WILDFIRE IMPACT MARKER PANELS

VOLATILE MARKERS - BASIC PANEL

ETS has been offering the Basic Panel of volatile ("free") markers, comprised of guaiacol and 4-methylguaiacol since 2008.

Despite its apparent simplicity, this panel has an excellent track record for assessing wildfire impact with pre-harvest grape samples, small-scale fermentation samples (micro-ferments), and production wines before any contact with oak.

The value and efficacy of this panel was confirmed by the 2018 Mendocino Complex Fire Lake County Winegrape Commission Study, with the participation of the University of California Cooperative Extension (UCCE) and the Australian Wine Research Institute (AWRI) (1). Also, volatile guaiacol results on grape samples are typically requested by crop insurance providers.

This Basic Panel was our "fall back" panel during the 2020 Harvest due to the unprecedented number of samples received during the catastrophic wildfire events. For the 2021 harvest, ETS also is offering an Expanded Volatile Markers Panel and a Glycosylated Markers Panel.

VOLATILE MARKERS - EXTENDED PANEL

This extended panel of volatile markers complements our basic panel. In addition to guaiacol and 4-methylguaiacol (guaiacols), this extended panel measures secondary markers of impact including o-cresol, m-cresol, p-cresol, phenol, syringol and 4-methylsyringol.

This extended panel is available for grapes, small-scale fermentation samples (micro-ferments), and wines. Compared to the Basic Panel, additional markers allow more complete assessments of wildfire impact, and provide useful information with moderately oaked wines (e.g. from so-called "neutral" barrels, which often contribute low amounts of guaiacols making interpretation of results very difficult).

GLYCOSYLATED MARKERS PANEL

In 2020, ETS further perfected a unique panel of glycosylated ("bound") smoke markers using a state-of-the-art combination of solid phase extraction, liquid chromatography, and triple quadrupole mass spectrometry (SPE/HPLC/MS/MS – QQQ.

ETS has worked behind the scenes with the Wine Institute Technical Committee, the AWRI and several major wineries to reach an agreement on a common list of glycosylated markers. We also joined forces to have pure reference compounds (and their isotopic analogues) synthesized for each of the markers in that list. This goal was achieved in December 2020. These analytical standards allow extremely reproducible quantitative results that are now comparable between laboratories. ETS has offered an updated panel employing these standards since January 2021 for wines, and will offer it for grapes and micro-ferments during the 2021 harvest season.

Our new Wildfire Glycosylated Markers panel includes, for each of the volatile markers listed in our Expanded Panel, its main glycosylated (sugar-bound) form – See figure 1. Note that glycosylated compounds are not contributed by toasted oak, making them particularly useful to assess wildfire impact in oaked wines. They are not directly odor-active, but may contribute lingering aftertastes often experienced with impacted wines. It is also possible (but not yet substantiated by data) that they hydrolyze in wine, slowly releasing volatile "free" forms and causing wildfire flavors to become more noticeable with time.

Volatile ("Free") Markers	Glycosylated ("Bound) Forms
Guaiacol	Guaiacol Rutinoside
4-Methylguaiacol	4-Methylguaiacol Rutinoside
ortho-, meta- and para- Cresol	Cresol Rutinoside
Phenol	Phenol Rutinoside
Syringol	Syringol Gentiobioside
4-Methylsyringol	4-Methylsyringol Gentiobioside

Figure 1: Compounds included in the ETS Wildfire Expanded Volatile Markers (left column) and their main glycosylated (sugar-bound) forms included in the Glycosylated Markers panel (right column).

(1) 2018 Mendocino Complex Fire Lake County Winegrape Commission Study – with the participation of the University of California Cooperative Extension (UCCE) and the Australian Wine Research Institute (AWRI) – see "From Blaze to Bottle: Smoke Gets in Your Wine" by Glenn McGourty in the January 2020 Issue of Wine Business Monthly.

WILDFIRE IMPACT: WHAT SAMPLES TO TEST?

Wildfire impacts in grapes and wines are caused by a wide range of volatile phenols found in wildfire smoke. These compounds are absorbed and accumulate in berries. They eventually end up in wine where they can cause unwanted flavors. These off-flavors, described as "smoky", "bacon", "campfire" and "ashtray", are usually long lasting and linger on the palate even after the wine is swallowed.

Wildfire impact in wine was identified as a serious problem after the 2003 wildfires in Australia and British Columbia. The California wine industry was also affected following the wildfires of summer 2008, and wildfire impact has been recognized as a concern for growers and wineries ever since. Here is a review of the various types of samples that may be submitted for testing:

1. BERRIES

During the 2008 California wildfires, ETS developed an analytical tool to screen grapes for the risk of wildfire impact. The analysis measures trace levels of free guaiacol and 4-methylguaiacol in whole berries. Knowing the levels of these indicators in berries enables winemakers to assess the risk of wildfire smoke impact and choose an appropriate course of action to mitigate the effects in their wines. In 2021, an extended panel of volatile ("free") wildfire markers and a glycosylated markers panel is also available for berry samples.

Exposure of vines to wildfire smoke can widely vary within a small geographic area, depending mainly on proximity with the fires and wind conditions. In effect, getting representative samples can be challenging. Mixing grape varieties in composite samples should be avoided, as grape cultivars often react differently to a similar exposure to smoke. Syrah grapes contain naturally occurring guaiacol and should never be mixed with grapes from other varieties.

Submit 200 to 300 loose berries, keeping them cold and undamaged as much as possible (do not crush them). When shipping samples, use hard plastic containers with icepacks in an insulated package. Avoid submitting cluster samples, which trigger additional fees and may delay getting results. . A typical strategy for berry sampling is to collect berries from at least 50 clusters, taking four berries from each cluster from the top, bottom, front and back of the cluster.

Berry samples are often requested by crop insurance providers, although micro-ferment samples have been more widely accepted during the 2020 fire events. It is advisable to keep backup samples in a freezer.

2. JUICES

It is possible to measure wildfire impact markers in juice samples, but since compounds are mostly located in skins, whole berry testing is the preferred method for pre-harvest screening. Please avoid submitting fermenting samples which may constitute a safety hazard.

3. SMALL SCALE FERMENTATIONS (MICRO-FERMENTS)

In order to complement pre-harvest grape tests, wines from small-scale fermentations ("micro-ferments" or "bucket ferments") may be tested for volatile wildfire markers (basic or extended panels), and for glycosylated markers. The pros and cons of both tests are outlined in Figure 2.

	Grape Samples	Micro-ferments
Sample Preparation Time (before sending to the laboratory)	Immediate	>1 Week
Sensory Evaluation	Not very useful	Useful, but difficult (need for multiple trained tasters including sensitive individuals)
Analysis Turnaround Time	1-2 days	1-2 days
Prediction of Smoke Characters in Production Wines	Indirect (variable "multipliers" between grape and wine results)	Reds: more direct (but delayed) Whites: uncertain (ferment with skins for "worst case scenario"?)

Figure 2: Grape Samples vs. Small Scale Ferments (Micro-ferments) as pre-harvest testing options for wildfire smoke impact.

3. PRODUCTION WINES

Analyzing immediately after completion of primary fermentation allows a first assessment of wildfire impact in production wines. It is preferable to sample and analyze wines that have not come in contact with oak or oak-derived products, which can contribute volatile smoke markers.

With barreled wines, it is still possible to get useful information from the volatile markers by choosing the extended panel of volatile ("free") smoke markers rather than the basic volatile marker panel (guaiacols only), and taking samples from the most "neutral" barrels available. Keep in mind that there is no issue analyzing oaked wines for glycosylated markers, as oak or oak products do not contain these compounds. Analyzing for glycosylated markers in wine is always relevant regardless of contact with oak.

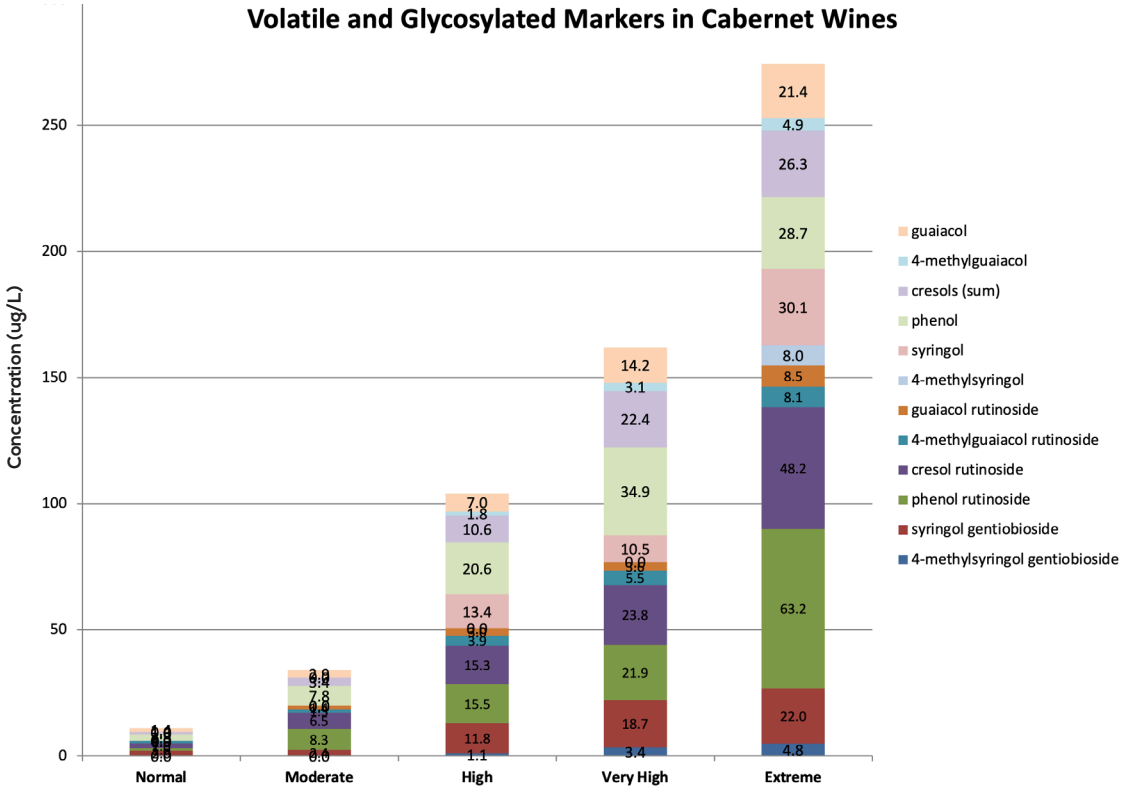
EXTENDED VOLATILE MARKERS AND GLYCOSYLATED MARKERS:

WHAT LEVELS AND PATTERNS TO EXPECT IN WINES?

Following the 2018 Mendocino Complex Fire Study (1), the selection of volatile ("free") and glycosylated ("bound") markers currently offered by ETS and the AWRI has shown to be relevant in the context of a major California wildfire event (with the exception of volatile syringol and 4-methylsyringol, which did not appear closely related to the level of exposure to wildfire smoke).

The strength of correlations observed between markers, however, left the following question open: "Is testing for other markers than "free" guaiacol and 4-methylguaiacol really useful from a practical standpoint?"

After having analyzed to date more than a thousand wines for both volatile extended markers and glycosylated markers, we can answer this question. So, let's present examples of results, typical and less typical, that we have observed with 2020 wines from California and Oregon.



	Normal	Moderate	High	Very High	Extreme
guaiacol	1.4	2.9	7.0	14.2	21.4
4-Methylguaiacol	<1.0	<1.0	1.8	3.1	4.9
Cresols (sum)	1.0	3.4	10.6	22.4	26.3
Phenol	2.5	7.8	20.6	34.9	28.7
Syringol	<5.0	<5.0	13.4	10.5	30.1
4-Methylsyringol	<5.0	<5.0	<5.0	<5.0	8.0
Guaiacol Rutinoside	0.0	1.4	3.0	3.6	8.5
4-Methylguaiacol Rutinoside	1.0	1.3	3.9	5.5	8.1
Cresol Rutinoside	2.0	6.5	15.3	23.8	48.2
Phenol Rutinoside	1.0	8.3	15.5	21.9	63.2
Syringol Gentiobioside	2.0	2.4	11.8	18.7	22.0
4-Methylsyringol Gentiobioside	<1.0	<1.0	1.1	3.4	4.8

Figure 3: Range of volatile and glycosylated markers observed in 2020 Cabernet Sauvignon wines, from normal baseline to extreme levels resulting from severe exposure to wildfire smoke.

Please note, these are examples of data collected from 2020 wine samples. Levels and ranges observed for the various markers measured may or may not apply to current or future events.

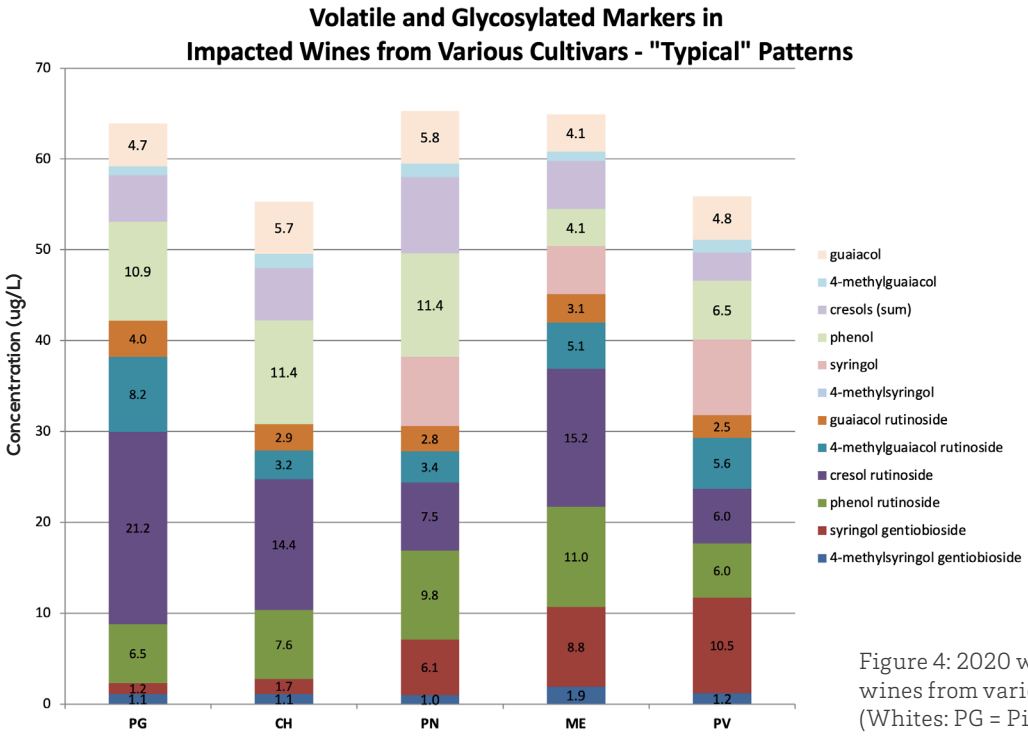
TYPICAL… AND LESS TYPICAL PATTERNS

To put it simply… as a general rule when volatile markers are high, glycosylated markers are also high. This is illustrated figure 3, which shows a range of levels observed in Cabernet Sauvignon wines, from normal low baseline levels observed without exposure to wildfires, to extreme levels resulting from severe exposure. According to our observations so far, this range can be expected with most red wines, while levels in white and rosé wines are generally lower due to reduced skin contact.

A notable exception is of course Syrah, now well known to contain naturally significant baseline levels of volatile guaiacol. With this variety however, baseline levels of other volatile markers appear to be normal, making them useful to assess wildfire smoke impact.

Another oddity is Petit Verdot, which doesn't appear to contain naturally unusual baseline levels of wildfire markers, but seems year after year to be much more sensitive to wildfire smoke. With Petit Verdot wines, the observed range is much larger than with any other variety. We have measured volatile guaiacol exceeding 200 ug/L, and combined levels of glycosylated markers approaching 1,000 ug/L.

Examples of "typical" patterns, meaning with volatile marker levels accompanied by relatively expectable levels of glycosylated markers, can be observed in impacted white, rosé or red wines from various varieties. Figure 4 shows a few examples of wines all containing volatile guaiacol in the 4-6 ug/L range, usually associated with noticeable characters from the sensory standpoint, for which glycosylated markers match this most commonly observed pattern.



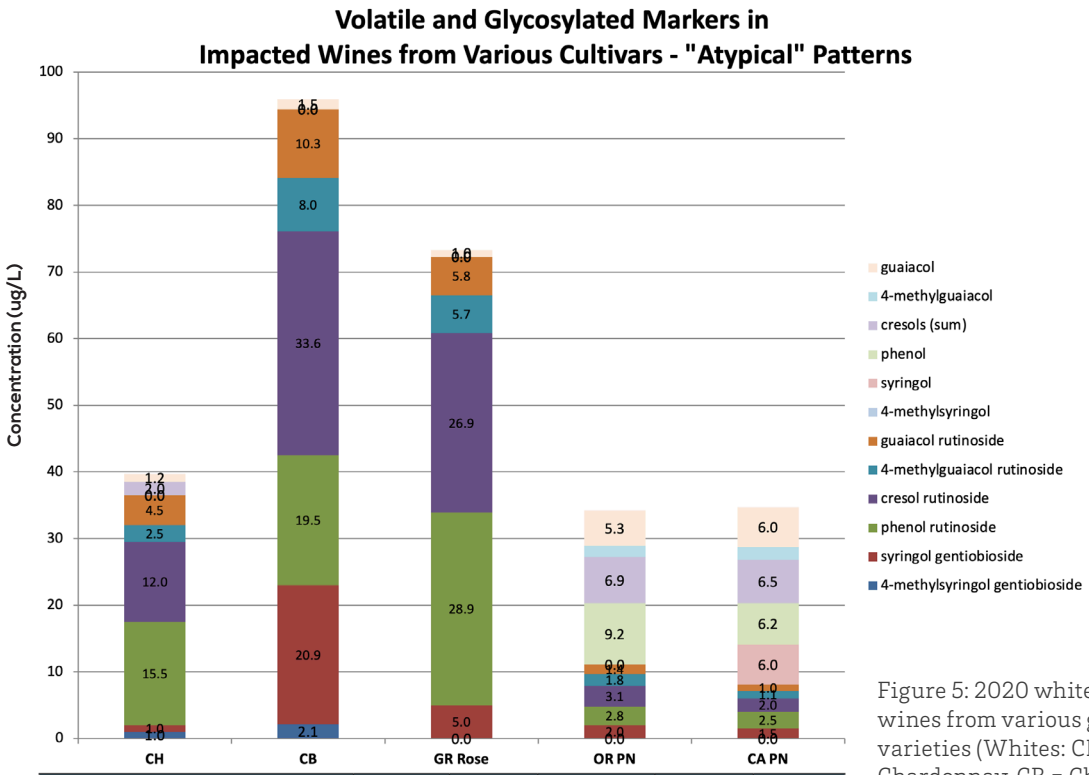
	PG	CH	PN	ME	PV
guaiacol	4.7	5.7	5.8	4.1	4.8
4-Methylguaiacol	1.0	1.6	1.5	1.0	1.4
Cresols (sum)	5.1	5.8	8.4	5.3	3.1
Phenol	10.9	11.4	11.4	4.1	6.5
Syringol	<5.0	<5.0	7.6	5.3	8.3
4-Methylsyringol	<5.0	<5.0	<5.0	<5.0	<5.0
Guaiacol Rutinoside	4.0	2.9	2.8	3.1	2.5
4-Methylguaiacol Rutinoside	8.2	3.2	3.4	5.1	5.6
Cresol Rutinoside	21.2	14.4	7.5	15.2	6.0
Phenol Rutinoside	6.5	7.6	9.8	11.0	6.0
Syringol Gentiobioside	1.2	1.7	6.1	8.8	10.5
4-Methylsyringol Gentiobioside	1.1	1.1	1.0	1.9	1.2

Figure 4: 2020 white and red wines from various grape varieties (Whites: PG = Pinot Grigio, CH = Chardonnay, Reds: PN = Pinot Noir, ME = Merlot, PV = Petit Verdot) displaying a typical pattern between volatile and glycosylated markers. Note that all these wines contain volatile guaiacol in the 4-6 ug/L range, usually associated with noticeable sensory wildfire characters.

Please note, these are examples of data collected from 2020 wine samples. Levels and ranges observed for the various markers measured may or may not apply to current or future events.

Less typical are patterns where volatile markers are relatively low, sometimes barely suggesting wildfire impact, or even no impact at all, co-occurring with relatively high levels of glycosylated markers. These patterns are quite uncommon, but particularly worrisome to winemakers, due to the fact that glycosylated ("bound") compounds, which are odorless, are suspected to degrade with time, releasing volatile ("free") odor-active compounds. Examples of such patterns are presented in figure 5 - three examples on the left.

On the other hand, opposite patterns, showing high volatile markers but relatively low levels of glycosylated markers, are observed with some regularity, especially with 2020 Pinot Noir wines – see figure 5 - two examples on the right. We do not know at this point if this is a recurrent occurrence with Pinot Noir, which might be observed year after year. Low levels of glycosylated markers may be caused by a weakened defense mechanism from the plant against smoke volatiles. This could be related to the severe heat wave we experienced all across the Western United States in mid-August 2020, causing a slowed or even "shut-down" metabolism with Pinot Noir grapes during most of their exposure to wildfire smoke.



	CH	CB	GR Rose	OR PN	CA PN
guaiacol	1.2	1.50	1.0	5.3	6.0
4-Methylguaiacol	<1.0	<1.0	<1.0	1.7	1.9
Cresols (sum)	2.0	<3.0	<3.0	6.9	6.5
Phenol	<2.0	<2.0	<2.0	9.2	6.2
Syringol	<5.0	<5.0	<5.0	<5.0	6.0
4-Methylsyringol	<5.0	<5.0	<5.0	<5.0	<5.0
Guaiacol Rutinoside	4.5	10.3	5.8	1.4	1.0
4-Methylguaiacol Rutinoside	2.5	8.0	5.7	1.8	1.1
Cresol Rutinoside	12.0	33.6	26.9	3.1	2.0
Phenol Rutinoside	15.5	19.5	28.9	2.8	2.5
Syringol Gentiobioside	1.0	20.9	5.0	2.0	1.5
4-Methylsyringol Gentiobioside	1.0	2.1	<1.0	<1.0	<1.0

Figure 5: 2020 white and red wines from various grape varieties (Whites: CH = Chardonnay, CB = Chenin Blanc, Rosé: GR = Grenache, Reds: PN = Pinot Noir) displaying atypical patterns between volatile and glycosylated markers. The three wines on the left have very low levels of volatile markers with high glycosylated markers, while the two wines on the right show opposite patterns.

Please note, these are examples of data collected from 2020 wine samples. Levels and ranges observed for the various markers measured may or may not apply to current or future events.

To conclude, additional volatile markers and glycosylated markers clearly allow more complete assessments of wildfire impact in production wines. They have the potential to provide useful and actionable information when measured in grapes and micro-ferments before harvest.

FREQUENTLY ASKED QUESTIONS

Q: I WANT TO TEST GRAPES FOR WILDFIRE IMPACT, WHAT KIND OF SAMPLE SHOULD I SUBMIT?

A: The preferred sample for red or white grapes is a representative 200-300 loose berry sample, with berries as intact as possible. Transport in small rigid "sandwich boxes" works well – this is necessary if you are shipping samples. Avoid submitting cluster samples: the additional sample preparation time in the lab will delay results. We do not recommend submitting juice samples.

Q: SHOULD I CRUSH BERRIES AND LET THEM SOAK IN THEIR JUICE BEFORE BRINGING THEM?

A: We do not recommend doing so. When we have control of the sample preparation, our interpretation guidelines are more applicable.

Q: WITH WHITE GRAPES, SHOULDN'T I BRING JUICE SAMPLES?

A: Since wildfire impact compounds are mostly located in skins, we still prefer whole berry samples for white grapes. Our risk interpretation guidelines are similar for white and red grapes. Of course with white grapes, the risk of off characters materializing in wine can be mitigated to some degree by minimizing skin contact (with red grapes, making rosé by the direct pressing method can be a successful approach too), but in case of significant maceration with skins (e.g. following machine harvesting or intentional skin contact) the risk level may be equivalent.

Q: CAN I MIX BERRIES FROM DIFFERENT VARIETIES AND BRING A COMPOSITE SAMPLE?

A: This is not advisable. Over the years we've seen drastic differences in pick-up of wildfire impact compounds between grape varieties. For example, Petit Verdot is often much more impacted than other Bordeaux cultivars. In 2020 we saw very substantial differences in behavior between Chardonnay and Pinot Noir.

Q: CAN YOU TEST SYRAH GRAPES?

A: Syrah naturally contains variable amounts of guaiacol, the main marker. This makes it impossible to assess wildfire impact based upon guaiacol only, whether with grapes or micro-ferments. The only but very imperfect strategy available so far has been to use other varieties grown next to Syrah blocks as "proxies". The availability of the extended volatile markers panel and the glycosylated markers panel should be a great help assessing directly Syrah samples.

Q: CAN YOU TEST FOR MORE THAN JUST "FREE" GUAIACOLS (GUAIACOL AND 4-METHYLGUAIACOL)?

A: Yes. We have offered our enhanced and extended volatile (free) markers panel for wines in November 2020, and in December 2020 we began offering an updated version of our glycosylated (bound) glycosylated markers panel. For the 2021 harvest we will offer both tests for grapes, micro-ferments, and production wines.

Q: DOES ETS MEASURE "TOTAL" MARKERS, OR ALL FORMS OF "BOUND" MARKERS?

A: Some laboratories offer testing after various acid and heat treatments, in an attempt to measure "Total" or "Bound" forms in their entirety. We have serious reservations about such tests and do not offer them. This concern is shared by our international colleagues such as Australian Wine Research Institute (AWRI) and they do not offer them either. Instead, for each of the volatile markers listed in our Expanded Panel, we can measure directly by HLPC/MS/MS its main glycosylated (sugar-bound) form (see figure 2). This is the strategy also adopted by the AWRI.

Q: WHAT IS THIS UPDATED ANALYSIS FOR GLYCOSYLATED ("BOUND") MARKERS AT ETS?

A: In 2019-2020 ETS enhanced its own method for the determination of glycosylated (sugar-bound) smoke markers, but quantitative results had to be expressed as "equivalent" ug/L units - a strategy commonly used when exact reference compounds are not available (for example just as tannins are commonly expressed as catechin or epicatechin equivalents). Following the 2020 firestorms, ETS joined resources with the AWRI and others from around the world to have pure reference compounds synthesized, so that results can now be reported as "true" concentrations (see Fig. 6). Accurate, quantitative and reproducible results (comparable over a long period of time and between different laboratories) are now achievable and a reality at ETS.

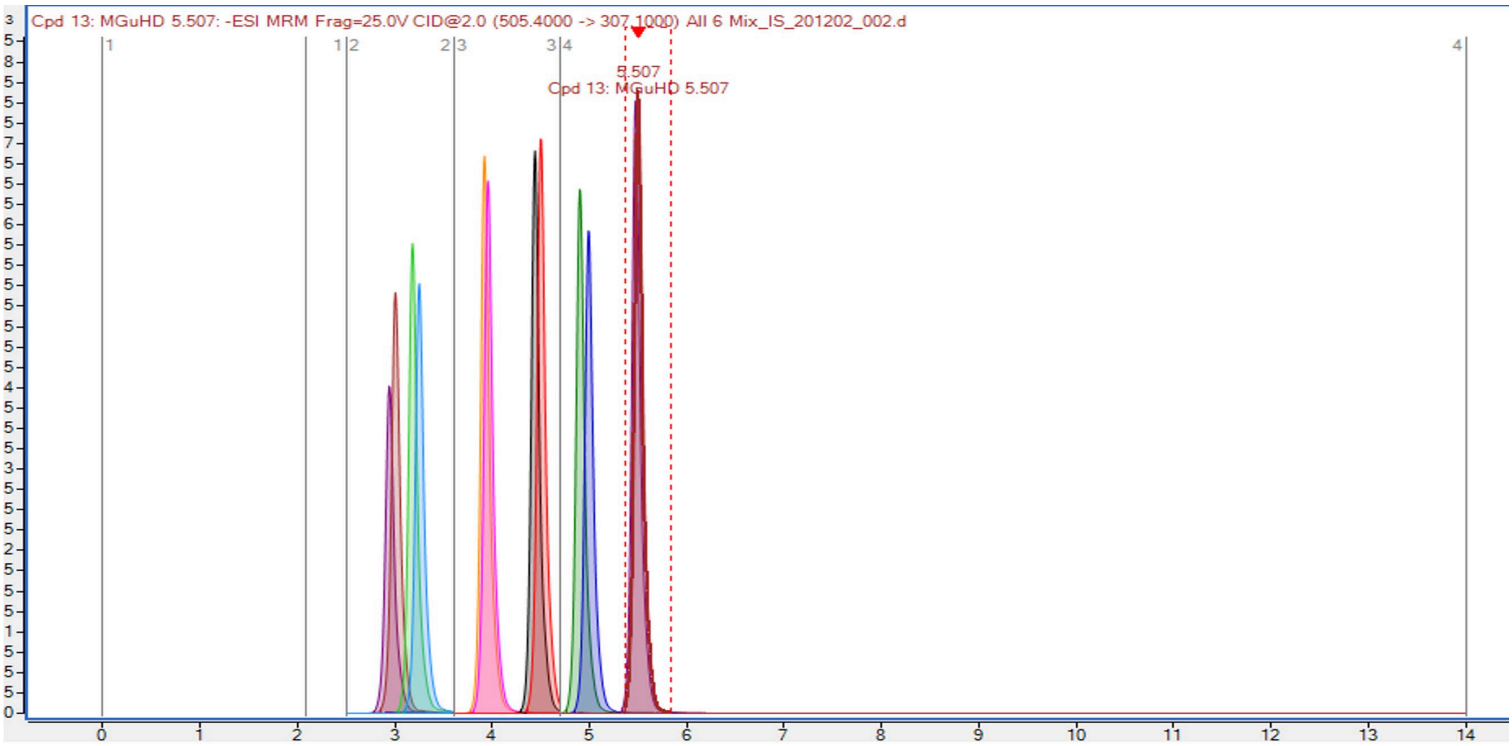


Figure 6: Glycosylated Wildfire Markers and their isotopic analogues detected by LC/MS/MS (ETS Laboratories December 2020). These standards make quantitative, accurate and reproducible results (comparable over a long period of time and between different laboratories) possible.

Q: I'D LIKE TO HAVE MICROFERMENTS TESTED. WILL ETS PREPARE THEM FOR ME?

A: Small-scale fermentations or "micro ferments" can be a good test for smoke exposure, from which wildfire characters can be detected by sensory evaluation and by confirmed by quantitative laboratory analysis. Unfortunately, due to practical constraints, we cannot offer to perform micro-ferments. For guidance, we recommend watching the tutorial "Step-by-Step: How to do small-scale fermentations for the evaluation of grape smoke exposure risk" available on the UC Davis website. Another good resource is the small-lot fermentation method protocol as specified by the Australian Wine Research Institute ("AWRI"). At completion of fermentation, transfer the fermented wine into a bottle, let settle in fridge for a few hours, decant and submit sample in a 60 mL plastic tube. If a crop insurance claim is considered, check with your insurance provider to determine if they accept results from micro-ferments.

Q: WHEN IS THE BEST TIME TO BRING SAMPLES?

A: For grape samples the typical recommendation is about 7-10 days prior to harvest. Keep in mind that the impact of wildfires is cumulative and that "negative" results early in the season may give a false sense of security, especially if more exposure happens. Of course with micro-ferments several days need to be accounted for after grape sampling, in order to produce fermented samples that can be analyzed.

Q: WILL YOU HELP ME WITH RESULTS INTERPRETATION?

A: Of course. Although each fire event is unique, our interpretation guidelines based on volatile (free) guaiacol derived from our experience since 2008 have stood the test of time, and have proven to be quite robust (Fig. 7). The presence of additional markers, with the extended volatiles marker panel, and the availability of a glycosylated markers panel, allow refining and confirming diagnostics. Always feel free give us a call for assistance with result interpretation.

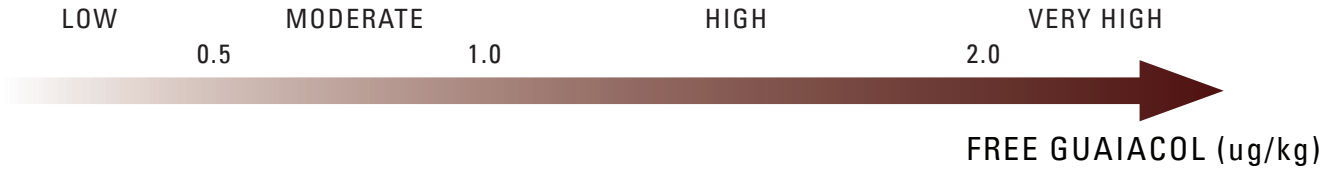
A step-by-step procedure for small-scale fermentations (micro-ferments) is available on the UC Davis Website:

https://wineserver.ucdavis.edu/multimedia/step-step-how-do-small-scale-fermentations-evaluation-grape-smoke-exposure-risk

The AWRI protocol is found at:

https://www.awri.com.au/wp-content/uploads/small_lot_fermentation_method.pdf

INTERPRETATION GUIDELINES FOR WHOLE BERRY TESTS (EXCLUDING SYRAH):
ANTICIPATED RISK OF WILDFIRE IMPACT IN WINE



INTERPRETATION GUIDELINES FOR MICROFERMENTS AND UNOAKED WINES (AGAIN EXCLUDING SYRAH):
PREDICTABLE SENSORY IMPACT

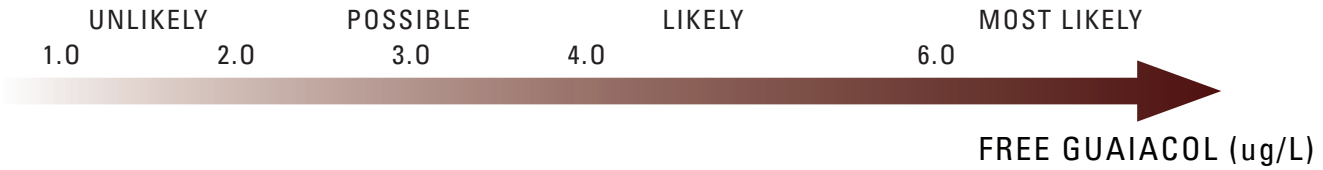


Figure 7: Interpretation guidelines for volatile (free) guaiacol, the main marker of wildfire smoke impact, in grapes and micro-ferments and unoaked wines. Additional markers in the extended volatile panel as well as glycosylated markers allow refining and confirming assessments.

IMPORTANT: THESE GUIDELINES ARE FOR GUAIACOL, NOT SUM OF COMPOUNDS. GUIDELINES ARE SIMPLY OBSERVATIONS BASED UPON PAST EVENTS, AND MAY OR MAY NOT APPLY TO CURRENT OR FUTURE EVENTS. ETS DOES NOT, AND WILL NOT, PROPOSE ACCEPTANCE OR REJECTION CRITERIA.

Q: WHAT ARE NORMAL "BASELINE" LEVELS FOR GLYCOSYLATED MARKERS?

A: Grapes and wines may naturally contain low "baseline" levels of glycosylated markers, variable by grape variety and geographic origin. ETS has been engaged for years in building a database for grapes not exposed to wildfire smoke, as well as wines also from grapes not exposed to wildfire smoke. The good news is that despite the fact that this project suffered a setback last year, with fires raging in the western states for a good part of the growing season, we are confident that our current database is sufficient to help our clients with result interpretation.

Q: MY GRAPES (OR MICRO-FERMENTS) TESTED "POSITIVE". SHOULD I HARVEST?

A: Our interpretation guidelines are related to incremental risk scales, as there is no "magic number" below which no risk is present and above which wines are guaranteed to be impacted. Choosing to harvest or not will always be complex risk management decisions, loaded with painful consequences for both growers and wineries. Analytical results only help in making these difficult decisions.

Q: DOES ETS RECOMMEND KEEPING PRE-HARVEST BACKUP SAMPLES?

A: Grape samples and micro-ferments serve two distinct purposes: helping with harvest decisions and serve as proof of wildfire impact for insurance claims. Especially in the second case, it makes sense to keep backup samples, especially in the context of catastrophic wildfires.

Q: WHAT IS YOUR TURNAROUND TIME?

A: First, we want to thank the University, State, and other labs that stepped-in during the 2020 fire season. For the 2021 season, we have expanded yet again our GC/MS, GC/MS/MS and LC/MS/MS analytical capabilities. We also have the options of transferring excess samples for rapid analysis by certain trusted partner laboratories under ISO 17025 guidelines. For the most accurate turnaround time we recommend taking a look at our website (Analyses - Smoke Markers). In the case of extraordinary fire events, we will keep our turnaround time information for grape, micro-ferment and wine tests prominently displayed and updated regularly.