Cluster-zone leaf removal refers to deliberate removal of selected leaves around grape clusters. It has been widely used in the vineyards of cool-climate viticultural regions to improve air circulation and sunlight exposure as well as decrease disease pressure.\textsuperscript{10,44,56}

Canopy microclimate is important in determining fruit and wine quality.\textsuperscript{18,55} A dense canopy with inadequate sunlight exposure can result in poor-quality grapes.\textsuperscript{34} On the contrary, sunlight-exposed grapes are generally higher in total soluble solids, anthocyanins and phenolics and lower in titratable acidity and malate compared to shaded fruit—although climate conditions may have an important impact.\textsuperscript{7,21,22}

Overexposure to sunlight also leads to greater than optimal berry temperature, resulting in fruit sunburn and inhibition of color development.\textsuperscript{52} Therefore, determining appropriate levels of leaf removal for optimum sunlight exposure is important for producers to consider when managing vines to yield high-quality grapes.

Grape-derived volatile secondary metabolites play important roles in fruit and wine aroma quality, since they reflect the particular variety, vineyard, regional climate and soil type.\textsuperscript{41} Only a small portion of grape volatile compounds are present in their free forms, and the majority exist in non-volatile, glycosidically bound forms or other precursor forms.\textsuperscript{16,59} However, these non-volatile precursors can be converted to the volatile form through enzymatic or chemical hydrolysis during vinification and aging, thus contributing to wine aroma.\textsuperscript{16,28,29}

Little research has been done on volatile composition with leaf removal in red grape cultivars. Many studies investigated the influence of leaf removal on grape-derived terpenoids of white grape varieties; however, results are still inconclusive. For example, in research conducted in British Columbia (Canada), basal leaf removal increased both free- and bound-form terpenoids in Gewürztraminer grapes.\textsuperscript{44} However, research on Riesling has shown only increases in bound-form terpenoids with leaf removal.\textsuperscript{45,64} Conversely, in central Europe, wine made from Riesling grapes grown with leaf removal showed no differences in free and bound forms of terpenoids compared to those with no leaf removal, while increases in free- and bound-form terpenoids were observed in Sauvignon Blanc wine by leaf removal.\textsuperscript{24} Conflicting results may be due to vineyard location, seasonal climate, cultivar, rootstock, timing and severity of leaf removal.

The effects of leaf removal on grape-derived C$_{13}$-norisoprenoids have not been sufficiently studied. C$_{13}$-norisoprenoids constitute an important part of the volatile compounds of “neutral” type grapes such as Cabernet Sauvignon,\textsuperscript{3} Syrah,\textsuperscript{39} Sauvignon Blanc\textsuperscript{30} and Pinot Noir.\textsuperscript{12} These compounds can be formed by direct degradation of carotenoids, or they can be stored as glycoconjugates that release their volatile aglycone during fermentation via enzymatic and acid hydrolysis processes.\textsuperscript{2,50,59}

Sunlight exposure has been speculated to influence levels of C$_{13}$-norisoprenoids in grapes. R. Ristic et al. reported that after acid hydrolysis, Shiraz wine made from shaded fruit had decreased levels of β-damascenone and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) compared to those made from typically exposed fruit.\textsuperscript{46} Other research has reported either an increase or no change of β-damascenone in shaded grapes.\textsuperscript{25,27}

Fruit-zone shading is a concern in the Pinot Noir vineyards of Oregon’s Willamette Valley due to the high vegetative growth common in the region. Excessive vine growth results from the region’s ample winter and spring rainfall combined with the high water-holding capacity of soils. Therefore, basal leaf removal is commonly applied post-fruit set in vineyards throughout the region. However, it is unclear what level of leaf removal is required to achieve optimal quality. A three-year study was designed...
to investigate varying intensities of basal leaf removal on Pinot Noir grape quality with a focus on volatile compounds and their precursors.

Materials and methods

Vineyard experimental design

A leaf-removal trial was conducted from 2010 to 2012 in two vineyards located in Oregon’s Willamette Valley. In 2010, the trial was conducted at a commercial vineyard in Dayton, Ore. The vineyard was planted in 1995 to a vine density of 1,248 vines per acre with Pinot Noir clone 115 grafted to 330C rootstock. The vine rows are oriented north-south with tractor row by vine spacing of 1 meter by 1.5 meters.

In 2011 and 2012, the trial was conducted at Oregon State University’s Woodhall Research Vineyard in Alpine, Ore. This vineyard was planted in 2006 to a vine density of 1,383 vines per acre with Pinot Noir clone Pommard grafted to 101-14 rootstock. The vine rows are oriented north-south with tractor row by vine spacing of 1.4 meters by 2.1 meters.

Both vineyards were cane-pruned to a bi-lateral Guyot system and vertically shoot-positioned. Standard vineyard-management practices including pest and canopy management were performed each year with the exception of leaf removal.

Basal leaves were removed from vines with four different intensities. Treatments included: 1) 0% leaf removal; 2) 50% leaf removal, where every other leaf along the shoot was removed starting at the basal node and working up to the node above the top-most cluster; 3) industry standard (IS) leaf removal, where only leaves that covered the clusters on the eastern (morning sun) side of the vine canopy were removed, and 4) 100% of leaves removed from both the east and west side of the cluster zone, starting from the base of the shoot up to the node above the top-most cluster.

The IS treatment was evaluated in 2011 and 2012 to compare treatments with commercial practices in vineyards. Leaf removal was imposed at one point in the growing process, the pea-sized stage of berry development, on six-vine plots in a randomized complete block design with five field replicates. At the time of leaf removal in the 100%, 50% and IS treatments, all lateral shoots in the cluster zone were removed.

Weather data

Weather data were collected onsite for each growing season. Data for daily temperature were logged and used to calculate growing degree-days and the mean daily temperature. Growing degree-day (GDD$_{50}$) units were calculated using the daily mean of $T_{\text{max}}$ and $T_{\text{min}}$ with a minimum threshold of 50°F and no maximum threshold applied. Daily precipitation was recorded for each growing season.

Vine growth and cluster exposure

Canopy size and density, photosynthetically active radiation, yield and dormant pruning weights were measured annually. In 2010, the amount of leaves removed were determined by collecting all primary and lateral leaves removed during treatment application, bringing them to the lab and scanning them on a leaf-area meter.

In 2011 and 2012, more detailed leaf-area quantification was conducted. Leaf areas were measured after treatment application and at véraison. After applying leaf-removal treatments, both total leaf area removed and remaining on the vine were measured using a non-destructive quantification method described in Schreiner et al. The percentage of leaf area removed was calculated and compared between leaf removal treatments.

Vine leaf area was quantified at véraison each year (2010, 2011, 2012) using the non-destructive template method described above. The template was used to measure all leaves on one randomly selected shoot from each of the six experimental vines per plot. The shoot leaf area was multiplied by shoot count to calculate whole vine leaf area.

Incident light in the cluster zone was quantified shortly after véraison, in the early ripening stages, each year on a clear, cloudless day by using a LP-80 ceptometer. This device measures photosynthetically active radiation (PAR), and it was quantified at 10 a.m., solar noon and 2:30 p.m. by placing the sensor rod parallel to the vine row at the height of the cluster zone on the east and west side of north-south-oriented vine rows.

Analysis of grape maturity parameters at harvest

A seven-cluster sample was randomly selected from grapes harvested from each plot, transported to the lab and kept cool (42.8°F) until analysis. All clusters were measured for cluster size metrics (cluster weight, berry weight and berries per cluster). The berries from the seven-cluster sample were pressed to juice to measure total soluble solids (TSS), pH and titratable acidity by titration.

A subset of harvested clusters were transported to the lab and immediately frozen at -176°F.

BASIC FRUIT MATURITY AT HARVEST RELATIVE TO LEAF REMOVAL

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>TSS (Brix)</th>
<th>pH</th>
<th>TA* (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010 (Dayton, Ore.)</td>
<td>None</td>
<td>20.9*</td>
<td>3.14 a</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>20.4*</td>
<td>3.08 b</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>20.3*</td>
<td>3.09 ab</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td>n.s.</td>
<td>0.0362</td>
<td>n.s.</td>
</tr>
<tr>
<td>2012 (Alpine, Ore.)</td>
<td>None</td>
<td>19.8*</td>
<td>3.05</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>19.9*</td>
<td>3.06</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>19.4*</td>
<td>3.00</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>25.0*</td>
<td>3.21</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>25.3*</td>
<td>3.27</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>25.1*</td>
<td>3.24</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>25.1*</td>
<td>3.19</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>$p$</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Means are presented (n=5); different letters indicate a difference in means between treatments using Tukey HSD mean separation at α=0.05.; n.s.: indicates no statistical differences. a: TA refers to titratable acidity shown in g/L of tartaric acid equivalents. Leaf removal treatments include the following: None (no leaf removal), 100% (all leaves in the cluster zone removed), 50% (half of the leaves in the cluster zone removed) and IS (industry standard where leaves are only removed in the cluster zone on the east side of the canopy).
After two days, the grapes were manually destemmed while frozen. Berries from different clusters were pooled and sealed in storage bags for each field-replicate respectively and stored at -80°C until analysis of secondary metabolites.

Results and discussion

Weather and vine performance

Weather conditions: Weather data varied between years and the two trial sites. Focus was placed on seasonal weather comparisons of the Alpine vineyard, as the trial was replicated at that site for two years. The 2011 season was cooler than 2012, with mean daily temperatures being 0.36°F cooler in 2011 and heat unit accumulation 63 GDD$_{50}$ lower than in 2012.

Precipitation in the 2011 growing season was more than double that of 2012. Consistent with the different weather conditions, vine phenological development exhibited temporal variation between years and sites.

The number of days between bud break and harvest was nearly two weeks longer at the Dayton site (2010) than at the Alpine site in 2011 and 2012. At the Alpine site, warmer temperatures and less precipitation in 2012 led to advanced fruit ripening and an earlier harvest compared to 2011. In 2012, fruit reached about 25° Brix at 45 days post-véraison, while fruit reached about 20° Brix at 45 days post-véraison in 2011. (See table “Basic Fruit Maturity at Harvest Relative to Leaf Removal” on page 51.)

Vines were delayed in development in 2011 due to a cool spring and summer, and fruit was not allowed to remain on the vine as long to achieve higher TSS.

Vine growth: No differences were found in the number of shoots and clusters per vine, yield or cluster weight relative to leaf-removal treatments within each year. This is to be expected, as vines were managed to typical commercial standards with standardized number of buds per vine at pruning, and shoot and cluster thinning practices resulted in uniform shoot and cluster counts in the vineyard.

Leaf-removal treatments effectively resulted in different amounts of leaves removed from the cluster zone. In 2010, the 100% leaf removal had 641 cm² more leaf area per vine than the 50% leaf removal treatment ($p = 0.0181$), for a total of seven more leaves removed per vine.

More basal leaves were removed in the 100% treatment than the 50% and IS treatments in 2011 ($p<0.0001$) and 2012 ($p=0.0004$). The 100% leaf-removal treatment removed 25% to 27% of leaves per vine when treatments were applied in 2011 and 2012, respectively. The 50% and IS treatments had similar leaf area removed (15% to 16%) in 2011 and 2012.

Despite differences in the amount of leaves removed, whole-vine leaf area did not differ by treatment when measured at véraison in 2010 or 2011. This is likely because the amount of leaves removed at the pea-size stage is only a small fraction of the total leaf area present on the vine at véraison, since grapevine canopies continue to grow in shoot length and lateral shoot development until véraison.$^{58}$

Similarly, S. Poni et al. found no differences in total leaf area later in the season, with leaf removal conducted pre-bloom because of increased lateral leaf shoot growth.$^{37}$

There was a minor difference in vine leaf area at véraison in 2012, with 100% and IS treatments having 23% and 9% less leaf area, respectively, than the control (None). C. Intrieri et al. found differences in total shoot leaf area with leaf removal in Sangiovese, but results were due to differences in lateral leaf area, not main shoot leaf area.$^{17}$

The 2012 lateral shoot number differed by treatment ($p = 0.0129$), with the 100% treatment having fewer total shoots and the smallest total leaf area. It is possible that the differences in leaf area between 2011 and 2012 were due to the differences in precipitation from bloom to véraison, with 0.3 inches in 2012 compared to 5.7 inches in 2011.

Basal leaf removal treatments in this study did not remove enough leaves to compromise vine function, as yield, pruning weights
and crop load (yield weight:pruning weight) were not different in any year that they were measured. When similar timing and severity of leaf removal treatments were applied by S. Staff et al., yield and pruning weights were reduced in 50% and 100% leaf-removal treatments compared to no leaf removal due to a lack of shoot growth at the time of leaf removal.53

In studies where leaf removal has altered vine growth, the treatments were applied in earlier growth stages such as pre-bloom and/or had more significant leaf area removed, thereby affecting vine carbohydrate assimilation and physiological development.1,37,57 As long as leaf removal is applied post-fruit set with only basal leaves removed, studies have shown no effect on vine yield, pruning weights or crop load.26

Cluster exposure: Leaf removal effectively altered the percent of ambient PAR measured in the cluster zone at 10 a.m. (2010 and 2011) and 2:30 p.m. (all three years), but there was no effect at solar noon. The percent of PAR was highest in vines with 100% leaf removal (p<0.0001), and the vines with 50% and IS leaf removal had similar levels of PAR (see “Cluster Zone Sunlight Exposure” on page 52). The lack of difference in incident light measured in the cluster zone at solar noon was expected and is in agreement with other research conducted on vertically shoot-positioned canopies, since the sun is positioned directly overhead, causing blockage of light to the fruit zone by the wall of canopy.38

Grape chemical composition

Fruit maturity parameters: When comparing
Other research suggests that changes in vine microclimate such as increased sunlight exposure and temperature increases anthocyanin compounds. Despite a difference in cluster exposure and PAR in 2011, there were no differences in any anthocyanins measured. Other studies have shown yearly variability in anthocyanin with cluster exposure, and the difference may be due to temperature and/or light conditions that year. Leaf removal affected the level of quercetin glycosides each year of the study. The 100% leaf-removal treatment consistently had the highest quercetin glycoside concentration in berries compared to 0% leaf removal in all years. The IS and 50% leaf-removal treatments had a similar concentration of quercetin glycosides as the 100% treatment in 2011 and 2012. The concentration of quercetin glycosides increased with increasing cluster exposure and increasing PAR in all three years. Others have shown increased quercetin glycosides with cluster exposure. Quercetin glycosides have been associated with anthocyanin polymerization in wine and can enhance wine color stability and quality. The combined effect of basal leaf removal on increasing anthocyanins and quercetin glycosides may allow for both greater color intensity and stability in wine aging, potentially leading to overall enhanced wine quality.

No differences were observed for the flavan-3-ols, including catechin and epicatechin, among leaf-removal treatments. These findings are in agreement with other research on flavan-3-ol monomers of Pinot Noir, Syrah and Cabernet Sauvignon grapes.

**Grape volatiles and their precursors**

C6 compounds: The C6 alcohols—namely 1-hexanol, trans-2-hexenal, trans-3-hexenol, and cis-3-hexenol—were present in both free and bound form. However, the C6 aldehydes hexanal and trans-2-hexenal were found in free form only. Leaf-removal treatments had no influence on concentration of the C6 compounds (free and bound forms) in any of the three years.

Other researchers have linked C6 compounds with berry maturity, demonstrating decreases in C6 compounds with increasing fruit maturity. Since the fruit did not vary in basic ripeness or crop load, it is understandable why there were no differences in the C6 compounds at harvest.

Terpenoids: Both free-form and bound-form terpenes were influenced by leaf-removal treatments, but results were variable by year and vineyard. In 2010, 100% leaf removal had 13% and 10% higher free-form linalool and geraniol and 58% to 105% greater bound-form terpenoids, respectively (p<0.05), compared to no leaf removal. In 2011, 100% leaf removal resulted in 48% and 33% greater concentrations of trans-linalool oxide and linalool, respectively (p<0.05). When three years of data were combined, there was a positive correlation between total bound-form terpenoid concentrations and PAR in the cluster zone (r² = 0.7178, p<0.0001).

Light exposure has been shown to increase terpenoid concentrations in grapes, especially bound-form terpenoids, while free-form terpenoids tend to be less responsive to sunlight exposure. Given the increased incident light exposure by leaf removal in this study (see “Cluster Zone Sunlight Exposure” on page 52), it can be hypothesized that cluster exposure to light mediated the accumulation or biosynthesis of bound-form terpenoids.

In 2012, leaf removal did not influence bound-form terpenoid concentrations despite differences in cluster zone PAR. The lack of differences may be a result of greater fruit ripeness that year and higher seasonal temperatures, potentially leading to losses to volatility.

Other studies have shown terpenoid concentrations to vary by differences in temperature across years or sites. The mean daily temperature from véraison to harvest in 2012 was 1.98° F greater than 2011, and berries were harvested at approximately 5° Brix higher in 2012. Other studies suggest that more moderate exposure may enhance volatile aroma compound concentration.

C13-norisoprenoids: One of the most important C13-norisoprenoids for Pinot Noir, β-damascenone, was found in both free and bound form; bound-form β-damascenone was approximately 10 times higher in concentration than the free form in all three
years (see “β-Damascenone Concentration in Grapes”).

Previous studies proposed that β-damascenone in grapes were formed from carotenoid degradation, and instead of forming free-form β-damascenone, most of the degradation products were transformed to β-damascenone glycoside conjugates which would be released chemically or enzymatically during vinification and wine aging.64,65

The concentrations of β-damascenone varied between years. Since 2010 samples were collected from a different vineyard than in 2011 and 2012, it is not possible to compare as clone, vine age and vine growth differences existed between the sites. However, higher concentrations of β-damascenone were observed in 2012 compared to 2011 at the Alpine site. This may be due to the difference in berry ripeness and the weather in those years, particularly temperatures in the ripening phase (see table on page 51).

The 100% leaf-removal treatment had higher bound-form β-damascenone than the no-leaf removal treatment in all three years.

### β-Damascenone Concentration in Grapes

#### Concentrations of free-form (A) and bound-form β-damascenone (B) in Pinot Noir grapes with different vineyard leaf-removal treatments from 2010 to 2012, and Mean±SD are presented (n=5). Different letters indicates a difference in means between treatments using Tukey HSD mean separation at √=0.05. Leaf removal treatments include the following: None (no leaf removal), 100% (all leaves in cluster zone removed), 50% (half of the leaves in cluster zone removed) and IS (industry standard where leaves are only removed from the cluster zone on the east side of the canopy).
When comparing light and fruit composition data each year, a positive correlation was found between levels of free- and bound-form β-damascenone and PAR in the cluster zone (see “Correlation Between β-Damascenone and Cluster Zone Sunlight Exposure” above) in all three years. This provides good evidence for the relationship of increased sunlight exposure and free-form β-damascenone in two of the three years (p<0.05). (See “β-Damascenone Concentration in Grapes” on page 55.) No other leaf-removal treatments had a consistent influence on free- and bound-form β-damascenone.

When comparing light and fruit composition data each year, a positive correlation was found between levels of free- and bound-form β-damascenone and PAR in the cluster zone (see “Correlation Between β-Damascenone and Cluster Zone Sunlight Exposure” above) in all three years. This provides good evidence for the relationship of increased sunlight exposure and β-damascenone in this cool climate.

Other research has shown an increase in berry norisoprenoid concentration with increasing sun exposure of both red and white grape cultivars, although other researchers have reported no change or a decrease of β-damascenone level in sun-exposed grapes. The impact is highly dependent on grape cultivars or warmer climate regions.

F. Yuan and M.C. Qian studied the carotenoid composition and the evolution of β-damascenone in Pinot Noir grapes during berry development and found that β-damascenone concentration increased from the early stage of berry development until harvest. Results of this study suggest that basal leaf removal may be altering the accumulation or biosynthesis of primary metabolites that affect metabolite biosynthesis later in the ripening process.

It is generally accepted that carotenoid synthesis starts in the first stage of berry development and continues until véraison, after which the compounds degrade to C13-norisoprenoids. As a group of photosynthetic pigments, carotenoids are affected by environmental factors such as sunlight. It has been reported that sunlight exposure increases the levels of carotenoids in unripe grapes compared to shaded grapes. But in the ripening process, grapes exposed to sunlight show a significant decrease in carotenoids compared to grapes under shade conditions.

The increase of β-damascenone with leaf removal in our study may be related to either increased carotenoids’ availability, resulting from more active photosynthesis in pre-véraison berries or due to post-véraison cluster sunlight exposure that accelerates carotenoid degradation. This area of study is currently under research at the genomic level. Studies that pair gene function with environmental factors will assist in understanding berry aroma enhancement through vineyard-management practices.

**Conclusion**

Cluster-zone leaf removal conducted at the pea-size berry stage effectively modified canopy microclimate (sunlight) and influenced berry composition. The level of leaf removal implemented in this study is feasible for application in commercial vineyards. The increased intensity of leaf removal (100%) did not reduce vine productivity (canopy growth or yield) or alter canopy:yield ratios, both of which can influence ripening directly.

The greatest leaf removal treatment (100%) effectively improved Pinot Noir grape quality through the increase of phenolics (anthocyanins and quercetin glycosides) and grape-derived volatile compounds and their precursors (terpenoids and C13-norisoprenoids) without causing fruit sunburn.

Results of this work could help grapegrowers manage their vine canopies more effectively to optimize Pinot Noir fruit and wine quality in Oregon’s cool climate.

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