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Research Article

Persistence of Elemental Sulfur Spray Residue on Grapes during Ripening and Vinification

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Abstract: Elemental sulfur (S^0) is commonly used to control powdery mildew in vineyards, but S^0 residues in musts have been correlated with increased H_2S and sulfurous off-aroma formation during fermentation. As a consequence, S^0 is often used sparingly late in the season, but defining appropriate pre-harvest intervals for S^0 sprays has been challenging due to limited data on S^0 persistence in vineyards and during pre-fermentation operations. Utilizing a new quantification method, S^0 residues were monitored in the vineyard over 3 years of field studies. Treatments varied in commercial formulation, application rate, and timing of the last application before harvest, all of which affected S^0 concentrations on the fruit at harvest. Residue levels generally were lower for a wettable powder versus a micronized formulation applied at the same rate and timing, and increased proportionally to the application rate when timing and formulation were constant. In all years, ceasing application ≥35 days prior to harvest resulted in S^0 residues below the 10 μg/g concentration associated with increased H_2S production in several previous studies. S^0 residues >1 μg/g correlated with increased H_2S production in our current work and were observed on all fruit sprayed within 56 days of harvest. However, clarification decreased S^0 in must by >95% prior to fermentation in all treatments. Furthermore, fermentation on treated skins increased H_2S

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formation nearly 3-fold relative to fermentations without skin contact. Collectively, these results indicate that S^0 residues are likely of low concern in white winemaking whereas residue levels in red fermentations can exceed levels associated with increased H_2S production when some S^0 sprays are applied within 8 weeks of harvest.

Key words: pesticide, fungicide, reduced, aroma, quantification, powdery mildew

Introduction

Various commercial formulations of elemental sulfur (S^0) are used for control of the most common disease of grapes worldwide, powdery mildew (PM), caused by the fungus *Erysiphe necator* (syn. *Uncinula necator*) (Gadoury et al. 2011). The advantages of S^0 as compared to alternatives include its low cost, good efficacy, and low risk of resistance development, as well as its acceptability within various "organic" and "biological" production systems, where it is arguably the most efficacious material available for control of PM (Savocchia et al. 2011). However, S^0 residues remaining at harvest can be reduced to hydrogen sulfide (H_2S) during fermentation, and its use in the vineyard has long been tied to reduced sulfur characters in some finished wines made from treated grapes (Rankine 1963, Acree et al. 1972, Schutz and Kunkee 1977). While the aforementioned studies indicate that increased H_2S production occurs when must S^0 concentrations exceed 10 mg/L (or ~10 µg/g of harvested fruit when fermented with skins) there is disagreement as to the impact of S^0 residues at lower concentrations, with some finding levels as low as 1mg/L significantly increasing H_2S production (Thoukis and Stern 1962, Wenzel et al. 1980). The S^0 concentration necessary to cause problems is not well agreed upon, in part because H_2S production is affected by factors other than S^0 concentration. H_2S is produced during fermentation as a byproduct of amino acid synthesis during normal yeast

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(*Saccharomyces cerevisiae*) metabolism (Jiranek et al. 1995), and this pathway can lead to differences in H_2S production in the absence of S^0 residues, related to differences in juice nutrient status (Ugliano et al. 2009), must turbidity (Rankine 1963), yeast strain (Rankine 1963), and fermentation temperature (Schutz and Kunkee 1977).

Unfortunately, there are few data available concerning the persistence of S^0 in the vineyard or during pre-fermentation vinification practices, and the limited number of studies that have attempted to quantify S^0 residues following field treatments show conflicting data. For example, Thomas et al. (1993b) working in California found that applications of 10 to 17 kg/ha of S^0 formulated as dust resulted in residues <14 μg/g on fruit 1 day after application; that these had declined to <4 μg/g within 2 additional weeks; and that final concentrations at harvest (6 weeks after the last application) were 1 to 3 μg/g. In contrast, Wenzel et al. (1980) working in Germany found residue levels as high as 8 μg/g at harvest when applications of a sprayable S^0 formulation ceased 7 weeks beforehand (Wenzel et al. 1980) although application rates were not disclosed. In this and a previous study (Wenzel and Dittrich, 1978), the same group also demonstrated that clarification of white wine must can greatly lower S^0 levels therein, leading to lower H_2S production during fermentation (Wenzel and Dittrich 1978). As a result of these conflicting observations, growers and winemakers cannot objectively assess the risk that late season applications will yield deleterious residues on berries, sometimes resulting in arbitrary commercial restrictions and conflicting recommendations regarding late-season sulfur use. A poor understanding of this relationship increases the likelihood of economic losses resulting from (i) an unnecessary overreliance on more expensive alternatives to S^0, which also increases the probability of compromised disease control following the eventual development of pathogen

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74 resistance to many of the substituted materials; or, at the other extreme, (ii) the production of
75 faulted wine as a result of S^0 application too close to harvest.

76 A major impediment to studies requiring quantification of S^0 residues has been the lack of
77 an affordable technology to do so in complex matrices such as grape juice and must, as standard
78 elemental analysis techniques measure total sulfur, including not only S^0 but also sulfur from
79 endogenous sulfates, S-amino acids, etc. Thomas et al. (1993b) circumvented this limitation by
80 washing sulfur dust residues from the surface of intact clusters and measuring total S in the
81 rinsate. Nevertheless, we were unable to apply this technique successfully in our own initial field
82 studies, as the sprayable formulations of S^0 utilized in many regions (and which are standard in
83 humid climates such as New York) left visible residues on the fruit after repeat washings, and
84 measured S levels in the rinsate were unexpectedly low. However, we recently reported the
85 development of a rapid, inexpensive technique for measuring S^0 in complex matrices, based upon
86 its quantitative reduction to H_2S *in situ* and simultaneous colorimetric quantification using
87 commercially available detection tubes (Kwasniewski et al. 2011). The present report details the
88 subsequent use of this technique to study the effect of fungicide formulation, rate, and application
89 timing on the persistence of S^0 residues on grape clusters in the field and their transfer to the must
90 after harvest and crushing. Additionally, we report upon the influence of vinification factors such
91 as whole-cluster pressing, length of skin contact, and must clarification on the proportion of S^0
92 transferred into the must.

93 ## Materials and Methods

94 **S^0 persistence following field applications.** Three years of field trials were conducted in test
95 vineyards at the New York State Agriculture Experiment Station in Geneva, NY (lat.: 42°52'43";

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long.: -77°00'56"), to determine the effect of pre-harvest spray interval, product formulation, and application rate on S^0 persistence. In 2009 and 2010, these trials were conducted on vines of *Vitis vinifera* cv. Chardonnay, and in 2011 on *V. vinifera* cv. Riesling. All vines were planted in 2004 on 3309C rootstock, and were trained to a vertical shoot-positioned system with 3-m row spacing and 2-m vine spacing. Vines were sprayed and fertilized according to normal commercial practices for the region, except that no S^0 sprays were applied other than those in the variable treatment regimens. S^0 treatments were applied to test vines using a custom-built over-the-row, hooded boom sprayer operating at a pressure of 2070 kPa and delivering a water volume of 935 L/ha through seven hollow cone nozzles on each side of the boom. Cumulative temperature and rainfall data for each intervening period between S^0 applications, and between the final application and harvest, are provided in Table 1.

Two commercial elemental sulfur products were applied over the course of this study, a micronized formulation (Microthiol Disperss 80DF, Cerexagri Inc., King of Prussia, PA) and a wettable powder formulation (Yellow Jacket Wettable Sulfur, Georgia Gulf Sulfur Corp., Valdosta, GA). Particle size for these formulations was quantified using a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK). The median particle diameter of the micronized formulation was 4.7 µm with 90% of particles between 2.6 and 8.4 µm, and the median particle diameter of the wettable powder was 32.0 µm with 90% of particles falling between 9.0 and 73.5µm.

In 2009, a single application of the micronized formulation was made either 68, 40 or 12 days pre-harvest, at a rate of either 2.69 or 5.38 kg/ha of S^0. Each of the seven treatments, including a control in which no S^0 was incorporated, was applied to six replicate four-vine panels arranged in a randomized complete block design. Fruit was harvested 14 October.

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In 2010, all treatments were initiated on 12 August (veraison), with additional sprays applied at approximately 2-wk intervals and continuing until either 50, 35, 22, or 8 days before harvest (1 October 2010), depending on the treatment. Vines in the 50-day pre-harvest treatment received only a single application of micronized sulfur at a rate of 2.69 kg/ha of S^0, whereas those in the latter three timing regimens received applications of either (i) wettable sulfur, at a 2.69 or 5.38 kg/ha rate of S^0; or (ii) micronized sulfur, at the 5.38 kg/ha rate. Individual plots consisted of two consecutive four-vine panels for each of the 11 treatments (including control), arranged in a randomized complete block design with three replications. For each treatment, five clusters were randomly sampled for S^0 residue analysis from all panel replicates at 32, 30, 28, 24, 20, 16, 7, 2 and 0 days before harvest.

In 2011, individual plots again consisted of two consecutive four-vine panels with the 11 treatments (including control) arranged in a randomized complete block design with three replications. Vines received 4.48 kg/ha of S^0 in either micronized or wettable powder formulation, beginning on 10 August and continuing at approximately 2-wk intervals until 54, 38, 25, or 12 days before harvest (16 October), for a maximum of 5 possible applications. An additional treatment was included that received micronized sulfur at 4.48 kg/ha in the first applications and 2.24 kg/ha in the final two applications 54 and 38 days before harvest. For all treatments, five clusters were randomly sampled for S^0 residue analysis from each of the two-panel plots in each of the three replicate blocks at 62, 53, 47, 40, 31, 24, 17, 9 and 0 days before harvest. In all experiments, S^0 residues were determined as described below and treatment means were first compared within a given sampling date using two-way ANOVA, followed by parametric testing within a sampling period using Tukey HSD.

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Quantification of S^0 residues. The method described in Kwasniewski et al. (2011) was followed for S^0 residue quantification. Briefly, for grape samples from the field, a whole cluster (fresh or frozen) was first blended with an equal weight of water using an immersion blender; juice and must samples obtained after pressing were used without initial preparation. Each sample was heated in PEG 400 (Fisher Scientific, Pittsburgh, PA) to disperse S^0, diluted with water, and subsequently de-aerated and adjusted to pH 6 through the addition of a pharmaceutical antacid tablet (Alka-Seltzer, Bayer Healthcare, Morristown, NJ). The 2.95-g antacid tablets consist of 0.32g acetylsalicylic acid, 1.63g Sodium Hydrogen Carbonate and 0.97g Citric Acid Anhydrous as well as <0.04g of the following: povidone, dimeticone, calcium silicate, docusate sodium, sodium benzoate and, sodium saccharin. Following de-aeration, dithiothreitol (Fisher Scientific, Pittsburgh, PA) was added to reduce S^0 to H_2S, and the H_2S sparged through either a Gastec 4L or 4LL model H_2S gas detection tube (Fisher Scientific, Pittsburgh, PA) via sequential addition of two additional antacid tablets. The S^0 concentration was determined by relating the distance of color change on an H_2S detection tube to that observed for calibration standards.

Basic vinification procedure. All wines were vinified in triplicate using the following procedure commonly applied to white wines, unless otherwise noted. Grapes from a given treatment were hand harvested, crushed-destemmed, then pressed in a hydraulic basket press. The collected juice was treated with 50 mg/L SO_2 and allowed to settle for 24 hr. Following settling, juice was inoculated with *Saccharomyces cerevisiae* strain DV10 (Lallemand, Petaluma, CA) previously rehydrated in 10 mg/L GoFerm (Lallemand) according to the manufacturer's instructions. Nutrient analysis was conducted and soluble solid content was determined by refractometry. Ammonia and alpha-amino acid were quantified enzymatically prior to inoculation using Unitab reagents and a ChemWell multiscanner (Unitech Scientific, Hawaiian Gardens,

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CA.). If necessary, nutrients were added at inoculation to raise yeast available nitrogen to 300 mg/L. Additions were in the form of Fermaid K (Lallemand, Petaluma, CA), to a maximum concentration of 25 mg/L of this product, with the reminder provided as $(NH_4)_2HPO_4$. Wines were fermented at 10°C to dryness as determined by Clinitest (Bayer, West Haven, CT), cold stabilized at -4°C, and bottled under Stelvin closures (Waterloo Container, Waterloo, NY). Following primary fermentation, wine transfers (i.e., racking and bottling) were made under N_2 gas.

In 2009, the vinification procedures described above were amended due to berry desiccation from powdery mildew development. Water was added at a rate of 200 mL/L of must to reduce the soluble solids and titratable acidity from 30.4(±0.5) Brix and 14.8(±0.3) g/L, respectively, to 24.6(±0.5) Brix and 11.4(±0.2) g/L, respectively. Nitrogen levels were tested and adjusted following amelioration.

In 2010, clusters with visible late-season Botrytis bunch rot problems were removed prior to crushing-destemming. Soluble solids and titratable acidity of juice produced from sorted fruit were 20.8 Brix (±0.4) and 8.4 g/L (±0.3), respectively, with a mean pH value of 3.35 (±0.1). Due to poor yield resulting from a combination of late spring frost events and losses due to sorting, there was insufficient fruit to vinify all treatments. Thus, triplicate 1-L fermentations were made with fruit from all timings of the 5.38 kg/ha micronized sulfur treatments, as well as from the other treatments that ceased 8 days prior to harvest.

No amendments were necessary prior to fermentation in 2011. Each treatment yielded triplicate 20-L batches, which were fermented to dryness. H_2S production was monitored daily using detection tubes as described above. S^0 residues were measured on the intact fruit prior to processing as well as in the juice prior to and at various points during the pre-fermentation

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187 settling process. In 2011, juice clarity levels were determined by measuring the turbidity of must
188 samples taken 30 cm below the surface with a wine thief, using a Hach 2100Q Turbidimeter
189 (Hach Company, Loveland, CO); all clarified musts obtained a turbidity of <20 NTU after 24 hr
190 of settling and racking. After racking, the sediment fraction consisted of the 2 L left in the carboy
191 after removing the clarified must. In earlier years the determination of final clarity prior to
192 fermentation was made visually.

193 In 2010 and 2011, H_2S produced during fermentation was monitored daily by measuring
194 the escaping gas with a Gastec 4H or 4HH model H_2S detection tube (Fisher Scientific,
195 Pittsburgh, PA) fitted into the fermentation airlock (Park 2000, Ugliano and Henschke 2010). In
196 these years, H_2S was also quantified in duplicate 80-mL samples of all wines produced, using the
197 apparatus described above for elemental sulfur quantification. For this purpose, two antacid
198 tablets were utilized for carrier gas generation (Kwasniewski et al. 2011), and H_2S was quantified
199 using H_2S gas detection tubes as described by Park (2008).

200 **Effects of skin contact time on S^0 persistence and H_2S production.** In 2010, a trial was
201 conducted to investigate the effect of skin contact duration prior to or during fermentation on S^0
202 persistence into fermentation and attendant H_2S production. Fruit was sourced from a commercial
203 vineyard of cv. Cabernet franc located near Geneva, NY (lat.: 42°50'40"; long.: -77°0'13"),
204 which was established in 2005 on 3309C rootstock with 3-m row spacing and 2-m vine spacing.
205 Following cessation of the grower's standard fungicide program, on 22 September all test vines
206 received a single application of micronized sulfur, providing 2.69 kg/ha of S^0, using the spray
207 equipment and technique described above. Fruit was harvested by hand on 3 October and
208 processed the following day.

209 Five different vinification treatments were imposed in triplicate upon this single source of

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fruit, as follows: (i) whole-cluster pressed; (ii) crushed-destemmed and pressed; (iii) crushed-destemmed and pressed following 24 hr skin contact; (iv) crushed-destemmed and pressed following a 1-wk maceration on the skins; or (v) crushed-destemmed and pressed following a 2-wk maceration on the skins. The basic wine making protocol described above was used except for the changes described below. The whole-cluster treatment (i) was imposed upon approximately 20% of the fruit from each of five harvest bins, which was removed immediately upon arrival from the field and pooled, separated into vinification replicates (n=3), pressed, and settled for 24 hr before racking and inoculation. The remaining fruit was homogenized, crushed, and destemmed; then, it was divided among 12, 60-L stainless steel tanks to accommodate three replicates of each of the four remaining treatments, with 30 kg of macerate per tank. The macerate in treatment (ii) was pressed immediately after crushing-destemming whereas that in treatment (iii) was allowed to remain in contact with the skins at 4°C for 24 h before pressing. Following pressing, vinification of treatments (ii) and (iii) proceeded according to the basic protocol above. Treatments (iv) and (v), simulating typical red wine fermentation conditions, were inoculated following crushing-destemming and division into fermentation replicates. The macerate for each replicate of treatments (iv) and (v), were placed into an individual 25-L plastic pail with airtight lid, and the buckets remained closed during the ensuing 7- or 14-day maceration period while the skins were integrated by swirling. After the given period of maceration, the wines were hand pressed through cheesecloth and transferred into a glass carboy. Yeast inoculum for all treatments was *S. cerevisiae* strain ICV-GRE (Lallemand, Petaluma, CA).

S^0 residue levels were quantified in the juice before and after settling as well as in wine post-fermentation and in the lees. H_2S produced during fermentation and remaining in the finished wines thereafter was quantified as described above.

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Statistics. JMP version 9.0.2 (SAS, Cary, NC) and Minitab 17 were used for statistical analyses. An assessment of equal variance by Levene's test was first conducted on Minitab. When the assumption of equal variance was met, one-way or two-way ANOVA was conducted (setting $p<0.05$ for both) followed by parametric mean testing using Tukey HSD on JMP. When equal variance was not determined, the following measures were taken to guard against type-I error: i) a Welch's ANOVA was used in one-way testing ($p<0.05$) or the p-value required in two-way ANOVA analysis was lowered to $p<0.01$; ii) parametric comparisons were conducted by Games-Howell, using Minitab. Linear regressions were conducted using JMP.

Results

Residue levels on grapes at harvest. In 2009, applications of S^0 continuing to 12 days of harvest resulted in residues more than 10-fold greater than those on berries last treated 4 or 8 wk earlier (Figure 1). Applications that ceased 40 days pre-harvest resulted in residues significantly higher than those on the control vines (no measurable residues), but an order of magnitude below the concentration of 10 mg/L demonstrated to increase H_2S production in fermentations (Acree et al. 1972). Only fruit treated until 12 days before harvest resulted in residue level in excess of this threshold. S^0 was detectable on some samples from the 68-day pre-harvest interval (PHI) treatment, but the mean concentration could not be differentiated statistically ($p > 0.05$) from that of the control. A two-way ANOVA showed that the timing of the S^0 application was a contributor to the variance ($p <0.0001$) whereas the application rate (2.69 or 5.38 kg/ha of S^0) was not.

In 2010, both the S^0 treatment (formulation-rate) and PHI impacted final residue levels ($p <0.001$) (Figure 1). All treatments applied to until 8 days before harvest resulted in residues exceeding 10 μg/g, although concentrations following applications of S^0 at 2.69 kg/ha in a

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wettable powder (WP) formulation were only about one-third the level of those following applications at 5.38 kg/ha in a micronized form. Residues following applications at this higher rate of the WP formulation were intermediate between those of the two other treatments and all three means were significantly different from one another (p <0.05, Figure 1). When sprays ceased 22 days before harvest, residues resulting from applications of the WP formulation at the lower rate averaged 6.4 ± 2.6 μg/g, whereas applications of either formulation at the higher rate resulted in significantly higher levels (p <0.05), well in excess of 10 μg/g (Figure 1). At a 35 day PHI, all three S^0 treatment residues were below 10 μg/g (0.6 to 4.6 μg/g), and at a 50 day PHI, the mean residue level on the one treatment imposed (the lower rate of the micronized formulation) was < 0.5 μg/g (Figure 1).

In 2011, both the duration of the PHI and the S^0 formulation affected residue levels on grapes at harvest. For both the wettable and micronized formulations applied at a constant S^0 rate of 4.48 kg/ha, residues were inversely proportional to the length of the PHI, with the exception that there was no significant (p< 0.05) difference between the 38- and 54-day PHI for the micronized form (Figure 1). Residues were above 1 μg/g for all treatments and near or well above 10 μg/g when either formulation was applied until either 25 or 12 days before harvest; those resulting from the micronized formulation were significantly (p< 0.05) greater than those from the wettable powder given the shorter PHI, whereas the converse was true for the longer PHI.

Persistence and accumulation in the vineyard. In 2010 and 2011, vines subjected to an S^0 treatment with the same formulation and application rate but designated for different pre-harvest withholding periods had experienced identical spray regimes at early time points (Figs. 2

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and 3). Therefore, for the following data summation, residue values were pooled for all treatments that had received undifferentiated S^0 applications up to a particular sampling time. Furthermore, although samples from control panels in which no S^0 was applied were quantified at every time point in both years, residue levels were always below the limit of detection (0.01 µg/g) for the methodology used; hence, no additional data are presented for the control treatment.

In 2010, S^0 residue levels 32 days before harvest (i.e., 3 days after the most recent application) averaged 27 µg/g for all plots receiving the micronized formulation at 5.38 kg/ha, 34 µg/g for the WP at this same rate, and 20 µg/g for the WP at 2.69 kg/ha. At 30 days before harvest, the mean levels for these three treatments had decreased to 21, 17, and 10 µg/g, respectively; at 28 days they were 28, 10, and 8 µg/g, respectively; and at 24 days, they were 14, 10, and 7 µg/g, respectively (Figure 2). Differences among rates and formulations were more pronounced immediately following an application and appeared to be cumulative over time. For example, across all vines treated 22 days before harvest, residues on fruit sampled 2 days later averaged 50 µg/g for the micronized formulation applied at 5.38 kg/ha, 56 µg/g for the WP formulation applied at this same rate, and 28 µg/g for the WP at 2.69 kg/ha when. One day following the subsequent application (as shown on the 8-day PHI vines), these values were 67, 86, and 30 µg/g, respectively (Figure 2). However, differences between the two S^0 formulations were inconsistent in 2011. e.g., residues were higher for the micronized formulation shortly after the final treatment and at harvest when applications ceased 12 days before harvest, whereas the converse was true on vines in the 25-day PHI treatment. As in 2010, residue levels typically spiked immediately after treatment, declining by about one-half after approximately 1 week (Figure 3). Detailed data on 2009-2011 S^0 residue concentrations are provided in supplemental data available online (Table S1).

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300 **Residue fate during pre-fermentation operations.** In 2009, there was a dramatic
301 reduction in S^0 residue levels measured in the clarified must versus those on the harvested fruit.
302 Residues were approximately 10 to 25% of those on the fruit, and the greatest absolute reductions
303 occurred in treatments with the highest initial concentrations. The H_2S concentration was
304 measured only in the finished wines that year, with all levels below the sensory threshold of 1
305 µg/L (Siebert et al. 2009) and no significant differences among treatments.
306 S^0 residue levels were compared among spray treatments on whole berries, and in both
307 unclarified and clarified juice in 2010 and 2011; they also were monitored at various times during
308 the cold-settling process in 2011. In 2010, mean residue levels for all treatments decreased from a
309 range of 4.6 to 60.8 µg/g on the harvested grapes down to 1.5 to 15.5 µg/g in the unclarified juice
310 immediately after pressing. S^0 residue levels in the juice declined substantially further after
311 settling, to between 0.43 and 1.75 µg/g. Following clarification, the majority of the S^0 residues
312 appeared to reside in the sediment fraction, which contained substantially greater concentrations
313 of S^0, 23.9 to 174.1 µg/g. The S^0 residue levels on the grapes correlated well with those in
314 unclarified juice (R^2=0.90, p=0.014; Figure 4), but not with those in the clarified juice (R^2=0.37,
315 p=0.28; data not shown). Similarly, S^0 residues on the harvested grapes did not correlate well
316 with the amount of H_2S produced during fermentation (R^2=0.45, p=0.21; data not shown),
317 whereas S^0 concentrations in the settled must were good predictors of total H_2S production during
318 its subsequent fermentation (R^2=0.69, p<0.001; Figure 5).
319 A similar pattern of the fate of S^0 residue on grapes following crushing and pressing was
320 observed in 2011, with residue levels on grapes again being a good predictor of those in the
321 unsettled must (R^2=0.74, p=0.002; Figure 4). Initial S^0 residues in the musts ranged from a mean
322 of 1.52 to 12.82 µg/g across S^0 application treatments, but declined to 0.14 to 0.28 µg/g after they

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had settled to a turbidity level of <20 NTU (Figure 6). There was no relationship between these low S^0 concentrations after settling and H_2S production during subsequent fermentation ($p=0.64$, Figure 5). Thus, S^0 residues in grapes, unclarified juice, and clarified juice were not good predictors of H_2S formation in the 2011 fermentations of clarified juice.

Skin contact effect on S^0 persistence and H_2S production. At harvest, Cabernet franc clusters used in the vinification trials had S^0 residue levels of 11.4 ±1.2 μg/g. By the time of inoculation, mean must S^0 levels ranged from 0.05 to 0.20 μg/g in those treatments that were pressed and settled first, whereas those undergoing an initial 1- or 2-week maceration had S^0 levels of 10.8 and 11.1 μg/g, respectively (Table 2)**.** Subsequent fermentation on the skins produced mean levels of H_2S two- to three-fold greater than those for treatments where juice was pressed off the skins and settled before inoculation (Table 2).

Discussion

Several reports have shown that ≥10 μg/g S^0 in must results in increased H_2S production during fermentation (Rankine 1963, Acree et al. 1972, Schutz and Kunkee 1977). However, less work has gone into understanding S^0 persistence in the vineyard and defining application regimes that will avoid excess residues in the fermentation. Two previous studies quantified S^0 that could be rinsed from intact clusters using either a water-detergent mixture (Thomas et al. 1993) or petroleum ether (Wenzel et al. 1980), although neither approach appears to have been validated using recovery experiments. During method development, we found the former technique to be inadequate for quantitative removal under our experimental conditions; we did not explore petroleum ether extraction, as it is a poor solvent for S^0 (Chen et al. 1973). Instead, we opted to blend whole cluster samples for subsequent quantification with a newly validated assay that

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345 allows quantification of S^0 in the presence of other sulfur-containing compounds (Kwasniewski et
346 al. 2011). These methodological differences may explain why we observed residue levels as high
347 as 86 μg/g berry weight on some clusters immediately after application of S^0, whereas Thomas et
348 al. (1993b) reported maximum levels <14 μg/g immediately post-application when utilizing rates
349 approximately two to four times greater than those we employed. Wenzel et al. (1980) observed a
350 maximum S^0 residue of 5.37 μg/g immediately after a single application (rate not specified),
351 declining to 0.83 μg/g at harvest 51 days after treatment; their greatest concentration at harvest
352 was 3.89 μg/g, following eight sequential S^0 applications that concluded 51 days earlier.
353 Although the difference in S^0 formulation used by Thomas et al. (1993b) relative to our study
354 (dusting versus sprayable, respectively) may have contributed to the differences in our findings,
355 Wenzel et al. (1980) used a colloidal formulation similar to ours and also found far lower levels at
356 harvest than we report. These differing results, consistent with our initial inability to remove all
357 visible residues with a dilute detergent solution in preliminary experiments, may reflect an
358 underreporting of the total S^0 on fruit when only the residue in rinsate is quantified. Additional
359 research is needed to ascertain whether the increased S^0 concentrations that we report from
360 blended clusters versus those reported by previous workers from rinsate (Wenzel et. al 1980,
361 Thomas et al. 1993b) may be due at least in part to incomplete recovery of S^0 using the latter
362 technique, resulting from its adsorbance to the waxy cuticle of the fruit.
363 Of the limited studies on S^0 persistence in the vineyard, Thomas et al. (1993b) determined
364 that residues would not exceed levels ultimately detrimental to wine quality if applications ceased
365 by the time that fruit had matured to the point of veraison. This developmental stage was chosen
366 as a point to cease application based on the then-current belief that berries lose their susceptibility
367 to new PM infections soon thereafter. Although it is now known that berries are resistant to new

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368 infections far before this point of development, continued control of PM after veraison may
369 nevertheless be necessary as the rachis and new shoot growth remain susceptible (Gadoury et al.
370 2011). In our studies, S^0 residue did not exceed 4.6 µg/g when applications ceased by 35 to 38
371 days before harvest, and were typically near or below the value of 3 µg/g previously shown to
372 provide no increase in H_2S production during fermentation (Thomas et al. 1993a). However,
373 residues consistently exceeded the 10 µg/g threshold when S^0 was applied within 25 days of
374 harvest, and in all 3 years only those treatments ceasing ≥50 days from harvest were below 1
375 µg/g. In addition to the timing of the final application, S^0 formulation and application rate also
376 affected residue levels and persistence, both at harvest and throughout the season. For example, in
377 2010, applications of the WP formulation at 2.69 kg/ha with a 22 day PHI resulted in residue
378 concentrations at harvest comparable to those for the same material applied at a rate of 5.38 kg/ha
379 with a 33 day PHI. Furthermore, concurrent applications of a WP versus a micronized
380 formulation at the same rate of S^0 typically resulted in higher residue levels for the latter
381 treatment. Thus, limiting the application rate and utilizing a WP rather than micronized
382 formulation in later sprays may help to minimize the PHI necessary to attain a given level of
383 residue on harvested fruit.

384 While vineyard treatments can have a significant influence on S^0 residue levels on fruit,
385 pre-fermentation decisions involving factors such as skin contact and settling time will exert a
386 strong influence on S^0 concentrations in must. In both 2010 and 2011, S^0 residues on harvested
387 Chardonnay and Riesling clusters, respectively, were a good predictor of S^0 residues in
388 unclarified juice following crushing and destemming, but did not correlate well with S^0 residues
389 in clarified juice. Examination of the post-clarification sediment fraction produced from these
390 trials and from a separate trial involving Cabernet franc vinified as a white wine indicated that

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391 most of the S^0 present in the unclarified must could be found in the sediment. Considering that
392 over 95% of residues were removed during settling, achieving must S^0 concentrations >10 μg/g
393 following settling would require initial S^0 residues of >200 μg/g, a level far exceeding any
394 residues detected immediately after spraying. Thus, in agreement with the finding by Wenzel et al
395 (1980), highly clarified musts (<20 NTU) appear to be at minimal risk for containing S^0 residues
396 sufficient to produce increased H_2S during fermentation. However, because our current work
397 looked at only a single target turbidity, we have not established general guidelines for the
398 relationship between NTU and S^0 residue loss.

399 In the previous discussion, we used must S^0 concentrations of ≥10 μg/g as a threshold for
400 increased H_2S production during fermentation. However, some authors have reported increased
401 H_2S with S^0 residues as low as 1 μg/g (Thoukis and Stern 1962, Wenzel et al. 1980) whereas
402 another group reported that residues as high as 3.0 μg/g generally had no effect while also noting
403 an interaction among S^0 concentration, fermentation medium, and yeast strain on H_2S (Thomas et
404 al. 1993). In vinifications of Chardonnay from the 2010 spray treatments, S^0 residue levels (<0.01
405 to 2.2 μg/g) were linearly correlated with the quantity of H_2S produced during fermentation,
406 whereas there was no such correlation within a lower range of S^0 residues (<0.01 to 0.3 μg/g)
407 examined from the Riesling treatments in 2011 (Figure 5). Thus, under these particular
408 fermentation conditions, our results agree with previous reports that S^0 residues above 1 μg/g can
409 increase H_2S production (Thoukis and Stern 1962, Wenzel et al. 1980). However, at low S^0
410 levels other factors such as juice nutrient status (Ugliano et al. 2009) likely have a larger role in
411 explaining differences in H_2S production. Additionally, yeast strain will not only affect H2S
412 production, but also the conversion efficiency of S^0 to H_2S (Acree et al. 1972).

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413 Fermentation treatments on Chardonnay and Riesling grapes simulated typical white
414 winemaking conditions in which fruit is pressed and the resulting juice clarified prior to
415 fermentation. To evaluate the effects of using typical red versus white winemaking practices,
416 different pre-fermentation treatments were applied to Cabernet franc clusters. Pressing and
417 settling prior to fermentation resulted in negligible S^0 residues (0.05 to 0.2 μg/g), even when a 24-
418 hr cold soak was introduced. However, skin-fermented treatments (involving 1- and 2-week
419 macerations to simulate typical red winemaking conditions) had pre-fermentation S^0 must
420 concentrations nearly identical to residue levels on the intact berries, i.e., one to two orders of
421 magnitude greater than those in clarified musts from the same lot of fruit. Skin-fermented
422 Cabernet franc treatments also produced two- to threefold more H_2S during fermentation than
423 treatments pressed prior to fermentation. It should be noted, however, that control treatments with
424 undetectable S^0 residues were not included, and we cannot exclude the possibility that differences
425 in H_2S production resulted from some other unknown factor associated with skin fermentation
426 rather than variable S^0 residues.
427 Lastly, this study did not attempt to determine the impact of potential variables that might
428 influence S^0 loss in the vineyard, including temperature, precipitation, spray application
429 technique, or canopy management and variety. Further work is needed to understand what roles
430 these factors may play in S^0 accumulation and persistence, perhaps leading to an improved ability
431 to predict S^0 residues at harvest. However, monitoring S^0 residue levels with the assay used in
432 this study is a viable option for producers looking to inform their viticultural and vinification
433 decisions relative to this factor.
434

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Conclusion

S^0 plays an important role in powdery mildew management due to its cost, efficacy, low resistance risk, and cachet as a natural product, but developing guidelines for pre-harvest withholding periods has been hindered by a paucity of data relating vineyard use patterns to residue levels on harvested fruit and their potential contribution to increased H_2S production during fermentation. We found that ceasing sprays no later than 35 days before harvest resulted in S^0 residues on harvested fruit below 10 µg/g, a concentration consistently shown in previous literature to increase H_2S production when present at inoculation. A more conservative threshold for S^0 residue in must (1 µg/g) was exceeded even with a 56-day pre-harvest interval in some treatments. Although S^0 residue levels in unclarified musts were strongly correlated with those on the grapes prior to crushing, pre-fermentation clarification reduced residues in the juice by >95%, such that S^0 contamination should be of concern only for skin-fermented wines (i.e., when utilizing red-winemaking conditions) under most circumstances. Because S^0 persistence on fruit in the vineyard was affected by application rate and formulation as well as vintage, an accurate determination of vineyard residues is best determined by measuring samples from a given site, which is relatively easy and inexpensive using the newly described methodology. Potentially, this information could also be useful in determining when S^0 needs to be reapplied, or to evaluate the selectivity of a sprayer for targeting the canopy vs. the fruit. Finally, future work could attempt to better link the kinetics of S^0 disappearance to weather phenomena, with the goal of generating predictive models that will negate the need for growers to individually measure S^0. Expanding beyond the single site used in this study, to survey studies of S-residues across multiple sites, with known spray schedules, could be used to construct confidence intervals for recommended S-spray cessation times to ensure grapes are at safe levels with respect to potential wine defects at harvest.

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Table 1 Accumulation of heat units and precipitation during the periods between sulfur applications in field experiments, 2009-2011.

2009

Treatment date (PHI)[a]	Degree days (10 C)[b]	Precipitation (mm)[c]
7 Aug (68)	-	-
4 Sep (40)	522	69.0
2 Oct (12)	267	145.0
Harvest	28	33.0

2010

Treatment Date (PHI)	Degree Days (10 C)	Precipitation (mm)
11 Aug (50)	-	-
26 Aug (35)	272	96.0
9 Sep (22)	263	13.2
23 Sep (8)	127	34.8
Harvest	88	25.7

2011

Treatment Date (PHI)	Degree Days (10 C)	Precipitation (mm)
16 Aug (54)	-	-
1 Sep (38)	305	103.4
14 Sep (25)	236	57.7
27 Sep (12)	136	32.3
Harvest	102	63.0

[a]PHI = pre-harvest interval (days).
[b]Accumulated degree days (base 10°C) since previous sulfur application.
[c]Accumulated precipitation (mm) since previous sulfur application.

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Table 2 Transfer of S^0 from Cabernet franc clusters into must and subsequent evolution of H_2S during fermentation, as affected by vinification method

	S^0 content before settling[b]		**S^0 content at inoculation[c]**		**H_2S produced during fermentation**	
Treatment[a]	**Mean (µg/g)**	**SD**	**Mean (µg/g)**	**SD**	**Mean (ng/mL)**	**SD**
Whole-cluster pressed	1.24	0.2 b[d]	0.2	0.1 b	70.5	5.1 a
Crushed-destemmed	0.6	0.0 a	0.05	0.0 a	67.8	3.2 a
24-hour skin contact	1.92	0.2 c	0.18	0.1 b	75.6	8 a
1-week maceration	NA	-	10.8	0.8 c	140.6	9.4 b
2-week maceration	NA	-	11.1	1.1 c	179.2	35 b

[a]Grapes for all vinification treatments received an application of micronized sulfur at a rate of 2.69 kg/ha 10 days before harvest, resulting in S^0 residues of 11.4 ±1.2 µg/g on harvested clusters; variable treatments were imposed upon a single lot of fruit in the winery.
[b]Samples were obtained immediately after pressing; treatments fermented on the skins had not been pressed at this time.
[c]For treatments processed as white wines, must concentrations were determined at the time of inoculation after pressing, settling, and racking.
[d]Values represent the means of three replicate vinifications per treatment. Means within a column not followed by a common letter are significantly different ($p < 0.05$) according to Games-Howell analysis, following Welch's ANOVA.

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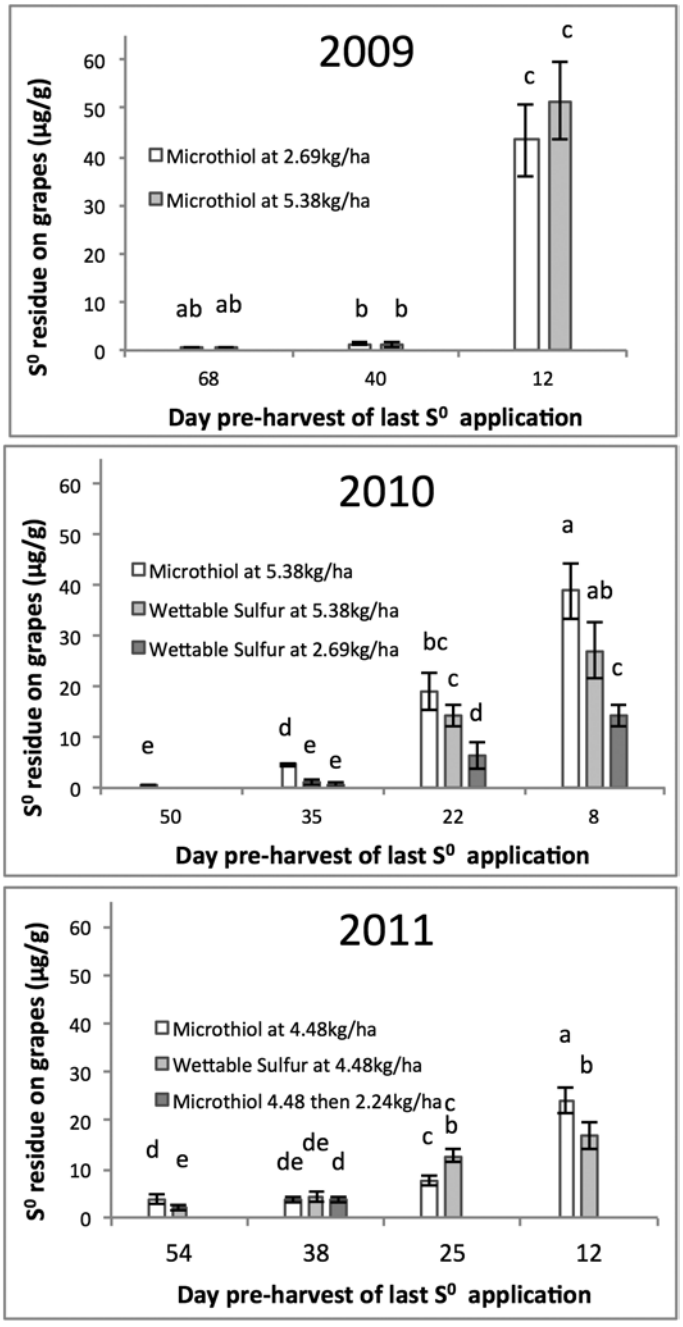

Figure 1 S^0 residues on Chardonnay (2009, 2010) and Riesling (2011) clusters at harvest. Data are grouped by days before harvest of the final S^0 application, with each bar representing the mean value for a five-cluster sample taken from each of six treatment sampling units (two per replicate plot). Means not labeled with a common letter are significantly different (Games-Howell $p < 0.05$). No residue was detected on any samples obtained from a control treatment in which S^0 was not applied (data not shown). The "*" denotes a treatment which received micronized sulfur at 4.48 kg/ha in the first three applications and 2.24kg/ha in the final two.

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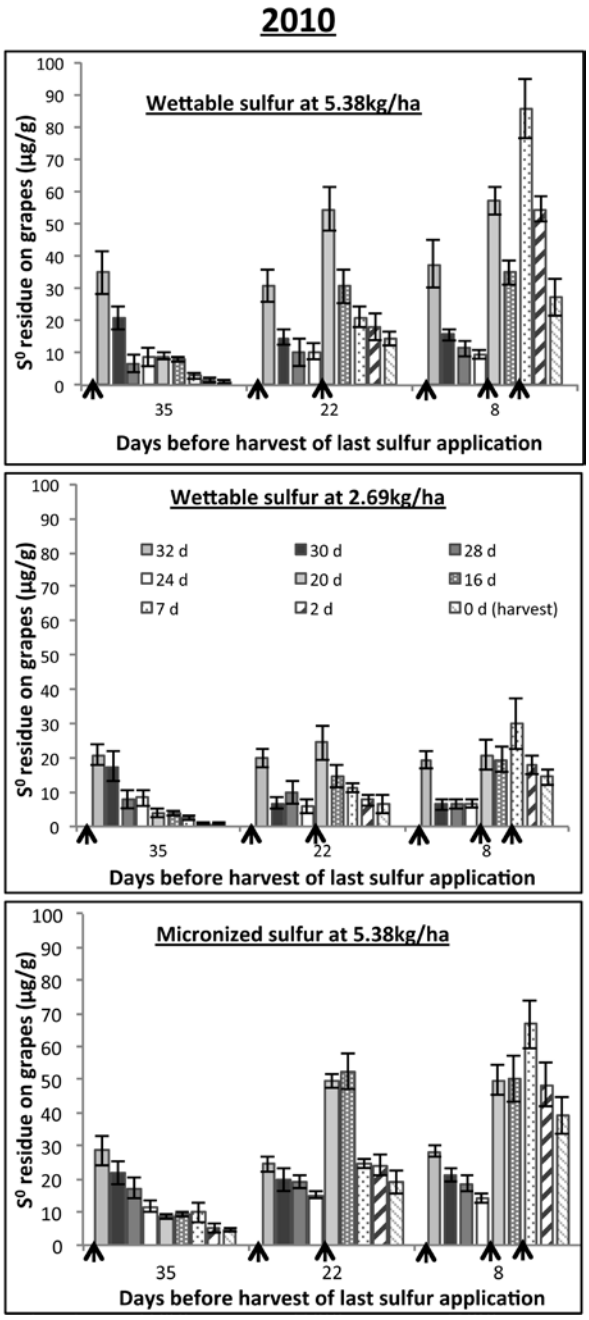

Figure 2 Elemental sulfur (S^0) residue on Chardonnay grape clusters sampled throughout the 2010 season. Sequential sprays of commercial sulfur formulations were applied starting 50 days before harvest and continuing at approximately 2-wk intervals, ceasing a variable number of days before harvest on designated vines as denoted on the x-axis. S^0 residue data are grouped by sulfur treatment, with each bar representing the mean value for a five-cluster sample taken from each of six treatment sampling units (two per replicate plot). The legend denotes the number of days before harvest that samples were obtained, and arrows signify when a S^0 application was made within the sampling period for each group of vines. No residue was detected on samples from control treatment vines to which S^0 was not applied (data not shown).

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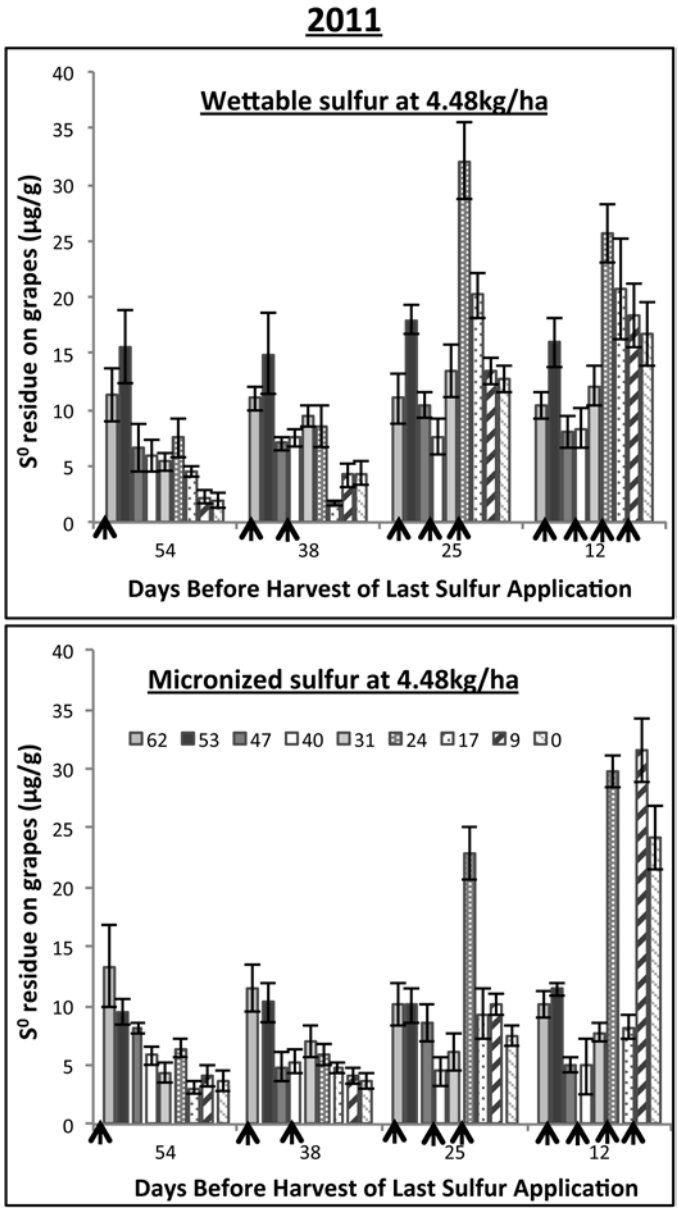

Figure 3 S^0 residues on Riesling grape clusters sampled throughout the 2011 season. Sequential sprays of commercial sulfur formulations were applied starting 81 days before harvest and continuing at approximately 2-wk intervals, ceasing a variable number of days before harvest on designated vines as denoted on the x-axis. S^0 residue data are grouped by sulfur treatment, with each bar representing the mean value for a five-cluster sample taken from each of six treatment sampling units (two per replicate plot). The legend denotes the number of days before harvest that samples were obtained, and arrows signify when a S^0 application was made within the sampling period for each group of vines. No residue was detected on samples from control treatment vines to which S^0 was not applied (data not shown).

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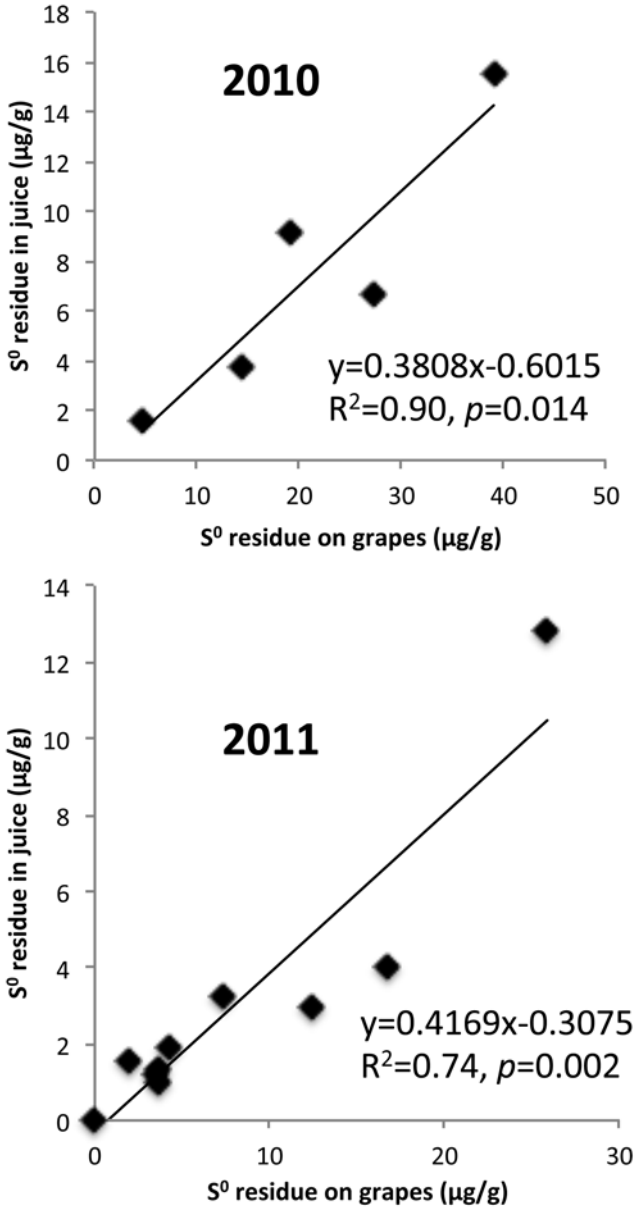

Figure 4 S^0 residues in unsettled Chardonnay (2010) and Riesling (2011) juice after pressing as a function of residues measured on grapes at harvest. Each data point represents the mean value for six replicate measures of grape residues and three replicate measure of residues in the expressed juice, per treatment.

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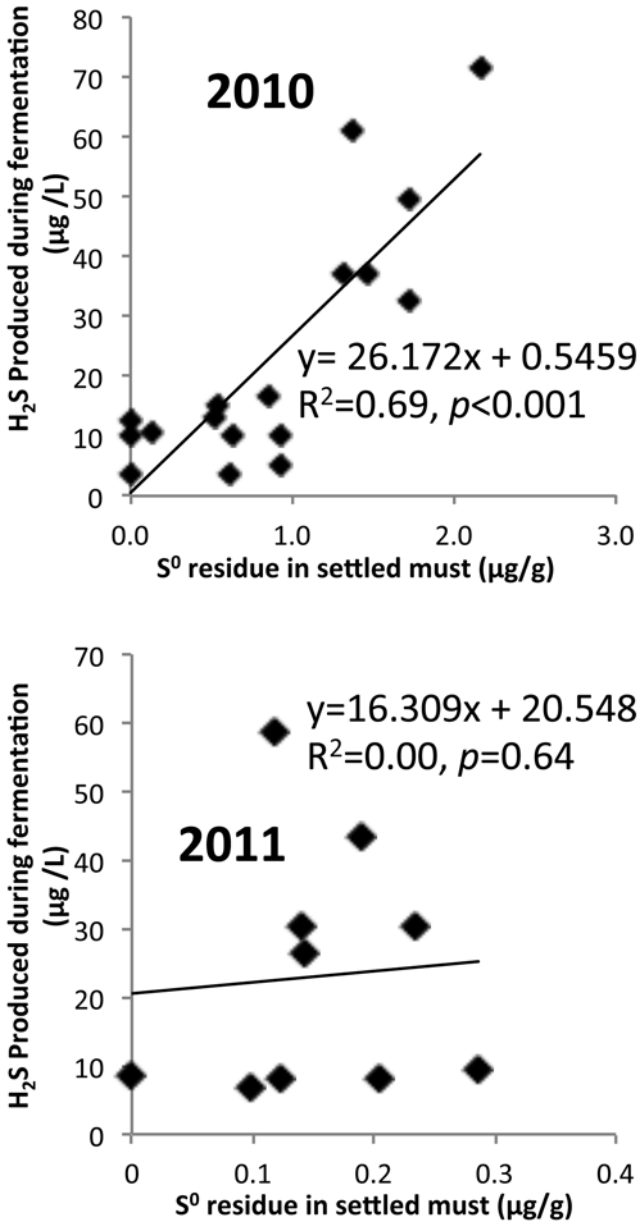

Figure 5 Hydrogen sulfide production during fermentation as a function of S^0 residues in the initial, settled Chardonnay (2010) and Riesling (2011) musts. In 2010, fermentation replicates were racked for the same period of time but turbidity was not measured and residue concentrations differed among fermentation replicates; data points represent values for individual replicates. In 2011, all samples were settled to a turbidity of >20 NTU and fermentation replicates were divided after racking; data points represent averages for three fermentation replicates per treatment.

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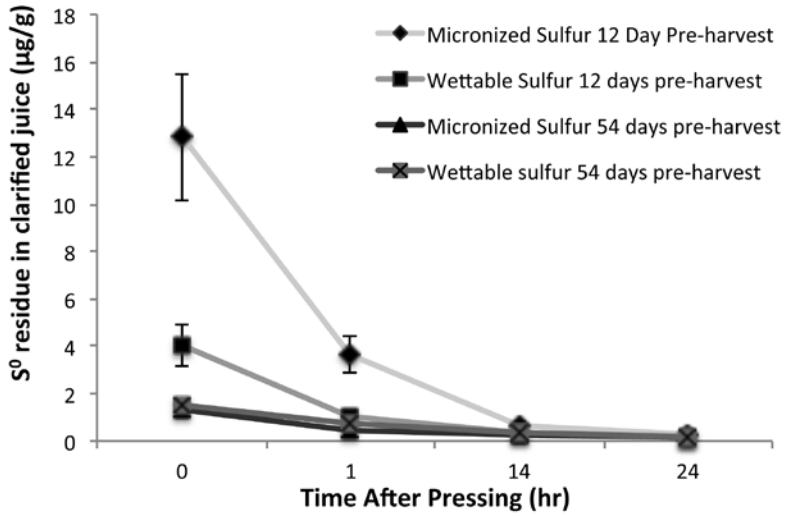

Figure 6 Elemental sulfur (S^0) residue present in juice pressed from fruit that received sequential applications of two commercial formulations (4.48 kg/ha S^0) during the 2011 season, ceasing either 54 or 12 days pre-harvest. Samples were obtained from 30 cm below the juice surface in a 20-L carboy, at the post-pressing time intervals indicated. Data points represent the means for three replicate fermentations per treatment, with error bars indicating standard deviation. All means for 38- and 25-day PHI treatments were intermediate between those for the 12- and 54-day extremes but are omitted from the graph for clarity.

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Supplemental Table 1 Sulfur residue levels on Chardonnay grape clusters taken during the 2009 season.

Sulfur residue in Chardonnay must and on Grapes (µg/L)

			Unsettled must[b]			Fruit		
Treatment number	**Microthiol application Rate (kg/ha)**	**Days before harvest[a]**	**Mean (µg/g)[c]**	**SD**		**Mean (µg/g)**	**SD**	
0	-	Control	0	0	a[d]	0	0	a
1	2.69	68	0.1	0.1	a	0.2	0.2	ab
4	5.38	68	0	0	a	0.2	0.7	ab
2	2.69	40	0.4	0.1	b	1.5	0.4	b
5	5.38	40	0.4	0.3	a	1.3	0.7	b
3	2.69	12	6.8	0.7	c	43.4	7.5	c
6	5.38	12	5.2	0.9	c	51.6	8.1	c

[a]A single application of a micronized formulation of S^0 Microthiol Dispers® was made either 12, 40 or 68 days pre-harvest, at a rate of either 2.69 or 5.38 kg/ha.

[b]Sulfur residue levels for "unsettled must" were taken immediately after pressing fruit.

[c]Mean values give are for sulfur residue measured on 5-cluster samples taken from each of the 6 treatment panel replicates.

[d]Means within a column not followed by a common letter are significantly different ($p < 0.05$) according to the Games-Howell test. Games-Howell analysis was performed following confirmation by 2-way ANOVA that variables contributed to differences at a significant level ($p < 0.01$).

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Supplemental Table 2 Sulfur residue levels on Chardonnay grape clusters taken through out the 2010 season.

					Sample Date[a]															
					30-Aug		1-Sep		3-Sep		7-Sep		11-Sep		15-Sep		24-Sep		29-Sep-12		1-Oct	
					Days before harvest															
					32		30		28		24		20		16		7		2		0	
Treatment number	Last application date[b]	Days before harvest	Formulation	Rate (kg/ha)	Mean[c]	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	12-Aug	50	Microthiol	2.69	3.5	± 2.0 e[d]	4.0	± 1.7 D	4.2	± 1.4 e	3.0	± 1.0 e	2.8	± 0.7 d	1.4	± 0.8 g	0.8	± 0.5 e	0.3	± 0.4 d	0.2	± 0.2 e
2	27-Aug	35	Microthiol	5.38	28.6	± 4.5 abc	21.9	± 3.6 a	17.3	± 3.3 abc	11.8	± 1.8 abc	8.8	± 0.9 c	9.3	± 0.7 de	10.0	± 3.0 c	5.1	± 1.5 c	4.6	± 0.5 d
3			Kumulus	5.38	34.9	± 6.6 a	20.8	± 3.7 a	6.9	± 2.8 de	8.7	± 2.9 bcd	9.0	± 1.1 c	7.9	± 0.9 e	2.7	± 0.7 d	1.5	± 0.8 d	1.2	± 0.7 e
4			Kumulus	2.69	20.9	± 3.1 bcd	17.5	± 4.4 ab	7.9	± 2.6 de	8.3	± 2.3 cd	4.0	± 1.3 d	3.6	± 0.6 f	2.6	± 0.9 de	0.7	± 0.4 d	0.6	± 0.3 e
5	9-Sep	22	Microthiol	5.38	24.5	± 2.3 abcd	19.9	± 3.5 a	19.2	± 1.9 a	15.2	± 1.1 a	49.5	± 2.2 a	52.5	± 5.4 a	28.3	± 6.2 b	24.2	± 3.1 b	19.1	± 3.7 bc
6			Kumulus	5.38	30.8	± 5.0 ab	14.8	± 2.2 ab	10.4	± 4.4 bcde	10.5	± 2.6 abcd	54.7	± 7.0 a	30.7	± 5.2 b	20.9	± 3.2 b	18.2	± 4.2 b	14.3	± 2.2 c
7			Kumulus	2.69	19.9	± 2.7 cd	6.8	± 1.6 cd	10.0	± 3.4 cde	5.7	± 2.0 de	24.5	± 5.1 b	14.7	± 3.2 cd	11.5	± 1.4 c	7.7	± 1.6 c	6.4	± 2.6 d
8	23-Sep	8	Microthiol	5.38	28.3	± 1.8 abc	21.3	± 2.0 a	18.7	± 2.7 ab	14.1	± 1.4 ab	49.7	± 4.5 a	50.2	± 6.8 a	66.7	± 7.2 a	48.4	± 6.6 a	39.0	± 5.6 a
9			Kumulus	5.38	37.6	± 7.3 a	15.6	± 1.7 abc	11.5	± 2.5 cd	9.5	± 1.3 cd	57.0	± 4.2 a	35.1	± 3.9 b	85.9	± 9.3 a	54.6	± 3.8 a	27.2	± 5.6 ab
10			Kumulus	2.69	19.6	± 2.6 d	6.5	± 1.7 bcd	6.6	± 1.6 de	6.6	± 1.0 d	20.8	± 4.5 b	19.5	± 3.6 c	30.0	± 7.5 b	17.8	± 2.6 b	14.3	± 2.2 c

[a]All treatments were sampled at each sampling date.

[b]Sequential sprays were applied to designated vines on 12 Aug, 27 Aug, 9 Sep, and 23 Sep , with the final application for each treatment as noted . Within a timing regime, treatments varied by S^0 formulation and application rate.

[c]Mean values represent sulfur residue measured on 5-cluster samples taken from each of the 6 replicate treatment panels per treatment. Measurements are given in µg of S^0 per gram of cluster weight.

[d]Means within a column not followed by a common letter are significantly different ($p < 0.05$) according to the Games-Howell test. Games-Howell analysis was performed following confirmation by 2-way ANOVA that variables contributed to differences at a significant level ($p < 0.01$).

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Supplemental Table 3 Sulfur residue levels on Riesling grape clusters taken through out the 2011 season

					Sample Date[a]																	
					15-Aug		24-Aug		30-Aug		6-Sep		15-Sep		22-Sep		29-Sep		9-Oct		16-Oct	
					62		53		47		40		31		24		17		9		0	
Treatment number	Last application date[b]	Days before harvest	Formulation	Application rate	Mean[c]	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	23-Aug	54	Microthiol	4.48kg/ha	13.3	± 3.5 a[d]	9.4	± 1.0 bc	8.1	±0.5 b	5.8	±0.8 abc	4.3	±0.8 e	6.4	±0.8 d	3.1	±0.5 de	4.0	±0.9 d	3.7	±0.9 d
2			Kumulus	4.48kg/ha	11.3	± 2.3 a	15.6	± 3.2 ab	6.6	±2.1 abcd	6.0	±1.5 abc	5.4	±0.8 de	7.5	±1.7 c	4.5	±0.4 c	2.2	±0.6 d	1.9	±0.6 e
3	8-Sep	38	Microthiol	4.48 and 2.24kg/ha[e]	11.1	± 2.4 a	8.4	± 0.9 c	2.4	±0.3 be	4.3	±0.6 c	3.8	±1.2 e	3.8	±0.5 c	2.8	±0.5 d	3.9	±0.6 d	3.7	±0.6 de
4			Microthiol	4.48kg/ha	11.5	± 1.9 a	10.3	± 1.6 bc	4.8	±1.3 cde	5.3	±1.0 abc	7.0	±1.4 bcd	5.9	±0.9 c	4.7	±0.5 c	4.1	±0.7 d	3.7	±0.7 de
5			Kumulus	4.48kg/ha	11.0	± 1.1 a	15.0	± 3.5 abc	7.0	±0.6 bc	7.5	±0.8 ab	9.4	±1.0 ab	8.6	±1.9 c	1.7	±0.3 e	4.2	±1.1 d	4.3	±1.1 d
6	21-Sep	25	Microthiol	4.48kg/ha	10.1	± 1.8 a	10.0	± 1.5 bc	8.5	±1.5 ab	4.5	±1.2 bc	6.1	±1.5 cde	22.9	±2.2 b	9.3	±2.1 b	10.1	±0.9 c	7.5	±0.9 c
7			Kumulus	4.48kg/ha	11.0	± 2.3 a	18.0	± 1.3 a	10.4	±1.1 a	7.6	±1.5 ab	13.5	±2.4 a	32.1	±3.5 a	20.1	±2.0 a	13.4	±1.2 b	12.7	±1.2 b
8	6-Oct	12	Microthiol	4.48kg/ha	10.1	± 1.1 a	11.4	± 0.6 bc	5.0	±0.6 d	4.9	±2.4 abc	7.7	±0.8 bc	29.7	±1.3 a	8.2	±1.0 b	31.5	±2.7 a	24.2	±2.7 a
9			Kumulus	4.48kg/ha	10.4	± 1.3 a	16.0	± 2.2 a	8.1	±1.5 ab	8.4	±1.9 a	12.1	±1.8 a	25.7	±2.7 ab	20.6	±4.4 a	18.3	±2.9 b	16.7	±2.9 b

[a]All treatments were sampled at each sampling date.

[b]Sequential sprays were applied to designated vines on, 10 Aug, 23 Aug, 8 Sep, 21 Sep, and 6 Oct with the final application for each treatment as noted. Within a timing regiment, treatments varied by S^0 formulation and application rate.

[c]Mean values represent sulfur residue measured on 5-cluster samples taken from each of the 6 replicate treatment panels per treatment. Measurements are given in µg of S^0 per gram of cluster weight.

[d]Means within a column not followed by a common letter are significantly different ($p < 0.05$) according to the Games-Howell test. Games-Howell analysis was performed following confirmation by 2-way ANOVA that variables contributed to differences at a significant level ($p < 0.01$).

[e]The treatment received Microthiol applications at 4.48kg/ha for the first application and 2.24kg/ha on 23 Aug and 8 Sep.