

# Development and Evaluation of a Vineyard-Based Strategy To Mitigate Smoke-Taint in Wine Grapes

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## Supporting Information

**ABSTRACT:** Smoke-taint is a wine defect that may occur when ripening grape crops absorb volatile phenols (VPs), compounds associated with the negative sensory attributes of smoke-taint, due to exposure of grapes to wildfire smoke. This study examined potential methods to reduce the impact that smoke-exposure has on wine grapes. Specifically, agricultural sprays normally used to protect grapes from fungal pathogens and a spray used to prevent cracking in soft-fleshed fruits were assessed for their capacity to inhibit increases in VP concentrations in wine grapes following on-vine smoke-exposure. The results indicated that an artificial grape cuticle applied 1 week before exposure to simulated forest fire smoke (at 1–2 weeks after veraison) can significantly hinder an increase in VP concentrations in smoke-exposed grapes at commercial maturity. This reduction in VP concentrations may mitigate crop losses experienced globally by the wine industry due to exposure of grapes on-vine (at key phenological stages) to wildfire smoke.

**KEYWORDS:** smoke-taint, smoke, grapes, fire, wine

## INTRODUCTION

Wines are characterized and distinguished by their appearance, mouthfeel, odors, and flavors. These sensory attributes depend on a wide array of factors,<sup>1</sup> including a complex mixture of grape and fermentation-derived and aging-related chemical compounds.<sup>2</sup> Alterations to the makeup of this chemical mixture can lead to wines with sensory defects (e.g., geranium-taint,<sup>3</sup> cork-taint,<sup>4,5</sup> etc.). One of the more prominent examples of such a sensory defect in recent years has been smoke-taint, which can occur when grapes are exposed to wildfire smoke during key stages of phenological development.<sup>6</sup> Wine made from these grapes can possess smoky or ashy sensory attributes,<sup>7</sup> resulting in significant revenue losses due to consumers finding such attributes objectionable. Such financial losses are expected to increase over time, as climate change models predict an increase in the length and intensity of forest fire seasons.<sup>8</sup> It is therefore critical to the sustainability of the global wine industry that methods to mitigate the impact of forest fire smoke be developed.

The development of smoke-taint is strongly correlated with an increase in the concentration of volatile phenols (VPs; Figure 1) that may be present in wine.<sup>1,9</sup> VPs in wines can originate from a variety of sources, including the aging of wine in oak barrels,<sup>5</sup> the presence of the spoilage organism *Brettanomyces bruxellensis*,<sup>10,11</sup> or biosynthesis within grapes.<sup>12</sup> In addition, smoke-derived VPs, which are the product of the incomplete combustion of lignin present in plants,<sup>13</sup> may also be present in wines made from grapes exposed to forest fire smoke. VPs are observed in their free forms in grapes and wine, but more dominantly, they are chemically converted to a variety of glycosidic derivatives.<sup>7,14,15</sup> It is critical to evaluate both free and bound forms of VPs when studying smoke-taint, with an increase in bound-VP concentrations correlating to an increase in smoke-taint-related flavors.<sup>16</sup>

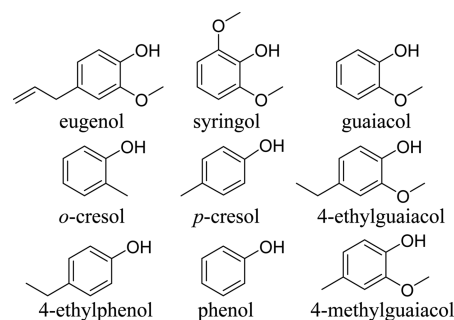


Figure 1. Volatile phenols (VPs) examined in this study.

To date, much of the research on smoke-taint has focused on its detection and prediction in grapes and wines.<sup>14,17–19</sup> However, the mitigation of smoke-taint has also been an area of concern, and a variety of methods, both preventive and ameliorative, have been examined. The method of winemaking employed has an impact on perceptible smoke-taint, with techniques such as cold maceration having been shown to reduce the extraction of VP glycosides.<sup>20</sup> Smoke-taint has previously been mitigated from afflicted wines through reverse osmosis.<sup>21</sup> Unfortunately, perceptible smoke-taint has been found to return over time, which appears to be a consequence of changes in the sensory profile of wine as it ages.<sup>2</sup> In addition, reverse osmosis can remove desirable compounds alongside the VPs. Fining agents have been used to reduce VP concentrations (with activated carbon showing the greatest

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efficacy),<sup>22</sup> while the addition of oak chips and tannins has been seen to mask smoke-taint in wine.<sup>20</sup> To date, no ameliorative methods have been proven to be universally effective at reducing the perception of smoke-taint in impacted wines.

Regarding preventative measures (i.e., those that seek to limit the uptake of smoke-borne VPs or limit their transfer into primary fermentation), harvesting grapes by hand has been shown to reduce the concentration of guaiacol in grapes when compared to mechanical harvesting, as fewer grape skins (where VPs are preferentially sequestered<sup>9</sup>) are ruptured.<sup>23</sup> More recently, van der Hulst et al. examined the effect of kaolin clay on VP uptake during smoke exposure. Their results demonstrated a statistically significant decrease in VP concentrations for kaolin-treated Merlot grapes.<sup>24</sup>

The application of kaolin represents one form of crop protection that has otherwise gone unexplored within the smoke-taint literature: agricultural sprays that are already applied to fruit crops for other purposes. While the uptake of VPs by epicuticular waxes appears to be the primary form of VP absorption by grape berries, it was also shown that the removal of these waxes corresponded to an increase in VP concentration in grape berries.<sup>25</sup> These contradictory findings suggest that such waxes have dual functions regarding smoke-taint—both as an insulator against exogenous VP ingress and as a facilitator of such ingress mechanisms. It follows then that agricultural sprays with similar physical properties to the grape cuticle could mimic these effects. This study set out to determine the effect that three commercially available agricultural sprays (Table S1) had on the concentration of VPs in smoke-exposed grapes, with the intent of identifying a prophylactic smoke-taint treatment that grape growers could apply ahead of a possible smoke-exposure event. It was hypothesized that if one of these three sprays could be shown to enhance the natural protective properties of the cuticle, this could provide a promising base for a defensive measure against smoke-taint. Additionally, if any of the sprays served to worsen the effects of smoke-taint, that information would also be very valuable to the wine industry.

## MATERIALS AND METHODS

**Chemical and General Details.** Hexane, ethyl acetate, chloroform, HPLC-grade methanol (MeOH), isopropyl alcohol (IPA), hydrochloric acid (HCl), guaiacol, *d*<sub>3</sub>-guaiacol, syringol, eugenol, phenol, *o*-cresol, *p*-cresol, 4-ethylguaiacol, *d*<sub>3</sub>-4-ethylguaiacol, 4-methylguaiacol, 4-ethylphenol, and *d*<sub>4</sub>-4-ethylphenol were all purchased from Sigma Aldrich (St. Louis, USA) and used as received. The *d*<sub>7</sub>-*o*-cresol and *d*<sub>7</sub>-*p*-cresol internal standards (ISTDs) were purchased from Toronto Research Chemicals (Toronto, Canada). All chemicals were used as received. Synthetic details for the *d*<sub>3</sub>-syringol ISTD are reported elsewhere.<sup>17</sup> A synthetic, wax-based biofilm (Cultiva, LLC, Las Vegas, USA) and two oil-based fungicides (Intelligro, Mississauga, Canada; Syngenta, Basel, Switzerland) were purchased from the manufacturers.

All samples and extracts were stored at  $-20\text{ }^{\circ}\text{C}$ . Depending on sample volume, centrifugation was performed using either an Allegra X-12R Centrifuge (Beckman-Coulter, Mississauga, Canada) or a Spectrafuge 24D microcentrifuge (Mandel, Guelph, Canada). An AdventurePro AV264 analytical balance (Ohaus Corporation, Pine Brook, NJ, USA) was used to prepare standards and weigh samples. A Barnstead E-Pure water purification system (Thermo Fisher Scientific, Waltham, USA) was used for all water unless noted.

VP and ISTD stock solutions were prepared in IPA from 1 to 20 mg/L and stored at  $-20\text{ }^{\circ}\text{C}$  for up to 12 months. Calibration standards were prepared fresh daily as per Noestheden et al.,<sup>7</sup> with the

following exceptions: the calibration range for all analytes was 1–200 ng/g; calibration samples were prepared using a 1:1 (v/v) hexane:ethyl acetate extract of Merlot whole berry homogenate (prepared on a 500 mL scale<sup>18</sup>) containing ISTD (50 ng/g) as the diluent.

**Study Design.** At each of the three vineyards, samples were collected from a total of 28 Pinot noir vines (see Table 1 for varietal

**Table 1. Viticultural Details of the Three Blocks of Pinot noir Used for Field Trials<sup>a</sup>**

parameter	V1 <sup>b</sup>	V2	V3
latitude/ longitude	49.7694° N/ 119.5306° W	49.7604° N/ 119.5418° W	49.8428° N/ 119.5661° W
spray	oil 1	oil 2	Biofilm
clone		67	828
rootstock		101/14	SO4
year planted		2000	2006
row orientation	NW-SE	N-S	N-S
yield (tons)/ acre	2.8	2.6	1.5
clusters/vine	20–30	10–15	15
°Bx <sup>c</sup>	23.4	23.6	25.4
TA <sup>d</sup> (g/L) <sup>c</sup>	8.1	6.7	8.5
pH <sup>c</sup>	3.48	3.35	3.47

<sup>a</sup>All vines were trained to vertical shoot positions with 1.3 m spacing between vines. <sup>b</sup>Age, clone, and root stock details were not available for V1. <sup>c</sup>°Bx, TA, and pH for all treatment groups at a given vineyard were equivalent ( $p > 0.05$  for a one-sided Student's *t* test with unequal variance), so aggregate values were reported. <sup>d</sup>Total acidity.

and viticultural details). These vines were divided into two treatment groups of 14 unsmoked vines and 14 smoke-exposed vines, with each set further divided into seven vines sprayed with tap water and seven vines sprayed with one of the three evaluated commercial sprays (Figure S1). The first of the sprays investigated, Biofilm, is an artificial phospholipid cuticle designed to prevent fruit-cracking in soft-fleshed fruits (e.g., cherries and blueberries). The other two studied sprays were oil 1, a broad-spectrum organic fungicide derived from a petrochemical distillate, and oil 2, another broad-spectrum fungicide derived from tea tree oil. Both of the fungicide sprays are currently used on wine grapes during the production of wine, but Biofilm is not. Unsmoked vines were separated from the smoked vines by a full row or two panels, if the vines were on the same or a directly adjacent row. Vines were sprayed 7 days before the first smoke application using handheld, pressurized applicators. This 1 week difference between spraying and smoking was chosen as a reflection of the need for wine-producers to be able to preventatively spray crops for smoke exposure. The sprays were diluted to a concentration of 1% (v/v) with tap water, as recommended by a local grower's supply company. Each vine was sprayed to the point of complete fruit and foliar coverage.

One bunch of grapes was collected from each vine at five time points, starting approximately 7 days after the onset of veraison (as determined by the viticultural staff at each partner vineyard) and continuing to commercial maturity (Table 2). Collected grape samples were stored in separate polyethylene bags on ice during transport and were processed (vide infra) and stored at  $-20\text{ }^{\circ}\text{C}$  on the same day as sample collection.

**Application of Artificial Forest Fire Smoke.** The smoked vines were surrounded by a modular enclosure constructed of polyvinyl-chloride tubing and steel with a polyethylene covering, as detailed by Noestheden et al.<sup>18</sup> The smoke-producing fire was fueled by a mixture of material collected from *Pinus ponderosa* forests (located near 49.7832 °N, 119.5163 °W and 49.9438 °N, 119.4026 °W). Fuel mixtures were composed of 20% pine needles (w/w), 30% bark (w/w; 3 cm pieces), and 50% soil organic matter (w/w). Vines were smoked

**Table 2.** Time Points Collected at Each Vineyard and a Description of Each Time Point

time-point description	days relative to veraison <sup>a</sup>				
	t1	t2	t3	t4	t5
vineyard 1	+7	+14	+14	+16	+32
vineyard 2					+30
vineyard 3					+21

<sup>a</sup>Veraison and commercial maturity decisions were made by the staff at each partner vineyard.

twice for a period of 1 h, with 48 h between smoke applications. A total of 1.5 kg of fuel was consumed for each smoke application.

**Sample Preparation.** All berries were destemmed immediately after collection. Twenty berries were separated from each treatment group and time point for cuticle extraction (vide infra); these berries were stored in polyethylene bags at  $-20\text{ }^{\circ}\text{C}$  until extraction. The remainder of the berries was homogenized (Magic Bullet, Homeland Housewares LLC, Los Angeles, CA, USA). The resulting whole berry homogenate (HMG) was transferred to 50 mL polyethylene centrifuge tubes and centrifuged at  $3000 \times g$  for 30 min at  $4\text{ }^{\circ}\text{C}$ , and the supernatant was stored at  $-20\text{ }^{\circ}\text{C}$  until analysis. After homogenization, extracts of both free and acid-labile VP conjugates were assessed using the method described by Noestheden et al.<sup>18</sup>

**Cuticle Extraction from Biofilm Treatment Groups.** To remove the cuticle, berries were submerged individually in 5 mL of chloroform (fortified with 50 ng/g VP/CHCl<sub>3</sub> ISTD) for 20 s, after which the berry was removed from the chloroform and another from the sample set was added such that the same 5 mL of chloroform was used to extract the cuticle from 20 berries for each biological replicate at each time point in each vineyard. The extracts were stored in 15 mL conical tubes at  $-20\text{ }^{\circ}\text{C}$  until analysis. Chloroform extractions were analyzed by GC–MS/MS without further workup.

After the berries had their cuticles removed, they were homogenized and prepared for free VP analysis. Due to the smaller amount of HMG sample, volumes of added components were 10-fold less than previously used, so 500  $\mu\text{L}$  of berry HMG was used instead of 5 mL, 200  $\mu\text{L}$  of 1:1 hexane:ethyl acetate was used instead of 2 mL, and 0.004 g of isotopically labeled VP ISTD was used instead of 0.04 g. Samples were centrifuged at  $16300 \times g$  for 2 min, before 100  $\mu\text{L}$  of sample was transferred to glass vial inserts contained within borosilicate glass autosampler vials, and analyzed without further workup. All data were weight-corrected (Table S2).

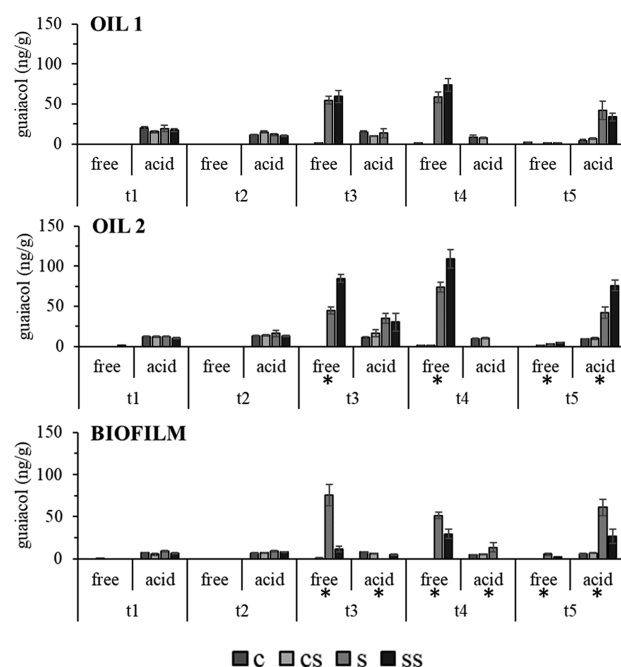
**GC–MS/MS.** The GC–MS/MS instrument used for analysis was a TSQ 9000AEI Triple Quadrupole GC–MS/MS equipped with a Trace 1310 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA). The GC–MS/MS analytical conditions were described by Noestheden et al.<sup>26</sup> except that 1  $\mu\text{L}$  was injected, a 5:1 split ratio was used, and the column was a ZB SemiVolatiles ( $30 \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ; Phenomenex, CA, USA).

**Data Processing.** GC–MS/MS data was acquired and processed with the Chromeleon (version 7.1) software package (Thermo Fisher Scientific). Data reduction and statistical calculations were performed using Microsoft Excel (Microsoft Corporation; Redmond, WA, USA) and R ( $\times 64$ , version 3.5.2; R Foundation for Statistical Computing). All statistical comparisons were done using a Mann–Whitney U test ( $\alpha = 0.05$ ), unless otherwise noted.

## RESULTS AND DISCUSSION

**Effect of Simulated Wildfire Smoke.** Artificially increasing the VP concentrations was essential for the determination of the effect of the three sprays being examined. Due to environmental conditions at the site of field trials (i.e., numerous forest fires occurred during the 2018 grape-growing season), all vines were exposed to smoke from regional wildfires in the critical period around veraison.<sup>6,27</sup> As such, all

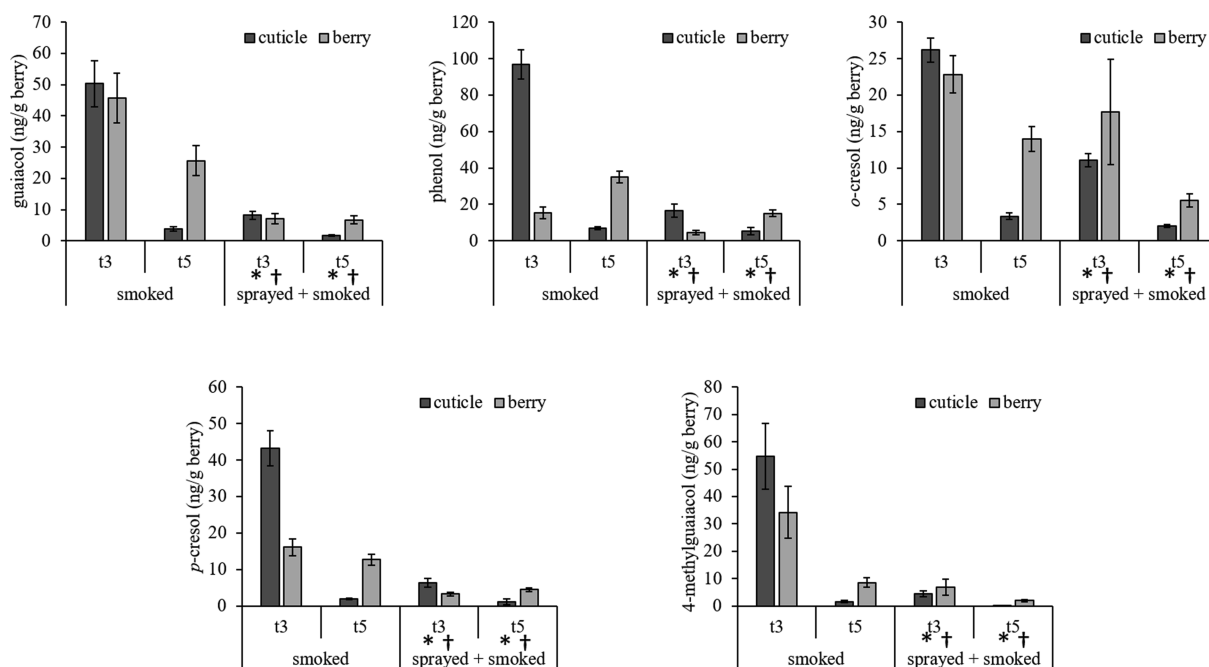
treatment groups were expected to have elevated concentrations of VPs. Therefore, to ensure a difference between treatment conditions, artificial wildfire smoke was applied to half the vines at each vineyard (the 14 smoked vines; see Figure S1). At all three vineyards, the smoke-exposed treatment groups displayed generally higher concentrations of free and total VPs (Mann–Whitney U Test,  $\alpha = 0.05$ ) than their respective unsmoked controls (Figure 2, Tables S3–S8), which was consistent with previous studies.<sup>17,18,27</sup>



**Figure 2.** Free and acid-labile guaiacol concentrations (ng/g) observed across all time points and treatment conditions in whole berry homogenates. Where no values are given, VPs were either not detected or were below their LOQs. Data are reported as mean  $\pm$  1 SEM and were tabulated from up to seven biological replicates (see Tables S8–S10). Asterisk indicates a statistical difference between respective smoked vines under control and sprayed treatment groups (Mann–Whitney U Test,  $\alpha = 0.05$ ). C – Control; CS – control + spray; S – smoked; SS – smoked + spray.

**Oil 1.** As an oil-based, fungicidal petrochemical distillate, oil 1 has a mechanism of action that relies on its ability to coat grape vines. Viticultural application of such products are often necessary during forest fire seasons, as anecdotal evidence suggests that, during prolonged smoke-exposure, there is a higher incidence of powdery mildew issues. Using guaiacol as an example (Figure 2), the data for smoke-exposed vines that went unsprayed against those that were treated with oil 1 demonstrated no significant change (Mann–Whitney U Test,  $\alpha = 0.05$ ) in guaiacol concentrations (free or total). Similar observations were made for all other VPs evaluated herein after treatment with oil 1 (Tables S3 and S4). While these data did not provide support for a protective effect, they do indicate that using oil 1 during acute smoke-exposure events to combat fungal infections is unlikely to exacerbate the uptake of smoke-taint-associated compounds.

**Oil 2.** Much like oil 1, the fungicidal properties of the tea tree oil derived oil 2 depend in part on the spray's coating of a grape vine—the terpenes in this spray also have fungicidal properties that facilitate efficacy. Smoke-exposed vines that



**Figure 3.** Free VP concentrations (ng/g) in Biofilm-treated grapes after the first smoke exposure (t3) and at commercial maturity (t5) in smoke-exposed berry cuticles and in the same berries after cuticle extraction. Data are reported as mean  $\pm$  1 SEM and were tabulated from up to seven biological replicates (see Table S11). Asterisk indicates a statistical difference between respective smoked vines under control and sprayed treatment groups in cuticles. Dagger indicates a statistical difference between respective smoked vines under control and sprayed treatment groups in berries after removal of cuticle (Mann–Whitney U Test,  $\alpha = 0.05$ ).

were treated with oil 2 had a significantly higher concentration (Mann–Whitney U Test,  $\alpha = 0.05$ ) of free guaiacol than their unsprayed matched controls at all three post-smoking time points (Figure 2, Table S5). For the acid-labile guaiacol fraction, the trend toward increased guaiacol concentrations was only statistically significant at commercial maturity (Figure 2, Table S6). This is likely a result of guaiacol being glycosylated over time, as the glycoside concentrations can require weeks to stabilize.<sup>28</sup> Similar to the guaiacol data, there was an increased concentration of *p*-cresol, phenol, and 4-methylguaiacol in the oil 2 sprayed analyses at the three time points after smoke-exposure for both free and total VPs (Tables S5 and S6). Collectively, these compounds represent those VPs most frequently correlated with smoke-taint.<sup>9,29</sup> The observed increase in concentration of these VPs suggests that the application of oil 2 may increase the uptake of VPs by wine grapes, which could result in an exacerbation of smoke-taint in wines made from treated grapes. Given this evidence, it would be prudent to avoid the use of such fungicides in grape growing regions that are prone to forest fire smoke exposure.

**Biofilm.** For vines treated with Biofilm that were subsequently exposed to smoke, free and total guaiacol concentrations showed a consistent difference when compared with vines that only received smoke treatment (Figure 2), with effect sizes of  $-245$  and  $-197\%$ , respectively, at commercial maturity. Similarly, the concentrations of the other free and total VPs were observed to be significantly lower (Mann–Whitney U-test,  $\alpha = 0.05$ ) in the samples sprayed with Biofilm than the unsprayed samples taken from the three time points after smoke-exposure (Tables S7 and S8). Effect sizes in these instances ranged from decreases of  $125$ – $317\%$ . The cresols showed similar results, with the concentrations of both total and free *o*-cresol and *p*-cresol decreasing after Biofilm application (Tables S7 and S8). As mentioned above, these

three VPs are among the most correlated with smoke taint.<sup>9,29</sup> It follows then that these data strongly suggest that Biofilm may insulate wine grapes from the impacts of forest fire smoke, with demonstrated effect sizes that are likely to impact the perception of smoke-taint in the resulting wines.

**VPs after Cuticle Extraction.** To investigate the mechanism behind the apparent protective effect of Biofilm regarding smoke-taint marker compounds, the grape cuticles were removed by chloroform extraction<sup>30</sup> and the concentration of VPs were determined in the cuticular extracts and in the berry material remaining following cuticle extraction. Smoke-exposed berries treated with Biofilm demonstrated a significant decrease (Mann–Whitney U-test,  $\alpha = 0.05$ ) in guaiacol concentration observed in both berry and cuticle when compared to their unsprayed, matched controls (Figure 3) after the first smoking and at commercial maturity. This decrease was also noted in phenol, *o*-cresol, *p*-cresol, and 4-methylguaiacol, all of which are highly correlated with smoke-taint and have been observed in smoke produced from the *Pinus ponderosa* plant material<sup>17</sup> (as used herein to generate simulated forest fire smoke). As this marked difference is observed directly following smoke application, this serves as an indication that Biofilm acts as a barrier preventing VPs from infiltrating into either the cuticular waxes or the berry interior.

When taken in conjunction with the decreased VP uptake in Biofilm-treated grapes, these data raise questions on how Biofilm functions to provide this inhibitive effect and how oil 2, conversely, resulted in an increase in VP concentrations (Figure 2). All three sprays consist primarily of hydrophobic waxes.<sup>31–34</sup> Biofilm is distinguished from the other two sprays because it consists of phospholipids rather than being lipid-based. The increase in VP concentrations caused by oil 2 is hypothesized to be the result of the additional lipid coating on the grapes presenting an increased effective volume for smoke-

borne VPs to partition into. While this may not inherently change the partition coefficient of the VPs for the grape cuticle, it would lead to an overall increase in the VP burden in the cuticles that could then diffuse into the grapes (presumably, oil 1 is not as retentive as oil 2 in this regard and consequently does not increase the VP burden in the same way). Meanwhile, Biofilm more likely serves to shield the grape from infiltration by VPs (Figure 3), possibly as a result of the phospholipid headgroups limiting the interaction between VPs and the berry cuticle; the lipophilic character of the berry cuticle<sup>35,36</sup> would favor an orientation where the polar phospholipid headgroups would be distal from the cuticle (e.g., exposed to the atmosphere). Indeed, such a mechanism is, at least in principle, consistent with the known dichotomous function of the native grape cuticle as it pertains to smoke-taint.<sup>25</sup>

Similar to Biofilm, van der Hulst et al. have reported<sup>24</sup> that kaolin clay provides some manner of protection from VP uptake. However, of the three cultivars that kaolin was tested on, only one (Merlot) demonstrated this effect, and its efficacy was not evaluated for free VPs. Therefore, much like Biofilm itself, kaolin must be subjected to additional testing before it can be widely applied.

Before Biofilm can confidently and rationally be applied as a protective measure, future studies need to be done to confirm its mechanism of action and also to evaluate the most effective application program that (1) protects the grapes from the impacts of forest fire smoke, (2) provides grape growers adequate lead time to protect their crops (i.e., the spray should be applied preventatively in regions prone to forest fire smoke during key developmental stages; how the spray's protective effect varies when applied at differing developmental stages must also be considered), (3) investigates the application rate to determine the most efficacious and cost-effective treatment program (the economics of Biofilm application will be dependent on factors such as duration of protection, dose-response, and the impact of environmental conditions such as rain), and (4) evaluates wines made from grapes treated with Biofilm to ensure no fermentation or sensory impacts are noted. Fieldwork aimed at answering these questions is currently being pursued.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.9b05859>.

Field trial design and metrics, spray details, and volatile phenol concentrations (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS AND NOMENCLATURE

HMG, whole berry homogenate; IPA, isopropyl alcohol; ISTD, internal standard;  $m/z$ , mass-to-charge ratio (in mass spectrometry); MeOH, methanol; SEM, standard error of the mean; SOM, soil organic matter; VPs, volatile phenols

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