

Terpene drift from *Cannabis sativa* L. (hemp) and the implications for *Vitis vinifera* (wine grapes) planted in close proximity

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Abstract

Industrial Hemp (*Cannabis sativa* L.) is one of the most versatile agricultural crops in the United States (US). However, until 2018, hemp had not been cultivated on a large scale in the US in over 80 years. With the recent re-authorization of hemp cultivation, the acreage under cultivation has increased tremendously while the knowledge base regarding hemp cultivation practices and interaction with other field crops has remained static. Hemp like other agricultural plants (e.g. *Vitis vinefera*, Eucalyptus, Lavandula, and *Arabidopsis*) produce copious amounts of volatile organic compounds (VOCs) such as terpenes. There are concerns about hemp VOCs tainting other agricultural crops. In this study, we examined the potential of hemp terpenes in tainting wine grapes planted in close proximity to a hemp field. Wine grape samples were collected from the vineyard over a five-week period when both the hemp plants and wine grapes were nearing harvest. Overall, the hemp plants contained high levels of terpenes. However, using a headspace GC-MS, there were no detectable levels of hemp terpenes on the wine grapes or the resultant wine made from the vineyard in this study. While the findings of this study are significant, we believe that more research is warranted to fully understand how other variables could influence hemp terpene emission and potential wine grape taint.

Introduction

Cannabis sativa L. (hemp) is one of the oldest sources of food, textile fibres, medicine, building materials, and paper. Industrial hemp is typically a dioecious plant, and it is one of the two most popular plants in the *Cannabaceae* family. Breeders have recently developed monoecious cultivars of industrial hemp that are suitable for producing dual- or tri-crops (fibres, seeds, and oil). Because of its myriad uses, hemp has become an economically viable crop for farmers across the world. Hemp was outlawed in the United States (US) in the mid 1930s after the adoption of the Uniform State Narcotic Drug Act, which was aimed at regulating cannabis (marijuana). However, over the past eight decades, researchers and breeders outside of the US have continued working on understanding hemp's chemical composition and secondary uses. The Agricultural Act of 2014 (Federal Farm Bill) established guidelines for farmers to partner with higher education institutions to cultivate hemp for research purposes. However, it failed to support the commercial cultivation of hemp, which, at that time, was still considered to be a scheduled drug. The Agriculture Improvement Act of 2018 (Farm Bill) reclassified hemp as an agricultural commodity and hence legalized commercial cultivation across the US. With the de-scheduling of hemp, there has been increased interest in its cultivation as a crop with a high dollar value.

Prior to the Marijuana Tax Act of 1937, US farmers cultivated hemp primarily for fibre. There was therefore little knowledge or interest in hemp cultivated for cannabidiol (CBD) in the US. Between 1937 and 2014, hemp cultivation was illegal in the US, and domestic research on this crop was dormant during this period. However, European nations have been able to conduct extensive research on hemp, and over the last century, they have developed both CBD and fibre varieties. The hemp research projects that were implemented as a result of the 2014 Farm Bill have led to a better understanding of effective cultivation and management practices for hemp across the US. However, there is still a lack of knowledge regarding hemp cultivation and processing in the US. Furthermore, there is limited research related to the chemical composition of hemp compounds and their uses.

There are three main phytochemical classes of hemp extract: cannabinoids, terpenes, and phenolic compounds¹⁷. In recent years, there has been a keen focus on the medicinal benefits of hemp CBD. Medicinal cannabis has been touted for the health benefits associated with CBD and delta-9-tetrahydrocannabinol (THC). The US Food and Drug Administration (FDA) distinguishes hemp from marijuana based on the content of THC, a psychoactive compound. In the US, hemp is described as a plant from the *Cannabaceae* family that is rich in non-psychoactive cannabinoids, with less than 0.3% of THC, while cannabis plants with a THC content greater than or equal to 0.3% are classified as marijuana.

Although hemp has been de-scheduled and is now considered an agricultural commodity in the US, there is a lot of resistance toward hemp cultivation in many parts of the country. In particular, some residents in neighbourhoods near hemp fields have complained about the pungent smell of terpenes emitted by hemp plants. Additionally, in some regions of the US, some vineyard owners have reported fears regarding the impact that hemp terpenes may have on their wine grapes. These concerns have been raised by local lawmakers in different jurisdictions¹⁵. On the other hand, traditional farmers have been exploring hemp as a means to augment their overall farm revenue. While both pro- and anti-hemp arguments are legitimate, there is no empirical research to support the position of some vineyard owners. Specifically, there has been little discussion about the properties of terpenes and their interactions with wine grapes or the possible methods of terpene transfer from one crop to another. In

the next section, we discuss the types of terpenes in hemp and wine grapes and the methods of terpene transfer between plants.

Terpenes in hemp

Terpenes are the compounds responsible for hemp's aroma, and they are primarily found in the tips of the plant's shoot system. The main volatiles are monoterpenes and sesquiterpenes, with β -myrcene and β -caryophyllene as the most representative monoterpene and sesquiterpene, respectively². CBD hemp varieties have more complex volatiles than fibre hemp varieties. The phenolic compounds in CBD hemp varieties are found in large amounts in the flowers. Hence, the terpene production of CBD hemp varieties is amplified between flowering and maturity of the plants¹

Terpenes in wine grapes

The aromas of wine grape varieties and wines have long been of interest to researchers due to the complex flavour profiles they present. Significant contributors to the flavour and aromatic characteristics of wine grape varieties are the numerous terpene compounds produced through viticultural management and oenology processes, which include vine and fruit management, plant nutrition, harvest protocols, biosynthesis in grapes, enzyme activation during grape crushing, grape fermentation, and wine maturation²⁵. The most prominent terpene compounds found in Muscat and related aromatic grapes and wines are linalool, geraniol, nerol, terpineol, and hotrienol. Several researchers⁶ have found that longer maceration periods were related to greater terpene content in wines. Furthermore, certain yeast species (e.g., *Saccharomyces cerevisiae*) were shown to be capable of enzymatically producing citronellol from geraniol and nerol, thereby transforming the aromatic profile of wine²⁶. The characteristic flavours and aromas of grapes and wines are dynamic due to the plethora of transformations inherent to the complex biochemical mechanisms involved in grape cultivation and wine production.

Wine grapes develop complex aromas from both natural processes and transformations during the winemaking process, and it is important to determine how these processes and transformations affect the flavour of wines. Most vineyards are planted close to other crops that may produce high levels of aromatic compounds. Hence, it is not prudent to assume that hemp is the only crop whose volatile terpenes could affect the quality of agricultural commodities. Several researchers^{12,24,42} have explored the potential transfer of volatile terpenes among plants. A handful of plants (Eucalyptus, Lavandula, and *Arabidopsis*) have been documented to emit volatile terpenes⁴⁰. Volatile terpenes in *Arabidopsis* are biosynthesized to monoterpenes and sesquiterpenes, which are among the major volatile terpenes in hemp. The three major terpenes found in *Arabidopsis* are limonene, β -myrcene, and β -ocimene¹². β -myrcene and β -ocimene are among the most abundant terpenes in hemp varieties. Hence, *Arabidopsis* and hemp varieties may emit similar terpenes. The impact of eucalyptus terpenes on wine grapes in Australia is often cited by vineyard owners in Sonoma County in the US as a reason to worry about the potential impact of hemp terpenes on wine grapes. However, few details are known about the process by which eucalyptus trees taint wine. In the next section, we review studies that have examined eucalyptol and its potential for tainting wine grapes.

Terpenes in eucalyptus

The compound 1,8-cineole (eucalyptol), which is a monoterpene, is the most abundant terpene in eucalyptus. It is also found in hemp and a large number of wine grape varieties. Some winemakers have surmised that eucalyptol contributes minty, herbal, and camphorous aromas that could lead to consumer rejection of wines.

There are several theories regarding the process by which eucalyptol ends up in finished wine. One theory posits that eucalyptol is introduced into wine grapes in vineyards within close proximity to eucalyptus trees via mechanical means of transport. Several researchers^{9,10,26} have studied finished wines made with grapes from a single vineyard and found that the eucalyptol concentration was 15.5 ppb in grapes grown within 50 meters of eucalyptus trees, whereas grapes grown outside of this range showed negligible levels of eucalyptol. In a similar study that focused on aromatic compounds in French red wines, several researchers³⁶ found that eucalyptol concentrations decreased significantly as the wine grape berries ripened, even though the eucalyptol concentrations in wine grape samples were as high as 18 µg/kg at their peak. This suggests that these compounds are endogenous and indicative of maturity rather than the result of exposure to exogenous terpene sources. In another study, several researchers^{21,38} examined the potential of eucalyptus plants to taint wine grapes planted in close proximity; the results of this study were inconclusive, as eucalyptol was not found in wine grapes planted in close proximity to eucalyptus plants. This suggests that the presence of eucalyptol in wines cannot be definitively explained by terpene drift from exogenous sources.

Terpene transport mechanisms

Several researchers^{21,32,41,42} have suggested a few physical mechanisms by which terpenes could be transferred from one crop to another. Air and soil have been identified as the two primary media for volatile terpene transfer.

Transport through air

It has been suggested that terpenes could be volatilized into the air and then deposited on and absorbed through the epidermis of leaves or grape skins. Plants emit volatile terpenes from their shoot systems into the atmosphere, and once these terpenes are emitted, they travel through the air until they encounter a target surface. If the target surface is a plant's foliage, the terpenes may be absorbed by the plant. Terpene transport through the air is facilitated by wind speed, wind direction, and temperature^{5,43}.

The rate of terpene absorption on the surface of foliage depends on the foliage's structure, the foliage's lipid content, and the plant species. Thinner leaves have been shown to have higher absorption rates for volatile terpenes in the air³⁰. A study of the terpene content in the air at various distances from a hemp field would help shed light on this phenomenon, and a separate study of the absorption rates of leaves at a range of terpene concentrations in the air would allow for the modelling of maximum absorption rates. A better understanding of potential terpene transport from the leaves of the vine into the grapes is also needed.

Transport through soil

Terpenes emitted from one plant could accumulate in the topsoil and potentially be absorbed by a target plant's roots^{4,31,32}. Volatile organic compounds (VOCs) such as terpenes are found both above and below ground. Microbial decomposition of plant material in the soil is a major source of terpene emissions. VOCs can also be emitted through plant roots. However, VOC emissions from plant roots could be mitigated by microbial activities in the soil. Some microbes in the soil break down plant litter and increase VOCs, while others consume VOCs that are produced via decomposition. Hence, net VOC emissions from the soil could be diminished by the aforementioned microbial processes.

VOC emissions in the soil could also be affected by the type of soil particles and the depth of each soil horizon. VOC deposition in the rhizosphere is affected by the distance from the VOC source.

Furthermore, the volume of VOCs deposited in the rhizosphere could be influenced by the length of time that the plants emitting the VOCs are in the field. For example, pine trees planted close to an open field are more likely to deposit high levels of VOCs in the rhizosphere as a result of long-term emission accumulation^{24,32}. The rate of terpene emission from the soil into the atmosphere is far lower than the rate of terpene absorption from the atmosphere into the soil. Soil acts as a sink for VOCs that are deposited and absorbed in the rhizosphere^{3,20}.

It is unclear whether soil is a viable source of terpene emissions from hemp plants that are in a field for only 90 to 120 days. Further, the two plants that have been studied extensively as potential sources of terpene drift for wine grapes (eucalyptus and pine trees) are both perennials.

Objectives

A variety of plants (including wine grapes and hemp) produce copious amounts of volatile terpenes. However, it is unclear whether terpene transfer through the soil could be a viable source of terpene drift in wine grapes planted in close proximity to hemp plants. A detailed review of the literature has revealed no documented peer-reviewed research related to cannabis terpene drift in the US. Despite the lack of evidence to support the claim that hemp terpenes can taint wine grapes, several jurisdictions across the US are considering banning hemp cultivation because of this concern.

Therefore, the purpose of this study was to determine whether volatile terpenes from a CBD hemp field planted in close proximity to a vineyard could taint the wine grapes and the wines made from those grapes.

Materials and Methods

The research plot (located in Sonoma County, CA, USA) was planted with two varieties of CBD hemp: Boax and Cherry Wine Boax. The field was planted with 360 clones of the Boax variety in six beds and 240 seedlings of the Cherry Wine Boax wine variety in one bed. The vineyard in this study was an established student vineyard comprising 13 blocks and 39 wine grape varieties. The hemp field was located 68.5 feet (20.9 metres) from the vineyard. Sonoma County ordinance currently stipulates that hemp cultivation must occur 200 feet from property lines or 600 feet from residences and businesses.

Field Site

The experiment was conducted in 2019 at Shone Farm in Forestville, CA, USA (38° 30' 18" N, 122° 52' 20" W). The soil characteristics of the experimental plot are shown in Table 1.

Table 1. Characteristics of the top 12 inches of soil in the experimental plot.

Parameter	North Field	South Field
Sand (%)	45	47
Silt (%)	28	30
Clay (%)	26	22
Overall soil type	Loam	Loam

Organic matter (%)	3.5	4.3
pH	6.5	6.4
Nitrogen (ppm)	37	38
Active phosphorus (ppm)	36	49
Exchangeable potassium (ppm)	364	374
Calcium	1,293	1,329
Cation exchange capacity	10.0	10.5

Cherry Wine Boax seeds were sown in 50 cell trays in Pindstrup medium on June 14, 2019. Boax clones were delivered from a certified nursery on June 18, 2019. The seedlings and clones were kept in a greenhouse on mist benches with timers. The clones were moved into four-inch pots with a custom peat moss mix two weeks later. The clones and seedlings received two foliar applications of nitrogen using BioLink®. The seedlings and clones also received one foliar application of calcium-magnesium during the first three weeks of growth. The clones and seedlings were transplanted into the field on July 12, 2019, and July 26, 2019, respectively.

The experimental field consisted of seven raised beds that were 300 meters long and four feet wide. The clones were planted on six beds with five feet between plants. The seedlings were planted on one bed with three feet between plants. For odour mitigation and physical barriers from the rest of the farm, two additional beds were planted on the north side of the hemp field (one bed of corn and one bed of sunflowers), and another two were planted on the south end of the field (Figure. 1).

Figure 1. Layout of the experimental field.



Looking into hemp plot from Eastern edge.



Sativa-dominant hemp plant in flower.



View of entire hemp plot from Eastern edge. Note buffer plantings on both sides



View of buffer planting of Corn and Sunflower. Approximately 8 ft wide.



Looking from vineyard block corner towards corner of hemp plot 68.5 ft away.



SW Corner of vineyard block, 68.5 ft from Hemp plot

Each bed in the hemp field was fitted with one line of drip tape for irrigation. Each drip tape line delivered 250 gallons of water daily during a three-hour irrigation period. At the onset of flowering, each bed was fitted with an additional line of drip tape (increasing the number of lines per bed to two). The beds were irrigated every other day until two weeks before harvest. Although the hemp field was not certified organic, the entire farm was farmed organically, as a large portion of the farm was, in fact, certified organic. Hence, only pesticide and herbicide certified for organic farming were used.

Data Collection

Plant Material Collection

Plant material was collected from both the hemp field and vineyard once a week for four weeks (between September 20, 2019, and October 18, 2019) between the hours of 08:00 and 09:00. Sample collection started five weeks before harvest and ended a week before harvest. The hemp and wine grapes were harvested during the same week.

Hemp plant tissue samples were randomly collected using the California state hemp sampling protocol (composite sampling). The samples were stored in breathable paper bags and transported to the lab within 30 minutes. Once at the laboratory, the samples were processed and analysed for cannabinoid content and terpene profile.

Grape cluster samples were collected from six specific blocks in the vineyard. Three samples of Zinfandel (red wine grapes) were collected from row 21: one from the area nearest to the hemp field (vines 43 to 48), another from the centre of the row (vines 21 to 26), and the last from the area farthest

from the hemp field (vines 1 to 6). A similar sampling technique was used to collect samples of white wine grapes from row 10. Samples were collected from different varieties of white wine grapes: Gewürztraminer (vines 1 to 3), Verdelho (vines 4 to 6), Viognier (vines 22 to 24), Sémillon (vines 25 to 27), Verdelho (vines 43 to 45), and Marsanne (vines 46 to 48). Once the samples were collected, they were placed in one-quart Ziplock bags, stored in an ice chest, and transported to the laboratory within an hour for terpene analysis.

Wine Samples

Finished wine samples were analysed to study the impact of hemp grown in close proximity to the wine grapes. Two sample groups of wine were made based on the proximity of the wine grapes to the hemp field and the addition of material other than grapes (MOG) in the wine.

The first sample group was based on the proximity of the wine grapes to the hemp field. Two batches of wine were made from this sample group. The first batch was made with grapes from the north end of the vineyard (row 21, vines 1 to 6), which was farthest from the hemp field. The second batch was made with grapes from the south end of the vineyard (row 21, vines 43 to 48), which was closest to the hemp field.

The second sample group was created by adding MOG to three batches of wine from south end of the vineyard. The amount of MOG added was based on industry standards. This portion of the study was conducted to assess the potential transmission of terpenes via other plant matter.

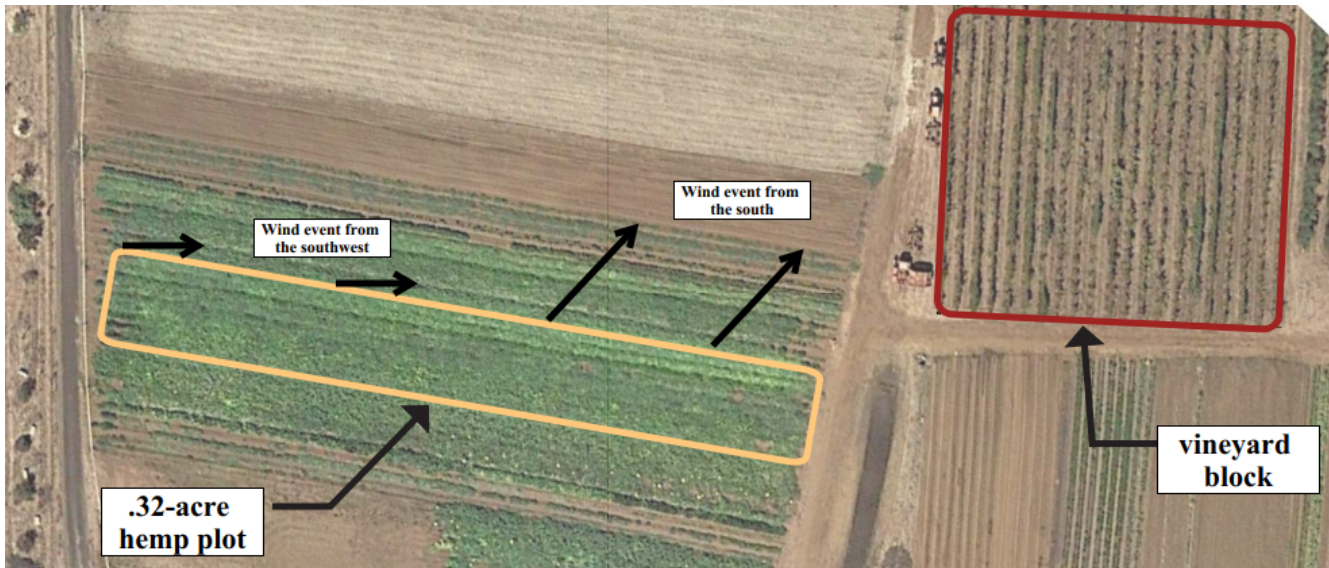
Approximately six kilograms of Zinfandel grapes were collected from row 21 (vines 43 to 48) on September 27, 2019. Leaves from the same vines were also collected and stored separately. These leaves were incorporated into the fermenters in different amounts to create three MOG samples (no leaves, 1% leaves by weight, and 3% leaves by weight). This was done to evaluate the effect of terpenes adsorbed into the leaves or absorbed onto the surface of leaves. The wines were produced at a commercial winery by an experienced winemaker using industry standards.

At the end of the winemaking process, one 750-mL glass bottle of wine was produced from each batch and sealed with a standard Diam wine cork.

Weather

Weather has a significant impact on plant growth, yield, physiologic expressions, and VOC emissions and drift^{42,43}. The researchers collected several weather data points every 15 minutes during the study period using the weather station located at Shone Farm. Data points included evapotranspiration (ET_o, in), relative humidity (RH, %), maximum and minimum RH (%), daily temperature (°F), daily maximum and minimum temperatures (°F), daily maximum wind speed (mph) and direction (°), and average wind speed (mph) and direction (°). Temperature, ET_o, and RH data were presented as weekly averages using a wind rose plot that was created to capture average wind speed and direction.

Figure 2. Aerial view of the experimental plot showing the proximity of the vineyard to the hemp field and wind direction.



Analysis

For analysis, the researchers used a multistep process that included analytical and olfactory testing. Several researchers^{18,37} have suggested that the best way to detect cannabis odours is through multidimensional gas chromatography (GC) in tandem with human olfaction. The terpene composition of the grape, hemp, and wine samples was determined using headspace gas chromatography-mass spectrometry (MS) (HS-20 GCMS-QP2010 SE; Shimadzu Corporation, Kyoto). The grape and hemp samples were analysed using headspace GC, and the wine samples were analysed using headspace GC and sensory analyses. Several researchers³³ have found that headspace GC provides a comprehensive method for analysing bioactive compounds in hemp.

Plant Materials

Plant material analysis showed the quantities of terpenes and cannabinoids as mass percentages. Approximately 100 mg of hemp inflorescence and 250 mg of grape mass were weighed into respective headspace vials. The gas chromatograph was fitted with a 30.0-m Rxi-624Sil MS column (Restek Corporation, Bellefonte, PA, USA). Helium was used as a carrier gas (1.64 mL/min 1 column flow). Oven settings were: 80 °C for 1 minute, steps of 12 °C/min up to 150 °C and then held at 150 °C for 1 min, and steps of 9 °C/min up to 250 °C and then held at 250 °C for 1 min with a run time of 20 min. The headspace was injected in split mode, and the split ratio was 1:50. Data acquisition was performed in selected-ion-monitoring mode using GC-MS real-time analysis software (Shimadzu Corporation, Kyoto, Japan). Terpene compounds were identified by comparing their mass spectra and retention times against reference standards.

Wine Samples

Six wine samples (one from each batch) were analysed using headspace GC-MS to quantitate terpene content. The purpose for this analysis was to determine whether terpenes from the hemp field diffused through the skin of the wine grapes during the growth period to the extent that they would be present in

wine. The headspace GC-MS used for this study had a limit of quantification of 10 ppm and a limit of detection of 2 ppm.

In addition to analytical testing, a general chemistry panel was conducted on the wine samples by a commercial lab. This panel assessed the wine's percent of alcohol by volume, pH, titratable acid (TA), malic acid (ML), residual sugar (RS), and volatile acid (VA). The pH and TA were determined by titration performed using a Mettler-Toldeo T90 auto-titrator (Greifensee, Switzerland) with LabX software (Ontario, Canada). RS, VA, and ML were quantified via enzymatic analysis performed using a Siemens Advia 1200 Chemistry Analyzer. The alcohol content of the samples was quantified using the Anton-Paar Alcoalyzer Wine Analysis System (Graz, Austria).

The aroma thresholds for terpene compounds in wines are generally around 100 ppb²⁵. In a published study²¹, the sensory detection threshold for eucalyptol, for example, was found to be as low as 3.2 ppb. This suggests that humans can sense much lower terpene levels than analytical instruments. Due to the potential limited sensitivity of GC-MS to detect ultra-trace levels of terpenes in grapes in the parts-per-billion range, a sensory analysis was also conducted to expand the range of detection. The purpose of the sensory analysis was to determine whether low levels of hemp terpenes may have transferred to the grapes and impacted the taste and aroma of the resultant wines. Sensory studies^{40,44} have been used to assess the impact of eucalyptol on the aroma of wine grapes planted in close proximity to eucalyptus plants but have shown a wide variance in the detection of aromas within and between sensory panels. However, sensory studies are still widely used to analyse wines.

Results and Discussion

Figures 3 and 4 show the quantified levels of the major terpenes in the plant material of the Boax and Cherry Wine Boax hemp varieties used in this study. The top three terpenes in the hemp varieties in this study were β -myrcene, α -ocimene, and β -caryophyllene. The production of these terpenes peaked in the hemp plants during the third week of data collection. If there is terpene drift, there should be a higher deposit of hemp terpenes in wine grapes during the same period.

Figure 3. Terpene profile evolution during the Cherry Wine Boax hemp growth cycle.

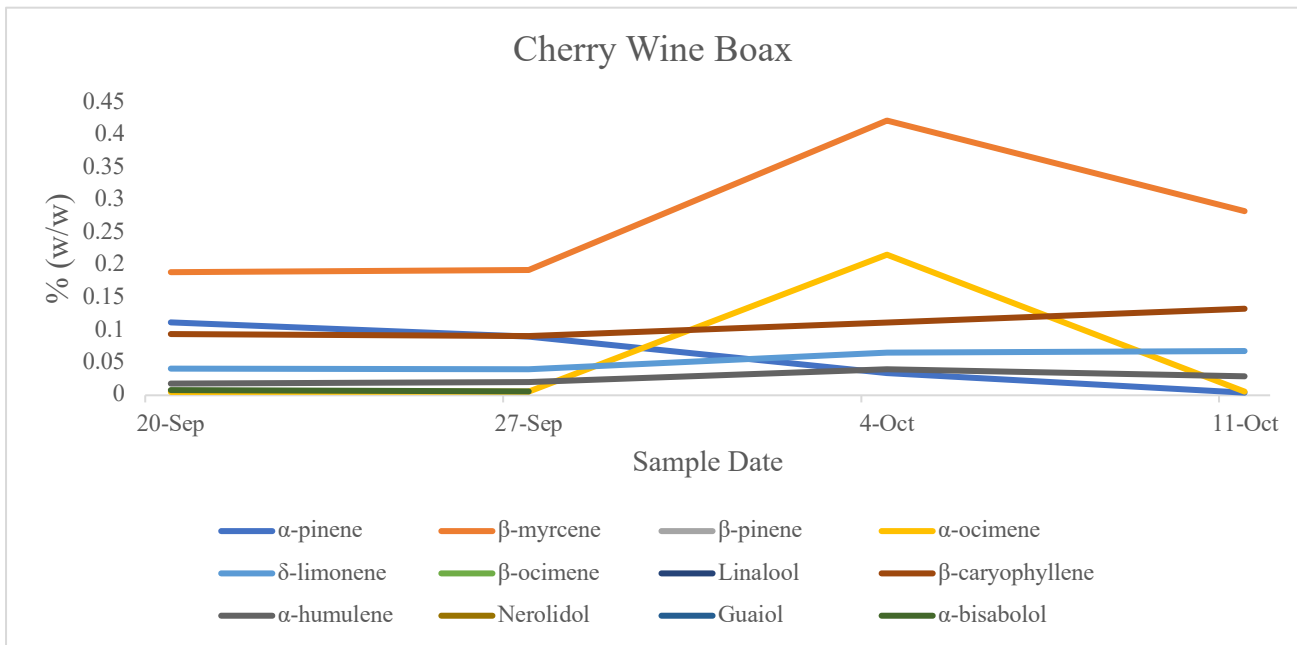
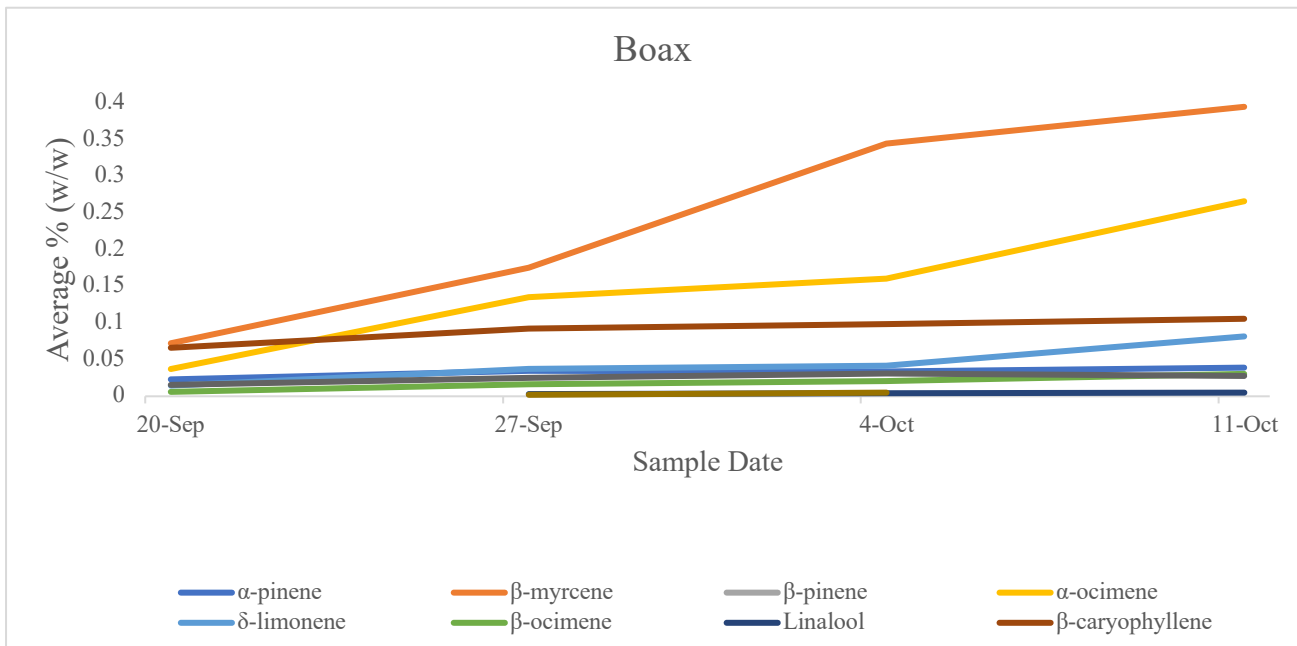


Figure 4. Terpene profile evolution during the Boax hemp growth cycle.



While the hemp plants' terpene levels peaked during the third week of data collection, no measurable level of hemp terpenes was found in the wine grapes during the same period. This is of particular significance because the hemp field was located 68.5 feet from the vineyard, while the local hemp ordinance requires a minimum of 200 feet easement from the property line. The distance required between these crops is nearly three times further than the conditions of this study. Figure 1 shows the corn and sunflower buffer plants on the north and south sides of the hemp field. The corn and the

sunflower plants were taller than the hemp plants, and it is likely that volatile terpenes could have been trapped by these buffer plants.

The wine grape terpene analysis revealed non-detects for all of the terpenes included in the assay, and the results are therefore not depicted here (Tables A.I and A.II). The wine grape samples were analysed using the same headspace GC-MS technique as the hemp samples. The instrument was unable to detect any of the terpenes shown in Figures 3 and 4. Chromatograms for the wine grapes are shown in the supplementary information section (Figures A.I., A.II and A.III). The work of several researchers ⁹ supports this study’s findings regarding the presence of hemp terpenes in wine grapes planted in close proximity to hemp plants.

Wine Analysis: Chemical

Samples of the wines made for this study were analysed at a commercial lab for the percent of alcohol by volume, pH, TA, RS, ML, and VA, which represents the combined concentration of acetic acid and ethyl acetate. The results are shown in Table 2.

Table 2. Chemical analysis of the wine samples.

Sample	Alcohol (% v/v)	pH	TA (g/100 mL)	RS (g/100 mL)	ML (mg/L)	VA (g/100 mL)
North	14.39	3.60	0.73	0.19	1692	0.071
South	11.79	3.33	0.71	0.02	372	0.023
3% MOG	14.81	3.44	0.75	0.01	1150	0.050
1% MOG	15.29	3.42	0.73	0.01	1125	0.048
No MOG	15.15	3.37	0.76	0.01	1028	0.061

The wines were all dry, although the north sample had slightly more RS. The wines were not inoculated with *Oenococcus oeni* after primary fermentation, as is customary in commercial production. Hence, ML concentrations were high. Furthermore, no sulphur dioxide or potassium metabisulfite were added to these wines during the winemaking process, so free and total sulphur dioxide concentrations were not analysed. These additions are often made during the commercial winemaking process to improve the palatability and stability of the wine.

The south sample’s ML concentration suggests that this sample went through “wild” or “spontaneous” malolactic fermentation, in which native lactic acid bacteria (typically *Pediococcus*, *Lactobacillus*, and *Leuconostoc*) converted the ML to lactic acid and carbon dioxide. When each of the wine bottles was opened for sampling, it was apparent that a significant amount of carbon dioxide accumulated in the

bottles, an indication that the wines went through at least a small amount of malolactic and/or primary fermentation in the bottle.

Wine Analysis: Sensory

The wines made for this study were analysed by experienced wine tasters (n = 10), who performed descriptive analyses of the various samples. The group consisted of four men and six women who were working, or who had worked, either as oenologists or winemakers. To prevent a biased response, the study objective (the potential of hemp terpenes to taint wine grapes) was not shared with the tasters. The wines were presented as two separate sample groups. The tasters were asked to compare the wine samples and characterize them, documenting any notable defects.

The sensory panel’s comments regarding the wines made with grapes from the north and south ends of the vineyard are shown in Table 3. These comments were consistent with the chemical analysis of the wines, which showed that the north sample had the highest RS level. The north sample also had the highest VA, which may have contributed to the intensity of fruity aromas when present in moderate amounts.

Table 3. Sensory descriptive analysis of the wine samples.

Sample	Sample Set	Aroma	Taste and Mouthfeel
North	1	Ripe/candied fruit, sweaty socks	Blueberry jam, sour cherry, sweet, juicy, slight alcoholic burn, herbal notes, slight effervescence, bitter seed tannin
South	1	Red fruit, sweaty socks	Black cherry, muted, disjointed, acidic, less fruity aromas than the north sample, herbal notes/brambly, mousy, chalky/drying tannins

In comparison, wine made with grapes from the south side of the vineyard was markedly less fruity than wine made with grapes from the north side. The sensory panellists described the south sample as dry, and the chemical analysis showed that the south sample had less RS, which is usually associated with less sweetness.

Both samples were noted to have a subtle herbal note that was balanced with the overall flavour profile of each wine sample. The subtle herbal note was described as “brambly,” a term that is commonly associated with the Zinfandel varietal²⁷ and less likely a result of that this character is the result of any extraneous conditions such as the hemp terpene.

The MOG wine samples had distinctly green (i.e., vegetal, herbal) flavours and aromas that rendered them non-useful for sensory analysis. These flavours and aromas are commonly associated with wines made with higher amounts of MOG and, therefore, they cannot be attributed to hemp⁴⁴. Due to these confounding factors, no sensory data are reported for this second sample set.

Weather

Wind, temperature, and RH could affect the drift of VOCs. At higher temperatures during the summer, the most volatile monoterpenes such as α -pinene and β -myrcene are emitted at higher levels. When high wind activity is coupled with high VOC emissions, the potential of terpene drift is increased. For these reasons, this study collected data to better understand the relationship between weather factors and terpene drift.

The weekly average temperature and RH data are shown in Figure 5. Temperatures generally ranged between 4C to 32C, and RH stayed between 45 and 65%. These conditions should allow for varying amounts of terpene volatilization, with significant amounts of terpenes likely emitted during periods of maximum temperature³⁵. Some of this temperature-dependent increase has been linked to elevated rates of terpene synthesis due to higher enzymatic activity in terpene-emitting plants²⁹. Higher RH and temperature have been found to be correlated with increased terpene emissions⁴³. Hence, in this study, data was collected for both factors to determine whether they were associated with terpene emissions from the hemp field.

Figure 5. Weekly relative humidity and temperature of the experimental field.

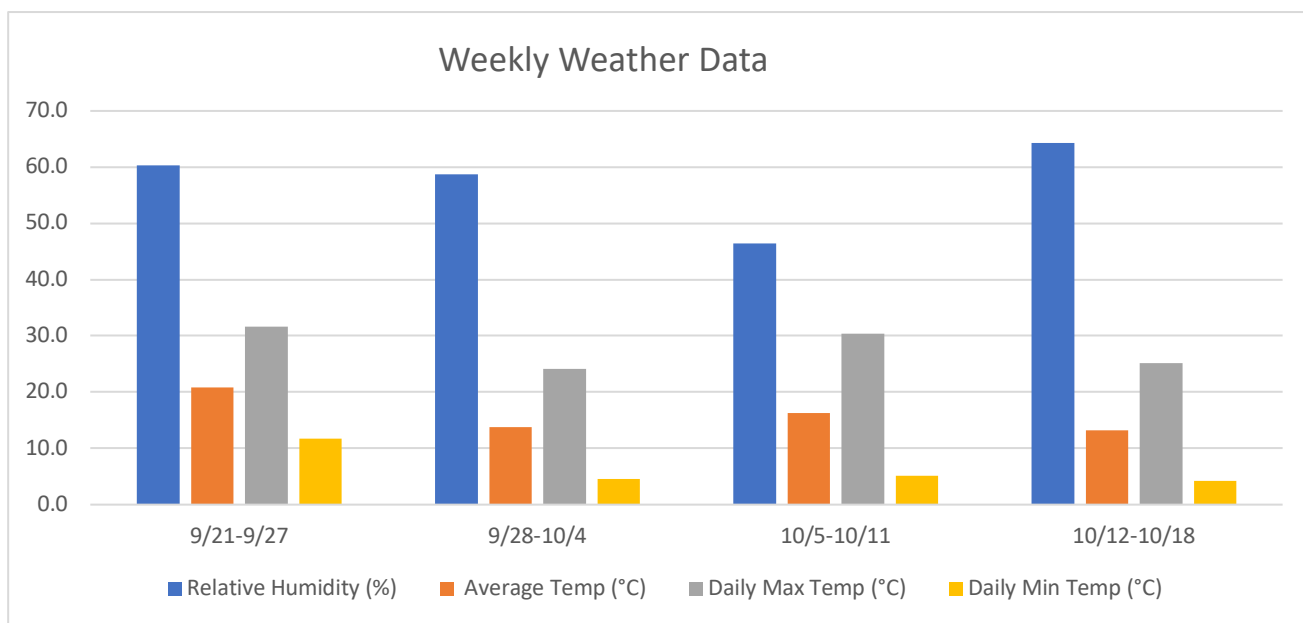


Table 4. Wind speed, wind direction, and evapotranspiration for the study period.

Week (2019)	Daily Max Wind Speed (mph)	Direction of Daily Max Wind Speed (°)	Average Wind Direction (°)	Daily Evapotranspiration (in)
9/21–9/27	14.0	341 NNW	209 SSW	0.15
9/28–10/4	17.1	95 E	246 WSW	0.14
10/5–10/11	11.0	134 SE	215 SSW	0.14
10/12–10/18	11.4	279 W	219 SW	0.12

The wind data in table 4 show that the wind was moving from the south and southwest directions most of the time, with many of the wind events blowing from the hemp field to the grapes. The wind speed and wind direction around the experimental field is also shown in a wind rose plot in the supplementary information section (Figure B). High temperatures are needed for increased terpene loss to the air. This suggests that there were weather conditions that were likely favourable for terpene emissions during a significant portion of the study period. Elevated temperature and RH coupled with higher-than-normal wind activity, with wind blowing directly over the vineyard, increased the likelihood of hemp terpenes tainting wine grapes. It is important to note that the physical odour barriers around the periphery of the hemp field (using beds of corn and sunflowers) could have mitigated potential terpene drift.

Conclusions

The experimental cultivation of hemp in close proximity to a vineyard was a unique opportunity that enabled the researchers to provide critical knowledge during the infancy of the hemp industry in the US. The future of hemp production in wine-growing regions of the US is dependent on a better understanding of the effects of hemp fields' proximity to established vineyards. We were able to record data on hemp's terpene profiles, the weather during the growing season of hemp and wine grapes, and a variety of sensory parameters pertaining to the finished wines in our study.

While this study cannot definitively determine the existence or absence of hemp terpenes in wine grapes planted in close proximity to hemp plants (bordered by plant barriers such as corn or sunflower), the researchers used current wine industry analytical instruments and wine sensory methods to show that hemp terpenes were not found in wine grapes or the resultant wines.

Limitations and Future Research

This study has several limitations. First, the experimental hemp field was not directly across from the vineyard, and it was not in the path of most of the wind activity. Second, the sensitivity of the analytical instruments used in the commercial wine labs did not have the capacity to quantitate the low terpene thresholds in the wine samples. Third, defects in the wines acted as confounding variables in the sensory analysis.

While this study has provided baseline data to inform farmers about the potential for hemp cultivation in wine regions, we believe that further research is warranted to move the hemp industry forward. We believe that if the aforementioned limitations are addressed, researchers will be able to more definitively determine hemp terpenes' potential to taint wine grapes.

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References:

1. Aizpurua-Olaizola, O., Soydaner, U., Öztürk, E., Schibano, D., Simsir, Y., Navarro, P., Etxebarria, N. & Usobiaga, A. Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis sativa Plants from Different Chemotypes. *Journal of Natural Products* **79**, 324-331(2016).
2. Andre, C.M., Hausman, J.F. & Guerriero, G. Cannabis sativa: The plant of the thousand and one molecule. *Frontier in Plant Science*. **7**, 1–1(2016).
3. Asensio, D., Penuelas, J., Llusia, J., Ogaya, R. & Filella, L. Interannual and interseasonal soil CO₂ efflux and VOC exchange rates in a Mediterranean holm oak forest in response to experimental drought, *Soil Biol. Biochem.* **39**, 2471-2484, doi:[10.1016/j.soilbio.2007.04.019](https://doi.org/10.1016/j.soilbio.2007.04.019) (2007).
4. Asensio, D., Owen, S. M., Llusia, J. & Penuelas, J. The distribution of volatile isoprenoids in the soil horizons around Pinus halepensis trees. *Soil Biol. Biochem.* **40**, 2937-2947(2008).
5. Baldocchi, D.D., Fuentes, J.D., Bowling, D.R., Turnipseed, A.A. & Monson, R.K. Scaling Isoprene Fluxes from Leaves to Canopies: Test Cases over a Boreal Aspen and a Mixed Species Temperate Forest. *J. Appl. Meteor.* **38**, 885–898 (1999).
6. Baron, M., Prusova, B., Tomaskova, L., Kumsta, M. & Sochor, J. Terpene content of wine from the aromatic grape variety ‘Israi Oliver’ (*Vitis vinifera* L.) depends on maceration time. *Open Life Sci.* **12**, 42-50 (2017).
7. Belda, I., Ruiz, J., & Esteban-Fernández, A. et al. Microbial contribution to wine aroma and its intended use for wine quality improvement. *Molecules.* **22**,1–29 (2017).
8. Bertoli, A.,Tozzi, S., Pistelli, L. & Angelini, L.G. Fibre hemp inflorescences: From crop-residues to essential oil production. *Ind. Crops Prod.* **32**, 329–337 (2010).
9. Capone, D.L., Jeffery, D.W. & Sefton, M.A. Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian red wine. *J. Agric. Food Chem.* **60**, 2281-2287 (2012).
10. Capone, D.L., Van Leeuwen, K.D. & Taylor, K. et al. Evolution and occurrence of 1,8-cineole (Eucalyptol) in Australian wine,” *Journal of Agricultural and Food Chemistry.* **59**,953–959 (2011).
11. Cascio, M.G., Pertwee, R.G. & Marini, P. The pharmacology and therapeutic potential of plant cannabinoids. In Cannabis sativa L.-Botany and Biotechnology (1st ed.). Chandra, S., Lata, H. & ElSohly, M.A. Springer International Publishing AG. Cham, Switzerland. ISBN 978-3-319-54563-9 (2017).
12. Chen, F., Tholl, D., D'Auria, J.C., Farooq, A., Pichersky, E. & Gershenzon, J. Biosynthesis and emission of terpenoid volatiles from Arabidopsis flower. *The Plant Cell.* **15**,481-494; doi: <https://doi.org/10.1105/tpc.007989> (2003).
13. Da Porto, C., Decorti, D. & Natolino, A. Separation of aroma compounds from industrial hemp inflorescences (*Cannabis sativa* L.) by supercritical CO₂ extraction and on-line fractionation. *Ind. Crops Prod.* **58**, 99–103 (2014)
14. ElSohly, M.A., & Slade, D. Chemical constituents of Cannabis: The complex mixture of natural cannabinoids. *Life Science.* **78**, 539-548 (2005).
15. Ezzone, Z. Cannabis Farm Applicant Presents Cannabis Terpenes Study, County Looks for Additional Information. Available: www.santamariasun.com/news/19255/cannabis-farm-applicant-presents-cannabis-terpenes-study-county-looks-for-additional-information/(2019).
16. Farina, L., Boido, E., Carrau, F., Versini, G., & Dellacassa, E. Terpene compounds as possible precursors of 1,8-cineole in red grapes and wines. *Journal of Agricultural Food Chemistry.* **53**, 1633-1636 (2005).

17. Flores-Sanchez, I. J. & Verpoorte, R. Secondary metabolism in Cannabis. *Phytochem. Rev.* **7**, 615–639 (2008).
18. Giese, M.W., Lewis, M.A. & Giese, L. et al. Development and validation of a reliable and robust method for the analysis of cannabinoids and terpenes in cannabis. *Journal of AOAC International.* **98**,1503-1522 (2015).
19. Girard, B., Fukumoto, I., Mazza, G., Delquis, P., & Ewert, B. Volatile terpene constituents in maturing Gewurztraminer grapes from British Columbia. *American Journal of Enology and Viticulture.* **53**, 99-109 (2002).
20. Hayward S., Muncey, R.J., James, A.E., Halsall, C.J. & Hewitt, C.N. Monoterpene emissions from soil in a Sitka spruce forest. *Atmospheric Environment.* **35**, 4081- 4087 (2001).
21. Herve, E., Price, S., & Burns, G. Eucalyptol in wines showing eucalyptus 388 aroma. In Proceedings of the VII^eme symposium international 389 d'Oenologie. Bordeaux, France. Actualites Oenologiques (Poster presentation) (2003).
22. Hodgson, M. Santa Barbara County Planning Commission Continues Appeal for Santa Ynez Cannabis Cultivation Permit. Available:santamariatimes.com/news/local/govt-and-politics/santa-barbara-county-planning-commission-continues-appeal-for-santa-ynez/article_469dfb56-d45e-531d-b4dd-e96fb3b1b257.html (2019).
23. Külheim, C., Padovan, A., Hefer, C., Krause, S.T., Köllner, T.G. & Myburg, A.A., et al. The Eucalyptus terpene synthase gene family. *BMC Genomics.* **16**, 450; doi: 10.1186/s12864-015-1598-x (2015).
24. Lin C., Owen S.M. & Peñuelas J. (2007) Volatile organic compounds in the roots and rhizosphere of *Pinus* spp. *Soil Biology & Biochemistry.* **39**, 951-960 (2007).
25. Marais, J. Terpenes in the aroma of grapes and wines: a review. *S. Afr. Journal of Enology and Viticulture.* **4**, 49-58 (1983).
26. Mateo, J.J., Jiminez, M. Monoterpenes in grape juice and wines. *Journal of Chromatography A*, **881**, 557-567 (2000).
27. Mobley, E. Zinfandel: California's heritage grape. *San Francisco Chronicle*. Available: <https://thepress.sfchronicle.com/article/wine-facts-zinfandel> (2016).
28. Molina, A., Reigosa, M.J. & Carballeria, A. Release of allelochemic agents from litter, through fall and topsoil in plantations of Eucalyptus globulus Labill in Spain, *Journal of Chemical Ecology.* **17**, 147-160 (1991).
29. Niinemets, Ü., Loreto, F. & Reichstein, M. Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends Plant Science.* **9**,180-186 (2004).
30. Noe, S.M., Copolovici, L., Niinemets, Ü. & Vaino, E. Foliar limonene uptake scales positively with leaf lipid content: non-emitting species absorb and release monoterpenes. *Plant Biology.* **9**, e86 (2007).
31. Ormeño, E., Baldy, V. & Ballini, C. et al. Production and Diversity of Volatile Terpenes from Plants on Calcareous and Siliceous Soils: Effect of Soil Nutrients. *J Chem Ecol.* **34**, 1219 (2008).
32. Ormeno, E., Fernandez, C., Bousquet-Melou, A., Greff, S., Morin', E., Robles, C., Vila, B. & Bonin, G. Monoterpene and sesquiterpene emissions of three Mediterranean species through calcareous and siliceous soils in natural conditions, *Atmos. Environ.* **41**, 629-639 (2007a).
33. Pellati, F., Brighenti, V., Sperlea, J., Marchetti, L., Bertelli, D. & Benvenuti, S. New Methods for the Comprehensive Analysis of Bioactive Compounds in Cannabis sativa L. (hemp). *Molecules.* **23**, 2639; doi.org/10.3390/molecules23102639 (2018).

34. Peñuelas J, Asensio D, Tholl D, Wenke K, Rosenkranz M, Piechulla B *et al.* Biogenic volatile emissions from the soil. *Plant Cell Environ.* **37**,1866-1891(2014).
35. Penuelas, J., Fillela, I., Seco, R. & Llusia, J. Increase in isoprene and monoterpene emissions after re-watering of droughted *Quercus ilex* seedlings. *Biologia Plantarum.* **53**, 351e354 (2009).
36. Poitou, X., Thibon, C. & Darriet, P. 1,8-Cineole in French Red Wines: Evidence for a Contribution Related to Its Various Origins. *Journal of Agricultural and Food Chemistry.* **65**, 383-393 (2017).
37. Rice, S. & Koziel, J.A. Characterizing the Smell of Marijuana by Odor Impact of Volatile Compounds: An Application of Simultaneous Chemical and Sensory Analysis. *PLoS ONE.* **10**,12; doi:10.1371/journal.pone.0144160 (2015).
38. Robertson, M.A., & Wilson, S. The eucalypt aroma of southern Australian 418 Pinot Noir wines. In Proceedings of the sixth international 419 symposium for cool climate viticulture and oenology. New Zealand, Christ church (Poster presentation) (2006).
39. Rothschild, M., Bergström, G. & Wängberg, S. Cannabis sativa: volatile compounds from pollen and entire male and female plants of two variants, Northern Lights and Hawaiian Indica. *Botanical Journal of the Linnean Society.* **147**, 387-397 (2005).
40. Saliba, A.J., Bullock, J. & Hardie, W.J. Consumer rejection threshold for 1,8-cineole (eucalyptol) in Australian red wine. *Food Quality Preference.* **20**, 500-504 (2009).
41. Sharkey, T. D., Singsaas, E. L., Lerdau, M. T. & Geron, C. Weather effects on isoprene emission capacity and applications in emissions algorithms. *Ecol. Appl.* **9**, 1132-1137(2000).
42. Sharkey, T.D. & Yeh, S.S. (2001b) Isoprene emission from plants. *Annu Rev Plant Physiol.* **52**, 407-436 (2001b).
43. Vallat, A., Gu, H. & Dorn, S. How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. *Phytochemistry.* **66**,1540-1550 (2005).
44. Ward, S.C., Petrie, P.R., Johnson, T.E., Boss, P.K., & Bastian, S.E.P. Unripe Berries and Petioles in *Vitis vinifera* cv. Cabernet Sauvignon Fermentations Affect Sensory and Chemical Profiles. *American Journal of Enology and Viticulture.* **66**, 435-443. doi:10.5344/ajev.2015.15016 (2015).
45. Williams, P.J., Strauss, C.R., Wilson, B. & Massy-Westropp, R.A. Novel monoterpene disaccharide glycosides of *vitis vinifera* grapes and wines. *Phytochemistry.* **21**, 2013-2020 (1982).
46. Yang, Y., Jin, G.J., Wang, X.J., Kong, C.L., Liu, J. & Tao, Y.S. Chemical profiles and aroma contribution of terpene compounds in meili (*vitis vinifera* L.) grape and wine. *Food Chemistry.* **284**, 155-161(2019).

Figure Legends

- Figure 1. Layout of the experimental field. Shows the experimental field with mature hemp plants and wine grapes. Photos were taken during the data collection for this study.
- Figure 2. Aerial view of the experimental plot showing the proximity of the vineyard to the hemp field and wind direction. The aerial view is Google image of the field that shows the size of the hemp field and the distance from the vineyard. It also shows the coordinates of the field and
- Figure 3. Terpene profile evolution during the Cherry Wine Boax hemp growth cycle. Chart shows the level of major hemp terpenes in the Cherry Wine X Boax hemp variety during the sample collection phase of the study. Each terpene is represented by a colour coded line.
- Figure 4. Terpene profile evolution during the Boax hemp growth cycle. Chart shows the level of major hemp terpenes in the Boax hemp variety during the sample collection phase of the study. Each terpene is represented by a colour coded line.
- Figure 5. Weekly relative humidity and temperature of the experimental field. Chart shows the relative humidity, average temperature, daily maximum and daily minimum temperature all represented by colour coded bars. These data points were collected concurrently with the plant material sample collection.

Figure A.I. Total ion chromatogram for sample H2756.

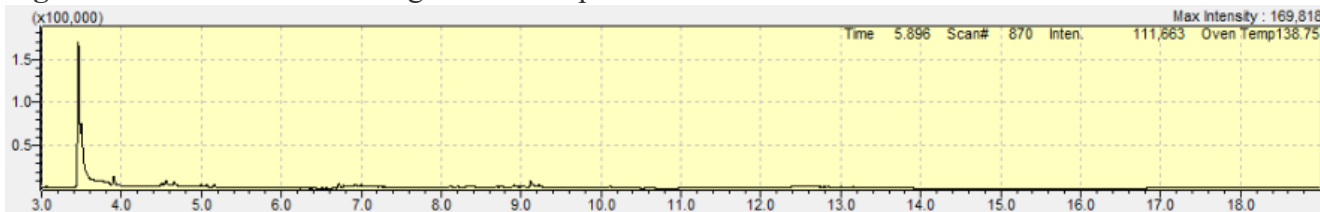


Figure A.II. Total ion chromatogram for sample H2762.

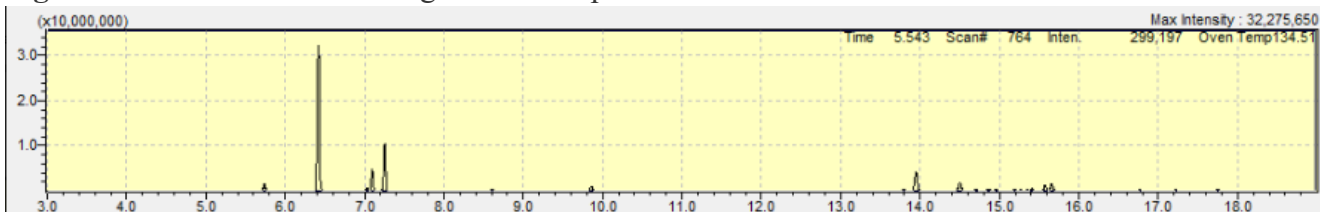
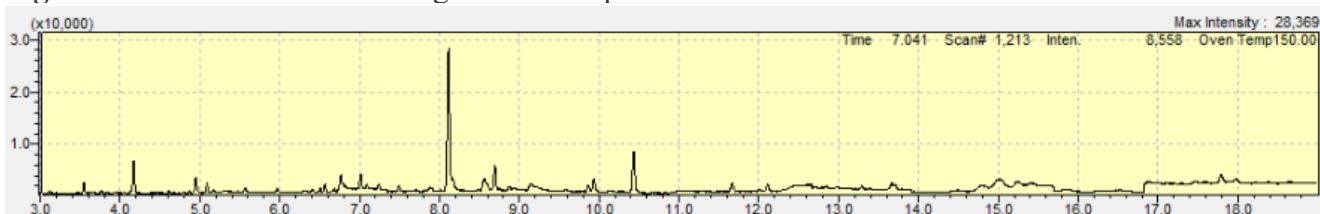


Figure A.III. Total ion chromatogram for sample H2764.



A wind rose plot was created to show the average wind speed from each direction (Figure B). The highest average speed was from the west-northwest direction, but most wind events were from between the west-southwest and southeast directions. The vineyard is nearly due north from the hemp field. Figure 2 shows examples of wind events from the southwest and south directions in relation to the two fields.

Figure B. Graphical representation of wind speed and wind direction.

