wine chemistry, with no Cook's distance value above 0.5 (data not shown). As a result of the potential impact of eliminating one of the three replications of the control treatment from the dataset on experimental balance and statistical analysis, the outlier was retained within the dataset.

Phenolic composition (Table 5) and wine color (Table 6) were not affected consistently by any of the thinning treatments, but all chromatic parameters as well as wine polymeric pigment, tannin, and non-tannin phenolic content were significantly affected by growing season. Polymeric pigments, formed by the covalent polymerization of anthocyanins with flavan-3-ols or tannins (Casassa & Harbertson, 2014) provide protection for anthocyanins against oxidation (Jurd, 1969; Chris Somers & Evans, 1977), which can be beneficial in wines made from cultivars lacking in phenolics, such as Pinot noir. However, as their formation may also lower wine saturation on accounts of their lower molar extinction coefficient relative to that of intact anthocyanins (Casassa, 2017), increasing polymeric pigments may result in comparative decreases in wine color saturation. Notwithstanding, polymeric pigments generally provide desirable mouthfeel properties as they are less astringent than tannins of the same molecular weight (Casassa & Harbertson, 2014). Polymeric pigments and tannins were lower in 2017, while non-tannin phenolics were higher (Table 18). While some parameters (total phenolics, non-tannin phenolics, L*, a*, chroma) were affected by thinning treatment in 2017 (Table 18), no consistent effect of treatment or treatment × growing season interaction was found in any wine phenolic or chromatic parameter. Polymeric pigments were likely higher in 2016 because of the increased level of tannins observed (Table 18), despite no difference in anthocyanin levels between growing seasons.

Wine CIE L*a*b* color parameters L*, b*, and hue angle were higher in 2017 than 2016, while a* and chroma were lower, indicating wine color was darker and bluer in the wines from the warmer growing season, but less saturated and red than wines from the cooler growing season. The color shift observed in 2017 wines is likely due to differences in wine pH and polymeric pigment content. The effect of pH on wine color and anthocyanin chromatic parameters is well established. As pH decreases, the equilibrium of anthocyanin forms shifts to favor the flavilium cation, which increases red hue, and as pH increases, the equilibrium shifts to favor the quinonoidal hydrobase, which increased blue hue (Heredia et al., 1998). Additionally, as pH increases, there is a linear decrease in chroma value observed (Heredia et al., 1998). As wine pH was generally higher in the 2017 wines (Table 16), it would follow that the color in 2017 wines, while not having statistically distinguishable anthocyanin concentration relative to 2016 wines, would have increased blue hue and lower chroma. Polymeric pigment formation may lower saturation (as indicated by chroma). Saturation may be lowered through the transformation (and subsequent reduction) of anthocyanins, or through the

modulation of the chromatic properties of the anthocyanin subunit following a reduction of the molar extinction coefficient relative to the native anthocyanin, although there is only indirect experimental evidence of this molar extinction coefficient reduction (Casassa, Larsen, et al., 2013; Weber et al., 2013).

The results of the overall sensory test performed in the 2016 wines generally mirrored previously uncovered trends in the basic, phenolic, and chromatic composition of the resulting wines. That is, none of the cluster thinning treatments produced wines that were distinguishable, from a sensory standpoint, from the control wines. Similar to what has been found in the present study, cluster thinning performed in Chardonnay Musqué grapes, while producing chemical differences in fruit, resulted in little sensory differences in the resulting wines (Reynolds et al., 2007). In another study, wine produced from cluster thinned Cabernet Sauvignon vines exhibited a small increase in perceived wine quality relative to wine produced from non-thinned vines (Ough & Nagaoka, 1984), although location was found to have a greater impact on sensory perception than the cluster thinning treatment, and the effect was not consistent across growing seasons. While wine chemical composition and perceived sensory attributes rarely follow linear correlations, without corresponding differences in chemical composition, it is unlikely that cluster thinning will have an impact on wine sensory perception. Therefore, the lack of sensory differences observed in the wines of the present study is unsurprising, and we hypothesize that any chemical differences in volatiles that may have occurred within the wines were below sensory thresholds, and therefore practically irrelevant. Unless cluster thinning is necessitated by the vine balance (i.e., the vine is overcropped) in cool climate Pinot noir, it is unlikely that there will be any sensory benefit to cluster thinning that would justify the economic impact.

In much of the previous research performed on cluster thinning, external factors independent of (although at times in combination with) vine crop load impacted berry and wine chemical composition to a greater degree than crop load. Factors such as climatic variation in growing season (Keller et al., 2005) and viticultural practices such as floor management (Reeve et al., 2018), deficit irrigation (Keller et al., 2005; Valdés et al., 2009), and leaf thinning (Burbola et al., 2017; Benoit Girard et al., 1997) have all been found to have a greater effect on fruit composition than cluster thinning. Indeed, in this study, no consistent effect of cluster thinning or cluster thinning timing was observed across two growing seasons, a cooler growing season and a warmer growing season, and growing season had a greater effect on variation in fruit and wine composition than thinning treatment for most parameters. A Ravaz Index range of 3–6 has been previously

proposed as an optimum cropload for Pinot noir grown in cool-climates (Kliewer & Dokoozlian, 2005). Based on the lack of differences observed in fruit Brix accumulation, wine composition, and wine sensory perception, Pinot noir vineyards on the central coast of California can, barring climatic conditions severely increasing crop set or severely limiting ripening potential, likely support higher crop levels than those of the vineyard utilized in this this study, which had Ravaz Index values of 3.23 across the non-thinned blocks. Considering previously proposed ranges and the results of the current study, a Ravaz Index value of 6 could be appropriate for Pinot noir on the central coast of California and should be examined and evaluated accordingly.

2.5. Conclusions

No positive effect of cluster thinning or the timing of it was observed across two growing seasons, a cooler growing season and a warmer growing season, for Pinot noir grapes and wines. In general, the growing season had a greater effect on variation than thinning treatment for most parameters. Few treatment × growing season interactions were found in wine composition parameters, indicating that rather than cluster thinning treatment being affected by seasonal variation, which has been reported previously, seasonal variation itself was the primary driver of differences in fruit and wine composition. No sensory difference was detected between the non-thinned control and any wines from cluster thinned treatments. However, on average, cluster thinning was associated with a 44% reduction in crop yields, and this reduction in crop load failed to produce a positive or discernible sensory effect on the resulting wines. Pinot noir vineyards on the central coast of California can support crop loads that result in Ravaz Index values larger than 3.23 and potentially up to 6 without concern for impacting ripening potential, barring a severe decrease in GDD accumulation. This study also suggests that in Pinot noir, balanced canopies with LA/Y ratios in tune with the prevalent seasonal conditions of the region would most likely yield quality fruit, with no discernible or marginal improvements in quality due to cluster thinning.

3. MULTI-YEAR STUDY OF THE EFFECTS OF CLUSTER THINNING ON VINE PERFORMANCE, FRUIT AND WINE COMPOSITION OF PINOT NOIR (CLONE 115) IN CALIFORNIA'S EDNA VALLEY AVA (USA)

The third chapter of this thesis consists of an article that was published in *Scientia Horticulturae* on October 15, 2019. This article was written as an evaluation of cluster thinning treatments incorporating all three years of experimental data, expanding upon the data written about in chapter two and increasing the parameters measured and discussed. Co-authors Dr. Jean C. Dodson Peterson and Dr. L. Federico Casassa. The article has been reformatted to integrate within this thesis.

3.1. Abstract

A three-year study was conducted at a commercial vineyard site in California's Edna Valley AVA to evaluate the physiological and agronomical effects of the timing of cluster thinning on Pinot noir (clone 115) grapevines. Vines were thinned to one cluster per shoot at three selected time-points during the growing season (bloom + 4 weeks, bloom + 8 weeks, bloom + 12 weeks), and fruit from each treatment was harvested and made into wine. Across all growing seasons, yield decreased 43% in thinned vines relative to non-thinned control vines. No effect of cluster thinning or interaction with growing season was found in vine shoot diameter, internode length, fruit zone light level, or cluster weight. The growing season significantly affected more fruit and wine parameters than did cluster thinning treatment, with interactions between treatment and growing season found in fruit Brix, titratable acidity, and anthocyanins, as well as wine anthocyanins and wine b* (yellow component). For example, bloom + 8 and bloom + 12 thinning treatments advanced Brix in 2017 but had no effect in 2018. Cluster thinning treatments respectively increased berry anthocyanins and total phenolics by 43% and 87% in 2017 and by 103% and 140%in 2018 relative to the non-control, with no significant differences found between the different thinning treatments. However, the wines from cluster thinning treatments showed no differences in the levels of anthocyanins and total phenolics. The fact that different cluster thinning treatments resulted in nil or minor effects on fruit and wine suggests that the vines tested were at or below a balanced crop load prior to the application of cluster thinning. Edna Valley AVA Pinot noir grapes could likely support crop loads (expressed as the

Ravaz Index) higher than 3.2 without negatively impacting fruit or wine composition and reducing crop load below that level is unlikely to increase fruit or wine quality.

Keywords: Cluster thinning; Yield manipulation; Vine balance; Crop load; Pinot noir; Central Coast of California

3.2. Introduction

The traditionally accepted yield paradigm in wine grapes (*Vitis vinifera* L.) is that of a negative linear relationship between yield and fruit quality (Gamero et al., 2014; Palliotti & Cartechini, 2000; Reynolds et al., 2007; Uzes & Skinkis, 2016). This relationship has been shown to be an oversimplification and may be more accurately represented by crop load (Bravdo et al., 1985; Jackson & Lombard, 1993). Crop load, expressed as the unitless ratio of fruit yield to dormant pruning weight in the Ravaz index (Ravaz, 1903; Smart et al., 1990), relates vine photosynthetic sinks, the fruit, to the photosynthetic capacity of the vine represented by the weight of canopy growth removed during dormant season pruning (Kliewer & Dokoozlian, 2005). Vine photosynthate production capacity and canopy growth is affected by cultivar (Kliewer & Dokoozlian, 2005), climate (Reeve et al., 2018), growing season (Frioni et al., 2017; Gamero et al., 2014; Reeve et al., 2018; Zhuang et al., 2014) site characteristics (Uriarte et al., 2015), rootstock (Santarosa et al., 2016), and cultural practices (Reeve et al., 2018). Given that the ability to ripen a set amount of fruit is dependent upon a sufficient vine capacity there is no single optimal yield for wine grapes across regions and cultivars (Kliewer & Dokoozlian, 2005; Reeve et al., 2018). Optimal crop load is considered to be between 5 and 10 on the Ravaz index (Ravaz, 1903) for most cultivars and regions (Bravdo et al., 1985). However it has been proposed that optimal crop load for small-clustered cultivars, such as Pinot noir, grown in cool climates should be between 3 and 6 on the Ravaz index (Kliewer & Dokoozlian, 2005).

In *Vitis vinifera* L. grapes, polyphenol content is modulated by cultivar (Dimitrovska et al., 2011; Harbertson et al., 2002), climate (Mateus et al., 2001), growing season (Downey et al., 2006; Guidoni et al., 2002), berry sun exposure (Lenk et al., 2007), canopy biomass (Cortell et al., 2005), vine water status (Mirás-Avalos et al., 2017), yield (Reynolds et al., 2018), and fruit maturity (Allegro et al., 2018). The phenolic profile of Pinot noir is quantitatively lower than other popular wine cultivars. Indeed, Pinot noir wines are typically relatively low in tannins (Harbertson et al., 2002), which are compounds responsible for wine astringency and mouthfeel (Casassa & Harbertson, 2014). Anthocyanins, compounds responsible for wine

color (Casassa & Harbertson, 2014) are also notoriously low in Pinot noir wines (Dimitrovska et al., 2011). Furthermore, the anthocyanin profile of Pinot noir is characterized by the lack of acylated anthocyanins (Dimitrovska et al., 2011). These peculiarities in the phenolic profile of Pinot noir manifest themselves as wines with lower tannin content and relatively low astringency (Harbertson et al., 2002) that have also lighter color (Cliff et al., 2007).

Cluster thinning is a common viticultural practice within Pinot noir production to reduce vine crop load. Cluster thinning is performed with the aim of either improving fruit quality or conforming with commercially expected crop loads (Uzes & Skinkis, 2016). As Pinot noir wines are typically lighter in color and astringency, and color is a primary component of perceived wine quality (Somers & Evans, 1974), the practice of cluster thinning is used to lower yields with the intention of increasing phenolic content and, by extension, wine quality (Gamero et al., 2014; Guidoni et al., 2002; Keller et al., 2005; Reynolds et al., 1994; Santesteban et al., 2011; Uzes & Skinkis, 2016; Valdés et al., 2009). For example, several studies have shown that cluster thinning increased the concentration of fruit anthocyanins in Pinot noir (Cañón et al., 2014), Cabernet franc (Frioni et al., 2017), Cabernet Sauvignon (Cañón et al., 2014; Palliotti & Cartechini, 2000), Sangiovese, and Merlot (Palliotti & Cartechini, 2000). However, when the effect of cluster thinning have been evaluated over multiple years, results have generally indicated no effect of cluster thinning on anthocyanin concentration in Cabernet Sauvignon (Keller et al., 2005) and inconsistent effects in Pinot noir, with thinning increasing berry anthocyanins in one season and no effect in two other seasons (Reynolds et al., 1994). These results suggest that cluster thinning is a practice that should be evaluated on not only a cultivar basis but on a season to season basis as well.

The timing of cluster thinning, while critical, is also subject to debate. For example, cluster thinning performed during the lag phase of berry development was suggested as the most appropriate time to perform this task, due to the popularity of using lag phase cluster weights to estimate yields ("Understanding vine balance," 2013) and the purported ability of pre-véraison thinning to affect fruit maturity (Jackson & Lombard, 1993). However, an experiment conducted in Cabernet Sauvignon reported that cluster thinning performed during lag phase of berry development had little to no impact on canopy growth, cluster yield components, and fruit maturity (Keller et al., 2008). It has also been hypothesized that removing crop earlier in the season, *e.g.* at bloom, may lead to lower leaf transpiration rates and therefore lower leaf photosynthesis

rates (Naor et al., 1997), which could negate the potential benefits of reduced crop load by reducing the photosynthetic capacity of the vine. A temporary photosynthetic reduction was observed after thinning at lag phase in Cabernet Sauvignon grapes (Wang et al., 2018), but photosynthetic capacity was not affected after véraison indicating a compensation effect of increased vegetative growth in response to cluster thinning. Cluster thinning performed during bloom has increased berry size in Pinot noir (Reynolds et al., 1994) as well as Cabernet Sauvignon, Chenin blanc, and Riesling grapes (Keller et al., 2005). In the latter study, this effect was only seen in one season and only thinning at bloom resulted in increased berry size, while véraison thinning resulted in berry size indistinguishable from the non-thinned control.

Winegrowing regions are quantified and compared by growing degree days accumulated during the vine's typical growing season (April 1 – October 31 in the northern hemisphere) and classified accordingly, spanning from the coolest region (I) to the warmest region (V) following the so-called "Winkler scale" (Jones et al., 2010; Winkler et al., 1974). Many areas of economic importance for Pinot noir production are classified as region I, II, and, less commonly, III (Robinson, 2006), which are considered cool climate (I and II) or moderately warm climate (III) areas according to the Winkler scale. Lower daytime temperatures result in lower leaf photosynthetic capacity and berry carbon assimilation, necessitating lower crop loads to achieve fruit maturity than would otherwise be required in warmer climates (Howell, 2001; Jackson & Lombard, 1993; Kliewer & Dokoozlian, 2005; Reeve et al., 2018).

Regardless of site-specific factors that affect vine capacity, growers may perform cluster thinning to reduce yields below what they believe is necessary to achieve commercially acceptable fruit ripeness in order to conform with winemaking expectations (Uzes & Skinkis, 2016). While the ability to ripen Pinot noir in cooler climates such as Oregon's Willamette Valley (northwest USA) require lower crop loads than those observed in warmer climates (Kliewer & Dokoozlian, 2005; Reeve et al., 2018)**,** these ratios are intrinsically tied to seasonal limitations (Frioni et al., 2017; Gamero et al., 2014; Reeve et al., 2018; Zhuang et al., 2014) and may not apply to Pinot noir grown in more moderate climates such as California's Central Coast. In cooler climate areas where cluster thinning effectively advanced ripening in one season, it has been observed that performing this practice during a subsequent, warmer season can have no impact on fruit maturation (Frioni et al., 2017). As there is considerable economic cost to the practice of cluster thinning associated with both yield reduction and increased labor, it would be beneficial for viticulturists to understand when

thinning can be applied to positively Pinot noir fruits and wines. In the Central Coast of California, Pinot noir represents over 7,000 hectares of the planted vineyards ("Grape acreage report," 2018) and in 2017, wine grapes were the most valuable crop produced in San Luis Obispo County, as found in the annual crop report ("Annual crop report," 2017).

The objective of this study was to investigate the effect of the timing of crop load reduction by cluster thinning on Pinot noir grapes (clone 115) grown in the Edna Valley American Viticultural Area (AVA) of the Central Coast (San Luis Obispo County) of California (USA) as well as in their resulting wines. The effects of crop load reduction on vine canopy structure, fruit composition, and wine composition were investigated over three consecutive seasons, with thinning applied at 3 different points during each of the growing season. Thinning treatments were applied at four weeks post-bloom (bloom + 4), eight weeks post-bloom (bloom + 8), and twelve weeks post-bloom (bloom + 12). The hypothesis of this study was that cluster thinning to one cluster per shoot would influence fruit physical and chemical parameters, particularly in the event of season-related source limitation. In accordance with previous literature, it was hypothesized that bloom + 4 thinning would have the highest magnitude impact on berry and cluster size, bloom + 8 thinning would have the highest magnitude impact on fruit chemical properties, and bloom + 12 thinning would have minimal impacts on fruit and wine composition due to the relative proximity to harvest.

3.3. Materials and Methods

3.3.1. Vineyard Site, Plant Material and Experimental Design

The study was conducted during the 2016, 2017, and 2018 growing seasons at a mature, drip-irrigated vineyard of Pinot noir grapevines (clone 115) planted in 1996 on 5C rootstock (*Vitis berlandieri x Vitis riparia*) in the Edna Valley (35°11'58.3" N 120°34'12.6" W), located within California's Central Coast AVA. Vine spacing was 2.75 x 1.52 m in north-south aligned rows planted in silty clay loam soil. Vines were pruned to either two or three 10-bud canes depending on the caliper of the previous year's canes, trained in a vertical shoot positioning (VSP) system with two catch-wires. To account for pruning variation, data from each treatment replication was collected from several sub-replicate vines and averaged to create the data points that were used for statistical analysis. Canes removed during dormant season pruning were collected and weighed on a per-vine basis to determine the vegetative growth of the 2017-2018 and 2018-2019 growing seasons. Precipitation and daily minimum and maximum temperatures were recorded from

California Irrigation Information Management System (CIMIS) weather station 52 (35°18'19.6" N 120°39'42.4" W), located 14.41 kilometers from the experimental site. Cumulative Growing Degree Days (GDD) were calculated using a baseline temperature of 10 °C and the average daily temperature from April 1 to October 31 of each year (Winkler et al., 1974). Grapevines were thinned to one cluster per shoot by removing all but the basal cluster on each shoot at different points during the growing season, approximating certain phenological growth stages. Bloom, as designated by the modified Eichhorn and Lorenz (E-L) scale as stage 23 (Coombe, 1995), occurred on June 1st in 2016, May 15th in 2017, and May 13th in 2018. Cluster thinning was applied at four weeks post-bloom (bloom + 4) approximating fruit set, eight weeks post-bloom (bloom + 8) approximating véraison, and 12 weeks post bloom (bloom + 12), shortly before harvest.

3.3.2. Vine Vegetative Growth and Photosynthetically Active Radiation

The experiment was designed as a randomized block design, with a total of five block replicates per treatment. Individual treatment replicates consisted of five rows, each row was 20 vines in length for a total of 100 vines per treatment replicate. Edge rows of each treatment block were eliminated from viticultural data collection to prevent capturing any interactions from neighboring treatments. Vine vegetative and photosynthetically active radiation measurements were restricted to the middle vines of the middle row for each treatment replicate, whereas all block rows replicated, excluding edge rows, within each treatment were utilized for winemaking purposes. After the vineyard plot reached 100% véraison (E-L stage 35), internode length and shoot diameter were measured. From each data collection vine, five shoots were evaluated. Measurements were taken of the distance between the diaphragms of node two and node three, the nodes directly above the first cluster on the shoot (internode length) and the diameter of the shoot at the thinnest point of the same internode (shoot diameter) using calipers (Neiko 01407A, Zhejiang Kangle Group, Wenzhou, China). Fifteen subsamples across each set of three vines were collected to represent one replicate (n = 5). Photosynthetically active radiation (PAR) in the fruit zone was measured with a ceptometer (AccuPAR LP-80, Meter Group, Pullman, Washington, USA) to determine the amount of light penetration through the canopy and into the fruiting zone. Ceptometer readings at midday were taken parallel to the fruiting canes within the fruiting zone in triplicate on all data collection vines in 2017 and 2018. The average of the nine individual measurements across each set of three vines were calculated to represent one replicate (n = 5). Intra-canopy ceptometer readings were standardized against a full-sun reading to determine the

percentage of PAR that was able to penetrate the canopy and reach the fruit zone and used as a proxy measurement of canopy density. Following pruning, pruned canes were collected and weighed on a per-vine basis to measure total seasonal vine vegetative growth. In 2017, pruned canes were collected and weighed at a central location using a commercial scale (CPWplus 150 weighing scale, Adam Equipment, Oxford, Connecticut, USA). In 2018, pruned canes were collected and weighed in the field using a hand-held scale (H-110 digital hanging scale, American Weigh Scales, Cumming, GA, USA). Individual vine fruit yields were compared to individual vine pruning weights to calculate yield : pruning weight ratios (Ravaz, 1903; Smart et al., 1990).

3.3.3. Carbohydrate Analysis

Trunk wood samples were collected following established protocols (K. Jones & Amore, 1960; Smith & Holzapfel, 2009) in 2018 and 2019 to measure starch and sugar content of vine tissue as a determination of stored carbohydrates. Trunk wood samples were collected as drillings to approximately mid-depth using a 12.6 mm spade drill bit (Black and Decker Inc, Baltimore, Maryland, USA). Wood samples were dried for 24 hours at 55-60 °C in a convection oven (PR305225M Precision Oven, Thermo Fisher Scientific, Waltham, MA, USA) and ground with a cutting mill (Model 4 Wiley Mini Mill, Thomas Scientific, Swedesboro, New Jersey, USA) until particles could pass through a 40-mesh screen. Subsamples of 0.5 g were suspended in deionized water and HCl (12 M) and heated in a flask with a reflux condenser for 2.5 hours. Samples were cooled and neutralized with NaOH (5 N). Sugar formed during hydrolysis was measured enzymatically as glucose + fructose concentration with Y15 automatic enzymatic analyzer (Biosystems, Barcelona, Spain) using commercial enzymatic analysis kits (Biosystems, Barcelona, Spain). Concentration of sugars was calculated as percentage of dry mass and presented as carbohydrate reserves. Carbohydrate reserves measured in 2018 were associated with the 2017 growing season, reserves measured in 2019 were associated with the 2018 growing season.

3.3.4. Yield and Berry Physical Properties

Fruit was harvested when a composite sample of all treatments (n = 20, 250 berries each) reached 22.5 Brix. Harvest dates were September 6, 2016, September 6, 2017, and September 24, 2018. Fruit was harvested manually from three independent vineyard replications of each treatment (n = 3). There was

sufficient fruit from the 80 vines that comprised the middle three rows of each treatment replication to gather the 80 kg needed for winemaking in each instance. Approximately 80 kg of fruit per replicate was harvested, for a total of 960 kg of fruit harvested in each season. Data collection vines provided fruit for harvest and clusters were weighed and counted on a per-vine basis. Berry weight, seed number, and seed weight were measured at harvest from random samples of 250 berries taken from each harvested replication (n = 3). Berry weights were measured from 200 berries using an analytical scale (Fisher Science Education ALF203 200 g scale, Thermo Fisher Scientific, Waltham, MA, USA); seeds were separated from 20 berries, counted, and weighed.

3.3.5. Fruit Chemistry

Following the measurement of berry physical properties, 200 berries were crushed by hand to extract juice and the Brix, titratable acidity, and pH of the juice product were measured. Brix was measured with a handheld refractometer (Vee Gee Scientific, Kirkland, WA, USA). In 2016 and 2017 titratable acidity was measured by titrating was measured by titrating a known quantity of juice (5 mL) in a deionized water solution against 0.067 N NaOH (Fisher Scientific, Waltham, MA, USA) to a pH endpoint of 8.2 in accordance with an established procedure (Iland, Bruer, Edwards, Chalogiris, & Wilkes, 2013). In 2018, titratable acidity was measured utilizing an auto titrator (Hanna Instruments, Woonsocket, RI, USA) following the same procedure. Juice pH was measured with a Benchtop pH meter (Orion Star A211, Thermo Fisher Scientific, Waltham, MA, USA). Yeast assimilable nitrogen (YAN) was measured enzymatically from juice utilizing an analyzer and commercially available kits (Biosystems, Barcelona, Spain) (Iland et al., 2013). In 2017 and 2018, 50 berries were homogenized to measure berry anthocyanins and total phenolics following a previously published protocol (Iland et al., 2013).

3.3.6. Winemaking

The three replicates of each of the five treatments were independently destemmed and crushed using a crusher–destemmer (Bucher Vaslin, Niederweningen, Switzerland), and placed separately in individual 60-L plastic fermenters (Speidel, Swabia, Germany), where alcoholic fermentation took place. Musts were inoculated with a commercial wine yeast (*Saccharomyces cerevisiae*, strain EC-1118, Lallemand, Rexdale, ON, Canada) at a rate of 30 g/hL. Musts were inoculated with commercial malolactic bacteria 48 hours after

crushing to ensure a standardized fermentation across treatments. In 2016, musts were inoculated with strain VP-41 (*Oenococcus oeni*, Lallemand, Rexdale, ON, Canada). In 2017 and 2018, musts were inoculated with strain ML Prime (*Lactobacillus plantarum*, Lallemand, Rexdale, ON, Canada). A standard addition of 250 mg/L diammonium phosphate was added to each fermentation one day after yeast inoculation. Temperature and Brix were followed daily during alcoholic fermentation using a density meter (Anton Paar, Graz, Austria) with temperature and sugar consumption curves showing good reproducibility within replicates of the same and different treatments (data not shown). Following 10 days of maceration, wines were drained off from solids, with free run wines immediately transferred to 20-L glass carboys fitted with airlocks until the completion of malolactic fermentation. Following the completion of malolactic fermentation, wines were adjusted to 0.3 mg/L molecular SO_2, and pad filtered (Buon Vino Superjet Filter Pad 0.2 µm, Buon Vino, Walnut Creek, CA, USA). Wines were subsequently bottled using a DIAM 5 microagglomerated cork closure (G3 Enterprises, Modesto, CA, USA) and kept in cellar-like conditions until analysis.

3.3.7. Wine Chemistry

Wine titratable acidity (TA) and pH were measured in the same method as juice TA and pH. Wine ethanol was measured with an alcoholyzer wine M/ME analysis system (Anton Paar, Graz, Austria); wine residual sugars, acetic acid, lactic acid, and malic acid were determined enzymatically with a Y15 automatic enzymatic analyzer (Biosystems, Barcelona, Spain) using commercial enzymatic analysis kits (Biosystems, Barcelona, Spain). Wine phenolics and color were measured at pressing and following the completion of malolactic fermentation (malic acid < 0.4 g/L). Anthocyanins, non-tannin phenolics, small polymeric pigments (SPP), large polymeric pigments (LPP), and total polymeric pigments (herein reported as SPP + LPP), were measured as previously described (Harbertson et al., 2003). Tannins in the wines were analyzed by protein precipitation (Harbertson et al., 2002).

3.3.8. Wine Color

Absorbance scans were taken using a spectrophotometer (Cary UV-VIS60, Agilent Technologies, Santa Clara CA, USA) and absorbance data was run through Cary WINUV Color module software (Agilent Technologies, Santa Clara, CA, USA) to extract CIE-L*a*b* tri-stimulus colorimetry values (D65 illuminant).

3.3.9. Statistical Analysis

Statistical analyses were carried out with JMP (SAS Institute, North Carolina, USA) for analyses of variance (ANOVA). Fisher's least significant difference test (LSD) was used for means separation. Two-way ANOVA models considering treatment and growing season, and their interaction, with replication as a blocking factor were carried out for all parameters with data from all growing seasons. One-way ANOVA models were carried out to assess differences between treatments for each parameter within each growing season. Statistical analysis on juice and wine pH was performed using the hydrogen ion concentration and presented as pH for clarity.

3.4. Results

3.4.1. Meteorological Conditions

7. Growing degree days (GDD), Winkler region classification, and precipitation for San Luis Obispo, California (USA).

Growing Season	Growing Degree Days[1]	Winkler Region	Annual Precipitation[2] (mm)	Seasonal Precipitation[3] (mm)
2016	2984	II	521.9	66.2
2017	3715	IV	733.6	65.1
2018	3936	IV	455.8	13.5

[1] Calculated from 1 April–31 October in Celsius units with a baseline of 10 °C; [2] Sum of precipitation from 1 January–31 December; [3] Sum of precipitation from 1 April–31 October.

The 2016-2018 growing seasons represent one cool growing season (2016) and two warm growing seasons (2017, 2018) based on GDD accumulation and Winkler region classification (Table 7). The 2017 growing season had the highest annual precipitation, with seasonal precipitation similar to 2016 (Table 7). The 2018 growing season had the lowest annual and seasonal precipitation as well as the highest degree day accumulation (Table 7).

8. Vine vegetative growth parameters at véraison and during dormancy of Pinot noir subjected to cluster thinning at different points of the growing season from 2016-2018. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 5). Significant *p-values* (< 0.05) are shown in bold fonts.

Growing season	Treatment	Internode length (mm)	Shoot diameter (mm)	Fruit zone PAR[1] (% penetration)	Dormant pruning weight (kg)	Dormant carbohydrate reserves[2] (% dry mass)
2016	Control	50 ± 2.1 a	6.1 ± 0.2 a	n.m.[3]	n.m.	n.m.
	Bloom + 4	52 ± 2.9 a	6.1 ± 0.2 a	n.m.	n.m.	n.m.
	Bloom + 8	54 ± 2.5 a	6.2 ± 0.2 a	n.m.	n.m.	n.m.
	Bloom + 12	47 ± 3.4 a	6.2 ± 0.1 a	n.m.	n.m.	n.m.
	p-value	0.32	0.97	n.m.	n.m.	n.m.
2017	Control	44 ± 2.4 a	6.3 ± 0.0 b	2.2 ± 0.6	0.86 ± 0.02 ab	2.2 ± 0.4 a
	Bloom + 4	42 ± 2.3 a	6.7 ± 0.1 ab	1.8 ± 0.4	0.73 ± 0.06 b	2.2 ± 1.4 a
	Bloom + 8	40 ± 3.2 a	7.0 ± 0.2 a	1.6 ± 0.2	0.91 ± 0.05 a	3.1 ± 1.2 a
	Bloom + 12	43 ± 2.0 a	6.6 ± 0.2 ab	1.8 ± 0.2	0.83 ± 0.04 ab	5.1 ± 1.1 a
	p-value	0.67	0.052	0.88	0.067	0.2698
2018	Control	36 ± 2.8 a	6.9 ± 0.3 a	5.2 ± 1.0 a	0.70 ± 0.05 a	2.5 ± 0.2 a
	Bloom + 4	42 ± 1.7 a	7.7 ± 0.5 a	6.0 ± 1.6 a	0.73 ± 0.06 a	2.3 ± 0.1 a
	Bloom + 8	39 ± 1.4 a	7.5 ± 0.6 a	5.0 ± 0.3 a	0.76 ± 0.03 a	2.3 ± 0.3 a
	Bloom + 12	39 ± 0.57 a	7.1 ± 0.2 a	4.4 ± 0.5 a	0.74 ± 0.04 a	3.1 ± 0.5 a
	p-value	0.32	0.64	0.36	0.77	0.2514
Treatment (T)		0.63	0.11	0.74	0.17	0.09
Growing Season (S)		**<0.0001**	**<0.0001**	**<0.0001**	**0.0052**	0.33
T x S Interaction		0.25	0.68	0.81	0.30	0.48

[1] PAR: Photosynthetically active radiation
[2] Measured as total soluble sugars following starch hydrolysis
[3] n.m.: not measured

3.4.2. Vine Vegetative Growth and Carbohydrate Reserves

No significant differences were observed in any vegetative growth parameter between treatments, indicating no effect of cluster thinning on vine vegetative growth. Significant differences were found between the growing seasons on vine internode length, shoot diameter, and PAR (Table 8). Internode length across all treatments was higher in 2016 than in 2017 and 2018 (Table 8). Shoot diameter increased successively with growing season, with 2017 being higher than 2016, and 2018 being higher than 2017 (Table 8). PAR infiltration was significantly higher in 2018 than 2017 (Table 8). Pruning weights were significantly higher in 2017 than 2018 (Table 8). No differences in vine carbohydrates were found between treatments or growing seasons. No treatment × season interaction was found for any vegetative growth parameter (Table 8).

9. Vine yield parameters and physical berry characteristics of Pinot noir subjected to cluster thinning at different points of the growing season from 2016-2018. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 3). Significant *p-values* (< 0.05) are shown in bold fonts.

Growing season	Treatment	Cluster Count	Vine Yield (kg)	Cluster Weight (g)	Ravaz Index[1]	Berry Weight (g)	Seed Weight (mg)	Seeds per Berry
2016	Control	33.0 ± 4.9 a	2.1 ± 0.3 a	63 ± 1.3 a	n.m.[2]	1.2 ± 0.0058 a	n.m.	n.m.
	Bloom + 4	22.4 ± 5.3 ab	1.3 ± 0.2 b	59 ± 7.2 a	n.m.	1.1 ± 0.036 ab	n.m.	n.m.
	Bloom + 8	20.1 ± 0.7 b	1.0 ± 0.2 b	51 ± 6.3 a	n.m.	1.0 ± 0.0088 b	n.m.	n.m.
	Bloom + 12	23.1 ± 2.7 ab	1.2 ± 0.0 b	55 ± 5.9 a	n.m.	1.1 ± 0.98 ab	n.m.	n.m.
	p-value	0.17	**0.046**	0.52	n.m.	0.10	n.m.	n.m.
2017	Control	32.3 ± 4.6 a	2.8 ± 0.6 a	86 ± 8.0 a	3.2 ± 0.8 a	1.0 ± 0.07 a	60 ± 6.9 a	1.4 ± 0.05 a
	Bloom + 4	19.1 ± 0.9 b	1.3 ± 0.2 b	73 ± 9.2 a	1.7 ± 0.2 b	1.0 ± 0.06 a	42 ± 8.7 a	1.2 ± 0.12 a
	Bloom + 8	23.7 ± 0.3 ab	1.6 ± 0.0 b	70 ± 2.1 a	1.9 ± 0.2 ab	0.9 ± 0.06 a	52 ± 0.25 a	1.4 ± 0.06 a
	Bloom + 12	21.1 ± 2.0 b	1.6 ± 0.3 b	77 ± 6.5 a	2.0 ± 0.3 ab	1.0 ± 0.11 a	51 ± 3.7 a	1.3 ± 0.11 a
	p-value	0.12	0.06	0.47	0.13	0.94	0.28	0.40
2018	Control	26.3 ± 1.7 a	2.1 ± 0.3 a	78 ± 6.3 a	2.9 ± 0.6 a	0.9 ± 0.08 ab	50 ± 6.5 ab	1.3 ± 0.13 a
	Bloom + 4	19.1 ± 1.2 a	2.0 ± 0.3 a	106 ± 19 a	2.8 ± 0.6 a	1.1 ± 0.05 a	54 ± 1.8 a	1.4 ± 0.08 a
	Bloom + 8	18.8 ± 2.0 a	1.6 ± 0.1 a	83 ± 2.4 a	2.1 ± 0.2 a	0.8 ± 0.03 b	40 ± 2.1 b	1.1 ± 0.11 a
	Bloom + 12	18.1 ± 4.3 a	1.5 ± 0.5 a	76 ± 10 a	1.9 ± 0.5 a	0.8 ± 0.1 b	46 ± 4.2 ab	1.4 ± 0.12 a
	p-value	0.16	0.49	0.28	0.46	**0.044**	0.19	0.28
Treatment (T)		**0.0058**	**0.0012**	0.29	0.051	**0.049**	0.56	0.87
Growing Season (S)		0.15	0.074	**0.00040**	0.57	**0.0016**	0.48	0.74
T x S Interaction		0.95	0.31	0.17	0.28	0.13	0.23	**0.029**

[1] Average of individual vine yield divided by individual vine pruning weight

[2] n.m.: not measured

3.4.3. Vine Yield, Crop Load, and Fruit Physical Properties

Cluster count and vine yield were significantly affected by the thinning treatments although there was no significant difference in cluster count between treatments within any single growing season (Table 9). No significant difference was found in Ravaz Index between treatments or growing seasons (Table 9), indicating no significant differences in vine crop load.

Across treatments, cluster weights were lower in 2016 than 2017 or 2018 (Table 9). No significant differences were found in cluster weight between treatments (Table 9). Berry weight generally decreased from 2016 to 2018 (Table 9), indicating that larger berries were produced in the cooler 2016 season. Bloom + 4 thinning, unlike the other thinning treatments, increased berry weight in 2018 (Table 9). Berry weight was significantly affected by cluster thinning only in 2018 (Table 9), where bloom + 4 berries were significantly higher than bloom + 8 and bloom + 12 with control berries being intermediate and statistically indistinguishable (Table 9). Lastly, seed weight was not affected by treatment or growing season (Table 9). No treatment × season interaction was found for any vine yield parameter (Table 9).

A treatment × season interaction was found in seeds per berry. However, neither individual effect was significant nor was there an effect of treatment within any individual growing season (Table 9).

10. Fruit chemistry of Pinot noir subjected to cluster thinning at different points of the growing season from 2016-2018. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 3). Significant *p-values* (< 0.05) are shown in bold fonts.

Growing Season	Treatment	Brix	pH	Titratable acidity (g/L)	L-malic acid (g/L)	Tartaric acid (g/L)	YAN[1] (mg/L)	Berry total phenolics (au*100/berry)[2]	Berry anthocyanins (mg/berry)
2016	Control	23 ± 0.43 a	3.48 ± 0.04 a	6.0 ± 0.17 b	n.m.[3]	n.m.	n.m.	n.m.	n.m.
	Bloom + 4	23 ± 0.44 a	3.44 ± 0.03 a	6.5 ± 0.19 b	n.m.	n.m.	n.m.	n.m.	n.m.
	Bloom + 8	23 ± 0.26 a	3.47 ± 0.02 a	6.6 ± 0.15 ab	n.m.	n.m.	n.m.	n.m.	n.m.
	Bloom + 12	22 ± 0.15 a	3.37 ± 0.05 a	7.5 ± 0.53 a	n.m.	n.m.	n.m.	n.m.	n.m.
	p-value	0.29	0.4030	**0.043**	n.m.	n.m.	n.m.	n.m.	n.m.
2017	Control	22 ± 0.27 b	3.60 ± 0.01 a	6.7 ± 0.13 a	2.7 ± 0.12 a	3.4 ± 0.15 a	1,300 ± 25 a	0.20 ± 0.016 b	0.56 ± 0.02 b
	Bloom + 4	22 ± 0.17 ab	3.61 ± 0.01 a	6.3 ± 0.15 ab	2.9 ± 0.052 a	3.2 ± 0.04 a	1,300 ± 20 a	0.34 ± 0.032 ab	0.70 ± 0.03 ab
	Bloom + 8	23 ± 0.15 a	3.62 ± 0.01 a	6.0 ± 0.04 bc	2.8 ± 0.075 a	3.5 ± 0.11 a	1,400 ± 120 a	0.38 ± 0.070 a	0.84 ± 0.99 a
	Bloom + 12	23 ± 0.19 a	3.59 ± 0.02 a	5.7 ± 0.28 c	2.7 ± 0.059 a	3.5 ± 0.36 a	1,300 ± 51 a	0.40 ± 0.053 a	0.86 ± 0.07 a
	p-value	**0.048**	0.2865	**0.018**	0.30	0.75	0.51	0.062	**0.037**

[1] YAN: Yeast Assimilable Nitrogen

[2] au: absorbance units

[3] n.m.: not measured

10 (cont.). Fruit chemistry of Pinot noir subjected to cluster thinning at different points of the growing season from 2016-2018. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 3). Significant *p-values* (< 0.05) are shown in bold fonts.

Growing Season	Treatment	Brix	pH	Titratable acidity (g/L)	L-malic acid (g/L)	Tartaric acid (g/L)	YAN[1] (mg/L)	Berry total phenolics (au*100/berry)[2]	Berry anthocyanins (mg/berry)
2018	Control	22 ± 0.33 ab	3.43 ± 0.04 b	5.3 ± 0.17 ab	1.7 ± 0.086 a	6.1 ± 0.11 a	750 ± 20 b	0.26 ± 0.083 b	0.8 ± 0.21 b
	Bloom + 4	21 ± 0.24 b	3.57 ± 0.03 a	6.0 ± 0.25 a	2.0 ± 0.087 a	5.8 ± 0.08 b	820 ± 20 a	0.67 ± 0.081 a	1.8 ± 0.19 a
	Bloom + 8	23 ± 0.37 a	3.52 ± 0.02 a	4.9 ± 0.13 b	1.8 ± 0.14 a	5.8 ± 0.11 b	750 ± 10 b	0.56 ± 0.052 a	1.4 ± 0.14 a
	Bloom + 12	22 ± 0.74 ab	3.51 ± 0.02 a	5.6 ± 0.38 ab	1.7 ± 0.037 a	5.8 ± 0.06 b	780 ± 27 ab	0.64 ± 0.088 a	1.5 ± 0.15 a
	p-value	0.071	**0.0148**	0.08	0.39	0.080	0.11	**0.021**	**0.018**
Treatment (T)		**0.035**	0.0755	0.20	0.14	0.34	0.70	**0.0025**	**0.0041**
Growing Season (S)		0.07	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.00040**	**<0.0001**
T x S Interaction		**0.047**	**0.0287**	**0.0015**	1.00	0.39	0.24	0.27	**0.044**

[1] YAN: Yeast Assimilable Nitrogen

[2] au: absorbance units

[3] n.m.: not measured

3.4.4. Fruit Chemistry

There was a significant treatment × season interaction found in Brix, with bloom + 8 and bloom + 12 thinning increasing Brix in 2017 but not in 2016 or 2018. Bloom + 4 thinning was indistinguishable from the non-thinned control in each year. After accounting for treatment and the interaction, growing season was not a significant factor in fruit Brix (Table 10).

Significant treatment × season interactions were found in fruit titratable acidity (TA) and pH (Table 10). In 2016, thinning treatments generally increased TA, but the opposite was observed in 2017, in which thinning treatments decreased TA. In 2018, there was no significant difference in TA between treatments. No significant difference was found in pH between treatments in 2016 or 2017 (Table 10). In 2018, all thinning treatments had higher pH than the control (Table 10). After accounting for growing season and the interaction, thinning treatment was not found to be a significant factor in fruit TA or pH (Table 10). Fruit YAN was 43% lower in 2018 than 2017 but was generally unaffected by cluster thinning treatment (Table 10).

Fruit total phenolics and anthocyanins were significantly higher for most thinning treatments relative to the control (Table 10) except for bloom + 4 in 2017, which was intermediate to and indistinguishable from the control and the other treatments (Table 10). No thinning treatment was significantly different from any other treatment in either 2017 or 2018 (Table 10).

11. Wine chemistry of Pinot noir subjected to cluster thinning at different points of the growing season from 2016-2018. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 3). Significant *p-values* (< 0.05) are shown in bold fonts.

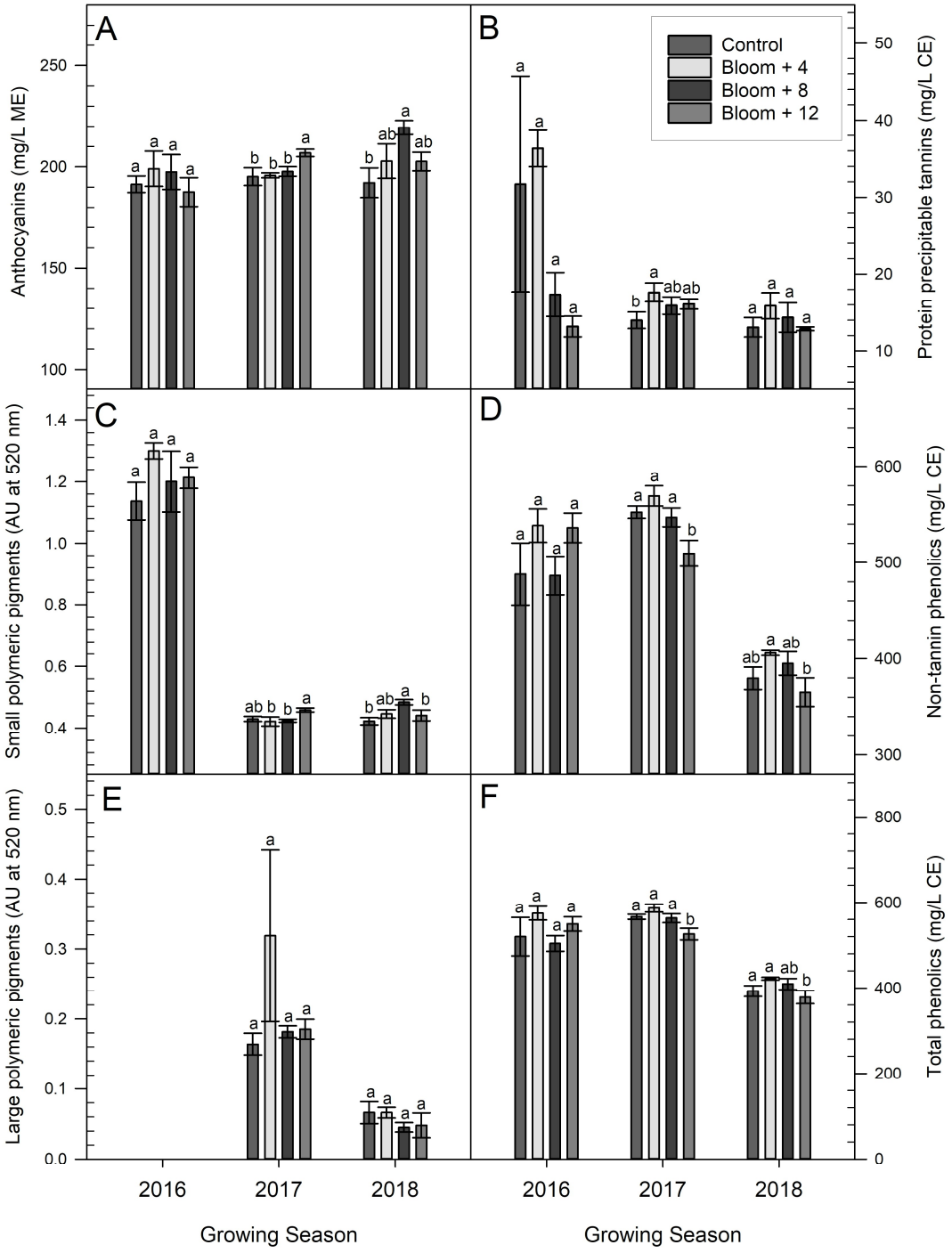
Growing season	Treatment	Ethanol (%v/v)	pH	Titratable acidity (g/L)	L-malic acid (g/L)	L-lactic acid (g/L)	Glucose-fructose (g/L)	Acetic acid (g/L)
2016	Control	13.2 ± 0.32 a	3.75 ± 0.01 a	5.7 ± 0.03 a	0.08 ± 0.00 a	1.1 ± 0.028 bc	0.48 ± 0.043 a	0.34 ± 0.0088 ab
	Bloom + 4	13.4 ± 0.14 a	3.79 ± 0.02 a	5.5 ± 0.07 b	0.07 ± 0.03 a	1.2 ± 0.0033 ab	0.46 ± 0.015 a	0.35 ± 0.012 a
	Bloom + 8	13.5 ± 0.16 a	3.77 ± 0.02 a	5.7 ± 0.03 a	0.07 ± 0.02 a	1.1 ± 0.0033 c	0.45 ± 0.027 a	0.35 ± 0.0058 a
	Bloom + 12	13.5 ± 0.02 a	3.73 ± 0.02 a	5.6 ± 0.06 ab	0.04 ± 0.01 a	1.2 ± 0.029 a	0.49 ± 0.021 a	0.32 ± 0.0067 b
	p-value	0.62	0.2335	0.07	0.66	**0.011**	0.80	0.06
2017	Control	12.9 ± 0.10 a	3.86 ± 0.09 b	5.1 ± 0.56 a	0.11 ± 0.06 a	1.4 ± 0.048 b	0.42 ± 0.038 ab	0.16 ± 0.0088 b
	Bloom + 4	12.7 ± 0.14 a	3.94 ± 0.07 c	4.4 ± 0.09 a	0.10 ± 0.04 a	1.5 ± 0.0088 a	0.39 ± 0.010 ab	0.26 ± 0.030 a
	Bloom + 8	12.9 ± 0.07 a	3.91 ± 0.07 bc	4.8 ± 0.26 a	0.01 ± 0.06 a	1.4 ± 0.018 ab	0.37 ± 0.010 b	0.28 ± 0.015 a
	Bloom + 12	13.0 ± 0.09 a	3.81 ± 0.02 a	4.9 ± 0.05 a	0.08 ± 0.06 a	1.4 ± 0.032 ab	0.45 ± 0.0058 a	0.16 ± 0.012 b
	p-value	0.51	**0.0052**	0.48	0.45	0.13	0.10	**0.0021**
2018	Control	12.4 ± 0.09 a	3.60 ± 0.02 a	5.1 ± 0.05 a	0.00 ± 0.00	1.0 ± 0.035 a	0.15 ± 0.0058 a	0.26 ± 0.012 a
	Bloom + 4	12.6 ± 0.10 a	3.66 ± 0.02 b	5.3 ± 0.08 a	0.00 ± 0.00	1.1 ± 0.032 a	0.14 ± 0.010 a	0.28 ± 0.020 a
	Bloom + 8	12.6 ± 0.10 a	3.62 ± 0.02 ab	5.2 ± 0.08 a	0.00 ± 0.00	1.0 ± 0.060 a	0.15 ± 0.012 a	0.26 ± 0.015 a
	Bloom + 12	12.1 ± 0.41 a	3.62 ± 0.09 ab	5.1 ± 0.17 a	0.00 ± 0.00	1.0 ± 0.060 a	0.14 ± 0.0067 a	0.25 ± 0.0033 a
	p-value	0.36	0.1242	0.47	---	0.57	0.87	0.38
Treatment (T)		0.68	**0.0003**	0.48	0.48	0.083	0.18	**<0.0001**
Growing Season (S)		**<0.0001**	**<0.0001**	**<0.0001**	**0.00040**	**<0.0001**	**<0.0001**	**<0.0001**
T x S Interaction		0.29	0.15	0.37	0.61	0.24	0.71	**0.0020**

3.4.5. Wine Chemistry

Growing season significantly affected all measured basic wine chemistry parameters, while cluster thinning treatment affected only wine pH and acetic acid (Table 11). Ethanol decreased from 2016 to 2018, although fruit Brix did not significantly differ between growing seasons (Table 10).

Wine pH was affected by treatment and growing season, although the treatment effect was only a significant factor in 2017 and no treatment × season interaction was found (Table 11). In 2017 bloom + 4 had the highest pH, bloom + 12 had the lowest pH and control was intermediate, with bloom + 8 indistinguishable from bloom + 4 or the control (Table 11). Acetic acid was highest in 2016, lowest in 2018, and intermediate in 2017 (Table 11).

Wine pH levels across treatments were highest in 2017 and lowest in 2018, with 2016 being intermediate (Table 11).



3. Mean values of Pinot noir wine anthocyanins (A), tannins (B), small polymeric pigments (C) non-tannin phenolics (D), large polymeric pigments (E), and phenolics (F) as a function of the different cluster thinning treatments. Error bars represent the standard error of the mean. Different letters within a single growing season indicate significant differences for Fisher's LSD and $p < 0.05$ (n = 3). au: absorbance units. CE: catechin equivalents. ME: malvidin equivalents.

3.4.6. Wine Phenolic Composition

After accounting for growing season, only anthocyanins (p = 0.0404) and total phenolics (p = 0.0362) were significantly affected by cluster thinning treatment (Table 25). All phenolic parameters measured were significantly affected by growing season, except anthocyanins, although there was a significant treatment × season interaction found in wine anthocyanins (Table 25). Despite increased berry anthocyanins found in most thinning treatments in 2017 and 2018 (Table 10), wine anthocyanins showed increases in two cluster thinning treatments: bloom + 12 thinning in 2017 and bloom + 8 thinning in 2018 (Figure 3). Wine anthocyanin content was not significantly different between growing seasons, despite fruit anthocyanins being significantly higher in 2018 than 2017. Wine total phenolics were significantly affected by cluster thinning treatment as well as growing season (Table 25). In the warm growing seasons (2017 and 2018), bloom + 12 thinning had diminished wine total phenolics: in 2017, bloom + 12 total phenolics were diminished relative to all other treatments and the control, in 2018 relative to the control and bloom + 4 thinning. In 2018, the total phenolics content of the bloom + 8 treatment were indistinguishable from any treatment or the control (Figure 3). 3).

12. Wine CIEL*a*b* color parameters of Pinot noir subjected to cluster thinning at different points of the growing season from 2016-2018. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 3). Significant *p-values* (< 0.05) are shown in bold fonts.

Growing season	Treatment	Wine L*	Wine a*	Wine b*	Wine hue angle	Wine chroma
2016	Control	80.6 ± 0.75 a	24.1 ± 0.69 a	-0.90 ± 0.12 a	-2.2 ± 0.32 a	24.1 ± 0.69 a
	Bloom + 4	80.6 ± 1.0 a	23.7 ± 1.4 a	-0.50 ± 0.40 a	-1.1 ± 0.92 a	23.7 ± 1.4 a
	Bloom + 8	80.9 ± 0.86 a	23.5 ± 0.90 a	-0.43 ± 0.35 a	-1.0 ± 0.81 a	23.5 ± 0.90 a
	Bloom + 12	80.5 ± 1.0 a	24.9 ± 1.3 a	-0.90 ± 0.12 a	-2.0 ± 0.18 a	24.9 ± 1.3 a
	p-value	0.99	0.81	0.52	0.51	0.81
2017	Control	90.1 ± 0.24 a	12.6 ± 0.43 b	5.9 ± 0.19 ab	0.44 ± 0.018 a	13.9 ± 0.40 b
	Bloom + 4	90.7 ± 0.54 a	11.6 ± 0.36 b	5.6 ± 0.11 b	0.45 ± 0.0046 a	12.9 ± 0.37 b
	Bloom + 8	90.8 ± 0.32 a	12.0 ± 0.39 b	5.7 ± 0.14 b	0.45 ± 0.074 a	13.3 ± 0.41 b
	Bloom + 12	88.2 ± 0.38 b	14.3 ± 0.45 a	6.3 ± 0.15 a	0.42 ± 0.013 a	15.6 ± 0.42 a
	p-value	**0.0053**	**0.0079**	**0.040**	0.29	**0.0062**
2018	Control	87.5 ± 0.73 a	10.7 ± 1.2 a	4.8 ± 0.20 a	0.43 ± 0.057 a	11.7 ± 0.98 a
	Bloom + 4	87.5 ± 0.81 a	9.3 ± 0.43 a	5.5 ± 0.35 a	0.54 ± 0.049 a	10.8 ± 0.18 a
	Bloom + 8	87.2 ± 0.75 a	11.0 ± 0.49 a	5.3 ± 0.19 a	0.45 ± 0.031 a	12.2 ± 0.37 a
	Bloom + 12	89.5 ± 0.67 a	10.2 ± 0.62 a	5.0 ± 0.16 a	0.46 ± 0.033 a	11.4 ± 0.53 a
	p-value	0.19	0.43	0.29	0.41	0.45
Treatment (T)		0.97	0.11	0.38	0.39	0.12
Growing Season (S)		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
T x S Interaction		0.11	0.52	**0.048**	0.52	0.48

3.4.7. Wine Color

No overall effect of treatment was found on any aspect of wine color after accounting for growing season, although there were differences between treatments in 2017 and all color parameters were affected by growing season (Table 12). In 2017, bloom + 12 wines had lower L*, higher a*, and higher chroma than all other treatments and the control, and higher b* than the other thinning treatments, indicating that wines were darker, redder, bluer, and more colorful than the other treatments or the control.

The 2016 wines had the highest a* and chroma, and the lowest L*, b*, and hue angle, indicating that 2016 wines were the reddest, bluest, darkest, and most color intense wines of any growing season. The 2017 wines had the highest L* and b*, and intermediate a*, hue angle, and chroma, indicating that 2017 wines were the lightest and most yellow of any growing season, with intermediate red hue and color intensity. The 2018 wines had the lowest a* and chroma*, and intermediate L*, b*, and hue angle, indicating that 2018 wines had the lowest color intensity, were the least red, and had intermediate lightness and blue hue relative to the other growing seasons

3.5. Discussion

The present study was conducted in the Edna Valley AVA of the Central Coast (San Luis Obispo County) of California (USA), to assess the effects of the timing of cluster thinning, and the resulting crop load reduction, on Pinot noir (clone 115) grapes and wines over three consecutive growing seasons. The study spanned one cool growing season (2016) and two warm growing seasons (2017, 2018) providing a unique opportunity to evaluate this viticultural practice over different growing conditions, particularly with regards to different temperature and degree-day accumulation conditions (Table 7).

The present study found differences in canopy parameters between growing seasons. These were likely a function of the pattern of growing degree day accumulation, with the cool growing season (2016) being associated with relatively longer internodes and smaller shoot diameters than the warm growing seasons (2017 and 2018). Higher PAR infiltration values in 2018 relative to 2017 indicate a lower canopy density. As no differences were found in pruning weight between 2017 and 2018, higher PAR infiltration values were most likely caused by lower leaf numbers, rather than fewer or less dense shoots. Temperature, along with sunlight, is directly related to grape composition and canopy growth. Increased (but not excessive) temperature was associated with increased Brix in Merlot fruit (Spayd et al., 2002) and increased pruning

weights in Shiraz (Moran et al., 2019). Similarly, increased sun exposure was associated with increased fruit anthocyanin content in Merlot (Spayd et al., 2002). Within the warm growing seasons, 2018 was a warmer, drier vintage (Table 7), which may have contributed to lower canopy density, shorter internodes, and larger shoot diameters by inducing water or heat stress. Vine nitrogen status correlates positively with canopy growth and is associated with fruit yeast assimilable nitrogen (YAN) (Bell & Henschke, 2005). While vine nitrogen status was not directly measured in this study, fruit YAN was 43% lower in 2018 than in 2017 (Table 10). This suggests lower vine nitrogen status in 2018, which could have contributed to lower canopy density, although YAN has been shown to be more sensitive than canopy growth to vine nitrogen status in Pinot noir (Schreiner et al., 2018). While 2016 and 2017 had seasonal precipitation of 66.2 and 65.1 mm, respectively, seasonal precipitation in 2-18 was lower at 13.5 mm (Table 7). Vintages with lower precipitation during the growing season have been shown to result in fruit with increased berry sugar and anthocyanin accumulation (van Leeuwen et al., 2004). However, the beneficial effects of limited seasonal precipitation are most likely related to water-deficit induced vine water stress, which was not measured in this study, and would be limited in an irrigated vineyard.

There were significant differences in vine yield between thinning treatments in 2016 but not in 2017 or 2018. The lack of significant differences in cluster count and vine yield within any single season could be caused by vineyard variation between replicates. Such variation could be the result of differences in bud fruitfulness, which would affect the both cluster number per shoot and cluster weight (Dry, 2000). Cluster number per vine, directly affected by pruning and bud fertility, has been shown to represent 60% of variation in grape yields (Martin, 2002). As cluster thinning targeted only the distal clusters, any shoot that had only a single cluster formed on the shoot would receive no thinning. Expectedly, there was a clear effect of cluster thinning on vine cluster count ($p = 0.0058$) and vine yield ($p = 0.0012$) when considering all three years of data within the model (Table 9). Deviation in cluster development between replicates within each year, potentially due to variation in soil composition or water holding capacity or pest and pathogen factors that were not accounted for in this study, could have confounded the effect of cluster thinning treatments. This, in turn, may have resulted in the lack of significant differences found within individual growing seasons despite clear trends of yield reduction with cluster thinning.

In agreement with previous research performed in Washington State (USA) on Cabernet Sauvignon, Riesling, and Chenin blanc, the effect of cluster thinning on berry size was inconsistent between growing seasons (Keller et al., 2005). However, contrary to previous studies that found increased berry size with bloom thinning (Keller et al., 2005; Reynolds et al., 1994), herein berry size was only increased in earlier season thinning relative to later thinning, with no increase found between any thinning treatment and the control. Warmer temperatures during bloom have been shown to decrease berry size but increase berry set (Kliewer, 1977), which could explain the general trend of decreasing berry size and increasing cluster weight between the cool growing season and the warm growing seasons. The bloom + 4 thinning treatment appears to have mitigated the effect of warmer temperatures on berry weight in 2018, although there was no corresponding effect on cluster weight.

Contrary to previous research indicating that cluster thinning increased Brix during cool seasons but not during warm seasons (Frioni et al., 2017), the current study found no effect of cluster thinning on Brix was found in 2016. However, some positive effects of cluster thinning were found on Brix in the warmer growing seasons of 2017 and 2018 (Table 10). That bloom + 4 thinning was indistinguishable from the control in each year of the study may be evidence for the hypothesis of downregulated photosynthetic activity resulting from early thinning (Keller et al., 2005; Naor et al., 1997; Wang et al., 2018). However, later season thinning treatments were only significantly different from the control in 2017, suggesting that the involvement of additional factors such as crop load and growing season meteorological conditions affected fruit Brix.

Fruit TA decreased from 2016 to 2017 and 2017 to 2018. However, pH was lower in 2018 than 2017, which is likely due to lower malic acid levels and higher tartaric acid levels in 2018 fruit (Table 10). Malic and tartaric acid were affected by growing season but not by the thinning treatments, and no significant treatment × season interaction was found. Fruit organic acid levels are affected by berry temperature and light exposure post-véraison, with increasing temperatures and light exposure corresponding to increased respiration and lower malate levels (Price et al., 1995; Rienth et al., 2016; Ruffner, 1982). As such, the decrease in malate, increase in tartrate, and corresponding decrease in pH from 2017 to 2018 is expected.

Fruit YAN is determined by vine nitrogen status, which is in turn dependent upon soil nitrogen availability, vine uptake, and fertilization practices (Bell & Henschke, 2005). Lower fruit YAN in 2018 may have been caused by decreased soil nitrogen. However, neither soil nitrogen or vine nitrogen status were

measured in this study, so it remains unclear whether YAN was impacted by low soil nitrogen, low vine nitrogen uptake, or another factor. Minimum YAN requirements for healthy fermentations in Pinot noir are in the range of 100 mg/L (Schreiner et al., 2018) to 140 mg/L (Bell & Henschke, 2005), although exact YAN requirements are dependent upon yeast strain (Bell & Henschke, 2005). With YAN values ranging from 749 mg/L to 1,443 mg/L, which are in clear excess of the suggested minimum ranges, any detrimental effects on wine composition associated with low YAN are unlikely.

Fruit phenolics (Table 10) were consistent with previously reported increases in Pinot noir berry phenolics with cluster thinning (Cañón et al., 2014) despite the lack of differences found in crop load and vine yield in the present study (Table 9). The absence of differences between cluster thinning treatments regardless of application timing may indicate that differences in berry phenolics are not due to a direct physiological response to cluster thinning and crop load manipulation but rather a response to differences in vine microclimate caused by cluster thinning. For example, in Merlot grapes, increasing sun exposure, independent of the effect of temperature, increased berry anthocyanins and phenolics (Spayd et al., 2002) and in Pinot noir grapes increased quercetin (Price et al., 1995), although the latter study found no effect on berry anthocyanins in Pinot noir. Pinot noir skin and seed tannins have also been shown to be higher in years with more heat accumulation (Pastor del Rio & Kennedy, 2006). Cluster thinning treatments, through the removal of all but the basal cluster, may have received increased average sun exposure relative to the control treatments which included fruit from more distal nodes that were more shaded by the canopy. Increased sun exposure in 2018 due lower canopy density relative to 2017 (Table 8) may explain the increased fruit anthocyanin and total phenolics in 2018, or the increase could simply be in response to higher GDD (Table 7).

The ethanol of the finished wines decreased from 2016 to 2018 (Table 11), although fruit Brix at harvest did not differ significantly between growing seasons (Table 10). Fermentation temperature driven differences in yeast alcohol conversion ratio are most likely responsible for the differences in wine ethanol content between 2016 and 2017, as fermentation temperatures were elevated in 2017 (Table 27). As fermentation temperature increases, *Saccharomyces cerevisiae* ethanol production decreases and volatilization of ethanol increases (Ough & Amerine, 1967). Fermentation temperatures in 2018 were similar to fermentation temperatures in 2016. However, average Brix in 2018 was 21.79 compared to 22.33 in 2017

and 22.5 in 2016 (Table 10), which, when combined with slight temperature differences, is likely responsible for the reduced ethanol content in 2018.

While fruit pH was not significantly different between treatments in 2017, lower average and maximum fermentation temperatures in 2017 corresponded with higher wine pH and acetic acid levels (Table 11, Table 27). As *Oenococcus* and *Lactobacillus* fermentation activity in wine is affected by temperature (Guerzoni et al., 1995), it is possible that fermentation temperature, combined with small differences in fruit pH and malic acid content, contributed to the differences found in wine pH. Although differences were found in wine acetic acid between growing seasons, the levels of acetic acid were relatively low across the treatments and are thus unlikely to have a sensory effect. Lower wine pH in 2018 could be due to the lower levels of lactic acid caused by the lower levels of malic acid at harvest (Table 10). Wine L-lactic acid levels were lower in 2018 than 2017 or 2016, likely due to lower malate levels at harvest as lactic acid content in wine is derived from the fermentation of malic acid to lactic acid. Although malic acid was not measured on fruit in 2016, differences between 2018 and 2016 could be caused by the different strains of malolactic bacteria used: in 2016, wines were inoculated with VP-41, (*Oenococcus oeni*), and in 2017 and 2018 wines were inoculated with ML-Prime, (*Lactobacillus plantarum*). Wine TA was only significantly affected by growing season (Table 11). Wine TA was highest in 2016, corresponding with fruit TA (Table 10), lowest in 2017, and intermediate in 2018 (Table 11). That 2017 had higher fruit TA and lower wine TA than 2018 is likely due to elevated levels of malic acid in 2017, which caused a larger reduction in TA in 2017 following malolactic fermentation. Regarding glucose and fructose, low levels are indicative of a complete alcoholic fermentation and thus all wines are considered dry from a technical and sensory standpoint.

The extraction of polyphenols from fruit into wine is modulated by many factors secondary to fruit native phenolic content, including the relative skin, seed, and pulp content of fruit (Sparrow et al., 2015), fermentation temperature (Lerno et al., 2015) and ethanol concentration (Casassa, Beaver, et al., 2013). Furthermore, different tissues within the grape berry have different rates of extraction. For example, less than 50% of fruit tannin is typically extracted and incorporated into wine (Harbertson et al., 2002). Although there were no significant differences in these factors between treatments, because winemaking conditions were kept strictly consistent during the three growing seasons it is possible that the combination of small

differences in berry composition and fermentation temperature had an additive effect on phenolic extraction, causing the observed inconsistency between fruit polyphenol composition and wine polyphenol composition.

Wine polymeric pigments are formed by the covalent polymerization of anthocyanins with flavanols or tannins (Casassa & Harbertson, 2014).Wine polymeric pigments are known to provide stable color (Somers, 1971) and are also associated with positive mouthfeel properties. Within polymeric pigments, large polymeric pigments can precipitate proteins, creating astringency and altering wine mouthfeel (Weber et al., 2013), whereas small polymeric pigment cannot precipitate proteins. Polymeric pigments, herein including small and large polymeric pigments, were not affected by cluster thinning treatment after accounting for growing season (Table 25), although there were compositional differences in polymeric pigments between growing seasons (Figure 3). The 2016 wines had only small polymeric pigments, whereas wines in 2017 and 2018 had predominantly small polymeric pigments but contained long polymeric pigments as well (Figure 3), suggesting potentially higher astringency in these wines. Total polymeric pigments were highest in 2016, when wine tannin was highest, intermediate in 2017, when total phenolics were highest, and lowest in 2018, when total phenolics were lowest, suggesting that polymeric pigment formation was related to the non-anthocyanin phenolic composition of the wine, even when anthocyanins were unchanged. Indeed, previous research has found that polymeric pigment formation is positively associated with tannin concentration (Casassa, Larsen, & Harbertson, 2016).

As there were no differences found in wine anthocyanins between growing seasons (Figure 3), wine color differences were driven by differences in pH, and polymeric pigment content. As pH increases, chroma decreases and anthocyanin hue shifts from blue to red (Heredia et al., 1998). Wine pH was lowest in 2018, highest in 2017, and intermediate in 2016, suggesting that there was an interaction between wine pH and another factor that influenced wine chroma and red color. Polymeric pigments may result in decreased wine chroma as they reportedly have a lower molar extinction coefficient relative to that of native anthocyanin (Weber et al., 2013), but the full chromatic properties of polymeric pigments are not yet known. Wines from 2017 and 2018, which contained large polymeric pigments, were lighter and yellower than 2016 wines that contained only short polymeric pigments, although 2016 wines had higher levels of total polymeric pigments than 2017 or 2018. Bloom + 12 wines in 2017 had more anthocyanins than all other wines, more polymeric

pigments than bloom + 4 and bloom + 8 wines (Figure 3), and lower pH than all other wines (Table 11), each of which contributed to the differences found in color profile.

3.6. Conclusions

A three-year study was conducted to evaluate the effects of cluster thinning applied at different points during the growing season on Pinot noir vegetative growth, fruit composition, and resulting wines. Due to low initial crop load combined with site yield variation, and despite an average yield reduction of 43% caused by cluster thinning, vine canopy, fruit, and wine chemistry characteristics were affected more by meteorological differences between growing seasons than by any of the cluster thinning treatments. When cluster thinning treatments did significantly affect fruit or wine composition, the direction and magnitude of the effects differed between growing seasons. We suggest that the observed inconsistency of the practice of cluster thinning on fruit and wine parameters between the three growing seasons were due to the differences in meteorological conditions found between growing seasons and the yield variation observed in the vineyard. Cluster thinning, regardless of the timing of it, generally increased anthocyanins and total phenolics in fruit. However, the effect did not carry through to wines and there were few differences found the phenolic profile and color of the resulting wines. The overall lack of effect of the various cluster thinning treatments in the present study suggests that the vines tested were at or below a balanced crop load prior to the application of cluster thinning. We conclude that Pinot noir grown in the Edna Valley AVA could likely support crop loads higher than 3.2 without negatively impacting fruit or wine composition and that reducing crop load below that level is unlikely to increase fruit or wine quality.

CHAPTER 4. CONCLUSIONS AND FURTHER RESEARCH DIRECTIONS

A three-year study was conducted at Chamisal Vineyards in California's Edna Valley AVA to evaluate the physiological and agronomical effects of the timing of cluster thinning on Pinot noir (clone 115) grapevines. Vines were thinned to one cluster per shoot at three selected time-points during the growing season (bloom + 4 weeks, bloom + 8 weeks, bloom + 12 weeks), and fruit from each treatment was harvested and made into wine. Across all growing seasons, yield decreased 43% in thinned vines relative to non-thinned control vines, although significant differences between yields were only found in one individual growing season due to inherent site variation.

Due to low initial crop load and site yield variations, the ability of the research team to study the effects of cluster thinning was limited. There were substantial differences in meteorological conditions during the three growing seasons studied, and in statistical analysis the growing season accounted for more variation than did the cluster thinning treatments. The magnitude and direction of cluster thinning effects differed between growing seasons, suggesting that there is no blanket application of cluster thinning that would be appropriate for every growing season. There were some differences observed between cluster thinning applications within growing seasons, suggesting that the timing of cluster thinning does play a role, but initial crop loads were not high enough to properly evaluate that role in this study. Future research should be conducted with higher initial crop loads to determine the effects of cluster thinning timing in Pinot noir, and to evaluate proper crop load guidelines for Pinot noir on the Central Coast of California.

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13. Vine fruit yield and fruit physical composition by treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's least significant difference (LSD) test at $p < 0.05$.

Growing Season	Treatment	Clusters per Vine	Vine Yield (kg)	Cluster Weight (g)	Berry Weight (g)	Seed Weight (g)	Seeds per Berry	Pruning Weight (kg)	Ravaz Index
2016	Control	33 ± 4.93 a	2.10 ± 0.34 a	63.18 ± 1.31 a	1.18 ± 0.01 a	n.d.[1]	n.d.	n.d.	n.d.
	Bloom + 4	22.4 ± 5.29 ab	1.27 ± 0.25 b	58.86 ± 7.21 a	1.08 ± 0.04 ab	n.d.	n.d.	n.d.	n.d.
	Bloom + 8	20.1 ± 0.77 b	1.04 ± 0.16 b	51.35 ± 6.33 a	0.97 ± 0.01 b	n.d.	n.d.	n.d.	n.d.
	Bloom + 12	23.1 ± 2.74 ab	1.24 ± 0.03 b	54.70 ± 5.87 a	1.12 ± 0.10 ab	n.d.	n.d.	n.d.	n.d.
	p-value	0.1701	**0.0461**	0.519	0.0986	n.d.	n.d.	n.d.	n.d.
2017	Control	32.30 ± 4.64 a	2.76 ± 0.59 a	86.42 ± 8.04 a	0.98 ± 0.07 a	0.060 ± 0.007 a	1.37 ± 0.05 a	0.86 ± 0.06 a	3.23 ± 0.79 a
	Bloom + 4	19.10 ± 0.95 b	1.27 ± 0.19 b	72.77 ± 9.23 a	1.01 ± 0.06 a	0.042 ± 0.009 a	1.19 ± 0.12 a	0.73 ± 0.08 a	1.67 ± 0.17 b
	Bloom + 8	23.70 ± 0.30 ab	1.63 ± 0.05 b	70.02 ± 2.07 a	0.95 ± 0.06 a	0.052 ± 0.000 a	1.44 ± 0.06 a	0.91 ± 0.06 a	1.91 ± 0.15 ab
	Bloom + 12	21.10 ± 2.03 b	1.64 ± 0.26 b	77.12 ± 6.45 a	1.01 ± 0.11 a	0.051 ± 0.004 a	1.30 ± 0.11 a	0.83 ± 0.06 a	2.01 ± 0.34 ab
	p-value	0.1179	0.0616	0.4665	0.9446	0.2773	0.3983	0.1921	0.1352

[1] n.d.: Not determined.

14. Fruit chemical composition by treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font. TA—titratable acidity.

Growing Season	Treatment	Fruit Brix	Fruit pH	Fruit TA (g/L)
2016	Control	22.73 ± 0.43 a	3.48 ± 0.04 a	6.03 ± 0.17 b
	Bloom + 4	22.67 ± 0.44 a	3.44 ± 0.03 a	6.47 ± 0.19 b
	Bloom + 8	22.70 ± 0.26 a	3.47 ± 0.02 a	6.63 ± 0.15 ab
	Bloom + 12	21.87 ± 0.15 a	3.37 ± 0.05 a	7.53 ± 0.53 a
	p-value	0.2908	0.4030	**0.0433**
2017	Control	21.83 ± 0.27 b	3.60 ± 0.01 a	6.68 ± 0.13 a
	Bloom + 4	22.17 ± 0.17 ab	3.61 ± 0.01 a	6.28 ± 0.15 ab
	Bloom + 8	22.73 ± 0.15 a	3.62 ± 0.01 a	6.08 ± 0.04 bc
	Bloom + 12	22.57 ± 0.19 a	3.59 ± 0.02 a	5.65 ± 0.28 c
	p-value	**0.0475**	0.2865	**0.0184**

15. Fermentation Temperature by treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Growing Season	Treatment	Average Temperature (°C)	Maximum Temperature (°C)
2016	Control	21.10 ± 0.06 c	26.23 ± 0.15 b
	Bloom+4	21.52 ± 0.08 a	26.57 ± 0.03 b
	Bloom+8	21.44 ± 0.12 ab	27.07 ± 0.07 a
	Bloom+12	21.18 ± 0.12 bc	26.20 ± 0.25 b
	p-value	**0.0456**	**0.0117**
2017	Control	25.08 ± 0.12 a	31.17 ± 0.50 a
	Bloom+4	24.69 ± 0.10 b	29.20 ± 0.35 b
	Bloom+8	24.58 ± 0.04 b	28.97 ± 0.23 b
	Bloom+12	25.28 ± 0.06 a	30.73 ± 0.27 a
	p-value	**0.0014**	**0.0049**

16. Wine chemical composition post-malolactic fermentation by treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Growing Season	Treatment	L-Malic (g/L)	L-Lactic (g/L)	Residual Sugar (g/L)	Acetic Acid (g/L)	EtOH (% *v/v*)	pH	Titratable acidity (g/L)
2016	Control	0.08 ± 0.00 a	1.12 ± 0.03 bc	0.48 ± 0.04 a	0.34 ± 0.01 ab	13.18 ± 0.32 a	3.75 ± 0.01 a	5.67 ± 0.03 a
	Bloom+4	0.07 ± 0.04 a	1.18 ± 0.00 ab	0.46 ± 0.02 a	0.35 ± 0.01 a	13.35 ± 0.14 a	3.79 ± 0.02 a	5.47 ± 0.07 b
	Bloom+8	0.07 ± 0.02 a	1.11 ± 0.00 c	0.45 ± 0.03 a	0.35 ± 0.01 a	13.48 ± 0.16 a	3.77 ± 0.02 a	5.67 ± 0.03 a
	Bloom+12	0.04 ± 0.01 a	1.23 ± 0.03 a	0.49 ± 0.02 a	0.32 ± 0.01 b	13.50 ± 0.02 a	3.73 ± 0.02 a	5.60 ± 0.06 ab
	p-value	0.6627	**0.0111**	0.8018	0.062	0.6209	0.2335	0.0672
2017	Control	0.11 ± 0.06 a	1.35 ± 0.05 ab	0.42 ± 0.04 ab	0.16 ± 0.01 b	12.88 ± 0.10 a	3.86 ± 0.01 b	5.05 ± 0.56 a
	Bloom+4	0.10 ± 0.04 a	1.46 ± 0.01 a	0.39 ± 0.01 ab	0.26 ± 0.03 a	12.74 ± 0.14 a	3.94 ± 0.01 a	4.35 ± 0.09 a
	Bloom+8	0.01 ± 0.01 a	1.40 ± 0.02 a	0.37 ± 0.01 b	0.28 ± 0.01 a	12.87 ± 0.07 a	3.91 ± 0.01 a	4.75 ± 0.26 a
	Bloom+12	0.08 ± 0.06 a	1.42 ± 0.03 b	0.45 ± 0.01 a	0.16 ± 0.01 b	12.98 ± 0.09 a	3.81 ± 0.02 c	4.90 ± 0.05 a
	p-value	0.4522	0.1326	0.1003	**0.0021**	0.5106	**0.0052**	0.4772

17. Wine chromatic parameters post-malolactic fermentation by treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Growing Season	Treatment	L*	a*	b*	Hue	Chroma
2016	Control	80.63 ± 0.75 a	24.13 ± 0.69 a	-0.90 ± 0.12 a	−2.17 ± 0.32 a	24.13 ± 0.69 a
	Bloom + 4	80.60 ± 1.01 a	23.73 ± 1.39 a	-0.50 ± 0.40 a	−1.13 ± 0.92 a	23.73 ± 1.39 a
	Bloom + 8	80.93 ± 0.86 a	23.47 ± 0.90 a	-0.43 ± 0.35 a	−1.00 ± 0.81 a	23.47 ± 0.90 a
	Bloom + 12	80.50 ± 1.03 a	24.90 ± 1.26 a	-0.90 ± 0.12 a	−1.97 ± 0.18 a	24.90 ± 1.26 a
	p-value	0.988	0.8069	0.5211	0.5131	0.8069
2017	Control	90.05 ± 0.24 a	12.61 ± 0.43 b	5.91 ± 0.19 ab	0.44 ± 0.02 a	13.94 ± 0.40 b
	Bloom + 4	90.74 ± 0.54 a	11.61 ± 0.36 b	5.60 ± 0.11 b	0.45 ± 0.00 a	12.89 ± 0.37 b
	Bloom + 8	90.76 ± 0.32 a	11.96 ± 0.39 b	5.72 ± 0.14 b	0.45 ± 0.01 a	13.26 ± 0.41 b
	Bloom + 12	88.24 ± 0.38 b	14.25 ± 0.45 a	6.31 ± 0.15 a	0.42 ± 0.01 a	15.59 ± 0.42 a
	p-value	**0.0053**	**0.0079**	**0.0402**	0.2863	**0.0062**

18. Wine Phenolic profile post-malolactic fermentation by treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD at $p < 0.05$. *p-values* below 0.05 are shown in bold font.

Growing Season	Treatment	Total Anthocyanins (mg/L Malvidin Equivalents)	Total Polymeric Pigments (Absorbance at 520 nm)	Total Tannins (mg/L Catechin Equivalents)	Total Phenolics (mg/L Catechin Equivalents)	Non-Tannin Phenolics (mg/L Catechin Equivalents)
2016	Control	191.41 ± 4.09 a	1.13 ± 0.15 a	31.70 ± 14.01 a	519.61 ± 45.10 a	487.91 ± 32.40 a
	Bloom+4	199.07 ± 8.70 a	1.16 ± 0.05 a	36.35 ± 2.36 a	574.75 ± 16.31 a	538.40 ± 17.35 a
	Bloom+8	197.44 ± 8.52 a	1.04 ± 0.07 a	17.36 ± 2.86 a	503.71 ± 18.50 a	486.35 ± 20.19 a
	Bloom+12	187.57 ± 7.13 a	1.07 ± 0.06 a	13.19 ± 1.34 a	549.21 ± 16.60 a	536.03 ± 15.42 a
	p-value	0.6755	0.7662	0.1500	0.3215	0.2481
2017	Control	195.17 ± 4.37 b	0.59 ± 0.01 a	14.00 ± 1.08 b	566.31 ± 5.92 a	552.31 ± 6.52 a
	Bloom+4	195.78 ± 1.22 b	0.74 ± 0.13 a	17.63 ± 1.22 a	586.96 ± 9.42 a	569.32 ± 10.49 a
	Bloom+8	197.67 ± 2.37 b	0.61 ± 0.01 a	15.92 ± 1.15 ab	562.89 ± 10.46 a	546.97 ± 9.83 a
	Bloom+12	206.86 ± 1.85 a	0.64 ± 0.02 a	16.13 ± 0.67 ab	525.71 ± 13.49 b	509.59 ± 13.52 b
	p-value	0.0522	0.4078	0.1905	**0.0171**	**0.0203**

19. Monthly weather data from California Irrigation Information Management System (CIMIS) weather station 52 in San Luis Obispo during the 2016 and 2017 growing seasons. Monthly average air temperature, minimum air temperature, and maximum air temperature.

Month	2016 Average Air Temperature (°C)	2016 Minimum Air Temperature (°C)	2016 Maximum Air Temperature (°C)	2017 Average Air Temperature (°C)	2017 Minimum Air Temperature (°C)	2017 Maximum Air Temperature (°C)
April	15	8.6	22.2	15.6	10.2	22.2
May	14.7	10.2	20.6	15.9	10.9	22.4
June	18.3	11.4	26.5	18.9	12.9	27.1
July	17.4	11.5	25.8	19.6	13.7	28.5
August	16.7	12.6	24	19.9	15	28.1
September	18.2	11.8	26.7	20	14.4	27.6
October	17.5	11.7	24.8	19.3	12.9	28.2

20. Two-way ANOVA *p-values* corresponding to the main effects and interaction between cluster thinning treatments and growing season on Pinot noir vine vegetative growth. Significant *p-values* (< 0.05) are shown in bold fonts.

Effect	Internode Length	Shoot Diameter	Fruit Zone PAR[1]*	Pruning Weight*
Treatment	0.63	0.11	0.74	0.17
Year	**<0.0001**	**<0.0001**	**<0.0001**	**0.0052**
Interaction	0.25	0.68	0.81	0.30

[1] PAR: Photosynthetically Active Radiation

*Data only from the 2017 and 2018 growing seasons

21. Two-way ANOVA *p-values* corresponding to the main effects and interaction between cluster thinning treatments and growing season on Pinot noir vine yield and fruit physical properties. Significant *p-values* (< 0.05) are shown in bold fonts.

Effect	Cluster Count	Vine Yield	Cluster Weight	Ravaz Index	Berry Weight	Seed Weight *	Seeds per Berry*
Treatment	**0.0058**	**0.0012**	0.29	0.051	**0.049**	0.56	0.87
Year	0.15	0.074	**0.0004**	0.57	**0.0016**	0.48	0.74
Interaction	0.95	0.31	0.17	0.28	0.13	0.30	0.029

* Data only from the 2017 and 2018 growing seasons

22. Two-way ANOVA *p-values* corresponding to the main effects and interaction between cluster thinning treatments and growing season on Pinot noir fruit chemistry. Significant *p-values* (< 0.05) are shown in bold fonts.

Effect	Brix	Titratable Acidity	pH	YAN[1] *	Total Phenolics*	Anthocyanins*
Treatment (T)	**0.035**	0.20	0.0755	0.70	**0.0025**	**0.0041**
Growing Season (S)	0.070	**<0.0001**	**<0.0001**	**<0.0001**	**0.0004**	**<0.0001**
T × S Interaction	**0.047**	**0.0015**	**0.0287**	0.24	0.27	**0.044**

[1] YAN: Yeast Assimilable Nitrogen

* Data only from the 2017 and 2018 growing seasons

23. Two-way ANOVA *p-values* corresponding to the main effects and interaction between cluster thinning treatments and growing season on Pinot noir wine chemistry. Significant *p-values* (< 0.05) are shown in bold fonts.

Effect	Ethanol	pH	Titratable Acidity	L-Malic	L-Lactic	Glucose-Fructose	Acetic Acid
Treatment (T)	0.68	**0.0003**	0.48	0.48	0.083	0.18	**<0.0001**
Growing Season (S)	**<0.0001**	**<0.0001**	**<0.0001**	**0.00040**	**<0.0001**	**<0.0001**	**<0.0001**
T × S Interaction	0.29	0.15	0.37	0.61	0.24	0.71	**0.0020**

24. Two-way ANOVA *p-values* corresponding to the main effects and interaction between cluster thinning treatments and growing season on Pinot noir wine phenolics. Significant *p-values* (< 0.05) are shown in bold fonts.

Effect	Anthocyanins (mg/L ME[1])	Tannins (mg/L CE[2])	Total phenolics (mg/L CE)	Non-tannin phenolics (mg/L CE)	Short polymeric pigments (au[3] at 520 nm)	Long polymeric pigments (au at 520 nm)
Treatment	**0.040**	0.067	**0.036**	0.055	0.27	0.32
Year	0.092	**0.0052**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interaction	**0.046**	0.092	0.30	0.11	0.33	0.17

[1] ME: Malvidin Equivalents

[2] CE: Catechin Equivalents

[3] au: absorbance units

25. Two-way ANOVA *p-values* corresponding to the main effects and interaction between cluster thinning treatments and growing season on Pinot noir wine color. Significant *p-values* (< 0.05) are shown in bold fonts.

Effect	L*	a*	b*	Hue Angle	Chroma
Treatment (T)	0.97	0.11	0.38	0.39	0.12
Growing Season (S)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
T × S Interaction	0.11	0.52	**0.045**	0.52	0.48

26. Pinot noir wine fermentation temperature by cluster thinning treatment and growing season. Treatment means followed by standard error of the mean. Different letters within a column and growing season indicate significant differences between treatment groups for Fisher's LSD (n = 3). Significant *p-values* (< 0.05) are shown in bold fonts.

Growing Season	Treatment	Average Fermentation Temperature (°C)	Maximum Fermentation Temperature (°C)
2016	Control	21 ± 0.061 bc	26 ± 0.15 b
	Bloom + 4	22 ± 0.076 a	27 ± 0.033 b
	Bloom + 8	21 ± 0.12 ab	27 ± 0.067 a
	Bloom + 12	21 ± 0.12 c	26 ± 0.25 b
	p-value	**0.045**	**0.012**
2017	Control	25 ± 0.12 a	31 ± 0.50 a
	Bloom + 4	25 ± 0.10 b	29 ± 0.35 b
	Bloom + 8	25 ± 0.038 b	29 ± 0.23 b
	Bloom + 12	25 ± 0.058 a	31 ± 0.27 a
	p-value	**0.0014**	**0.0049**
2018	Control	21 ± 0.073 a	23 ± 0.20 ab
	Bloom + 4	21 ± 0.043 a	23 ± 0.13 ab
	Bloom + 8	21 ± 0.055 a	23 ± 0.12 a
	Bloom + 12	21 ± 0.067 a	23 ± 0.18 b
	p-value	0.59	0.15
Treatment (T)		0.10	0.12
Growing Season (S)		**<0.0001**	**<0.0001**
T x S Interaction		**<0.0001**	**<0.0001**