# Pennsylvania Wine Market & Research Promotion Program

# Progress Report

A financial status report and a project performance report will be required on a semi-annual basis. October and April reports are due. A final report may serve as the last semi-annual report due 30 days after completion of the contract. Grantees shall monitor performance to ensure that time schedules are being met and projected goals by time periods are being accomplished. Please submit reports to: RA-AGCommodities@pa.gov.

**SECTION 1 – SUMMARY INFORMATION**

Date of Report: July 7, 2021

Contract/PO#: 63019427 Fiscal Year: 2020-2021 Round of Grant: (i.e. Round 1, Round 2, etc) 3

Title of Paper: Boosting Polyfunctional Thiols and Other Aroma Compounds in White Hybrid Wines Through Foliar Nitrogen and Sulfur Application

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Progress Report: ☐ October ☐ April

☒ Final

Area of Focus: ☒ Research

☐ Marketing

## SECTION 2 –OBJECTIVES | TIMELINES | OUTCOMES | BUDGET

**(A comparison of actual accomplishments to the objectives for that period?)**

Nitrogen (N) is an essential mineral for plant growth, metabolism and health, and is commonly applied via soil and/or foliar treatments. Early season shoot growth is heavily dependent on reserve N and total nonstructural carbohydrates, and by the beginning of bloom, reserves are often depleted. Therefore, bloom through véraison and post-harvest periods are the two critical demand periods for vine nitrogen uptake, and consequently, the most efficacious time periods to supply nitrogen, mainly through soil application (Wolf 2008). However, these recommendations are mainly based on vegetative growth consideration rather than fruit yield and composition (in particular, yeast assimilable nitrogen; YAN). For example, a recent study conducted in Virginia showed that foliar application of nitrogen on Sauvignon blanc and Petit Manseng (*Vitis vinifera*) was more effective in increasing berry YAN than soil applied nitrogen (Moss 2015). Soil applied N was assimilated primarily into the vegetative components of the vine. Therefore, proper nitrogen management in the vineyard affects fermentation and, ultimately, wine quality, as adequate YAN in the grapes is critical to ensuring high quality fermentation (Leonardelli 2013).

In general, high N status in grapevines is associated with higher levels of glutathione and cysteinylated aroma precursors, including glutathione- and cysteine-linked precursors for the powerful polyfunctional thiols such as 3-mercaptohexanol (3MH), 3-mercaptohexyl acetate (3MHA), and 4-methyl-4-sulfanylpentan-2-one (4MSP) (Geffroy et al. 2017). These aroma compounds have exceedingly low aroma threshold values and contribute to varietal typicity in a number of cultivars, by imparting aromas of grapefruit, passionfruit, and blackcurrant. While Sauvignon blanc is most commonly associated with these beneficial varietal thiols, they are important to the aroma profiles of many other *Vitis vinifera* (e.g., Gewürztraminer, Colombard), hybrid (Cayuga White) and *Vitis labrusca* (Niagara) varieties (Dubourdieu & Tominaga 2009; Jeffrey 2016; Musumeci et al. 2015). Recent work in Europe (Lacroux et al. 2008; Geffroy et al. 2017) and in the US (Kelly 2017) on various *Vitis vinifera* varieties has demonstrated that the concentrations of these important aroma compounds can be increased through foliar N and sulfur (S) spraying. Combining N with S led to a more pronounced effect than the N foliar spray alone, most likely due to synergistic adsorption of N in presence of S (Tea et al. 2007).

In all of the above-mentioned studies, total N content, YAN and amino acid concentration in the juice, as well as glutathione and varietal thiols (3MH, 3MHA) in the wines, were observed to increase in foliar sprayed treatments compared to the untreated control, while the foliar treatments and the untreated control did not differ in canopy density, yield and juice Brix, pH and titratable acidity (TA). Evaluation of the treated wines (Lacroux et al. 2008; Geffroy et al. 2017) by wine experts revealed higher aroma intensities in grapefruit and tropical fruit while no reductive notes were perceived.

Taking all reports together, foliar spray applications of N and S present an interesting and potentially low-cost approach to enhancing wine quality through overexpression of important and desirable aroma active compounds (e.g., varietal thiols, terpenoids). This project was designed to test the applicability of foliar nitrogen and sulfur sprays on the white hybrid cultivar Traminette in order to increase levels of these desirable compounds.

Traminette, a hybrid of Gewürztraminer and Seyval blanc, is grown all over the Commonwealth, and produces wines with similar characteristics to Gewürztraminer. It is growing in popularity and

commercial significance (Vigna 2016), as it is well-suited to Pennsylvania's climate and its cold hardiness is superior than its Gewürztraminer parent (Reisch et al. 1996). Similar in aroma to its Gewürztraminer parent, Traminette grapes and wines are characterized by high levels of the varietal terpenols linalool, geraniol and nerol that are linked to pleasant floral aromas and flavors in wine (Ji & Dami 2008; Skinkis et al. 2008). Traminette also contains polyfunctional thiols at similar levels to that of Gewürztraminer (Roland et al. 2011) and thus, presents an excellent cultivar candidate for our pilot experiment. The proposed interdisciplinary study described here aims to characterize the effects on viticultural, chemical and sensory properties of Traminette juice and wine, using (i) foliar urea N spray, (ii) foliar micronized S spray, (ii) foliar urea N and micronized S spray, and (iv) an untreated control.

The following experimental treatments were applied in Years 1 and 2 of the study:

- Treatment 1: Control: no foliar nitrogen (N) or sulfur (S) application other than regular S applications for disease management
- Treatment 2: Nitrogen (N): 15 kg/ha N – applied as two equal foliar applications around veraison
- Treatment 3: Sulfur (S): 5 kg/ha S (MicroSulf) – applied as two equal foliar applications around veraison
- Treatment 4: N + S: 15 kg/ha N + 5 kg/ha S– applied as two equal foliar applications

**Final Methodology Used:**

Field and wine evaluations were conducted over the 2018 and 2019 growing seasons.

*Experimental layout*: A Traminette grower cooperator in Centre County was identified in January 2018 and his vineyard was used as the experiment site in both years (2018 and 2019) of the study. The experiment was set up as a randomized complete block design with 4 replications per treatment, each comprising of 12 contiguous vines. Treatment replicates within the row were separated by 3 to 4 guard vines. To standardize the number of shoots per vine, shoots were thinned to 16 shoots per meter of cordon shortly after bud burst.

The treatments consisted of

1. Control with no nitrogen or sulfur applications other than sulfur applied by the grower as part of a disease management spray program
2. 15 kg/ha of urea N (Coron®, Helena Chemical Co., Collierville, TN) split in two equal foliar applications, the first at the onset of véraison and the second one week later
3. 5 kg/ha of micronized sulfur (Micro Sulf®, Nufarm Americas Inc., Burr Ridge, IL) in two split applications at the onset of véraison and one week later
4. 15 kg/ha of urea N and 5 kg/ha of micronized sulfur in two split applications at the onset of véraison and one week later.

*Vegetative growth:* To assess the impact of the treatments on canopy density and microclimate of the fruiting zone, point quadrat analysis and light availability in the cluster zone (EPQA) was measured before treatments application and again after treatment application, shortly before harvest. Pruning

weight were collected during the winter season following treatments application. Vine crop load were estimated as Ravaz index (yield/pruning weight).

*Vine nutrient status:* Forty leaf petioles were sampled from each treatment replicate and analyzed for macro- and micronutrients concentration. Concurrently, leaf blades were used for determining total nitrogen concentration. Samples were collected at bloom (before treatments application) and again late in the summer, about 4 weeks after treatments applications.

*Disease rating:* Because N foliar application may increase fruit susceptibility to bunch rot disease, the severity (percentage of infected cluster area) and incidence (percentage of clusters infected) of *Botrytis* bunch rot were visually assessed using a scale from one to ten on 25 randomly selected clusters per each treatment replicate the day before harvest.

*Yield component and fruit composition*: Vines were harvested by hand one day prior to commercial harvest. The total number of clusters per vine were counted and the cumulative cluster weight per vine were measured. Five-hundred grams berry samples were randomly collected at harvest for berry chemistry analysis (total soluble solids, pH, TA) and average berry size was determined. Juice samples were also be analyzed for total yeast assimilable nitrogen (YAN) concentrations.

*Research winemaking:* Grapes were vinified in the Penn State Department of Food Science's Wet Pilot Plant facility using a standard pilot-scale (2018) or microscale (2019) winemaking protocol to eliminate potential sources of variation in the aroma profiles of the wines. In brief, fruit was whole cluster pressed and the resulting juice was allowed to settle for 3 days at 4°C before being racked to glass fermentation vessels. All fermentations were performed in duplicate. Following fermentation, all wines were transferred and cold settled at 4°C for 48 h prior to racking off the lees. The wines were then allowed to cold stabilize for an additional 14 days prior to racking and bottling with the addition of 30 mg/L $SO_2$ in 750 mL glass bottles with aluminum screw cap closures lined with foil. Basic wine chemistry (TA, pH, ethanol, free and total sulfur dioxide, residual sugar) was measured.

*Wine aroma analysis*: Aroma profiling of wine samples was analyzed by head space solid-phase microextraction gas chromatography/mass-spectroscopy (HS-SPME-GC/MS), with a method being adapted from previous studies. Analysis was performed using duplicate vials of triplicate wine samples. Sample preparation used 20 mL crimp top headspace vials (Restek, Bellefonte, PA) with 2 g of NaCl added to each vial along with 2 mL of wine sample and 50 µL of the internal standards 2-octanol and d8-napthalene (10 mg/L prepared in HPLC-grade methanol). Sample vials were sealed using a crimp cap with septum (Restek, Bellefonte, PA). A blank vial was prepared using an empty 20 mL headspace vial and crimp cap with septum (Restek, Bellefonte, PA). A standard vial containing 50 µL of the internal standards 2-octanol and d8-napthalene and 5 µL of alkane standard (C8-C20) was prepared in a 20 mL crimp top headspace vial with crimp cap and septum (Restek, Bellefonte, PA). A separate vial containing 10 µL of SPME-mix was prepared in a 20 mL screw top headspace vial with screw cap and septum (Restek, Bellefonte, PA). Vials were placed into the holding tray of the auto sampler in the order of blank, internal standard, wine samples, spme-mix. Wine samples were incubated using an autosampler (Gerstel Robotic, Linthicum, MD) for 5 minutes

at 30°C prior to extraction. Samples were then withdrawn with a 2 cm DVB/CAR/PDMS SPME fiber (Supelco, Bellefonte, PA) for 30 minutes at 30°C. The GC oven temperature program was 1 minute at 30°C and then ramped up at a rate of 10°C/min to 250°C and held for 5 minutes. Samples were then desorbed for 10 minutes into the inlet of an Agilent 7890 GC coupled to an Agilent 5977B single quadrupole MS (Agilent, Santa Clara, CA). The desorption occurred in splitless mode at 250°C and the purge valve opened at 1.2 minutes. The column was a Rtx-Wax 30m x 0.25mm x 0.25 µm (Restek, Bellefonte, PA). Spectra were collected at a rate of 8.1/sec by MS in scanning mode. Acquired raw GC/MS data was analyzed using PARADISe software (version 3.1) for data visualization, dividing data into retention time intervals, deconvolution of peaks, validation and extraction of deconvoluted peaks, and compound identification using NIST search engine and NIST mass spectral library. Compounds were then further validated by calculating linear retention indices (LRI) and concentrations of identified compounds are reported in internal standard equivalents.

Thiol extractions in model wine and Traminette wine were carried out according to Capone et al. In brief, 20 mL of wine was spiked with an internal standard of 4-methoxy-2-methyl-2-butanethiol (4MMB; 50 µL of 0.001 mg/mL) and extracted with 4,4'-dithiodipyridine (DTDP; 200 µL of 10 mM) with 50% acetaldehyde (80 µL) and EDTA (200 mg) for 30 minutes. Samples were filtered using C18 solid-phase extraction cartridges, dried under vacuum, and reconstituted in 200 µL ethanol. Multiple reaction monitoring (MRM) transitions and optimized collision energies were determined for each of the following compounds: 4-methoxy-2-methyl-2-butanethiol (4MMB; internal standard), 4-Mercapto-4-methylpentan-2-one (4-MMP), 3-Mercaptohexanol (3-MH), and 3-Mercaptohexyl acetate (3-MHA). Thiols were analyzed on a reverse-phase HPLC and quantified using a mass spectrometer. The aqueous solvent (A) consisted of 95% water, 5% acetonitrile, and 0.1% formic acid; the organic solvent (B) consisted of 100% acetonitrile and 0.1% formic acid. The following gradient was used at 0.5 mL/min: 0 mins, 15% B; 5 mins, 47% B; 5.5 mins, 79% B; 6.5 mins, 79% B; 6.6 mins, 15% B; 12 mins, 15% B. Peak areas were normalized with respect to the internal standard, and one-way ANOVAs were carried out comparing the relative abundances of 4MMP, 3MH, and 3MHA between control Traminette wine, Traminette wine with nitrogen, Traminette wine with sulfur, and Traminette wine with sulfur and nitrogen.

**Key Findings & Results:**

Treatment Key:

- Treatment 1: Control: no foliar nitrogen (N) or sulfur (S) application other than regular S applications for disease management
- Treatment 2: Nitrogen (N): 15 kg/ha N – applied as two equal foliar applications around veraison
- Treatment 3: Sulfur (S): 5 kg/ha S (MicroSulf) – applied as two equal foliar applications around veraison
- Treatment 4: N + S: 15 kg/ha N + 5 kg/ha S– applied as two equal foliar applications

Leaf samples were collected before treatment application to confirm that all vines had similar N concentration in plant tissue before foliar applications (data not shown). A second leaf sampling was conducted on, approximately four weeks after treatments application. For each experimental unit we

collected 40 leaves, 20 for each canopy side. Leaves of the same age (first fully expanded leaf from the shoot tip) were used for nitrogen analysis (Table 1).

**Table 1.** Effects of nitrogen (N) or/and sulfur (S) application on N concentration in the leaf blade and petiole and on yeast assimilable nitrogen (YAN) in the juice. Leaves were collected four weeks after the second N and S application; YAN was measured at harvest.

| Treatment | N- Leaf blade (%) | N -Leaf petiole (%) |
|---|---|---|
| ***2018*** | | |
| Control | 3.44 | 1.61 |
| Nitrogen | 3.49 | 1.66 |
| Sulfur | 3.42 | 1.61 |
| Nitrogen+ Sulfur | 3.33 | 1.63 |
| *P-value* | *0.316* | *0.423* |
| ***2019*** | | |
| Control | 2.87 ab | 1.17 |
| Nitrogen | **2.97 a** | 1.23 |
| Sulfur | **2.77 b** | 1.19 |
| Nitrogen+ Sulfur | **2.78 b** | 1.20 |
| *P-value* | 0.011 | 0.254 |

Fruit was hand-harvested and was sorted both in the field and at the winery to remove damaged and/or rotten berries. Given the abnormal growing conditions in 2018 (i.e., record rainfall and, in particular, an extremely wet post-veraison period), virtually every cluster in the control and treatment blocks were afflicted with at least some level of rot. Furthermore, sugar accumulation was lower than what was expected given a late September harvest date, with harvest soluble solids levels recorded at ca. 16 Brix. The potential confounding effects of these conditions on the study's results (and the conclusions that can be drawn) are discussed below. The 2019 growing season, however, was relatively normal, and fruit quality was significantly better than 2018 vis-à-vis fungal diseases and fruit chemical composition.

Standard white winemaking protocols were followed according to the methods described above, and the resulting juices were sulfited (30 mg/L $SO_2$) and treated with 1.5 mL/HL CinnFree (enzyme). For Year 1, juices were chaptalized with sucrose to 22 Brix (final) and inoculated with VIN13. The final volumes were 13.82 L of juice per fermentation replicate for Treatments 1 (Control), 3 (S), and 4 (N+S); the final volume for Treatment 2 (N) was 12.3 L per fermentation replicate. Once the wines reached zero Brix (as confirmed by enzymatic analysis), they were allowed to settle before being racking and before a final sulfite adjustment was made. The wines were transferred to 750 mL capacity bottles under gaseous nitrogen blanket and sealed using ROTE (screw cap) closures. Sample aliquots were taken at this time, which were transferred to 50 mL capacity centrifuge tubes

and frozen at -80 C until volatiles analysis could be performed. Wine samples stored in 750 mL glass bottles were maintained at 15 C until sensory analysis could be performed. For Year 2, juices were produced according to the same procedure used in Year 1, however, with reduced volumes (300 mL of juice obtained per treatment) in order to reduce overall waste with respect to fruit and final wine. Standard microvinification protocols were thus able to be used in Year 2.

## Analysis of Wine Volatiles by GC-MS

The volatile fraction of each wine sample was chromatographically separated and analyzed by headspace GC-MS according to the methodology described above. A representative GC-MS chromatogram is shown in Figure 1.

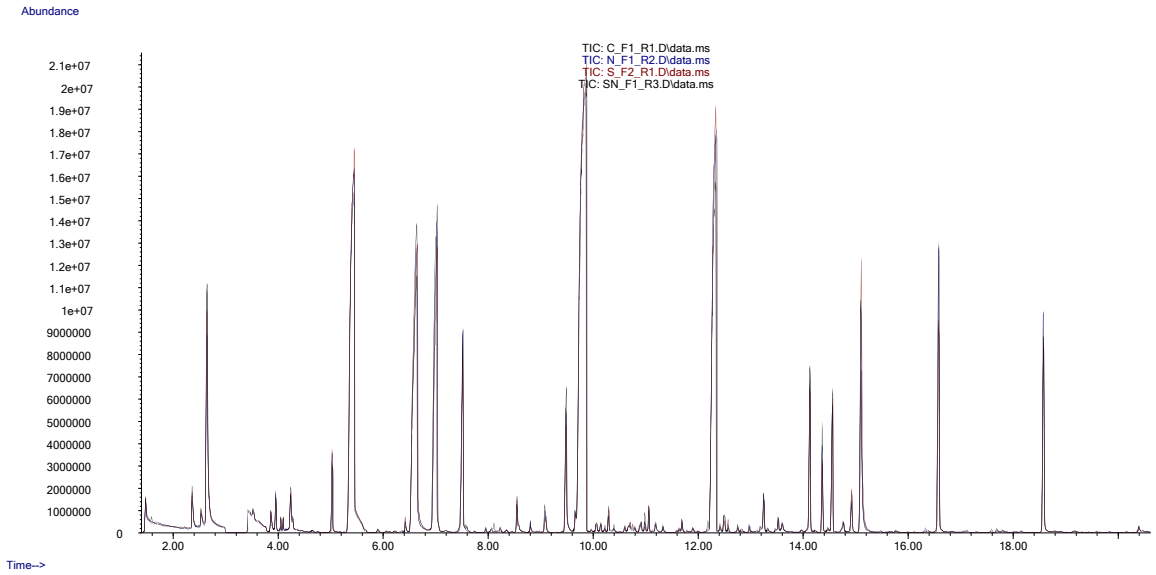


**Figure 1.** Representative gas chromatogram showing mass spectrum detection of key aroma active volatile compounds in control and treatment wines. All chromatograms are scaled to the same abundance intensity; shown are total ion current (TIC).

Particular attention was paid to both undesirable and desirable aroma-active, non-terpenoid, volatile compounds in the control and treatment wines (i.e., ethyl acetate, isoamyl acetate, isoamyl alcohol, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl decanoate, 2,phenylethyl acetate, hexanoic acid, ethyl dodecanoate, 2-phenylethyl alcohol, octanoic acid, and decanoic acid). We noted no significant differences between the control and treatment wines in terms of any of these aroma active compounds in Year 1 of the study, suggesting that the foliar spray application treatments had no influence on the presence/absence or final concentrations of these volatile compounds of interest; however, given that this growing season was highly unusual (i.e., record rainfall resulting in considerable decreases in fruit quality), it is difficult to generalize based on these findings. Therefore, research wines prepared from Year 2 grapes were analyzed using identical methodology

in order to compare aroma active volatiles across treatments. A summary of these data is presented in Table 4.

**Table 4**. Listing of aroma active volatiles detected and confirmed (based on NIST library) in treatment wines (Year 2) by SPME-HS-GC-MS. Concentrations are presented as relative abundances. Treatment (i.e., Nitrogen, Sulfur, or Nitrogen + Sulfur) means that are significantly different ($p > 0.05$) from the Control are denoted with a "**b**".

| Analyte | Sensory Descriptor | Reference | Control | Nitrogen | Sulfur | Nitrogen + Sulfur |
|---|---|---|---|---|---|---|
| Ethyl acetate | Etherial, fruity, sweet, grape and rum-like | Mosciano, Gerard P&F 19, No. 5, 79, (1994) | 0.377 ± 0.046 | 0.415 ± 0.015 | 0.405 ± 0.009 | 0.387 ± 0.021 |
| Isobutyl acetate | Sweet, fruity, ethereal, banana, tropical | Mosciano, Gerard P&F 15, No. 6, 35, (1990) | 0.046 ± 0.0004 | 0.045 ± 0.010 | 0.034 ± 0.009 | 0.039 ± 0.003 |
| Ethyl butyrate | Fruity, juicy fruit, pineapple, cognac | Luebke, William tgsc, (1985) | 0.156 ± 0.005 | 0.150 ± 0.006 | 0.172 ± 0.004 | 0.140 ± 0.004 |
| Isobutanol | Ethereal, winey | Luebke, William tgsc, (1985) | 0.203 ± 0.010 | 0.227 ± 0.013 | 0.216 ± 0.008 | 0.220 ± 0.010 |
| Isoamyl acetate | Sweet, banana, fruity with a ripe estry nuance | Mosciano, Gerard P&F 16, No. 6, 43, (1991) | 0.080 ± 0.032 | 0.941 ± 0.035 **b** | 0.089 ± 0.011 | 0.097 ± 0.010 |
| Methyl hexanoate | Ethereal, fruity, pineapple, apricot, strawberry, fruit, tropical, banana | Luebke, William tgsc, (2021) | 0.004 ± 0.002 | 0.007 ± 0.001 | 0.007 ± 0.002 | 0.006 ± 0.002 |
| Ethyl hexanoate | Sweet, fruity, pineapple, waxy, green, banana | Luebke, William tgsc, (1990) | 1.353 ± 0.065 | 1.537 ± 0.063 | 1.442 ± 0.025 | 1.325 ± 0.074 |
| Acetic acid hexyl ester | Green, fruity, sweet, fatty, fresh apple, pear | Mosciano, Gerard P&F 18, No. 2, 38, (1993) | 0.397 ± 0.018 | 0.585 ± 0.030 **b** | 0.414 ± 0.009 | 0.458 ± 0.028 |
| 1-Hexanol | Pungent, etherial, fusel oil, fruity and alcoholic, sweet | Mosciano, Gerard P&F 18, No. 2, 38, (1993) | 0.267 ± 0.026 | 0.425 ± 0.019 **b** | 0.349 ± 0.012 **b** | 0.379 ± 0.014 **b** |
| Methyl octanoate | Waxy, green, sweet, orange and aldehydic with vegetative and herbal nuances | Mosciano, Gerard P&F 19, No. 3, 51, (1994) | 0.036 ± 0.008 | 0.096 ± 0.013 **b** | 0.054 ± 0.015 | 0.072 ± 0.009 |
| Ethyl octanoate | Waxy, sweet, musty, pineapple | Mosciano, Gerard P&F | 2.946 ± 0.140 | 3.071 ± 0.186 | 3.411 ± 0.101 | 2.845 ± 0.206 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | and fruity with a creamy, dairy nuance | 22, No. 2, 69, (1997) | | | | |
| 2,3-Butanediol | Fruity, creamy, buttery | Mosciano, Gerard P&F 18, No. 2, 38, (1993) | 0.209 ± 0.021 | 0.149 ± 0.004 **b** | 0.143 ± 0.016 **b** | 0.154 ± 0.001 **b** |
| Linalool | Citrus, orange, floral, terpy, waxy and rose | Mosciano, Gerard P&F 21, No. 1, 33, (1996) | 0.042 ± 0.001 | 0.065 ± 0.0002 **b** | 0.052 ± 0.001 **b** | 0.059 ± 0.004 **b** |
| Methyl decanoate | Fruity, floral | Mosciano, Gerard P&F 18, No. 2, 38, (1993) | 0.022 ± 0.002 | 0.076 ± 0.004 **b** | 0.034 ± 0.007 | 0.058 ± 0.001 **b** |
| Hotrienol | Sweet, tropical, ocimene, fennel, ginger, myrcene | Luebke, William tgsc, (2007) | 0.005 ± 0.001 | 0.007 ± 0.001 | 0.006 ± 0.0003 | 0.007 ± 0.001 |
| Butanoic acid | Sharp, dairy-like, cheesy, buttery with a fruity nuance | Mosciano, Gerard P&F 19, No. 2, 55, (1994) | 0.019 ± 0.002 | 0.021 ± 0.001 | 0.020 ± 0.002 | 0.018 ± 0.0002 |
| Ethyl decanoate | Sweet, waxy, fruity, apple | Mosciano, Gerard P&F 15, No. 3, 51, (1990) | 1.187 ± 0.121 | 0.915 ± 0.116 | 1.633 ± 0.117 **b** | 0.954 ± 0.118 |
| Isovaleric acid | Cheese, dairy, acidic, sour, pungent, fruity, stinky, ripe | Mosciano, Gerard P&F 18, No. 5, 39, (1993) | 0.025 ± 0.000004 | 0.028 ± 0.004 | 0.029 ± 0.002 | 0.029 ± 0.0002 |
| 1-Propanol, 3-(methylthio)- | SuIfureous and onion-like | Mosciano, Gerard P&F 20, No. 1, 31, (1995) | 0.004 ± 0.0001 | 0.004 ± 0.002 | 0.004 ± 0.001 | 0.004 ± 0.0004 |
| Nerol | Floral, orange blossom, citrus, mandarin, herbal | Mosciano, Gerard P&F 21, No. 1, 33, (1996) | 0.011 ± 0.002 | 0.021 ± 0.002 **b** | 0.012 ± 0.001 | 0.013 ± 0.001 |
| 2-Phenylethyl ester | Sweet, honey, floral rosy, with a slight yeasty honey note with a cocoa and balsamic nuance | Mosciano, Gerard P&F 26, No. 1, 52, (2001) | 0.104 ± 0.029 | 0.097 ± 0.00004 | 0.106 ± 0.006 | 0.092 ± 0.011 |
| Hexanoic acid | Cheesy, fruity, phenolic, fatty, goaty | Luebke, William tgsc, (1987) | 0.412 ± 0.050 | 0.346 ± 0.008 | 0.382 ± 0.037 | 0.316 ± 0.002 |
| Phenylethyl alcohol | Sweet, floral, fresh and bready with a rosey honey nuance | Mosciano, Gerard P&F 18, No. 4, 51, (1993) | 0.011 ± 0.001 | 0.019 ± 0.003 **b** | 0.011 ± 0.001 | 0.013 ± 0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Nerol acetate | Floral, rosy, sweet, soapy, citrus, grapefruit and fruity with a tropical nuance | Mosciano, Gerard P&F 22, No. 3, 47, (1997) | 0.093 ± 0.006 | 0.207 ± 0.012 **b** | 0.128 ± 0.008 **b** | 0.148 ± 0.005 **b** |
| Citronellol | Floral, rosy, sweet, citrus with green fatty terpene nuances | Mosciano, Gerard P&F 16, No. 1, 31, (1991) | (nd) | 0.007 ± 0.001 **b** | (nd) | (nd) |
| Terpinen-4-ol | Woody, ceding, mentholic, citrus terpy, spicy | Mosciano, Gerard P&F 23, No. 4, 33, (1998) | 0.236 ± 0.012 | 0.203 ± 0.017 | 0.328 ± 0.016 **b** | 0.256 ± 0.019 |
| Citronellol acetate | Floral, rosy, green, fatty, citrus lemon and bois de rose-like | Mosciano, Gerard P&F 25, No. 6, 26, (2000) | 0.003 ± 0.001 | 0.008 ± 0.002 **b** | 0.004 ± 0.001 | 0.005 ± 0.0001 |
| Alpha terpineol | Pine, terpene, lilac, citrus, woody, floral | Mosciano, Gerard P&F 22, No. 4, 75, (1997) | 0.096 ± 0.014 | 0.222 ± 0.009 **b** | 0.082 ± 0.002 | 0.169 ± 0.021 **b** |
| Methyl dodecanoate | Oily, wine, fruity, floral | Luebke, William tgsc, (2007) | 0.260 ± 0.029 | 0.369 ± 0.041 | 0.364 ± 0.036 | 0.338 ± 0.021 |

Unlike Year 1, where no significant differences in aroma active volatiles were observed across treatments, we observed many significant differences between treatments in terms of several compounds that are important with respect to the aroma quality of white wines. When differences were observed, the application of foliar nitrogen, sulfur, and/or nitrogen + sulfur resulted in increases in aroma active compound abundances, and these compounds are generally considered to be desirable in the context of aromatic white wines.

The nitrogen-only treatment resulted in wines with **significantly higher** concentrations of isoamyl acetate ("sweet, banana, fruity with a ripe estery nuance"), acetic acid hexyl ester ("green, fruity, sweet, fatty, fresh apple, pear"), 1-hexanol ("pungent, etherial, fusel oil, fruity and alcoholic, sweet"), linalool ("citrus, orange, floral, terpy, waxy and rose"), methyl decanoate ("fruity, floral"), and nerol ("floral, orange blossom, citrus, mandarin, herbal"). The sulfur-only and nitrogen+sulfur treatments also resulted in wines with increased levels of aroma active volatiles, although apparently to a lesser extent compared to the nitrogen-only treatment. It should also be noted that the three foliar treatments led to wines with relatively **lower** concentrations of 2,3-butanediol, an aroma active glycol known to impact a "creamy" and "buttery" character, compared to the control. While the presence of this compound might be considered desirable in some styles of wine, we would consider this to be an undesirable compound in the context of an aromatic Traminette wine.

**Polyfunctional Thiol Analysis by LC-MS/MS**

All treatments (i.e., N, S, N+S) resulted in wines with elevated concentrations of 4MMP compared with the control. A significant difference between all four samples with respect to the amount of 3MH and 3MHA present was also observed, with the highest concentrations of 3MH and 3MHA

detected in wines prepared from N+S treated fruit, followed by the S treatment, the control, and the N treatment. For 3MH and 3MHA, all four treatments were significantly different from one another. **These results suggest that the N+S treatment, and the S treatment, increased the amount of 3MH and 3MHA in Traminette wines**. The nitrogen treatment resulted in lower amounts of 3MH and 3MHA than the control treatment.

**Table 5**. Listing of important grape-derived polyfunctional thiols detected and confirmed (based on authentic standards) in treatment wines (Year 2) by SPME-HS-GC-MS. Concentrations are presented as relative abundances. Treatment (i.e., Nitrogen, Sulfur, or Nitrogen + Sulfur) means that are significantly different ($p > 0.05$) from the Control are denoted with a "**b**".

| **Analyte** | **Sensory Descriptor** | **Reference** | **Control** | **Nitrogen** | **Sulfur** | **Nitrogen + Sulfur** |
|---|---|---|---|---|---|---|
| 3-mercapto-1-hexanol (3MH) | Floral, fruity, pear, tropical, passionfruit, blackberry, raspberry, black currant bud | Luebke, William tgsc, (2017) | 3878.43 ± 0.007 | 3205.24 ± 0.005 [b] | 12604.18 ± 0.018 [b] | 15492.37 ± 0.031 [b] |
| 3-sulfanylhexyl acetate (3MHA) | Floral, fruity, pear, tropical, passionfruit, blackberry, raspberry, black currant bud | Luebke, William tgsc, (2017) | 169.56 ± 0.001 | 57.62 ± 0.004 [b] | 396.32 ± 0.001 [b] | 735.91 ± 0.001 [b] |
| 4-methyl-4-mercaptopentan-2-one (4MMP) | Box tree, passion fruit, broom, black current | Coetzee et al, 2012; Tominaga et al, 1998; Ribéreau-Gayon et al, 2006; Lund et al, 2009 | 122.40 ± 0.001 | 252.02 ± 0.001 [b] | 220.75 ± 0.0008 [b] | 209.01 ± 0.0005 [b] |

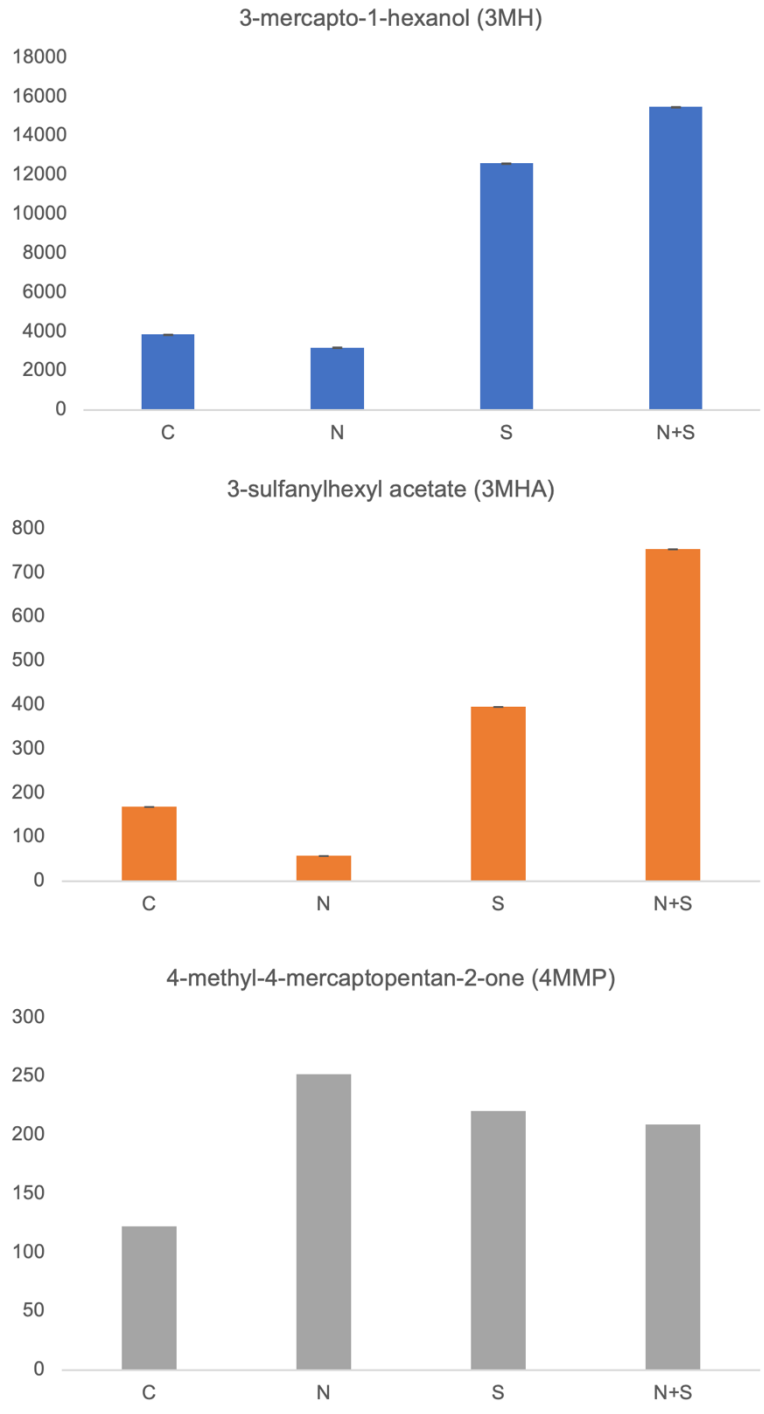


**Figure 2**. Effect of foliar treatment on final concentration of polyfunctional thiols (3MH, 3MHA, 4MMP) in Traminette wines. Means (n=3) represent abundances relative to an internal standard (4MMB), and error bar represent standard deviations.

## SECTION 3 – SCOPE OF WORK

**(Reasons why established objectives were not met, if applicable?)**

Due to travel restrictions imposed by the COVID-19 pandemic, we were unable to complete the following sub-aim: "Funds are requested to travel from University Park to the experimental vineyard site and is also requested to support the PIs travel expenses to disseminate extension and research outcomes at annual association meetings." Therefore, we will be returning all funds earmarked for "Travel". All other research objectives were accomplished, however.

Furthermore, due to COVID-19 restrictions, we were unable to recruit a graduate student in the final year to perform the bulk of the analysis and data interpretation; therefore, Dr. Centinari (Co-PI) generously offered to assist with these tasks in the spring and early summer (2021). We are grateful for her effort and contributions to the project in the final home stretch.

## SECTION 4 – DELAYS/RISKS

**(Reasons for any problems, delays, or adverse conditions which will affect attainment of overall program objectives, prevent meeting time schedules or objectives, or preclude the attainment of particular objectives during established time periods. This disclosure shall be accomplished by a statement of the action taken or planned to resolve the situation?)**

(n/a)

## SECTION 5 – SPECIAL NOTES

**(What objectives and timetables are established for the next reporting period? Etc.)**

(n/a)