

AN EXAMINATION OF METHODS TO IMPROVE THE EFFICACY AND UTILITY OF
SULFUR FOR MANAGEMENT OF GRAPEVINE POWDERY MILDEW

By

WALTER WILLIS SANDERS

(Under the Direction of Phillip M. Brannen and Marin T. Brewer)

ABSTRACT

Grapevine powdery mildew (GPM), caused by *Erysiphe necator*, is one of the most economically important diseases of *Vitis vinifera* grapes. Sulfur is the most common contact fungicide applied for GPM control. This thesis evaluated the potential of surfactants and air induction nozzles to enhance disease control afforded by sulfur. The capacity of sulfur to cause phytotoxicity during high heat and relative humidity conditions was also examined. Surfactants, when combined with sulfur, often increased GPM disease control over sulfur alone. However, the impact of adding surfactants varied by year. Air induction nozzles did not provide increased efficacy compared to the industry standard cone nozzles. Sulfur phytotoxicity was not observed when sulfur was applied in northern Georgia vineyards in mid- to late summer, as conducive conditions were not achieved. Historical data revealed that weather conditions conducive to phytotoxicity are exceedingly rare in the areas where *V. vinifera* grapes are grown in Georgia.

INDEX WORDS: *Erysiphe necator*, powdery mildew, grape, sulfur, *Vitis vinifera*

AN EXAMINATION OF METHODS TO IMPROVE THE EFFICACY AND UTILITY OF
SULFUR FOR MANAGEMENT OF GRAPEVINE POWDERY MILDEW

by

WALTER SANDERS

B.S., University of Georgia, Athens, GA, 2022

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GA

2024

© 2024

Walter Willis Sanders

All Rights Reserved

AN EXAMINATION OF METHODS TO IMPROVE THE EFFICACY AND UTILITY OF
SULFUR FOR MANAGEMENT OF GRAPEVINE POWDERY MILDEW

by

WALTER WILLIS SANDERS

Major Professors:	Phillip M. Brannen Marin T. Brewer
Committee:	Sarah R. Lowder Paul Severns

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2024

DEDICATION

This thesis is dedicated to Carl.

TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	x
 CHAPTER	
1 INTRODUCTION AND LITERATURE REVIEW	1
2 UTILITY OF SURFACTANTS AND AIR INDUCTION NOZZLES FOR IMPROVED SULFUR EFFICACY WHEN MANAGING POWDERY MILDEW OF GRAPE	27
ABSTRACT.....	28
INTRODUCTION	29
MATERIALS AND METHODS.....	32
RESULTS	38
DISCUSSION.....	40
LITERATURE CITED	48
3 ASSESSMENT OF SULFUR PHYTOTOXICITY ON VITIS VINIFERA GRAPEVINES IN NORTHERN GEORGIA.....	73
ABSTRACT.....	74
INTRODUCTION	75
MATERIALS AND METHODS.....	78
RESULTS	80

	DISCUSSION	82
	LITERATURE CITED	86
4	CONCLUSIONS.....	96

LIST OF TABLES

	Page
Table 2.1: Additional fungicides applied during efficacy trials conducted in the research vineyard at the Georgia Mountain Research and Education Center in Blairsville, GA	53
Table 2.2: List of materials used for experimental treatments.....	54
Table 2.3: Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew fruit severity (2023).	55
Table 2.4: Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew leaf severity (2023)	55
Table 2.5: Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2023).....	56
Table 2.6: Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew fruit severity (2024).	57
Table 2.7: Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew leaf severity (2024).	57
Table 2.8: Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2024).....	58
Table 2.9: Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease incidence (2023 and 2024).	59
Table 2.10: Analysis of the synergy of surfactants combined with sulfur for control of grapevine powdery mildew on leaves as derived through use of the Colby method (2023).....	60

Table 2.11: Analysis of the synergy of surfactants combined with sulfur for control of grapevine powdery mildew on leaves as derived through use of the Colby method (2024)	61
Table 2.12: Analysis of the synergy of surfactants combined with sulfur for control of grapevine powdery mildew on fruit clusters as derived through use of the Colby method (2024)....	62
Table 2.13: Factorial analysis of surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through fruit severity (2024).	63
Table 2.14: Factorial analysis of surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through leaf severity (2024)	63
Table 2.15: Analysis of organosilicone surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through leaf severity (2024).	64
Table 2.16: T-test comparison of percent area coverage of water sensitive cards following application of water through cone or air induction nozzles (2023).	65
Table 2.17: T-test comparison of percent area coverage of water sensitive cards following application of water through cone or air induction nozzles (2024)	65
Table 2.18: T-test comparison of percent area coverage of water sensitive cards following application of water through cone or air induction nozzles with or without a surfactant (2024)	66
Table 3.1: Effect of sulfur application in 2023 on phytotoxicity of grapes when applied at different times	89

Table 3.2: Effect of sulfur application in 2024 on phytotoxicity of grapes when applied at different times	90
---	----

LIST OF FIGURES

	Page
Figure 2.1: Rainfall and grapevine powdery mildew disease severity curves on untreated leaves and fruit (2023 and 2024).	67
Figure 2.2: Rainfall and grapevine powdery mildew disease incidence curves on untreated leaves and fruit (2023 and 2024).	68
Figure 2.3: Examples of spray cards showing differences in percent area coverage (below each card) and visual droplet size/pattern following application of water or water + surfactant and applied through either a cone or an air induction nozzle. For each application, the spray volume per ha was equivalent. For each panel, cards in the left, center, and right positions were attached to the upper surface of the same leaf (same spot on the same vine), respectively..	69
Figure 2.4: Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2023). Treatment dates were: 10 May (pre bloom), 24 May (bloom), 2 Jun (post bloom), 16 Jun (first cover), Jun 30 (second cover), 18 Jul (third cover). Percent fruit area covered by powdery mildew calculated from five clusters per plant. Percent leaf area covered by powdery mildew calculated from 25 leaves per plant. Means followed by the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$). Vertical black lines separate different analyses. Error bars represent standard error..	70

Figure 2.5: Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2024). Treatment dates were: 26 April (pre bloom), 13 May (bloom), 28 May (post bloom), 10 Jun (first cover), 25 Jun (second cover), 9 Jul (third cover). Percent fruit area covered by powdery mildew calculated from five clusters per plant. Percent leaf area covered by powdery mildew calculated from 25 leaves per plant. Bars with the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$). Vertical black lines separate different analyses. Error bars represent standard error.71

Figure 2.6: Analysis of organosilicone surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through leaf severity (2024). Treatment dates were: 26 April (pre bloom), 13 May (bloom), 28 May (post bloom), 10 Jun (first cover), 25 Jun (second cover), 9 Jul (third cover). Percent fruit area covered by powdery mildew calculated from five clusters per plant. Percent leaf area covered by powdery mildew calculated from 25 leaves per plant. Bars with the same letter are not significantly different when comparing each pair using Fishers protected LSD ($P \leq 0.05$). The untreated control was not utilized in the ANOVA or post-hoc test, but is provided or reference. Statistics not shown due to high variability and insignificant ANOVA F-test. Data shown for reference only. Vertical black lines separate different analyses. Error bars represent standard error.72

Figure 3.1: Twenty-one year daily maximum temperatures recorded when the maximum daily (same day) relative humidity (RH) $\geq$ 75% (Three Sisters Vineyard; Dahlonge, Georgia). Any day in which the temperature crosses the black horizontal line (30°C) is displayed in red, and the conditions of RH and temperature could have theoretically resulted in some

level of phytotoxicity on *Vitis vinifera* grapes. Only data from the summer months (June, July, and August) are displayed. Only rarely did these conditions occur for this site, and the duration of the simultaneous events was minimal.91

Figure 3.2: Twenty-one year daily maximum temperatures recorded when the maximum daily (same day) relative humidity (RH) $\geq 75\%$ (Georgia Mountain Research Station, Blairsville, Georgia). Any day in which the temperature crosses the black horizontal line (30°C) is displayed in red, and the conditions of RH and temperature could have theoretically resulted in some level of phytotoxicity on *Vitis vinifera* grapes. Only data from the summer months (June, July, and August) are displayed. These conditions only occurred once for this site.92

Figure 3.3: Damage scale used to assess phytotoxicity (scorch symptoms) on individual grape leaves. Numbers below leaves correspond to percent scorched (necrotic) tissue area.93

Figure 3.4: ‘Cabernet Franc’ vines one week after Microthiol Disperss was applied at 11.2 kg/ha – the maximum single application rate. From left to right: untreated control, 3:00 p.m. application (31.2°C and 55% relative humidity at time of application), and 7:00 p.m. application (26.8°C and 71% relative humidity at time of application). Sulfur applications were conducted on 24 July. Sulfur did not result in phytotoxicity.94

Figure 3.5: Various forms of phytotoxicity observed on hybrid grapes (*Vitis vinifera* × American grape species) following application of Microthiol Disperss applied at 11.2 kg/ha – the maximum single application rate. From left to right: yellowing of leaves of ‘Crimson Cabernet’, scorching on leaves of ‘UCD-07370-84’, and mild marginal necrosis or scorch of ‘Crimson Cabernet’.95

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

History of grape production. Grape production has been a part of human society for thousands of years. It is estimated that grapes were domesticated around 8000 BC and that humans started wide-scale grape production in the Caucasus region around 6000 BC (Gerrath et al. 2015). Today, grape production is a multi-billion-dollar industry with millions of tons produced all around the world (Alston and Sambucci 2019). Thousands of different grape cultivars exist, but by far, the predominant cultivars are derived from the European wine grape, *Vitis vinifera* (Khan et al. 2020). These cultivars are desirable for their high sugar content and excellent juice characteristics which can create very complex wines. However, these cultivars are also known for their disease susceptibility. The age of exploration in the 16th and 17th centuries brought European grapes to the American continent but also brought American diseases to the European continent (Wilcox et al. 2015). Many grapevine diseases wreaked havoc on European vineyards in the 19th century, and those same diseases are targets for disease management programs today. Though effective control options currently exist for most of these pathogens, disease pressure limits profitability of operations worldwide (Strohm et al. 2014). Within the United States, northern Georgia has a growing wine grape industry despite its high disease pressure, as a result high humidity and rainfall. In this region, vineyards and wineries create revenue through agritourism – also dramatically boosting the local economy. Within the past ten years, grapes in Georgia have increased from a farmgate value of \$8 million (USD) in 2011 to over \$31 million in 2021 (Wolfe) and, overall, the grape industry in Georgia generates roughly

\$5.3 billion in total economic activity (Dunham 2022). Though the outlook for expansion of the Georgia wine market is promising, the local climate results in substantial disease pressure, meaning that Georgia producers are constantly fighting an uphill battle relative disease management.

Grapevine powdery mildew. One such major disease is grapevine powdery mildew (GPM), caused by the ascomycete fungus *Erysiphe necator*, an obligate plant parasite that can devastate vineyards if not properly controlled. *E. necator* covers all green plant tissues with its distinctive white mycelium, interfering with light-harvesting for photosynthesis (Lakso et al. 1982). Specialized structures called haustoria penetrate plant tissues and extract nutrients from infected leaves, stems and berries. In addition, cracking can occur in berries following infection, and these open wounds allow for infection by secondary pathogens (Wilcox et al. 2015). GPM thereby generates off-flavors in wine due to both decreased amounts of sugars and soluble solids (Calonnec et al. 2004) and secondary rots. Off-flavors can occur from even limited infections (3-5% incidence), necessitating extensive spray programs for effective disease management (Iland et al. 2011).

GPM was first described in the United States in 1834 and was introduced to Europe in 1845 (Large 1940). In the years following its introduction to Europe, GPM reduced grape yields by as much as 80% in highly infected areas and negatively impacted the quality of the limited crop that could still be harvested. It was not until the discovery of effective control in the form of elemental sulfur applications that wine production was able to recover (Bioletti 1907). Today, powdery mildew is still a threat to wine-producing regions around the world, and elemental sulfur is still used for its control. Sulfur has become one of the most widely used chemicals in the vineyard, making up roughly 30% of fungicide applications (Oliver et al. 2024). One reason for

sulfur's popularity is its multi-site mode of action, making it a useful tool for fungicide resistance management. Its low toxicity to humans and beneficial insects, relative cost-effectiveness, as well as its labeling in some formulations as an organic fungicide, have further contributed to its utility as a major vineyard pesticide (Williams and Cooper 2004). However, elemental sulfur can have phytotoxic effects on grapes (Emmett et al. 2003a) and residual sulfur can impact the vinification process (Kwasniewski et al. 2014). In response, many producers avoid sulfur applications during the latter part of the season when GPM is often observed. In addition, its lower efficacy, as compared to newer systemic fungicide formulations (Warneke et al. 2020), has resulted in a producer bias against sulfur use for GPM management. For these reasons, producers in many parts of the world have recently relied more heavily on systemic, single-site fungicide formulations such as quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs). While these compounds are effective in moderation, their single-site mode of action presents a risk for fungicide resistance development in fungal populations. This risk has unfortunately become a reality, as the overuse of these compounds has led to the development of fungicide-resistant GPM populations in the United States (Miles et al. 2012; Warres 2021) and other countries (Beresford et al. 2016; Taksonyi et al. 2013). In addition, some fungicide classes are under pressure due to movements towards greater pesticide restrictions in light of environmental concerns (Hillocks 2012). Collectively, these issues present a challenge to future GPM management; therefore, efforts to improve the efficacy of sulfur are highly warranted. Sulfur, which does not lead to resistance development (Gallian et al. 2006), can supplement as a resistance-management tool for at-risk fungicide classes by delaying resistance development. As fungicide classes are removed from the market, either through resistance development or through

regulatory actions, the role of sulfur for GPM management will become even more important, as it could once again be one of few remaining active fungicides.

There are commercially applicable methods which have been shown to improve fungicide application, but which have yet to be extensively explored with sulfur. One potential method involves altering application techniques to increase fungicide coverage. Sulfur's contact activity depends on uniform coverage across the entire vine; therefore, improved coverage can correlate with improved efficacy. Several products have the potential to accomplish this, with one category being surfactants. Surfactants are soap-like, hydrophobic compounds which can enhance the spreading and adhesion of individual water droplets by decreasing the cohesion of water molecules. These products are typically tank-mixed with pesticides and result in more continuous and uniform leaf area coverage by the individual water droplets upon contact with the leaf surface (Ellis et al. 1997). Surfactants have been well-studied in relation to herbicide coverage, and though the same principle can be applied to fungicides, studies with surfactants and fungicides are much less prevalent, including those with sulfur (Jibrin et al. 2021).

Another potential avenue for increasing coverage is the use of air induction nozzles. Air induction nozzles increase droplet size by injecting air directly into droplets. This increases the average droplet size, thereby producing a coarser spray and resulting in increased canopy penetration and reduced fungicide drift (Önler et al. 2020). Additionally, these air-filled droplets have an 'exploding' effect when they come into contact with leaves which reduces the number of droplets that bounce off leaf surfaces (McGrath & Landers, 2001). Air induction nozzles could have synergistic effects when combined with surfactants, as the decreased cohesion between water molecules could spread out larger droplets more readily, creating better overall coverage.

The utility of sulfur could also be improved by increasing the number of sulfur sprays applied each growing season. Currently, growers often cease sulfur sprays after mid-summer due to concerns with potential phytotoxicity. The common belief is that spraying sulfur when the temperature exceeds 30°C will lead to phytotoxicity in any grape cultivar. However, recent anecdotal observations from research trials have brought the validity of these phytotoxicity concerns into question. Emmett et al. (2003a) determined that phytotoxicity occurred when relative humidities exceeded 75% and temperatures were above 30°C. However, limited spray trials in northern Georgia involving frequent sulfur sprays over multiple summers showed no phytotoxicity (Warres et al. 2022).

Based on these powdery mildew disease management issues within the winegrape industry, the following objectives have been proposed for my thesis research:

- 1) Determine the efficacy of different nonionic organosilicone surfactant products when combined with sulfur for GPM control,
- 2) Compare air induction and standard cone nozzles with regard to spray coverage and GPM control when using sulfur and two different surfactant products applied with a commercial air blast sprayer, and
- 3) Determine whether temperature and humidity associated with mid- and late-afternoon spray timings are likely to interact with sulfur treatments to cause leaf phytotoxicity in northern Georgia *Vitis vinifera* and hybrid grape vineyards

LITERATURE REVIEW

Powdery mildew and its presence in Georgia. *Erysiphe necator*, the causal pathogen of grapevine powdery mildew (GPM), is an ascomycete fungus which is highly aggressive towards

Vitis vinifera, with varying degrees of aggressiveness towards commercial hybrid grape cultivars, many of which are grown in Georgia (Wilcox et al. 2015). Northern Georgia is home to several species of native grapes, and it has been shown that *E. necator* populations can infect both native and cultivated varieties, to include the most common native grape variety in northern Georgia: *Vitis rotundifolia* (Frenkel et al. 2010). This species of grape, known commonly as muscadine, can often be found along the margins of northern Georgia vineyards, along with one of its close relatives, *Vitis aestivalis*. Both *V. rotundifolia* and *V. aestivalis* have been characterized as more resistant to GPM, though *V. rotundifolia* more so (Staudt, 2015). Nonetheless, powdery mildew populations can still infect these native grape species and closely related commercial cultivars (e.g. Norton and Lenoir [*V. aestivalis* heritage], and muscadines), and are likely undergoing early stages of speciation as a result of host specialization, especially in the case of *V. rotundifolia*-associated *E. necator* (Brewer and Milgroom 2010). Some isolates of *E. necator* have a currently uncharacterized genetic mutation that appears to circumvent the normal hypersensitive response of muscadine vines to *E. necator* spores (Frenkel et al. 2010). Additionally, it has been shown that *E. necator* that can infect *V. rotundifolia* can also infect all other *Vitis* species found in the Eastern U.S., such as *V. riparia*, *V. aestivalis*, and *V. labrusca* (Frenkel et al. 2010).

Due to the adverse effect on wine quality that even limited infections (3-5% incidence) can cause, the disease threshold for GPM is exceedingly low (Iland et al. 2011). GPM's potentially destructive nature makes it a disease of concern wherever grapes are grown, especially on the highly susceptible *V. vinifera* cultivars. *E. necator* overwinters as either mycelium within dormant buds or overwintering structures known as chasmothecia. Primary infections begin in the spring with the germination of ascospores that emerge from chasmothecia

and/or the opening of dormant buds containing mycelium. The primary overwintering structure, chasmothecia, release ascospores which are splash and wind-dispersed to establish primary infections. Chains of asexual conidia are then produced from infected tissues, and these conidia are then wind-dispersed, resulting in additional infections in plants throughout the vineyard. In addition, with bud break, overwintering mycelium from infected buds can grow to cover young shoots, also producing conidia. Secondary infections occur throughout the season. These polycyclic, secondary infections are the primary concern for disease control programs, as the pathogen can persist and multiply throughout the growing season. Although asexual conidia are primarily dispersed by wind, they can also be dispersed by mechanical means during maintenance hedging or other vineyard operations. The time at which chasmothecia are produced will vary from region to region, but they are sometimes observed by mid-summer in Georgia (Gadoury et al. 2012).

GPM control revolves around carefully timed fungicide applications. Sprays will start in the early- to mid-spring as shoots reach ~15.2 cm in length and will typically persist for the duration of the growing season, and even after harvest in humid growing areas. The most critical sprays are applied preventively during the initial infection period just before, during, and after bloom (Warneke et al. 2020). Clusters will develop ontogenic resistance to *E. necator* as they develop, thus early-season applications are crucial while clusters are susceptible to infection (Gadoury et al. 2003). Other aspects of fungicide programs will vary depending on location, as climatic conditions will affect spore germination and ultimately the ability of the fungus to spread (Essling et al. 2021; Gadoury et al. 2012). In Georgia, it is recommended that fungicides be applied at 7- to 14-day intervals until harvest (Brannen 2017). Additionally, post-harvest applications can be used to protect vine health and reduce overwintering inoculum (Redl et al.

2021). Growers typically use a combination of contact protectants and single-site systemic fungicides within their fungicide programs. Elemental sulfur, formulated in various commercial products, is the primary contact protectant used early in the season; its usage is typically reduced during mid-summer due to concerns with phytotoxicity. Other than sulfur, quinone-outside inhibitors (QoI, FRAC 11), sterol demethylation inhibitors (DMI, FRAC 3), succinate dehydrogenase inhibitors (FRAC 7), and azanaphthalenes (FRAC 13) are the primary modes of action used for GPM control in Georgia (Brannen, 2017). With the exception of azanaphthalenes, which are specific to GPM, these fungicides have broad-spectrum activity against several other fungi, and in the case of the QoI fungicides, oomycetes as well.

Due to the use of single-site fungicides, many powdery mildew populations have become resistant to these modes of action (Beresford et al. 2016; Taksonyi et al. 2013). This is true in northern Georgia, where previous surveys have confirmed widespread resistance to both DMIs and QoIs (Warres 2021). Molecular methods for detecting resistance alleles within *E. necator* isolates have been developed, but these methods require lab infrastructure and are mostly impractical for routine grower use. Miles et al. (2021) outlined methods using quantitative polymerase chain reaction (qPCR) to detect the primary mutation which confers QoI resistance, G143A in the cytochrome b gene. DMI resistance has been associated with mutations on the CYP51 gene (Rallo 2013). Moving forward, it is in our best interest to alter our fungicide programs to reduce the onset of fungicide resistance in fungal populations as much as possible. Alternative spray programs using a greater percentage of less vulnerable modes of action, such as that of sulfur, are needed to secure the longevity of single-site systemic formulations.

Sulfur fungicide. Sulfur is the oldest effective fungicide still in use today. Its use can be traced to the ancient Greeks, where Homer found utility in the ‘pest-averting’ properties of sulfur

fumigation (Williams and Cooper 2004). Egyptians also recognized sulfur for its unique medicinal and pesticidal properties, as well as its usefulness in dyes (Cunningham 1935). As the uses for sulfur expanded to gunpowder and then to industrial processes, the production, processing, and shipment of sulfur expanded to become a worldwide industry. As of 2018, the United States is ranked second only to China in sulfur production, and ~911 thousand metric tons of sulfur is associated with agricultural use (Apodaca and Bryden 2018). Agriculturally, it is used primarily as a pesticide but can also be used as a fertilizer and an acidifying soil amendment. Its spectrum of activity as a pesticide includes several species of mites (*Tetranychus urticae*, *T. turkestani*, *Aculops lycopersici*), scab of peach (*Monilinia fructicola*), scab of apple and pear (*Venturia inaequalis*, *V. pirina*), peanut leafspot (*Passalora arachidicola*), and many types of powdery mildews (*Erysiphe* spp., *Sphaerotheca fuliginea*) (Griffith et al. 2015). Though the spectrum of organisms that can be controlled with sulfur is wide, little is known about its specific mode of action. In regards to sulfur's activity against fungal pathogens, several theories have been proposed (Tweedy 1981); however, the most plausible suggests that sulfur inhibits the respiratory functions of the mitochondria within spores (Beffa et al. 1987). The precise cellular mechanisms by which this occurs is unknown, but the observation that GPM is unable to develop resistance to sulfur leads researchers to believe that the mode of action is multi-site (Cooper and Williams 2004). The mechanism by which sulfur is able to enter fungal spores is well described, and this occurs through the volatilization of sulfur deposits on leaf surfaces which pass through the permeable membrane of spores (Bent 1967). Despite sulfur acting as an essential phytoalexin in some plant systems (Williams and Cooper 2004), it does not appear to have systemic effects when taken up by the plant through leaves (Warneke et al. 2022). For this reason, sulfur is considered a contact fungicide.

There are several downsides to sulfur which prevent it from being used at critical spray periods, especially later in the season. Systemic fungicides are far more efficacious and have longer-lasting activity than the contact activity of sulfur. In Georgia (U.S.), sulfur must be applied within a 7- to 14-day spray period to provide effective GPM control on its own, while systemic fungicides are absorbed by the plant and provide protection for extended periods of time after the initial spray (Klittich 2014). As a contact fungicide, residual sulfur is readily washed from the plant surface by precipitation. In addition, sulfur has been reported to cause off-flavors in wine if applied too close to harvest. Residue on berries has been reported to persist and cause rotten egg aromas from hydrogen sulfide formation during the vinification process (Thoukis & Stern 1962). Though these claims are contested (Thomas et al. 1993), the threat alone is sufficient, and producers eliminate sulfur applications within the month prior to harvest (Brannen 2017).

Sulfur has also been reported to cause phytotoxicity at high temperatures and humidities (Emmett et al. 2003a). Fungicidal sulfur products come in many formulations including dusts, wettable powders, dry flowables, wettable granules, and liquid flowables (Emmett et al. 2003b). It is unknown whether different formulations have different effects on efficacy or phytotoxicity. Emmet et al. (2003) found that sulfur can cause phytotoxicity on grape leaves at temperatures exceeding 30°C and humidity exceeding 75%. Symptoms include marginal necrosis on leaf edges and premature defoliation of the vine. It is well-known among grape growers that some grape cultivars are more susceptible to sulfur phytotoxicity than others, primarily hybrid *Vitis* cultivars with American lineages including many cold climate cultivars (Wilcox et al. 2015; McManus et al. 2017). However, the phytotoxic sulfur sensitivity of the more popular *V. vinifera* cultivars has not been well-characterized. Recent studies involving extensive sulfur sprays at the

Georgia Mountain Experiment Station in Blairsville, GA have displayed a surprising lack of phytotoxicity during theoretically conducive conditions (Breedon et al. 2023). Based on current belief, temperature and humidity during sprays would often have been greater than needed for phytotoxicity formation. Additionally, agricultural surfactants were used in combination with sulfur sprays, which has been reported to increase the chance of phytotoxicity (Appah et al. 2020). However, no phytotoxicity was recorded in the preliminary trial. This led us to question whether phytotoxicity is truly a concern within the northern Georgia region. Given the current situation of single-site fungicide resistance within GPM populations, expanding the use of sulfur would be of great utility for fungicide resistance management. If phytotoxicity is found to be a non-issue, sulfur sprays could be extended to later in the season. This would reduce the use of single-site systemic fungicides and prolong their efficacy in the vineyard.

Agricultural surfactants. Agricultural surfactants are soap-like compounds used as adjuvants in spray tanks for improved coverage and absorption of chemicals (Miller and Westra 1998). The utility of surfactants in improving the efficacy of agricultural sprays has been known for more than 50 years (Parr and Norman 1965). The underlying mechanisms behind surfactants involve a complex interaction between water, the active chemical, the nozzle, and the surfactant being used. There are several different classes of surfactants formulated from different compounds, each altering spray characteristics differently. The effects of many popular surfactants have been characterized in lab settings (Holloway et al. 2000), however, the potential unique effects of different surfactant-nozzle-chemical combinations leaves many questions unanswered. Additionally, the properties of surfactants are generally studied in the context of industrial use or herbicide application, leaving gaps in our understanding concerning their utility

in fungicide applications. It is important to understand the basic mechanisms behind surfactants to answer questions concerning them.

Changes to liquid properties can have a multitude of effects on the overall quality of a spray (Ellis et al. 1997). Alterations will cause individual droplets to interact differently with the nozzle, air, and plant surface. Surfactants function by decreasing the cohesion between water molecules, which increases the area droplets spread when they encounter the leaf surface (Miller and Westra 1998). It has also been reported that surfactants can improve the absorption of chemicals into leaves and the retention of sprayed material in the canopy (Holloway and Western 2003; Yao et al. 2014). Many of the biological effects that accompany droplet alternations are not well understood, especially concerning fungicide sprays. This is in part due to research being conducted primarily on herbicide applications. A recent study by Jibrin et al. (2021) tested the interaction between fungicides and surfactants, examining their effects on disease control. They found a potential synergy between the two, though the effect varied depending on the surfactant used. Further study into the extent of these effects is warranted, given the potential utility for fungicide sprays.

Surfactants have promising implications in vineyard settings. Grapes require large fungicide inputs to remain economically viable, so even small changes in fungicide efficacy can translate to large increases in profit. Surfactants are relatively inexpensive and are easy to combine in tank mixes. They also have low reactivity with fungicidal chemicals. Previous research conducted at the Georgia Mountain Experiment Station suggested that surfactant adjuvants could have synergistic effects when combined with sulfur for the control of GPM (Breedon et al. 2023). This is most likely due to the contact activity of sulfur being enhanced by the increased spray coverage provided by the surfactant product. However, only one of the five

surfactant treatments seemed to provide additional GPM control. This inconsistency among surfactants when combined with fungicides is in concordance with the findings of Jibrin et al. (2021), which suggests that different surfactant formulations must be tested thoroughly before any conclusions can be made as to their utility for increasing disease control with sulfur. Given the lack of knowledge pertaining to the utility of surfactant products, there is a need for further research on this topic.

Calculating chemical synergy. Researchers studying the utility of adjuvants in fungicidal sprays have noted the numerical improvement in efficacy of treatments incorporating surfactants (Jibrin et al. 2021; Kierzek and Wachowiak 2003). However, those studies did not find the improved efficacy to statistically differ from treatments without surfactants. Small improvements can be difficult to discern statistically with the few replicates that are typical of agricultural research. Additionally, the interaction of multiple chemicals within a treatment can be confounding. Calculating synergistic effects can shed light on the interaction between different chemicals within a treatment. The methods of calculating chemical synergy outlined by Colby have been used extensively in herbicide research (Colby 1967). The following formula is used to calculate the synergy between two chemicals:

$$E = X + Y - \frac{XY}{100}$$

where X is the control achieved by one fungicide, Y is the control achieved by another fungicide, and E is the expected outcome of spraying the two chemicals combined. The expected outcome is calculated utilizing the outcomes measured through experimentation. When the actual outcome is larger than the expected outcome, the relationship is synergistic. When the actual outcome is smaller than the expected outcome, the relationship is antagonistic. This equation allows researchers to take a closer look at the relationship between chemicals and interactions that

would otherwise go unnoticed. For simple interactions of two chemical compounds in the field, the Colby equation can prove very useful for the prediction of synergy or antagonism, though it can also support the simple additive nature of combinations as well. With replicated trials, statistical analysis can be applied to Colby equation results to validly support the conclusions of synergy or antagonism.

Air induction nozzles. Air blast sprayers are a predominant method of fungicide application for grape growing operations. These sprayers apply fungicide by generating large gusts of wind that carry droplets through the air in the desired direction. Specialized nozzles attached to the outer edge of turbines deliver the product from the spray tank into the surrounding area. The most efficient configurations of this system have long been studied for applications in orchard and vineyard settings (Randall 1971). Adjustments with pressure, turbine speed, application speed, nozzle configuration, and droplet size are well characterized (Ferguson et al. 2016; Wilson et al. 1963). Their relationship with spray efficiency is often estimated through leaf coverage and biological efficacy, though, the effect of coverage will vary between different fungicide classes (fungicides with systemic activity will not be as affected by coverage as contact fungicides). Research involving sprayer coverage often involves water-sensitive cards that imitate leaves and allow visualization of the coverage across them. Analyzing spray patterns using cards has allowed researchers to predict the effectiveness of canopy penetration and overall plant coverage (Salyani et al. 2013).

Different configurations will result in different effects on coverage and, in the case of contact fungicides, biological efficacy. Higher pressures increase the velocity and number of droplets. If a droplet moves too fast, it will bounce off the leaf surface. Alternatively, if pressure is too low, some droplets will not have enough velocity to reach the plant tissue (Ferguson et al.

2016). Pressure will also affect droplet size, with higher pressures creating finer mists (Ranta et al. 2021). Changes in droplet size can also be made by choice of nozzle. Droplet size has several effects on the properties of the spray. Finer sprays (smaller droplets) often yield greater overall coverage, with the downside of off-target drift potential (Prokop and Veverka 2006). Coarser sprays (larger droplets) result in reduced coverage, but sprays can penetrate deeper into the canopies of trees and vines with less drift potential (Önler et al. 2020).

Nozzles are a simple and cheap method to alter spray characteristics without changing the components of the sprayer itself. Innovative nozzle technologies can potentially enhance spray quality. Air induction nozzles (AI nozzles) are engineered to produce large droplets with pockets of air encased within. This is accomplished by a small hole near the base of the nozzle which creates negative pressure and sucks air into the nozzle body (Doruchowski et al. 2017). AI nozzles combine the increased coverage of fine mists with the canopy penetration and drift reduction of coarse mists. Similar coverage to fine mists comes as a result of droplets exploding as they make contact with the leaf surface, covering surrounding tissue with product (McGrath and Landers 2001). Canopy penetration and drift reduction come as a result of the larger droplet size (Wenneker et al. 2005). AI nozzles have also been shown to increase the proportion of the total spray volume reaching the intended target (McArtney and Obermiller 2008). This shows potential for decreasing spray volume and increasing overall fungicide efficiency.

AI nozzles have promising implications within vineyard settings. Previous research has found a positive effect for both drift reduction (Ranta et al. 2021) and canopy penetration (Önler et al. 2020). However, the effect of AI nozzles on fungicide efficacy has never been assessed for grape-growing systems. The current state of systemic fungicide resistance within GPM populations creates an interest in increasing sulfur fungicide efficacy using this technology.

Additionally, AI nozzles have shown a potential synergistic relationship with surfactant adjuvants which could provide even greater effects on sulfur sprays (Kierzek and Wachowiak 2003). The potential benefits of AI nozzles are worthy of further research to identify a better alternative to standard nozzles. Additionally, improved fungicide efficiency has implications for sustainable agricultural practices.

To summarize, the reduction in the number of classes of efficacious synthetic fungicides, either through resistance development in fungal populations or through regulatory action, requires improved efficacy of multi-site chemicals such as sulfur. Resistance in *E. necator* has already, for all practical purposes, removed some fungicide classes for use in *E. necator* control. In addition, winegrape production is a target for new restrictive legislation, given the high fungicide input of vineyard spray programs. Research to improve the efficacy of sulfur is relevant to meet the current and future demands for disease control, especially for highly aggressive diseases such as powdery mildew. The efficacy of sulfur applications could be improved by using additives such as surfactants or by using modified nozzle designs. The utility of sulfur could also be improved among grape growers by providing them with empirical evidence of the potential for phytotoxicity that allows them to utilize sulfur with confidence throughout the growing season. The research presented in this thesis was developed with these goals in mind – ultimately providing winegrape producers with a more thorough understanding of how to best utilize and improve the efficacy of sulfur for control of powdery mildew of wine grapes in the northern Georgia climate.

LITERATURE CITED

- Alston, J. M., and Sambucci, O. 2019. Grapes in the world economy. *The grape genome*, 1-24.
- Apodaca, L., Bryden, B. 2021. 2018 Minerals Yearbook: Sulfur [Advance Release]. *US Geological Survey*.
- Appah, S., Jia, W., Ou, M., Wang, P., and Asante, E. A. 2020. Analysis of potential impact and phytotoxicity of surfactant-plant surface interaction in pesticide application. *Crop protection*, 127, 104961.
- Beffa, T., Pezet, R., and Turian, G. 1987. Multiple-site inhibition by colloidal elemental sulfur (S^0) of respiration by mitochondria from young dormant α spores of *Phomopsis viticola*. *Physiologia Plantarum*, 69(3), 443-450.
- Bent, K. J. 1967. Vapour action of fungicides against powdery mildews. *Annals of Applied Biology*, 60(2), 251-263.
- Beresford, R. M., Wright, P. J., Wood, P. N., and Agnew, R. H. 2016. Sensitivity of grapevine powdery mildew (*Erysiphe necator*) to demethylation inhibitor and quinone outside inhibitor fungicides in New Zealand. *New Zealand Plant Protection*, 69, 1-10.
- Bioletti, F. T. 1907. *Oidium or powdery mildew of the vine*. University of California, College of Agriculture, Agricultural Experiment Station Bulletin No. 186, February.

- Brannen, Phillip. 2017. Powdery and Downy Mildew Recommendations. *UGA Extension Viticulture Blog*, Pest management guidelines. <https://site.extension.uga.edu/viticulture/2017/06/powdery-and-downy-mildew-recommendations/#:~:text=Sulfur%20is%20an%20inexpensive%20and,of%20sulfur%20should%20be%20considered.&text=Strobilurin%20fungicides%20are%20locally%20systemic,excellent%20activity%20against%20downy%20mildew.>
- Breeden, S., W. Sanders, C. Hawkins, P. Brannen, C. Johnston, and R. Covington. 2023. Efficacy of Microthiol Disperss sulfur and various surfactants for control of powdery mildew of grape, 2022. *Plant Disease Management Reports* 17:PF016.
- Brewer, M. T., and Milgroom, M. G. 2010. Phylogeography and population structure of the grape powdery mildew fungus, *Erysiphe necator*, from diverse *Vitis* species. *BMC Evolutionary Biology*, 10(1), 1-13.
- Calonnec, A., Cartolaro, P., Poupot, C., Dubourdieu, D., & Darriet, P. 2004. Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. *Plant pathology*, 53(4), 434-445.
- Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. *Weeds*, 15(1), 20-22.
- Cooper, R. M., and Williams, J. S. 2004. Elemental sulphur as an induced antifungal substance in plant defence. *Journal of experimental botany*, 55(404), 1947-1953.
- Cunningham, W. A. 1935. Sulfur. I. *Journal of Chemical Education*, 12(1), 17.

- Doruchowski, G., Świechowski, W., Masny, S., Maciesiak, A., Tartanus, M., Bryk, H., and Hołownicki, R. 2017. Low-drift nozzles vs. standard nozzles for pesticide application in the biological efficacy trials of pesticides in apple pest and disease control. *Science of the Total Environment*, 575, 1239-1246.
- Dunham, John. 2022. Georgia Economic Impact Study 2022. Wineamerica.org.
<https://wineamerica.org/economic-impact-study/georgia-wine-industry/>
- Ellis, M. B., Tuck, C. R., and Miller, P. C. H. 1997. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. *Crop protection*, 16(1), 41-50.
- Emmett, B., Rozario, S., and Hawtin, J. 2003a. Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines. *Final Report to GWRDC, Project NumberL DAV*, 98(1), 220.
- Emmett, B., Wicks, T., and Magarey, P. 2003b. 3. Sulphur formulations, particle size and activity—a review. *Strategic Use of Sulphur in Integrated Pest and Disease Management (IPM) Programs for Grapevines*, 52.
- Essling, M., McKay, S., and Petrie, P. R. 2021. Fungicide programs used to manage powdery mildew (*Erysiphe necator*) in Australian vineyards. *Crop Protection*, 139, 105369.
- Ferguson, J. C., Hewitt, A. J., and O'Donnell, C. C. 2016. Pressure, droplet size classification, and nozzle arrangement effects on coverage and droplet number density using air-inclusion dual fan nozzles for pesticide applications. *Crop Protection*, 89, 231-238.

- Frenkel, O., Brewer, M. T., and Milgroom, M. G. 2010. Variation in pathogenicity and aggressiveness of *Erysiphe necator* from different *Vitis* spp. and geographic origins in the eastern United States. *Phytopathology*, 100(11), 1185-1193.
- Gadoury, D. M., Seem, R. C., Ficke, A., & Wilcox, W. F. 2003. Ontogenic resistance to powdery mildew in grape berries. *Phytopathology*, 93(5), 547-555.
- Gadoury, D. M., Cadle-Davidson, L. A. N. C. E., Wilcox, W. F., Dry, I. B., Seem, R. C., and Milgroom, M. G. 2012. Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular plant pathology*, 13(1), 1-16.
- Gallian, J., Miller, J. S., and Nolte, P. 2006. *Managing fungicide resistance* (Vol. 1130). University of Idaho Extension. <https://extension.okstate.edu/fact-sheets/fungicide-resistance-management.html>
- Gerrath, J., Posluszny, U., Melville, L., Gerrath, J., Posluszny, U., and Melville, L. 2015. Humans and grapes. *Taming the Wild Grape: Botany and horticulture in the Vitaceae*, 103-114.
- Griffith, C. M., Woodrow, J. E., and Seiber, J. N. 2015. Environmental behavior and analysis of agricultural sulfur. *Pest management science*, 71(11), 1486-1496.
- Hillocks, R. J. 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, 31(1), 85-93.

- Holloway, P. J., Ellis, M. B., Webb, D. A., Western, N. M., Tuck, C. R., Hayes, A. L., and Miller, P. C. H. 2000. Effects of some agricultural tank-mix adjuvants on the deposition efficiency of aqueous sprays on foliage. *Crop Protection*, 19(1), 27-37.
- Holloway, P. J., and Western, N. M. 2003. Tank-mix adjuvants and pesticide residues: some regulatory and quantitative aspects. *Pest Management Science: formerly Pesticide Science*, 59(11), 1237-1244.
- Iland, P., Dry, P., Proffitt, T., and Tyerman, S. 2011. *The grapevine: from the science to the practice of growing vines for wine*. Patrick Iland Wine Promotions.
- Jibrin, M. O., Liu, Q., Jones, J. B., and Zhang, S. 2021. Surfactants in plant disease management: A brief review and case studies. *Plant Pathology*, 70(3), 495-510.
- Johansen, N. S., Moen, L. H., and Egaas, E. 2007. Sterol demethylation inhibitor fungicides as disruptors of insect development and inducers of glutathione S-transferase activities in *Mamestra brassicae*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 145(3), 473-483
- Kierzek, R., and Wachowiak, M. 2003. Effect of nozzle types and adjuvants on the leaf coverage and biological efficacy of fungicides in potato. *Journal of Plant Protection Research*, 43(2).
- Khan, N., Fahad, S., Naushad, M., and Faisal, S. 2020. Grape production critical review in the world. Available at SSRN 3595842.

- Klittich, C. J. 2014. Fungicide mobility and the influence of physical properties. *Retention, uptake, and translocation of agrochemicals in plants*, 95-109.
- Kwasniewski, M. T., Sacks, G. L., and Wilcox, W. F. 2014. Persistence of elemental sulfur spray residue on grapes during ripening and vinification. *American Journal of Enology and Viticulture*, 65(4), 453-462.
- Lakso, A. N., Pratt, C., Pearson, R. C., Pool, R. M., Seem, R. C., and Welser, M. J. 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (powdery mildew). *Phytopathology*, 72(2), 232-236.
- Large, E. C. 1940. The advance of the fungi. *The advance of the fungi*.
- McArtney, S. J., and Obermiller, J. D. 2008. Comparative performance of air-induction and conventional nozzles on an axial fan sprayer in medium density apple orchards. *HortTechnology*, 18(3), 365-371.
- McGrath, M. T., and Landers, A. 2001. Evaluating New Nozzles and an Air Assist Sprayer for Improving Spray Coverage and Powdery Mildew Control on Underleaf Surfaces. *Final project report to the nys IPM program, agricultural IPM 200-2001*. <https://ecommons.cornell.edu/server/api/core/bitstreams/d993dd2b-b070-4bf4-8f92-3ac999440695/content>
- McManus, P. S., Kartanos, V., and Stasiak, M. 2017. Sensitivity of cold-climate wine grape cultivars to copper, sulfur, and difenoconazole fungicides. *Crop protection*, 92, 122-130.
- Miller, P., and Westra, P. 1998. How surfactants work no. 0.564. *Colorado State University Cooperative Extension, Crop Fact Sheet*.

- Miles, L. A., Miles, T. D., Kirk, W. W., and Schilder, A. M. C. 2012. Strobilurin (QoI) resistance in populations of *Erysiphe necator* on grapes in Michigan. *Plant disease*, 96(11), 1621-1628.
- Miles, T. D., Neill, T. M., Colle, M., Warneke, B., Robinson, G., Stergiopoulos, I., and Mahaffee, W. F. 2021. Allele-specific detection methods for QoI fungicide-resistant *Erysiphe necator* in vineyards. *Plant Disease*, 105(1), 175-182.
- Oliver, C., Cooper, M., Ivey, M. L., Brannen, P., Miles, T., Lowder, S., Mahaffee, W., and Moyer, M. M. 2024. Fungicide use patterns in select United States wine grape production regions. *Plant Disease*, 108(1), 104-112.
- Önler, E., Çelen, İ. H., and Avcı, G. G. 2020. Evaluation of Residue Distribution of Spraying Nozzles Produced for the Prevention of Spray Drift. *International Journal of Innovative Approaches in Agricultural Research 2020*, Vol. 4 (2), 242-250.
- Parr, J. F., and Norman, A. G. 1965. Considerations in the use of surfactants in plant systems: A review. *Botanical Gazette*, 126(2), 86-96.
- Prokop, M., and Veverka, K. 2006. Influence of droplet spectra on the efficiency of contact fungicides and mixtures of contact and systemic fungicides. *Plant Protection Science*, 42(1), 26-33.
- Rallos, L. E. E. 2013. *Characterizing resistance of Erysiphe necator to fungicides belonging to the quinone outside inhibitors and demethylation inhibitors* (Doctoral dissertation, Virginia Tech).

- Randall, J. M. 1971. The relationships between air volume and pressure on spray distribution in fruit trees. *Journal of Agricultural Engineering Research*, 16(1), 1-31.
- Ranta, O., Marian, O., Muntean, M. V., Molnar, A., Ghețe, A. B., Crișan, V., Stanila S., and Rittner, T. 2021. Quality analysis of some spray parameters when performing treatments in vineyards in order to reduce environment pollution. *Sustainability*, 13(14), 7780.
- Redl, M., Sitavanc, L., Hanousek, F., and Steinkellner, S. 2021. A single out-of-season fungicide application reduces the grape powdery mildew inoculum. *Crop Protection*, 149, 105760.
- Salyani, M., Zhu, H., Sweeb, R., and Pai, N. 2013. Assessment of spray distribution with water-sensitive paper. *Agricultural Engineering International: CIGR Journal*, 15(2), 101-111.
- Staudt, G. 2015. Evaluation of resistance to grapevine powdery mildew (*Uncinula necator* [Schw.] Burr., anamorph *Oidium tuckeri* Berk.) in accessions of *Vitis* species. *VITIS-Journal of Grapevine Research*, 36(3), 151.
- Strohm, K., Dirksmeyer, W., and Garming, H. 2014. International analysis of the profitability of wine grape production. In *8th International Conference of the Academy of Wine Business Research*. http://academyofwinebusiness.com/wp-content/uploads/2014/07/BM04_Dirksmeyer_Walter.pdf
- Taksonyi, P., Kocsis, L., Matyas, K. K., and Taller, J. 2013. The effect of quinone outside inhibitor fungicides on powdery mildew in a grape vineyard in Hungary. *Scientia horticulturae*, 161, 233-238.

- Thomas, C. S., Boulton, R. B., Silacci, M. W., and Gubler, W. D. 1993. The effect of elemental sulfur, yeast strain, and fermentation medium on hydrogen sulfide production during fermentation. *American Journal of Enology and Viticulture*, 44(2), 211-216.
- Thoukis, G., and Stern, L. A. 1962. A review and some studies of the effect of sulfur on the formation of off-odors in wine. *American Journal of Enology and Viticulture*, 13(3), 133-140.
- Tweedy, B. G. 1981. Inorganic sulfur as a fungicide. In *Residue reviews: residues of pesticides and other contaminants in the total environment* (pp. 43-68). New York, NY: Springer New York.
- Warneke, B., Thiessen, L. D., and Mahaffee, W. F. 2020. Effect of fungicide mobility and application timing on the management of grape powdery mildew. *Plant disease*, 104(4), 1167-1174.
- Warneke, B. W., Nackley, L. L., and Pscheidt, J. W. 2022. Management of grape powdery mildew with an intelligent sprayer and sulfur. *Plant Disease*, 106(7), 1837-1844.
- Warres, B. E. 2021. *Fungicide efficacy and resistance management for Erysiphe necator* (Doctoral dissertation, University of Georgia).
- Warres, B., S. Breeden, W. Sanders, T. Rios, A. Cheh, P. Brannen, D. Rogers, and R. Covington. 2022. Assessment of interactions between sulfur and DMI fungicides to control powdery mildew in the presence of a DMI-resistant *Erysiphe necator* population in Georgia, 2021. Plant Disease Management Reports 16:PF010.

- Wenneker, M., Heijne, B., and Van de Zande, J. C. 2005. Effect of air induction nozzle (coarse droplet), air assistance and one-sided spraying of the outer tree row on spray drift in orchard spraying. *Annual Review of Agricultural Engineering*, 4(1), 116-128.
- Wilcox, W. F., Gubler, W. D., and Uyemoto, J. K., eds., 2015. Compendium of grape diseases, disorders, and pests, 2nd Ed. APS Press, St. Paul, MN.
- Williams, J. S., and Cooper, R. M. 2004. The oldest fungicide and newest phytoalexin—a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathology*, 53(3), 263-279.
- Wilson, J. D., Hedden, O. K., and Sleesman, J. P. 1963. Spray droplet size as related to disease and insect control on row crops. *Ohio Agricultural Experiment Station*. Research bulletin 945.
- Wolfe, K.L., and Shepherd, T. 2012. 2011 Georgia Farm Gate Value Report. Athens, GA: Center for Agribusiness & Economic Development, University of Georgia.
- Wolfe, K.L., and Shepherd, T. 2022. 2021 Georgia Farm Gate Value Report. Athens, GA: Center for Agribusiness & Economic Development, University of Georgia.
- Yao, C., Myung, K., Wang, N., and Johnson, A. 2014. Spray retention of crop protection agrochemicals on the plant surface. In *Retention, Uptake, and Translocation of Agrochemicals in Plants* (pp. 1-22). American Chemical Society.

CHAPTER 2

UTILITY OF SURFACTANTS AND AIR INDUCTION NOZZLES FOR IMPROVED SULFUR EFFICACY WHEN MANAGING POWDERY MILDEW OF GRAPE

Sanders, W., Brewer, M., Lowder, S., Severns, P., and Brannen, P.M. 2024. To be submitted to *Plant Disease*.

ABSTRACT

Grapevine powdery mildew (GPM), caused by the fungal pathogen *Erysiphe necator*, is an economically important disease of *Vitis vinifera*. Elemental sulfur is a contact fungicide used extensively in vineyards for powdery mildew management. Three non-ionic organosilicone surfactants (Cohere, Hi-Wett, and Silwet L-77) were tank-mixed in a standard sulfur spray regimen in northern Georgia (U.S.) on a block of ‘Chardonnay’ vines in 2023 and 2024 to test their ability to increase disease suppression when combined with sulfur. In a separate trial on the same site using a block of ‘Merlot’ vines, sulfur was also applied with air induction nozzles to determine whether application of sulfur, with and without surfactants, through an air induction nozzle would provide better efficacy than a cone nozzle. Surfactants applied alone reduced GPM severity on both fruit and leaves by an average of 13.6% and 12.2%, respectively. When surfactants were tank-mixed with sulfur, results were variable, with Silwet L-77 providing the most consistent improvement in sulfur efficacy against GPM severity on leaves and fruit for both years. However, surfactants improved disease control over sulfur alone by an average of 3.5% on leaves and 14.5% on fruit. In 2023, there were positive synergistic interactions of sulfur and each surfactant for disease control on leaves, but these synergistic interactions were generally not observed in 2024. Silwet L-77 did show a synergistic interaction with sulfur for disease control on fruit in 2024. Applying sulfur through air induction nozzles provided less disease control than that provided by traditional cone nozzles and, though difficult to explain, surfactants provided more consistent improvement in disease control when using cone nozzles as well. Water-sensitive cards placed within a vine canopy were used to examine coverage patterns of nozzle types. Though the coverage provided by the nozzles was similar, the differing spray patterns

could explain the differences in observed efficacy. Overall, we found that sulfur efficacy for control of GPM was improved through tank-mixing with non-ionic organosilicone surfactants, while air induction nozzles did not improve GPM disease control over cone nozzles.

Keywords: sulfur, surfactant, grape, *Erysiphe necator*, disease management, fungicide

INTRODUCTION

Grapevine powdery mildew (GPM), caused by the obligate biotroph Ascomycota fungus *Erysiphe necator*, is an aggressive disease known for its destructive capabilities on *Vitis vinifera*. GPM is a polycyclic disease that can infect any green tissues, causing high yield losses if not properly controlled (Bioletti 1907). Incidence thresholds for this disease are often near zero due to the effect that even limited infections can have on berry quality (Iland et al. 2011). Therefore, disease management spray programs require incorporation of highly efficacious fungicides, and fungicides provide the primary control mechanism used in modern vineyard operations. It is a common practice to use fungicide classes with multiple modes of action to combat GPM. One of the most common contact materials applied for GPM control is sulfur, which is the world's oldest effective fungicide (Oliver et al. 2024). The first account of sulfur use as a fungicide came from the ancient Greeks when Homer mentioned the “pest-averting” qualities of sulfur fumigation (Williams and Cooper 2004). Sulfur was first found to be effective against GPM in the early 1850s, consequently saving the collapsing wine industry in Europe after GPM was introduced into France (Bioletti 1907). Sulfur is used today in conjunction with modern fungicide formulations, largely because of its low environmental toxicity and its effectiveness as a fungicide resistance management tool.

Fungicide resistance management is a primary concern among wine producers in northern Georgia, as well as throughout the world. Previous surveys of *E. necator* populations in northern Georgia have revealed widespread fungicide resistance to two major modes of action: quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs) (Warres 2021). Fungicides with these modes of action are crucial for effective disease management programs, and their loss of effectiveness is a threat to grape production in northern Georgia. Because sulfur-resistant populations of *E. necator* have never been detected, it could be a useful tool for resistance management. Though the mode of action for sulfur is not completely understood, it is suggested that sulfur interferes with the mitochondria of *E. necator* spores by inhibiting the oxidation of key metabolites and that the interaction is multi-site (Beffa et al. 1987; Williams and Cooper 2004).

Given the advantageous characteristics and importance of sulfur for GPM management, the efficacy of sulfur applications should be maximized in vineyard settings. There are several potential methods to accomplish this, one being the addition of adjuvants for routine sprays of sulfur. Agricultural adjuvants are formulations designed to enhance the activity or other properties of a pesticide mixture (Holland 1996). Typical spray properties targeted by different adjuvants include spreading, sticking, and/or improving uptake of pesticides (Emmett et al. 2003). Surfactants are a class of soap-like adjuvants that alter the physical characteristics of water by decreasing the cohesion between molecules. This can create a droplet-spreading effect, increasing the overall coverage across a plant (Miller and Westra 1998). Surfactants also have anti-microbial properties (Tawfik et al. 2015; Falk 2019). Though surfactants have proven useful in the application of herbicides (Kirkwood 1993), research concerning surfactant utility for fungicide application is limited (Abbott and Beckerman 2018; Emmett et al. 2003; Steurbaut

1993). Breeden et al. (2023) determined that surfactant adjuvants could have a positive effect when combined with sulfur for the control for GPM; however, only one of the five surfactant types tested, the organosilicone surfactant Hi-Wett, provided increased efficacy over sulfur alone. Surfactants can also provide independent and direct efficacy against powdery mildew (Jibrin et al. 2021), so studies with surfactants and fungicides, such as sulfur, should attempt to discern whether the impact of the surfactant is due to an interaction with the fungicide (e.g. spreading the fungicide on the plant surface), an additive effect, or both.

The Colby method (Colby 1967) can be used to detect synergistic or antagonistic relationships between two chemicals using the calculation: observed response – expected response. The observed response is the resulting percent disease control when two fungicides are tank mixed and sprayed together. The expected response is the projected percent disease control calculated from the resulting percent disease control of the two fungicides sprayed alone. The following equation is used to calculate the expected response: $E = X + Y - \frac{XY}{100}$, where X is the percent disease control of fungicide X and Y is the percent disease control of fungicide Y. When interpreting the results of the equation, a negative number represents an antagonistic response, and a positive number represents a synergistic response. This method is useful for determining synergistic interactions between fungicides (Gisi 1996), however, materials with multiple mechanisms of interaction can cause difficulties in interpretation. Surfactants, for example, can cause direct control through anti-microbial interaction (Tawfik et al. 2015; Falk 2019), as well as indirect control through fungicide spreading (Miller and Westra 1998).

Another potential technique to enhance efficacy through spray characteristics is the use of alternative spray nozzles. Typical vineyard operations will use air blast sprayers equipped with fitted nozzles to apply pesticides. Changes in pressure, turbine speed, application speed, nozzle

configuration, and droplet size will alter the coverage of the spray (Ferguson et al. 2016; Wilson et al. 1963). Droplet size can easily be changed by selecting sprayer nozzles that produce finer or coarser sprays, depending on the desired outcome. Finer sprays (smaller droplets) often yield greater coverage while increasing off-target drift potential (Prokop and Veverka 2006). Coarser sprays (larger droplets) often reduce coverage, but with the added benefits of greater canopy penetration and less drift potential (Önler et al. 2020). Air induction (AI) nozzles are specialized nozzles engineered to capture the canopy penetration ability of larger droplets with the coverage efficiency of smaller droplets. AI nozzles inject air into each droplet, which creates an ‘exploding’ effect when they make contact with leaves. This prevents droplets from bouncing off surfaces, resulting in similar coverage to finer sprays (McGrath and Landers 2001). AI nozzles have also been shown to increase the total proportion of the spray volume reaching the plant (McArtney and Obermiller 2008). The potential utility of spray alterations has promising implications in vineyard settings.

Sulfur has been an important fungicide for GPM management since the introduction of *E. necator* to Europe, but with resistance development in major fungicide classes, increasing the utility and efficacy of sulfur is important to sustainable production of wine grapes in Georgia and elsewhere. Moreover, the potential to improve spray programs with surfactants warrants further study. The objectives of this study were to: (1) evaluate the effectiveness of surfactants in improving GPM control in combination with a standard sulfur spray regimen; and (2) evaluate the effectiveness of air induction nozzles in comparison to standard cone nozzles in controlling GPM.

MATERIALS AND METHODS

Efficacy trials for evaluation of sulfur and surfactants

Based on the success of the combination of sulfur and the organosilicone surfactant used in the study by Breeden et al. (2023), three surfactant formulations were selected for testing with sulfur in field trials conducted in 2023 and 2024. Cohere (Helena Agri-Enterprises, LLC, Collierville, TN), Hi-Wett (Loveland Products, Inc., Loveland, CO), and Silwet L-77 (Helena Agri-Enterprises, LLC, Collierville, TN) surfactants were applied alone and in combination with the sulfur product Microthiol Disperss (United Phosphorus, Inc., Cary, NC). Trials were conducted at the Georgia Mountain Research Center (GMRC) located near Blairsville, GA on a block of mature ‘Chardonnay’ grapevines. The trial utilized a randomized complete block design with treatments arranged in a 2×4 factorial, and main effects included sulfur levels (with/without) and surfactants. Each individual treatment was replicated across five plots, and each plot consisted of a single vine. Unsprayed buffer plants prevented spray drift and allowed for increased powdery mildew disease pressure. Treatments were: (1) an untreated control [no material applied], (2) Microthiol Disperss [11.2 kg/ha], (3) Hi-Wett [0.69 L/ha], (4) Cohere [0.58 L/ha], (5) Silwet L-77 [1.17 L/ha], (6) Microthiol Disperss [11.2 kg/ha] + Hi-Wett [0.69 L/ha], (7) Microthiol Disperss [11.2 kg/ha] + Cohere [0.58 L/ha], and (8) Microthiol Disperss [11.2 kg/ha] + Silwet L-77 [1.17 L/ha]. All treatments were applied to run-off with a CO₂-pressurized backpack sprayer (R & D Sprayers, Opelousas, LA) equipped with a single TT11002 (TeeJet Technologies, Wheaton, IL) nozzle, delivering 475 L/ha total spray volume at 172.4 kPa. Different 2 L plastic bottles were used for each product, and these were thoroughly cleaned between uses. In 2023, treatment applications were made six times (10 May, 24 May, 2 June, 16 June, 30 June, and 18 July) corresponding to the pre-bloom (~E-L 17-18), bloom (~E-L 23), post-bloom (~E-L 27), first (~E-L 29), second (~E-L 31), and third (~E-L 33) cover sprays. In

2024, treatments were applied six times (26 April, 13 May, 28 May, 10 June, 25 June, and 9 July), each application corresponding to the same plant phenology in the first year. Cultural practices were representative of those used in commercial vineyards in northern Georgia (e.g. insecticides, weed control, vine training, etc.). To protect against other pathogens that would compromise the trial results, maintenance sprays (Table 2.1) were applied every two weeks from bud break until pre-harvest with a John Bean Redline 537T Air Blast sprayer (Durand-Wayland, Inc., LaGrange, GA) at 473 L/ha total spray volume.

Efficacy of air induction nozzles versus standard cone nozzles and interactions with surfactants

Two sets of airblast sprayer nozzles were evaluated for differences in powdery mildew control when spraying Microthiol Disperss and two surfactant products. A standard airblast sprayer manifold (CDP 100, Durand Wayland, LaGrange, GA) was equipped with eight TX-VK6 hollow cone spray tip nozzles (TeeJet Technologies, Wheaton, IL) on one side of the sprayer and eight AITXA80VK air induction spray tip nozzles (TeeJet Technologies, Wheaton, IL) on the other side. Treatments were applied by making one pass on each side of each treatment vine corresponding to the side of the tractor with the correct spray tip for the treatment. In addition to testing the impact of these nozzles on the efficacy of sulfur, the trial was also designed to review the interaction of nozzles with surfactants when applying sulfur. As such, the experimental design was a randomized complete block with treatments arranged in a 2×3 factorial (main effects of nozzle type and surfactants), with each individual treatment replicated across five plots. Products were tested at the Georgia Mountain Research Center on a block of ‘Merlot’ vines. Treatments included: (1) an untreated control [no sulfur applied and not part of

the factorial arrangement], (2) Microthiol Disperss [11.2 kg/ha] [with cone nozzle], (3) Microthiol Disperss [11.2 kg/ha] + Hi-wett [0.69 L/ha] [with cone nozzle], (4) Microthiol Disperss [11.2 kg/ha] + Cohere [0.58 L/ha] [with cone nozzle], (5) Microthiol Disperss [11.2 kg/ha] [with air induction nozzle], (6) Microthiol Disperss [11.2 kg/ha] + Hi-wett [0.69 L/ha] [with air induction nozzle], and (7) Microthiol Disperss [11.2 kg/ha] + Cohere [0.58 L/ha] [with air induction nozzle]. The tractor was calibrated to 1700 RPM and 150 PSI at a constant speed of 3.2 kph. Treatments were applied at the maximum labeled rates calculated to correspond with a 475 L/ha spray volume. Treatments were applied six times (26 April, 13 May, 28 May, 10 June, 25 June, 9 July) corresponding to the pre-bloom, bloom, post-bloom, first, second, and third cover sprays. Cultural practices were again representative of those used in commercial vineyards in northern Georgia, and additional pest management products were applied with an airblast sprayer at a rate of 475 liters per hectare. All relevant foliage and bunch diseases were controlled through multiple maintenance applications (Table 2.1).

Coverage of air induction nozzles versus standard cone nozzles on water-sensitive cards

To investigate differences between spray coverage of air induction and cone nozzles, 76.2 mm x 50.8 mm water-sensitive cards (TeeJet Technologies, Wheaton, IL) were used in trials conducted in 2023 and 2024. The additional effect of surfactants was tested in 2024 only. Trials were conducted at the GMRC on mature ‘Chardonnay’ vines. Nozzle configurations and sprayers, and tractor calibration were consistent with those of the efficacy trial. In the 2023 test, five vines were selected, and three cards per vine were attached to leaves within the canopy using clips. Card positions were selected to be near the center of the canopy with cards placed on the upper side of leaves. The vines were then sprayed with water through TX-VK6 hollow cone

spray tip nozzles at 475 L/ha. After the cards were removed and stored, new cards were attached in the same position on the same leaves and then sprayed with the same air blast sprayer equipped with AITXA80VK air induction spray tip nozzles.

For 2024, nozzle configuration and tractor calibration were consistent with the previous air induction nozzle test and spray coverage was assessed similarly, but the test was expanded to assess surfactant coverage when using each nozzle type. Ten cards were attached to leaves located both near the central canopy and near the outer edges of a single vine using clips. The vines were then sprayed with water, again at 475 L/ha, through TX-VK6 hollow cone spray tip nozzles. After the cards were removed and stored, new cards were attached in the same position on the same leaves and then sprayed with the same air blast sprayer equipped with AITXA80VK air induction hollow spray tip nozzles. This process was repeated using the same basic methodology, but with the surfactant Hi-Wett incorporated into the tank at a rate of 0.7 L/ha. Between treatment applications, sprayed cards were collected, vines were allowed to dry, and then new cards were placed in the same locations in the vine or on the scaffold. To determine percent area coverage on cards from both years, cards were scanned using Evernote scannable version 2.5 (Evernote Corporation, Redwood City, CA), and assessed using ImageJ 1.54g (National Institute of Health, USA).

Disease Assessment

Once GPM was established in the field, efficacy ratings were conducted for each trial in each year. For leaf ratings (destructive sampling), 25 representative leaves were collected from each plot, and GPM mean leaf incidence (% of leaves infected) and severity (% of leaf area infected) were recorded through visual assessment. For fruit ratings (non-destructive sampling),

five representative clusters were rated in each plot, and mean fruit incidence (% of fruit clusters infected) and severity (% of fruit cluster area infected) were recorded through visual assessment. All visual assessments were conducted by the same individual during the course of the trials, and GPM severity scales were used during analysis to reduce visual biases. In 2023, leaf and fruit ratings were both conducted on 20 July, 27 July, and 9 August. In 2024, leaf ratings were conducted on 28 June, 5 July, 12 July, and 19 July and fruit ratings were conducted on 14 June, 21 June, 28 June, 5 July, 12 July, and 19 July for both the surfactant/sulfur and nozzle/sulfur efficacy trials. At the end of each season, the area under the disease progress curve (AUDPC) in units of percent-days was generated for each variable using the following formula: $A_k =$

$$\sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i) \text{ (Simko and Piepho 2012).}$$

Data analysis

Data was tested for heteroscedasticity using Levene's test and, when necessary, transformed prior to analysis in order to make treatment variances homogeneous. For efficacy trials, statistical analyses were conducted in SAS v. 9.4 (SAS Institute, Cary, NC). An analysis of variance (ANOVA) was used to determine significant differences, and Fisher's protected least significant difference ($P \leq 0.05$) was used for a post-hoc analysis. In addition, positive or negative synergistic effects of sulfur in combination with surfactants was calculated using the Colby method (Colby 1967), and both chi-square and one-tailed t-tests were conducted in Excel (Microsoft Corp., Redmond, WA) to determine if the synergistic or antagonistic interactions were statistically significant. When comparing water-sensitive card data for the nozzle comparison trial, a one-tailed paired t-test was used to test the hypothesis that card coverage with a surfactant is greater than card coverage without a surfactant. A two-tailed, paired t-test analyses

was used to test the card coverage between nozzle types. All paired t-test analyses were conducted in Excel.

RESULTS

Surfactant efficacy

GPM disease levels were sufficient to separate treatments during both 2023 and 2024, but they were more elevated and appeared earlier during the 2024 season. Disease incidence on leaves on untreated vines reached an average of 97.2% and 99.2% by the end of the trials in 2023 and 2024, respectively (Table 2.9). All efficacy trials were conducted with factorial analyses, and factorial tables are presented for the AUDPCs for each of the specific field trials (Tables 2.3, 2.4, 2.6, and 2.7). The independent main effects data is also presented in separate tables for leaf and fruit severity and AUDPCs for each rating date (Tables 2.5, 2.8, and 2.9; Figs. 2.4 and 2.5), to show the impact of the individual treatments over time. Fruit incidence data is not presented, as fruit incidence was nearly 100% when data collection was initiated in all trials.

Treatments containing Microthiol Disperss (sulfur) provided significant efficacy against powdery mildew on leaves and fruit in both years ($P \leq 0.05$; Tables 2.5, 2.8, and 2.9; Figs. 2.4 and 2.5). For example, at the point at which sulfur sprays were terminated, sulfur had reduced disease severity on fruit by 69% and 41% in 2023 and 2024, respectively. When used alone, all three surfactants decreased the severity of powdery mildew on leaves and fruit. However, Silwet was consistently the most efficacious surfactant in solo applications, with disease control levels approaching those of sulfur in 2023 (Tables 2.5, 2.8, and 2.9; Figs. 2.4 and 2.5). When tank-mixed with sulfur, the addition of surfactants decreased disease incidence and severity over that of sulfur alone (Tables 2.3, 2.4, 2.6, and 2.7), though the degree of increased efficacy varied

among surfactants. Silwet provided the best and most consistent overall efficacy when mixed with sulfur, whereas Cohere, and to a lesser degree Hi-Wett were not as consistent for both years in their enhancement of GPM control. An analysis for synergistic or antagonistic interactions using the Colby method (Colby 1967), revealed that all surfactants tested had a synergistic interaction with sulfur for the 2023 data on leaves (Table 2.10). When comparing the differences in observed versus expected values for disease control, both chi-square and one-tailed t-tests showed some synergistic interactions of Cohere or Hi-Wett in combination with sulfur in 2023 (Table 2.10). For example, Hi-Wett in combination with sulfur synergistically reduced GPM incidence and severity on leaves. However, though Silwet provided the greatest overall efficacy when combined with sulfur, the synergistic interactions were not as great as those with Cohere and Hi-Wett, with Silwet only showing statically significant synergistic interactions with the chi-square test for disease incidence. The same analysis was conducted in 2024 for both leaf and fruit incidence and severity, but the only statistically significant synergistic interaction detected was that of fruit severity when Silwet was combined with sulfur ($P = 0.00012$ for chi-square and 0.019 for a one-tailed t-test; Table 2.12).

Air induction nozzle efficacy

The nozzle comparison trial was only conducted in 2024, but it also further examined the use of surfactants. Disease levels when applying sulfur sprays through air induction nozzles were greater than those observed when using standard cone nozzles ($P = 0.0016$; Table 2.14), regardless of whether surfactants were utilized. For example, when applied with cone nozzles, sulfur combined with either Hi-Wett or Cohere provided lower fruit disease severity levels than sulfur alone ($P = 0.0005$; Table 2.15; Fig 2.6), whereas disease levels were equivalent when

sulfur or sulfur with either surfactant was applied through air induction nozzles. The only exception was that of Hi-Wett, which appeared to have a significant effect on leaf severity when applied with an air induction nozzle ($P = 0.004$; Table 2.14). This effect was not seen with the Cohere surfactant.

Water-sensitive cards

Though methods varied, water-sensitive card area coverage data was collected and analyzed successfully in 2023 and 2024. The trial in 2023 tested only the effects of cone versus air induction nozzles, while the trial in 2024 tested these nozzles with and without an incorporated surfactant. The average relative humidity for the days tested were 81.3% and 75.5% for 2023 and 2024, respectively. Card coverage between nozzle types alone was similar during both years ($P = 0.6$ and 0.8 for 2023 and 2024, respectively; Tables 2.16 and 2.17). In 2023 and 2024, the cone or AI nozzle coverage was similar for cards collected from plant canopies. In 2024, the surfactant increased overall coverage regardless of nozzle type (Table 2.18). When Hi-Wett was added to water, the card area coverage with either nozzle type was statistically greater than that of water alone ($P = 0.007$ and 0.05 for cone and AI nozzles, respectively; Table 2.18). However, there were noticeable differences in spray patterns, with cone nozzles producing more, but smaller individual droplets and air induction nozzles producing fewer, but larger droplets with increased space among each droplet (Figure 2.4).

DISCUSSION

In this study, the effect of surfactant adjuvants on sulfur sprays for GPM disease management was tested in a vineyard setting. We found that the addition of surfactants improved

disease management, though the effect seemed to vary by product. When tank-mixed and applied through various sprayer configurations, surfactant adjuvants have enhanced coverage of pesticide chemicals (Gent et al. 2003). Theoretically, better coverage over a leaf surface should translate to enhanced disease control, but studies examining surfactants have found mixed results (Breedon et al. 2023; Jibrin et al. 2021). Ultimately, surfactants applied in agricultural systems should be inspected on a case-by-case basis to determine their utility. (Tables 2.3, 2.4, 2.6, and 2.7).

During both years, the combination of sulfur and a surfactant provided a statistically significant increase in GPM suppression over sulfur alone (Tables 2.5 and 2.8; Figs. 2.4 and 2.5). This improvement is most likely due to the greater coverage area resulting from the altered water properties provided by the surfactant (Emmett et al. 2003; Miller and Westra 1998). However, the efficacy provided by these surfactants, even when applied alone, suggests that enhanced efficacy of sulfur in combination with these surfactants may be explained by reasons in addition to enhanced coverage of sulfur on the leaf or fruit surface. For example, organosilicone surfactants have been known to increase absorption of fungicides into plant stomata (Stevens 1993). Given the effect of non-ionic organosilicone surfactants on water properties and their antimicrobial properties (Tawfik et al. 2015; Falk 2019), the organosilicone surfactants may have some direct activity against fungal mycelium or spores. Indeed, others have also observed direct effects of surfactants on pathogens and plant diseases (Abbott and Beckerman 2018; Jibrin et al. 2021), but surfactants can also have no direct impact on plant diseases as well (Abbott and Beckerman 2018). Though unlikely in these trials, the effect of water itself could also potentially confound identifying the direct effect of surfactants on GPM. When used at high volumes, water has been found to wash off conidia on leaf surfaces (Pscheidt 2019; Asalf et al. 2021). With that said, water has more often been found to have no effect on GPM development at all (Ehret et al.

2002; Crisp et al. 2006). The method of application used in this study utilized limited pressure and spray volumes, so a response from water is unlikely. Also, the high levels of disease observed in untreated plants that surrounded the plots allowed for continuous movement of conidia to uninfected surfaces. In addition, the 14-day interval between applications used in this study is very long. Therefore, we would conclude that water is not a factor in these experiments.

Whether enhanced disease control with sulfur and surfactant tank mixes is directly due to surfactant anti-microbial properties, better coverage, or a combination of both is complex and scarcely researched. Additionally, results would likely be specific to each pathogen and disease system due to variations in pathogen biology. Weather conditions could also have an effect on surfactant efficacy, causing regional variations. In these studies, we observed synergistic interactions of surfactants and sulfur in control of GPM in 2023, but the same interactions were not observed in 2024, with the exception of Silwet for a reduction in GPM fruit severity. Synergistic interactions of two fungicides indicates both provide better efficacy together than expected based on each fungicide applied individually in the same trial, and as compared to an untreated control (Colby 1967; Kosman and Cohen 1996). However, surfactant direct activity (e.g. the surfactant itself having efficacy) and indirect activity (e.g. spreading of sulfur over the leaf or fruit surface) can potentially be occurring simultaneously, and these two effects cannot be readily isolated. Therefore, we can state that synergistic interactions between sulfur and the surfactants were observed in 2023, but we do not know the cause of the synergistic interaction (i.e. direct surfactant efficacy, sulfur spread, or a combination of the two). The lack of significant synergistic interactions in 2024, with the exception of Silwet, may indicate that the surfactants provided were not as effective when sulfur was not washed off due to rainfall, and the 2024 season was much drier than the 2023 season. Additionally, surfactant activity could be

influenced by the high concentration of fungicide used in this study. A lower concentration of sulfur could increase sulfur-surfactant synergy, as improved coverage may have greater significance when there is less product.

Among the surfactant products tested, Hi-Wett and Silwet provided the most consistent disease control across years, while Cohere performed well in 2023 but provided little to no added control in 2024. Of these three, Silwet clearly provided the most consistent efficacy either alone or when combined with sulfur. Differences in performance could be related to chemical differences in the surfactant formulations. Moreover, as mentioned above, there were notable differences between the rainfall patterns in 2023 and 2024, and differences in weather patterns could have interacted with the surfactants and sulfur to produce varying results in disease control. In June 2023, there was 475 cm of rainfall compared to 90 cm in June 2024 (Figures 2.1, 2.2). Precipitation can wash off existing spray residues, so a lack of rain over a long period could account for the lack of improvement from a coverage-improving agent, as full coverage would have been maintained by prior applications. Application of sulfur at much lower rates than those utilized in our trials has provided residues that have been shown to provide disease reduction for as long as 21 days after application (Neill et al. 2015). Therefore, sulfur applications in this study, made during the drought conditions of 2024, would likely have still been efficacious at the end of a 14-day interval, diminishing the effect of re-application and the need for surfactant to improve efficacy. In contrast, consistent rainfall would continually wash off existing spray residue, accentuating the potential improvements of each re-application.

The lack of consistency in control efficacy among surfactant products is consistent with previous studies. Studies from the same research station in 2022 (Breedon et al. 2023) indicated that only one product enhanced sulfur activity against GPM – the organosilicone surfactant Hi-

Wett. Abbott and Beckerman (2018) observed differences in efficacy of different surfactants when applied with captan for control of apple diseases. However, Emmett et al. (2003) found that surfactant adjuvants when combined with sulfur did not improve GPM control. Our results indicate that, though variable, organosilicone surfactants can enhance the efficacy of sulfur for management of GPM. In addition, specific adjuvants such as Silwet can provide very consistent and positive enhancements of efficacy.

Air induction nozzles (AI nozzles) are designed to combine the canopy-penetrating effect of larger spray droplets with the coverage-enhancing effect of smaller spray droplets (McGrath and Landers 2001). This technology could have useful implications in vineyard settings, as canopy penetration is essential for complete leaf and cluster coverage. Additionally, the incorporation of surfactants with this technology has the potential to synergistically enhance spray characteristics (Kierzek and Wachowiak 2003). In this study, we found that the performance of air induction nozzles for improving the efficacy of sulfur was inferior to standard cone nozzles (Tables 2.13 and 2.14). Treatments sprayed with cone nozzles had consistently lower disease levels on both leaves and fruit compared to those sprayed with AI nozzles (Table 2.15; Fig 2.6). This was contrary to our hypothesis, which was that the penetration advantage of AI nozzles would improve spray characteristics, thus outperforming the less advanced cone nozzles. However, this result is not completely unexpected, as reduced fungicide efficacy is consistent with some previous studies (McGrath and Landers 2001). Similar reductions in efficacy have been reported for various diseases on apples, especially in years with high disease pressure (Doruchowski et al. 2017; McArtney and Obermiller 2008). Additionally, studies finding comparable efficacies between these nozzle types mainly examined either herbicidal applications, where AI nozzles are favored for their anti-drift capabilities (Wang et al. 2023), or

systemic fungicidal products, where coverage is not as critical (Lešnik et al. 2005; Gil et al. 2014). Notably, studies reporting antagonistic effects have examined cropping systems that require horizontal spray angles rather than downward spray angles (McArtney and Obermiller 2008). This could provide a potential explanation for the underperformance of AI nozzles, as droplets encountering upwards-facing leaves from a lateral position could lessen the “exploding” effect which creates the coverage-enhancing outcome.

A more interesting result concerning this trial was the performance of the surfactants. The efficacy-enhancing effect of the surfactants found in the ‘Chardonnay’ trial was also observed in the ‘Merlot’ nozzle trial, but only when the treatments were applied with a cone nozzle. Both Hi-Wett and Cohere provided significant advantages in powdery mildew control (Table 2.15; Fig 2.6). This consistency between trials, despite differences in application methods, provides insight into the effectiveness of organosilicone surfactants when combined with sulfur. However, this consistency was not detected in treatments sprayed with an AI nozzle. Though Hi-Wett appeared to provide some effect on leaves, control outcomes on fruit worsened with a surfactant. This suggests an interference with the physics behind the spray technology. Perhaps the decreased surface tension causes the larger droplets to interact differently with the differing textures of leaves and berries. Whatever the case, the finer mist of the cone nozzle was able to outperform the coarse AI nozzle.

Water-sensitive cards (TeeJet Technologies) were used to gain insight into the coverage-altering effects of one of the tested products, Hi-Wett. In grape canopies, we found that AI nozzles provide similar coverage to those of cone nozzles, without regard to whether or not surfactants are used (Tables 2.16 and 2.17). Our results align with those of McGrath and Landers (McGrath and Landers 2001), and our observations are consistent with previous findings which

indicate that AI nozzles have similar coverage to finer sprays despite their droplet size differences (Doruchowski et al. 2017). There were, however, visual differences in spray patterns provided by the two nozzle types, due to the differences in droplet size (Figure 2.4). The reduction in efficacy of sulfur when applied through an AI nozzle is difficult to explain, especially in light of the nearly identical coverage provided by cone and AI nozzles; however, the different spray patterns could provide an answer. The AI nozzle pattern provided fewer larger droplets with greater distance among the droplets as compared to more uniform coverage with a greater number of smaller droplets in the case of the cone nozzles. This could create a less-protected zone with AI nozzles, allowing more opportunity for fungal invasion. Lešnik et al. noted a similar result, stating that an uneven micro-distribution effected insect control (Lešnik et al. 2005). Though these findings align with the relative poorer efficacy provided with AI nozzles, they should be cautiously interpreted, as the trial was only conducted a single time.

When developing spray programs for disease management, adding a surfactant is an important consideration to make. There are many different surfactant classes that could be utilized in a tank mix, and within these classes, each surfactant can be different, as observed with the surfactants used in our studies (Tables 2.5, 2.8, and 2.9; Figs. 2.4 and 25). We have clearly shown the value of some organosilicone nonionic surfactants for the enhancement of sulfur control of GPM. However, to determine their utility, each surfactant needs to be tested with each chemical, disease, and plant system. Grape growers often tank-mix multiple products at the same time to increase disease control or target multiple pests and diseases in one spray, which further complicates any overall recommendation of surfactants. Moreover, surfactants have been known to either directly, or through interactions with chemicals, cause phytotoxicity, so the decision to add surfactants becomes even more complicated. However, for the purposes of GPM

management, we have shown that organosilicone surfactants provide a benefit when mixed with sulfur.

As fungicide restrictions become more stringent and fungicide resistance more widespread, it will become increasingly important to maximize the use of sulfur, a compound that has minimal impact on the environment and does not lead to resistance in fungal populations. Sulfur is one of the largest fungicide inputs in vineyard operations in Georgia, making even small increases in its efficacy valuable (Oliver et al. 2024). Organosilicone surfactants, though varying in efficacy, can increase the effectiveness of sulfur sprays targeted for GPM management. Application of sulfur through air induction nozzles did not improve efficacy, and though this is unfortunate, researchers should continue a review of new and improved application technologies for fungicides as they become available.

LITERATURE CITED

- Abbott, C. P., and Beckerman, J. L. 2018. Incorporating adjuvants with captan to manage common apple diseases. *Plant Dis.* 102:231-236.
- Asalf, B., Onofre, R. B., Gadoury, D. M., Peres, N. A., and Stensvand, A. 2021. Pulsed water mists for suppression of strawberry powdery mildew. *Plant Dis.* 105: 71-77.
- Beffa, T., Pezet, R., and Turian, G. 1987. Multiple-site inhibition by colloidal elemental sulfur (S^0) of respiration by mitochondria from young dormant α spores of *Phomopsis viticola*. *Physiol. Plant.* 69:443-450.
- Bioletti, F. T. 1907. Oidium or powdery mildew of the vine. University of California, College of Agriculture, Agricultural Experiment Station Bulletin No. 186, February.
- Breeden, S., W. Sanders, C. Hawkins, P. Brannen, C. Johnston, and R. Covington. 2023. Efficacy of Microthiol Disperss sulfur and various surfactants for control of powdery mildew of grape, 2022. *Plant Disease Management Reports* 17:PF016.
- Crisp, P., Wicks, T. J., Lorimer, M., and Scott, E. S. 2006. An evaluation of biological and abiotic controls for grapevine powdery mildew. 1. Greenhouse studies. *Aust. J. Grape Wine Res.* 12: 192-202.
- Doruchowski, G., Świechowski, W., Masny, S., Maciesiak, A., Tartanus, M., Bryk, H., and Hołownicki, R. 2017. Low-drift nozzles vs. standard nozzles for pesticide application in the biological efficacy trials of pesticides in apple pest and disease control. *Sci. Total Environ.* 575:1239-1246.

- Ehret, D. L., Utkhede, R. S., Frey, B., Menzies, J. G., and Bogdanoff, C. 2002. Foliar applications of fertilizer salts inhibit powdery mildew on tomato. *Can. J. Plant Pathol.* 24: 437-444.
- Emmett, B., Rozario, S., and Hawtin, J. 2003. Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines. Final Report to GWRDC, Project NumberL DAV. 98:220.
- Falk, N. A. 2019. Surfactants as antimicrobials: a brief overview of microbial interfacial chemistry and surfactant antimicrobial activity. *J. Surfactants Deterg.* 22:1119-1127.
- Ferguson, J. C., Hewitt, A. J., and O'Donnell, C. C. 2016. Pressure, droplet size classification, and nozzle arrangement effects on coverage and droplet number density using air-inclusion dual fan nozzles for pesticide applications. *Crop Prot.* 89:231-238.
- Gent, D. H., Schwartz, H. F., and Nissen, S. J. 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorption, and efficacy. *Plant Dis.* 87:591-597.
- Gil Moya, E., Gallart González-Palacio, M., Llop Casamada, J., Ercilla Montserrat, M., Domènech, F., and Masip, P. 2014. Effect of low-drift nozzles on the biological effectiveness to control powdery mildew in vineyards. *Proceedings of the 7th International Workshop on Grapevine Downy and Powdery Mildew.* pp: 51-54.
- Gisi, U. 1996. Synergistic interaction of fungicides in mixtures. *Phytopathology.* 86: 1273-1279.
- Holland, P. T. 1996. Pesticides report 36. Glossary of terms relating to pesticides (IUPAC Recommendations 1996). *Pure and Applied Chemistry.* 68:1167-1193.
- Iland, P., Dry, P., Proffitt, T., and Tyerman, S. 2011. The grapevine: from the science to the practice of growing vines for wine. Patrick Iland Wine Promotions. (Patrick Iland Wine Promotions: Campbelltown, SA, Australia).

- Jibrin, M. O., Liu, Q., Jones, J. B., and Zhang, S. 2021. Surfactants in plant disease management: A brief review and case studies. *Plant Pathol.* 70:495-510.
- Kierzek, R., and Wachowiak, M. 2003. Effect of nozzle types and adjuvants on the leaf coverage and biological efficacy of fungicides in potato. *J. Plant Prot. Res.* 43:181-189.
- Kirkwood, R. C. 1993. Use and mode of action of adjuvants for herbicides: a review of some current work. *Pestic. Sci.* 38:93-102.
- Kosman, E., and Cohen, Y. 1996. Procedures for calculating and differentiating synergism and antagonism in action of fungicide mixtures. *Phytopathology.* 86:1263-1272.
- Lešnik, M., Pintar, C., Lobnik, A., and Kolar, M. 2005. Comparison of the effectiveness of standard and drift-reducing nozzles for control of some pests of apple. *Crop Prot.*, 24: 93-100.
- McArtney, S. J., and Obermiller, J. D. 2008. Comparative performance of air-induction and conventional nozzles on an axial fan sprayer in medium density apple orchards. *HortTechnology.* 18:365-371.
- McGrath, M. T., and Landers, A. 2001. Evaluating new nozzles and an air assist sprayer for improving spray coverage and powdery mildew control on underleaf surfaces. Final Project Report to the NYS IPM Program, Agricultural IPM 200-2001. <https://ecommons.cornell.edu/server/api/core/bitstreams/d993dd2b-b070-4bf4-8f92-3ac999440695/content>
- Miller, P., and Westra, P. 1998. How surfactants work no. 0.564. Colorado State University Cooperative Extension, Crop Fact Sheet. http://www.thanyagroup.com/research/download/25530111_2.pdf
- Neill, T., Livesay, A., Albrecht, A., and Mahaffee, W. 2015. Seeking the right level of sulfur to fight powdery mildew on grapes. OSU Extension Service. <https://extension.oregonstate.edu>

- du/crop-production/wine-grapes/seeking-right-level-sulfur-fight-powdery-mildew-grapes#:~:text=Sulfur%20applications%20of%20less%20than,mildew%20on%20leaves%20and%20fruit.
- Oliver, C., Cooper, M., Ivey, M. L., Brannen, P., Miles, T., Lowder, S., Mahaffee, W., and Moyer, M. M., 2024. Fungicide use patterns in select United States wine grape production regions. *Plant Dis.* 108:104-112.
- Önler, E., Çelen, İ. H., and Avcı, G. G. 2020. Evaluation of Residue Distribution of Spraying Nozzles Produced for the Prevention of Spray Drift. *International Journal of Innovative Approaches in Agricultural Research.* 4:242-250.
- Prokop, M., and Veverka, K. 2006. Influence of droplet spectra on the efficiency of contact fungicides and mixtures of contact and systemic fungicides. *Plant Prot. Sci.* 42:26-33.
- Pscheidt, J. W. 2019. How to deal with a vineyard powdery mildew outbreak. OSU Extension Service. <https://extension.oregonstate.edu/crop-production/wine-grapes/how-deal-vineyard-powdery-mildew-outbreak>
- Simko, I., and Piepho, H. P. 2012. The area under the disease progress stairs: calculation, advantage, and application. *Phytopathology.* 102: 381-389.
- Stephenson, G. R., Ferris, I. G., Holland, P. T., and Nordberg, M. 2006. Glossary of terms relating to pesticides (IUPAC Recommendations 2006). *Pure Appl. Chem.* 78:2075-2154.
- Steurbaut, W. 1993. Adjuvants for use with foliar fungicides. *Pestic. Sci.* 38:85-91.
- Stevens, P. J. 1993. Organosilicone surfactants as adjuvants for agrochemicals. *Pestic. Sci.* 38:103-122.

- Tawfik, S. M., Zaky, M. F., Mohammad, T. G., and Attia, H. A. 2015. Synthesis, characterization, and in vitro antifungal activity of anionic and nonionic surfactants against crop pathogenic fungi. *J. Ind. Eng. Chem.* 29: 163-171.
- Wang, S., Li, X., Nuyttens, D., Zhang, L., Liu, Y., and Li, X. 2023. Evaluation of compact air-induction flat fan nozzles for herbicide applications: spray drift and biological efficacy. *Front. Plant Sci.* 14:1018626.
- Warres, B. E. 2021. Fungicide efficacy and resistance management for *Erysiphe necator* (Masters Thesis, University of Georgia).
- Williams, J. S., and Cooper, R. M. 2004. The oldest fungicide and newest phytoalexin – a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathol.* 53:263-279.
- Wilson, J. D., Hedden, O. K., and Sleesman, J. P. 1963. Spray droplet size as related to disease and insect control on row crops. Ohio Agricultural Experiment Station. Research Bulletin 945.

Table 2.1. Additional fungicides applied during efficacy trials conducted in the research vineyard at the Georgia Mountain Research and Education Center in Blairsville, GA

Phenology	Fungicides applied
Bud-Break	Manzate Pro Stick 3.4 kg/ha Microthiol Disperse 3.4 kg/ha
5.0 cm shoot growth	Manzate Pro Stick 3.4 kg/ha Microthiol Disperse 3.4 kg/ha
10.2 cm shoot growth	Manzate Pro Stick 3.4 kg/ha
Pre-bloom	Manzate Pro Stick 3.4 kg/ha
Bloom	Manzate Pro Stick 3.4 kg/ha Elevate 50WDG 1.1 kg/ha
Postbloom	Manzate Pro Stick 3.4 kg/ha
First Cover	Manzate Pro Stick 3.4 kg/ha
Bunch Closure	Captan 80WP 2.8 kg/ha Revus 0.58 L/ha Elevate 50WDG 1.1 kg/ha
Second Cover	Captan 80WP 2.8 kg/ha Zampro 1 L/ha
Veraison	Captan 80WP 2.8 kg/ha Revus 0.58 L/ha Elevate 50WDG 1.1 kg/ha
Pre-harvest	Captan 80WP 2.8 kg/ha Revus 0.58 L/ha Elevate 50WDG 1.1 kg/ha

Table 2.2. List of materials used for experimental treatments

Trade name	Active ingredients	Concentration	Manufacturer	Classification
Microthiol Disperss	sulfur	11.2 kg/ha	United Phosphorus, Inc	Multisite fungicide
Hi-Wett	polyoxyethylene-polyoxypropylene copolymer, polysiloxane polyether copolymer	0.7 L/ha	Loveland Products, Inc	Surfactant, non-ionic organosilicone
Silwet L-77	polyalkyleneoxide modified heptamethyltrisiloxane	1.2 L/ha	Helena Agri-Enterprises, LLC	Surfactant, non-ionic organosilicone
Cohere	alkanolamide surfactants, 1,2-propanediol, alkylaryl polyethoxyethanol sulfates	0.6 L/ha	Helena Agri-Enterprises, LLC	Surfactant, non-ionic organosilicone

Table 2.3. Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew fruit severity (2023)

Surfactant	AUDPC (%-days) for fruit severity ^{ac}		Mean ^b
	No sulfur	Sulfur	
None	1889.7	903.9	1608.1 a
Cohere	1437.1	357.8	897.5 bc
Hi-Wett	1595.5	819.0	1164.2 b
Silwet	836.6	422.1	698.5 c
Mean	1468.1 a	651.7 b	

^a Each value is derived from the mean of five replicates.

^b Means followed by the same letter are not significantly different based on a post-hoc Fisher's protected LSD test ($P = 0.05$). LSD = 256.2 for the main effect of sulfur. LSD = 363.5 for the main effect of surfactant.

^c No significant interactions were observed ($P = 0.3227$).

Table 2.4. Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew leaf severity (2023)

Surfactant	AUDPC (%-days) for leaf severity ^{ac}		Mean ^b
	No sulfur	Sulfur	
None	517.0	127.0	322.0 a
Cohere	387.5	65.0	226.3 b
Hi-Wett	352.2	44.7	198.5 b
Silwet	210.7	39.6	134.7 b
Mean	366.9 a	70.7 b	

^a Each value is derived from the mean of five replicates.

^b Means followed by the same letter are not significantly different based on a post-hoc Fisher's protected LSD test ($P = 0.05$). LSD = 66.2 for the main effect of sulfur. LSD = 93.7 for the main effect of surfactant.

^c No significant interactions were observed ($P = 0.1079$).

Table 2.5. Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2023)

Treatment ^a	Powdery mildew fruit severity ^b				Powdery mildew leaf severity ^c			
	20 Jul	27 Jul	9 Aug	AUDPC ^d	20 Jul	27 Jul	9 Aug	AUDPC ^d
Untreated	86.2 a ^e	96.6 a ^f	97.2 a	1889.7 a	13.3 a	24.0 a	35.5 a	510.0 a
Hi-Wett	59.7 a	82.9 ab	87.4 a	1595.5 a	9.5 ab	15.7 ab	24.9 ab	352.2 ab
Cohere	55.7 ab	69.1 bc	86.2 a	1437.1 a	14.3 a	20.0 a	21.1 b	387.5 ab
Silwet L-77	22.6 b	38.9 c	53.7 b	836.6 b	6.3 b	8.5 b	15.9 b	210.7 c
LSD ($P \leq 0.05$)	35.6	13.8	17.1	511.2	6.1	9.7	12.6	185.9
Microthiol	17.2 ^h	52.1 ^h	49.5 a	903.9 a	2.0 ^h	5.9 a ^g	8.8 a	127.0 a
Microthiol + Hi-Wett	25.0	41.8	48.1 a	819.0 ab	1.0	1.3 b	4.1 bc	44.7 b
Microthiol + Cohere	8.8	18.3	21.9 b	357.7 b	1.0	2.2 b	5.7 b	65.0 b
Microthiol + Silwet L-77	3.6	20.1	32.0 ab	422.1 ab	1.3	1.5 b	2.9 c	39.6 b
LSD ($P \leq 0.05$)	-	-	25.2	535.6	-	0.4	2.8	35.9

^a Treatment dates: 10 May (pre bloom), 24 May (bloom), 2 Jun (post bloom), 16 Jun (first cover), Jun 30 (second cover), 18 Jul (third cover).

^b Percent fruit area covered by powdery mildew calculated from five clusters per plant.

^c Percent leaf area covered by powdery mildew calculated from 25 leaves per plant.

^d Area under the disease progress curve.

^e Means followed by the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$).

^f An arcsin transformation was used for analysis for this date for the surfactant-only treatments. Back-transformed means are shown.

^g A square root transformation was used for analysis for this date for the sulfur with surfactant treatments. Back-transformed means are shown.

^h Statistics not shown due to high variability and an insignificant ANOVA F-test. Data shown for reference only.

Table 2.6. Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew fruit severity (2024)

Surfactant	AUDPC (%-days) for fruit severity ^{ac}		Mean ^b
	No sulfur	Sulfur	
None	3371.2	1525.7	2448.5
Cohere	2807.8	1546.4	2177.1
Hi-Wett	2999.5	1394.4	2196.9
Silwet	2787.9	820.2	1804.1
Mean	2991.6	1321.7	

^a Each value is derived from the mean of five replicates.

^b Means followed by the same letter are not significantly different based on a post-hoc Fisher's protected LSD test ($P = 0.05$). LSD = 169.6 for the main effect of sulfur. LSD = 239.9 for the main effect of surfactant.

^c Significant interactions were observed ($P = 0.0275$).

Table 2.7. Factorial analysis of surfactants and sulfur applications and their impact on grapevine powdery mildew leaf severity (2024)

Surfactant	AUDPC (%-days) for leaf severity ^{ac}		Mean ^b
	No sulfur	Sulfur	
None	1030.3	229.6	629.9
Cohere	951.5	235.8	593.7
Hi-Wett	607.6	160.7	384.2
Silwet	478.9	126.4	302.7
Mean	767.1	188.1	

^a Each value is derived from the mean of five replicates.

^b Means followed by the same letter are not significantly different based on a post-hoc Fisher's protected LSD test ($P = 0.05$). LSD = 64.6 for the main effect of sulfur. LSD = 91.4 for the main effect of surfactant.

^c Significant interactions were observed ($P = <0.0001$).

Table 2.8. Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2024)

Treatment ^a	Powdery mildew fruit severity ^b							Powdery mildew leaf severity ^c				
	14 Jun	21 Jun	28 Jun	5 Jul	12 Jul	19 July	AUDPC ^d	28 Jun	5 Jul	12 Jul	19 Jul	AUDPC ^d
Untreated	94.5 a ^{ef}	94.5 a	98.2 a ^f	98.4 a	94.3 a	97.9 a	3371.2 a	22.8 a	52.1 a	55.6 a	55.8 a	1030.3 a
Hi-Wett	74.2 b	79.6 b	84.5 b	88.2 b	92.8 a	95.6 ab	2999.5 b	11.4 b	28.5 b	32.4 b	40.0 b	607.6 b
Cohere	55.3 b	73.9 b	83.3 b	83.2 bc	87.8 a	91.4 bc	2807.8 b	17.8 a	47.3 a	48.9 a	61.4 a	951.5 a
Silwet L-77	66.2 b	70.8 b	82.9 b	81.4 c	85.9 a	89.0 c	2787.9 b	7.3 b	18.4 c	27.7 b	37.2 b	478.9 b
LSD (P≤0.05)	5.4	12.1	1.5	6.2	9.2	5.6	277.13	5.5	8.7	12.3	13.8	179.9
Microthiol	16.6 a	29.3 ab	43.0 a	51.2 a	53.1 a	65.7 a	1525.7 a	6.2 ab	9.4 a	13.5 a	13.2 ab	229.6 a
Microthiol + Hi-Wett	15.3 a	29.8 ab	44.7 a	41.7 a	46.6 a	57.2 a	1394.4 a	4.7 ab	7.6 ab	8.5 b	8.8 b	160.7 b
Microthiol + Cohere	11.9 ab	33.0 a	41.8 a	51.7 a	54.8 a	66.8 a	1546.4 a	6.5 a	10.9 a	12.0 ab	14.9 a	235.8 a
Microthiol + Silwet L-77	7.6 b	16.3 b	22.8 b	27.2 b	31.0 b	31.9 b	820.2 b	3.7 b	4.1 b	7.6 b	8.6 b	126.4 b
LSD (P≤0.05)	7.5	14.8	15.6	13.5	9.1	11.9	334.3	2.7	4.2	5.0	4.8	60.5

^a Treatment dates: 26 April (pre bloom), 13 May (bloom), 28 May (post bloom), 10 Jun (first cover), 25 Jun (second cover), 9 Jul (third cover)

^b Percent fruit area covered by powdery mildew calculated from five clusters per plant.

^c Percent leaf area covered by powdery mildew calculated from 25 leaves per plant.

^d Area under the disease progress curve.

^e Means followed by the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$).

^f An arcsin transformation was used for analysis for this date for the surfactant-only treatments. Back-transformed means are shown.

Table 2.9. Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease incidence (2023 and 2024)

Treatment ^a	Powdery mildew leaf incidence ^a								
	2023				2024				
	20 Jul	27 Jul	9 Aug	AUDPC ^b	28 Jun	5 Jul	12 Jul	19 Jul	AUDPC ^b
Untreated	72.2 a ^{ce}	94.8 a ^d	97.2 a	1817.2 a	96.4 a ^d	100.0	100.0 a	99.2 ^h	2084.6 ^h
Hi-Wett	64.3 a	89.8 ab	91.6 a	1687.6 ab	90.8 ab	96.0	97.2 c	99.2	2017.4
Cohere	67.6 a	90.6 a	91.6 a	1712.2 ab	95.6 ab	100.0	99.6 ab	100.0	2081.8
Silwet L-77	58.8 a	98.3 b	92.4 a	1472.2 b	86.8 b	91.2	98.0 bc	95.2	1961.4
LSD (P≤0.05)	1.8	7.5	7.1	248.1	9.5	-	1.7	-	-
Microthiol	29.2 a	50.4 a	62.0 a	1194.2 a	43.2 a	47.2 a	54.4 ^h	52.8 ^h	1047.2 a
Microthiol + Hi-Wett	24.8 a	26.8 b	54.8 a	711.0 b	44.8 a	47.6 a	62.8	54.4	1120.0 a
Microthiol + Cohere	18.8 a	30.0 b	58.8 a	748.0 ab	49.2 a	50.0 a	57.2	62.4	1141.0 a
Microthiol + Silwet L-77	35.5 a	36.5 ab	48.0 a	794.2 ab	49.6 a	44.0 a	55.6	64.4	1096.2 a
LSD (P≤0.05)	16.1	12.2	16.4	234.8	9.6	10.2	-	-	14.6

^a Percent of leaves with powdery mildew calculated from 25 leaves per plant.

^b Area under the disease progress curve.

^c Means followed by the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$).

^d An arcsin transformation was used for analysis for this date for the surfactant-only treatments. Back-transformed means are shown.

^e A log10 transformation was used for analysis for this date for the sulfur with surfactant treatments. Back-transformed means are shown.

^h Statistics not shown due to high variability and an insignificant ANOVA F-test. Data shown for reference only.

Table 2.10. Analysis of the synergy of surfactants combined with sulfur for control of grapevine powdery mildew on leaves as derived through use of the Colby method (2023)

Treatment	Rating type	Percent disease control on leaves ^{ab}		O-E	Chi-square test ^e	T-test ^f
		Observed ^c	Expected ^d			
Cohere + sulfur	Incidence	59.0	37.9	+21.1	1.32E-19	0.0059
	Severity	86.4	80.9	+5.5	0.62	0.1103
Hi-Wett + sulfur	Incidence	60.8	39.1	+21.7	8.60E-16	0.00046
	Severity	90.8	81.1	+9.7	0.0022	0.064
Silwet L-77 + sulfur	Incidence	56.0	49.7	+6.3	0.028	0.2612
	Severity	90.7	90.2	+0.5	0.46	0.46

^a Percent disease control is calculated from AUDPCs developed for grapevine powdery mildew disease incidence and severity for each surfactant plus sulfur treatment.

^b Sampling dates were 20 July, 27 July, and 9 August.

^c Average observed percent disease control by the specified surfactant product when combined with sulfur.

^d Expected disease control percentage as calculated through use of the actual disease control levels provided by the individual products and use of the Colby formula (Colby, 1967).

^e Chi-square test of the differences between observed and expected values ($P \leq 0.05$).

^f One-tailed t-test of the differences between observed and expected values ($P \leq 0.05$).

Table 2.11. Analysis of the synergy of surfactants combined with sulfur for control of grapevine powdery mildew on leaves as derived through use of the Colby method (2024)

Treatment	Rating type	Percent disease control on leaves ^{ab}		O-E	Chi-square test ^e	T-test ^f
		Observed ^c	Expected ^d			
Cohere + sulfur	Incidence	45.2	49.7	-4.5	0.32	0.06
	Severity	76.2	78.2	-2.0	0.34	0.36
Hi-Wett + sulfur	Incidence	46.2	51.4	-5.2	0.06	0.12
	Severity	84.1	86.2	-2.1	0.78	0.23
Silwet L-77 + sulfur	Incidence	47.4	52.7	-5.3	0.39	0.09
	Severity	87.2	88.5	-1.3	0.98	0.34

^a Percent disease control is calculated from AUDPCs developed for grapevine powdery mildew disease incidence and severity for each surfactant plus sulfur treatment.

^b Sampling dates were 28 June and 5, 12, and 19 July.

^c Average observed percent disease control by the specified surfactant product when combined with sulfur.

^d Expected disease control percentage as calculated through use of the actual disease control levels provided by the individual products and use of the Colby formula (Colby, 1967).

^e Chi-square test of the differences between observed and expected values ($P \leq 0.05$).

^f One-tailed t-test of the differences between observed and expected values ($P \leq 0.05$).

Table 2.12. Analysis of the synergy of surfactants combined with sulfur for control of grapevine powdery mildew on fruit clusters as derived through use of the Colby method (2024)

Treatment	Rating type	Percent disease control on fruit ^{ab}		O-E	Chi-square test ^e	T-test ^f
		Observed ^c	Expected ^d			
Cohere + sulfur	Severity	54.0	62.1	-8.1	0.11	0.10
Hi-Wett + sulfur	Severity	58.6	60.0	-1.5	0.52	0.42
Silwet L-77 + sulfur	Severity	75.6	62.3	+13.3	0.00012	0.019

^a Percent disease control is calculated from AUDPCs developed for grapevine powdery mildew disease incidence and severity for each surfactant plus sulfur treatment.

^b Sampling dates were 28 June and 5, 12, and 19 July.

^c Average observed percent disease control by the specified surfactant product when combined with sulfur.

^d Expected disease control percentage as calculated through use of the actual disease control levels provided by the individual products and use of the Colby formula (Colby, 1967).

^e Chi-square test of the differences between observed and expected values ($P \leq 0.05$).

^f One-tailed t-test of the differences between observed and expected values ($P \leq 0.05$).

Table 2.13. Factorial analysis of surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through fruit severity (2024)

Surfactant	AUDPC (%-days) for fruit severity ^{ac}		
	Cone	Air induction	Average ^b
None	1413.3	1379.1	1396.2
Cohere	1010.9	1605.9	1308.4
Hi-Wett	1046.6	1394.7	1220.6
Average	1156.9	1459.9	

^a Each value is derived from the mean of five replicates.

^b Means followed by the same letter are not significantly different based on a post-hoc Fisher's protected LSD test ($P = 0.05$). LSD = 178.4 for the main effect of nozzle. LSD = 218.4 for the main effect of surfactant.

^c Significant interactions were observed ($P = 0.0230$).

Table 2.14. Factorial analysis of surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through leaf severity (2024)

Surfactant	AUDPC (%-days) for leaf severity ^{ac}		
	Cone	Air induction	Average ^b
None	109.7	190.3	150.0 a
Cohere	85.7	187.8	136.8 ab
Hi-Wett	66.9	121.2	94.1 b
Average	87.4 a	166.4 b	

^a Each value is derived from the mean of five replicates.

^b Means followed by the same letter are not significantly different based on a post-hoc Fisher's protected LSD test ($P = 0.05$). LSD = 39.5 for the main effect of nozzle. LSD = 48.4 for the main effect of surfactant.

^c No significant interactions were observed ($P = 0.5951$).

Table 2.15. Analysis of organosilicone surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through leaf severity (2024)

Factor levels ^a		Powdery mildew fruit severity ^{bc}							Powdery mildew leaf severity ^{bc}				
		14 Jun	21 Jun	28 Jun	5 Jul	12 Jul	19 Jul	AUDPC ^d	28 Jun	5 Jul	12 Jun	19 Jul	AUDPC ^d
	Untreated	34.9	50.6	54.2	62.6	72.1	76.6	2067.3	5.9	18.8	16.7	24.4	355.1
Cone nozzle	Microthiol	11.4 a	25.5 a	34.1 a	49.3 a	54.7 a	65.3 a	1413.3 a	2.4 ^f	5.9 ^f	4.9 a ^e	6.8 ^f	109.7 ^f
	Microthiol + Hi-Wett	11.0 a	18.2 a	22.8 b	32.9 b	44.3 ab	52.4 b	1046.6 b	1.8	3.6	3.1 b	3.8	66.9
	Microthiol + Cohere	9.5 a	19.8 a	18.7 b	32.6 b	40.3 b	56.1 b	1010.9 b	1.8	4.1	5.0 a	4.2	85.7
	LSD (P≤0.05)	3.8	7.3	10.1	12.3	10.7	4.3	236.6	-	-	0.15	-	-
Air induction nozzle	Microthiol	14.8 a	30.8 a	32.6 a	42.7 a	52.7 a	61.7 a	1379.1 a	5.7 a	10.7 a	7.8 a	11.6 a	190.3 a
	Microthiol + Hi-Wett	19.0 a	28.0 a	33.4 a	45.0 a	49.4 a	67.6 a	1394.7 a	4.5 a	7.7 a	3.6 b	7.7 a	121.2 b
	Microthiol + Cohere	21.3 a	37.3 a	40.1 a	49.2 a	56.1 a	72.1 a	1605.9 a	5.4 a	10.6 a	8.1 a	10.9 a	187.8 a
	LSD (P≤0.05)	8.9	15.6	14.5	8.5	19.2	13.6	417.4	2.9	4.2	3.1	4.3	63.6

^a Treatment dates: 26 April (pre bloom), 13 May (bloom), 28 May (post bloom), 10 Jun (first cover), 25 Jun (second cover), 9 Jul (third cover)

^b Powdery mildew severity (% area of leaves covered by powdery mildew) was calculated from 25 leaves and 5 clusters per treated plant

^c Means followed by the same letter are not significantly different when comparing each pair using Fishers protected LSD ($P \leq 0.05$). The untreated control was not utilized in the ANOVA or post-hoc test, but is provided for reference.

^d Area under the disease progress curve.

^e An arcsin transformation was used for analysis for this date for the surfactant-only treatments. Back-transformed means are shown.

^f Statistics not shown due to high variability and insignificant ANOVA F-test. Data shown for reference only.

Table 2.16. T-test comparison of percent area coverage of water sensitive cards following application of water through cone or air induction nozzles (2023)

Nozzle type ^a	Mean	Standard deviation	t-value	Degrees of freedom	p-value ^b
Cone	9.5	13.8			
Air induction	10.7	14.1			
t-test			0.5	14	0.6

^a Prior to each application of water through each nozzle type, cone and air induction, three cards were attached inside the canopy middle of five vines, making 15 total cards utilized per nozzle application. Cards were attached to the upper surface of the same leaf (same spot on the same vine) for a pass with a cone nozzle and then an air induction nozzle. For each application, the spray volume per ha was equivalent.

^b Percent area coverage for cards were averaged and analyzed for statistical differences through use of a two-tailed paired t-test.

Table 2.17. T-test comparison of percent area coverage of water sensitive cards following application of water through cone or air induction nozzles (2024)

Nozzle type ^a	Mean	Standard deviation	t-value	Degrees of freedom	p-value ^b
Cone	12.8	19.4			
Air induction	11.6	14.7			
t-test			-0.2	9	0.8

^a Prior to each application of water through each nozzle type, cone and air induction, 10 cards were attached inside the canopy of a single vine, making 10 total cards utilized per nozzle application. Cards were attached to the upper surface of the same leaf (same spot on the same vine) for a pass with a cone nozzle and then an air induction nozzle. For each application, the spray volume per ha was equivalent.

^b Percent area coverage for cards were averaged and analyzed for statistical differences through use of a two-tailed paired t-test.

Table 2.18. T-test comparison of percent area coverage of water sensitive cards following application of water through cone or air induction nozzles with or without a surfactant (2024)

Nozzle type ^a	Mean	Standard deviation	t-value	Degrees of freedom	p-value ^b
Cone	12.8	19.4			
Cone w/ surfactant	28.9	31.0			
t-test			-3.0	9	0.007
Air Induction	11.6	14.7			
Air Induction w/ surfactant	29.7	27.7			
t-test			-1.9	9	0.05

^a Prior to each application of water through each nozzle type × surfactant permutation, five cards were attached inside the canopy middle of a single vines, making 10 total cards utilized per application. Cards were attached to the upper surface of the same leaf (same spot on the same vine) for each pass with each treatment. For each application, the spray volume per ha was equivalent.

^b Percent area coverage for cards were averaged and analyzed for statistical differences through use of a one-tailed paired t-test.

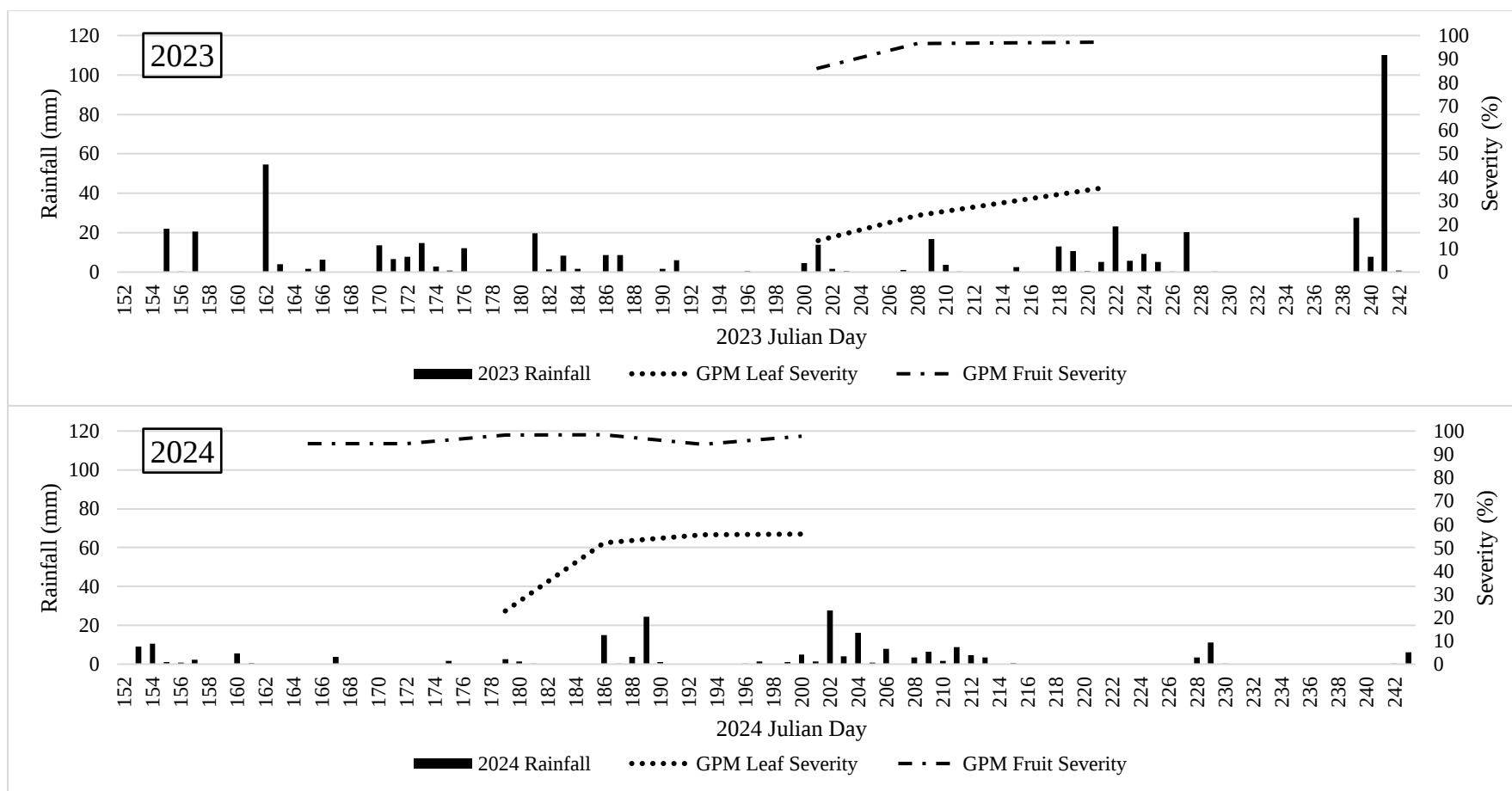


Figure 2.1. Rainfall and grapevine powdery mildew disease severity curves on untreated leaves and fruit (2023 and 2024)

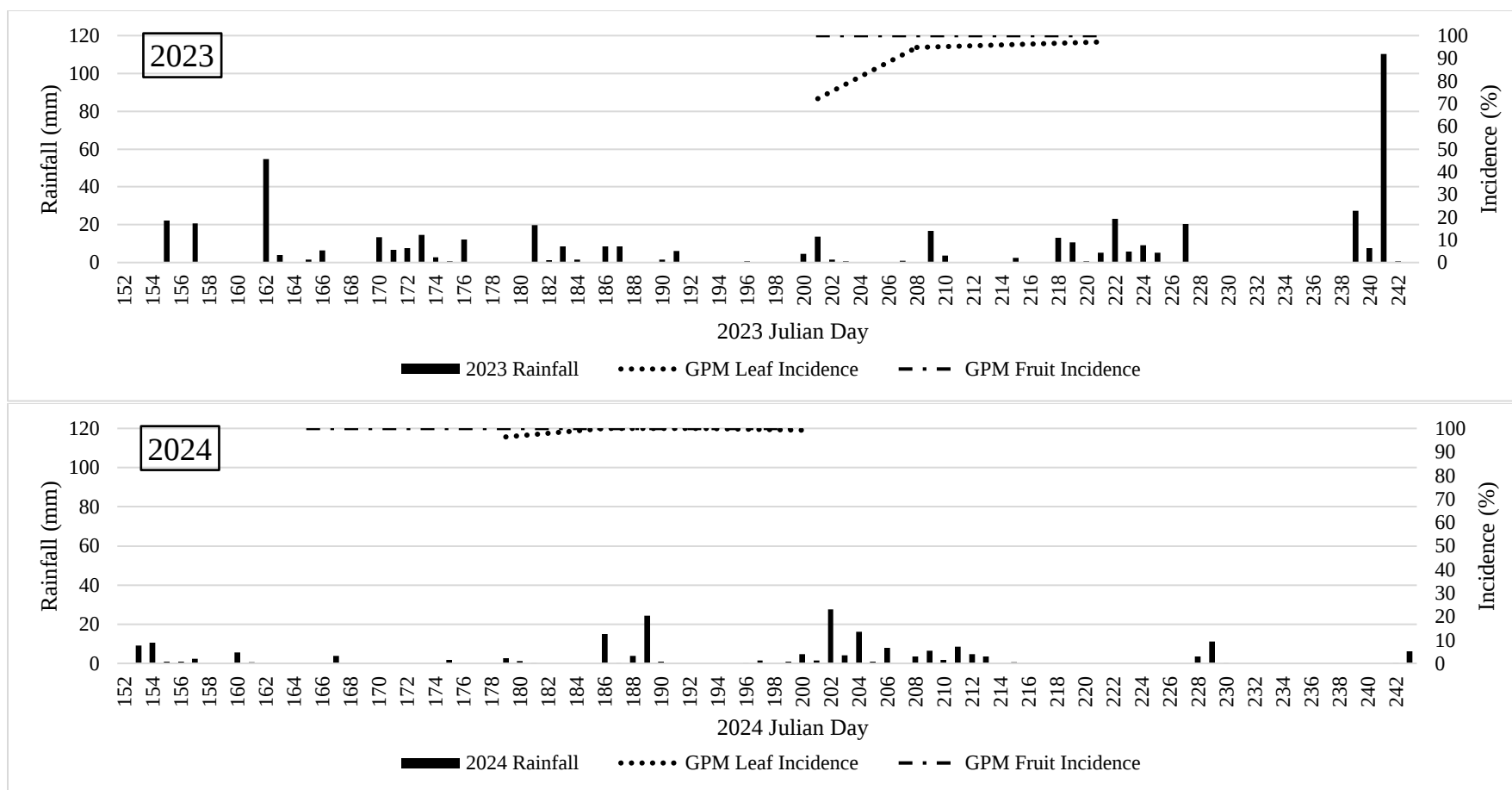


Figure 2.2. Rainfall and grapevine powdery mildew disease incidence curves on untreated leaves and fruit (2023 and 2024)

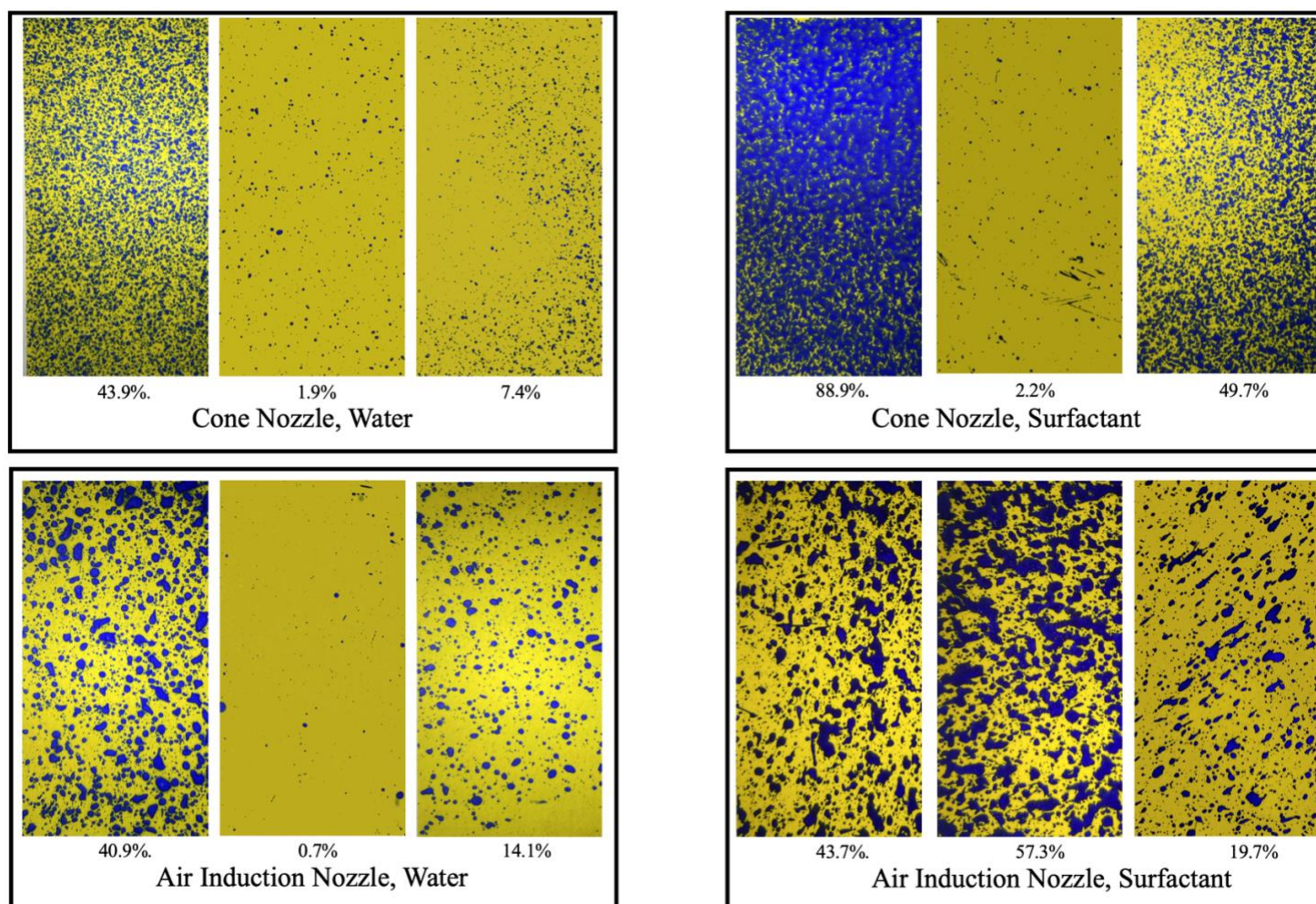


Figure 2.3. Examples of spray cards showing differences in percent area coverage (below each card) and visual droplet size/pattern following application of water or water + surfactant and applied through either a cone or an air induction nozzle. For each application, the spray volume per ha was equivalent. For each panel, cards in the left, center, and right positions were attached to the upper surface of the same leaf (same spot on the same vine), respectively.

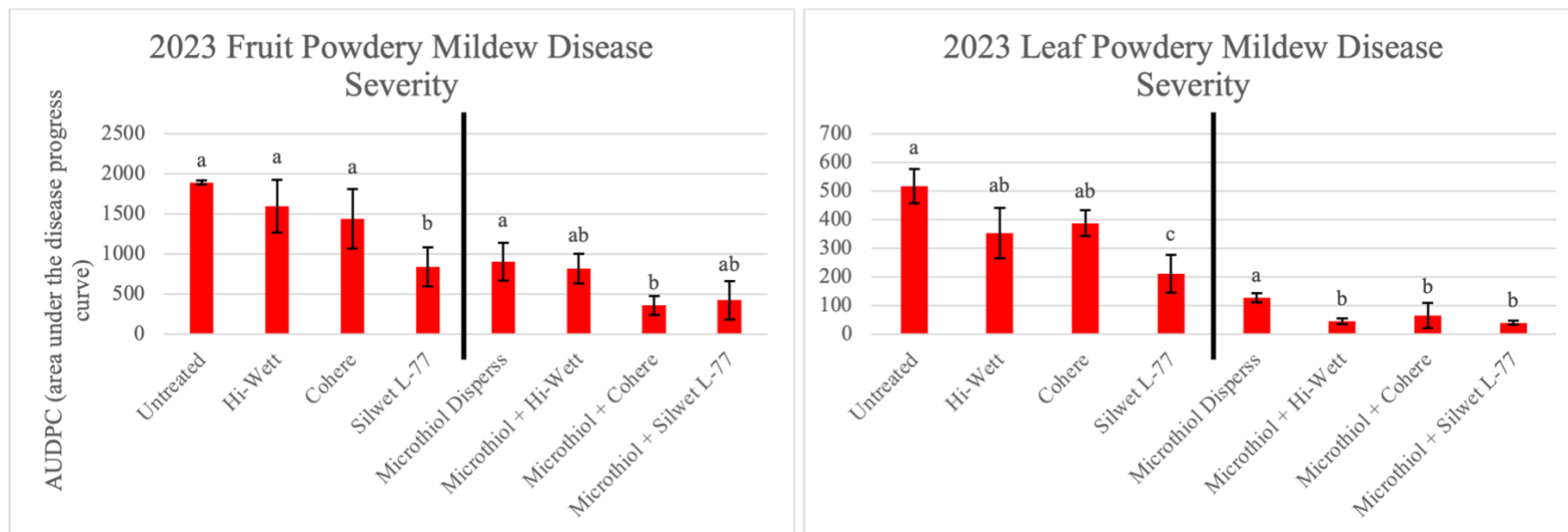


Figure 2.4. Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2023). Treatment dates were: 10 May (pre bloom), 24 May (bloom), 2 Jun (post bloom), 16 Jun (first cover), Jun 30 (second cover), 18 Jul (third cover). Percent fruit area covered by powdery mildew calculated from five clusters per plant. Percent leaf area covered by powdery mildew calculated from 25 leaves per plant. Means followed by the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$). Vertical black lines separate different analyses. Error bars represent standard error.

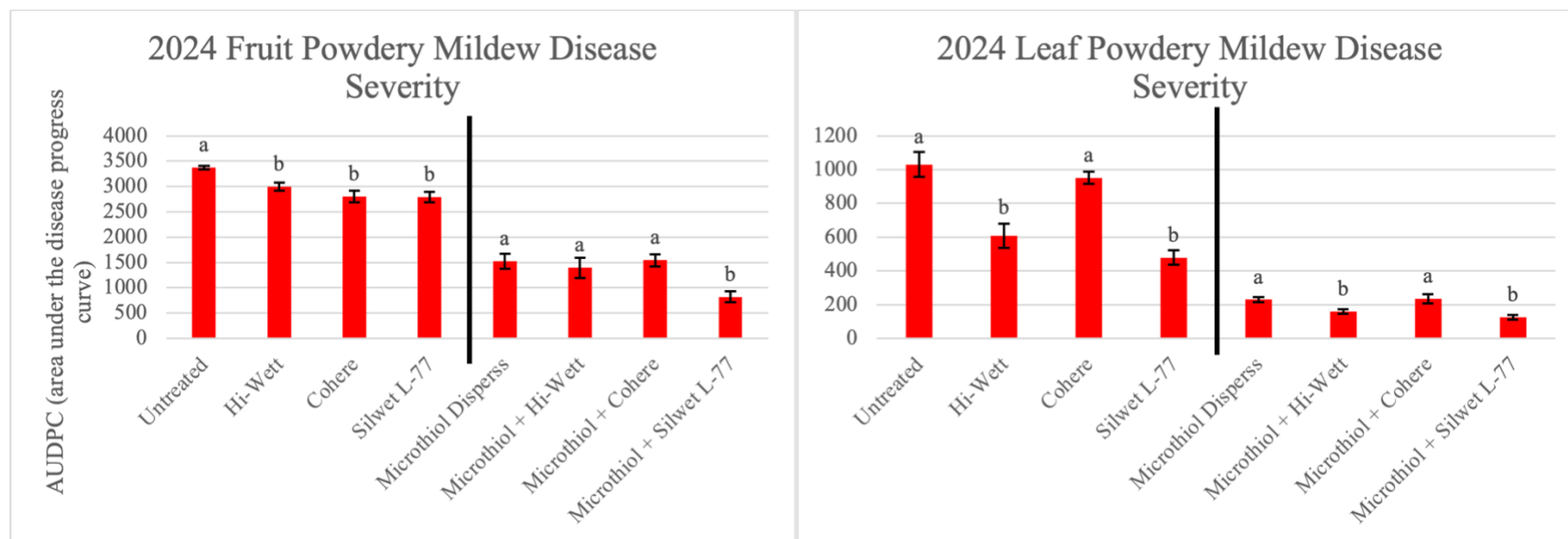


Figure 2.5. Effect of sulfur when combined with organosilicone surfactants on grapevine powdery mildew management as measured by disease severity (2024). Treatment dates were: 26 April (pre bloom), 13 May (bloom), 28 May (post bloom), 10 Jun (first cover), 25 Jun (second cover), 9 Jul (third cover). Percent fruit area covered by powdery mildew calculated from five clusters per plant. Percent leaf area covered by powdery mildew calculated from 25 leaves per plant. Bars with the same letter are not significantly different when using Fisher's protected LSD ($P \leq 0.05$). Vertical black lines separate different analyses. Error bars represent standard error.

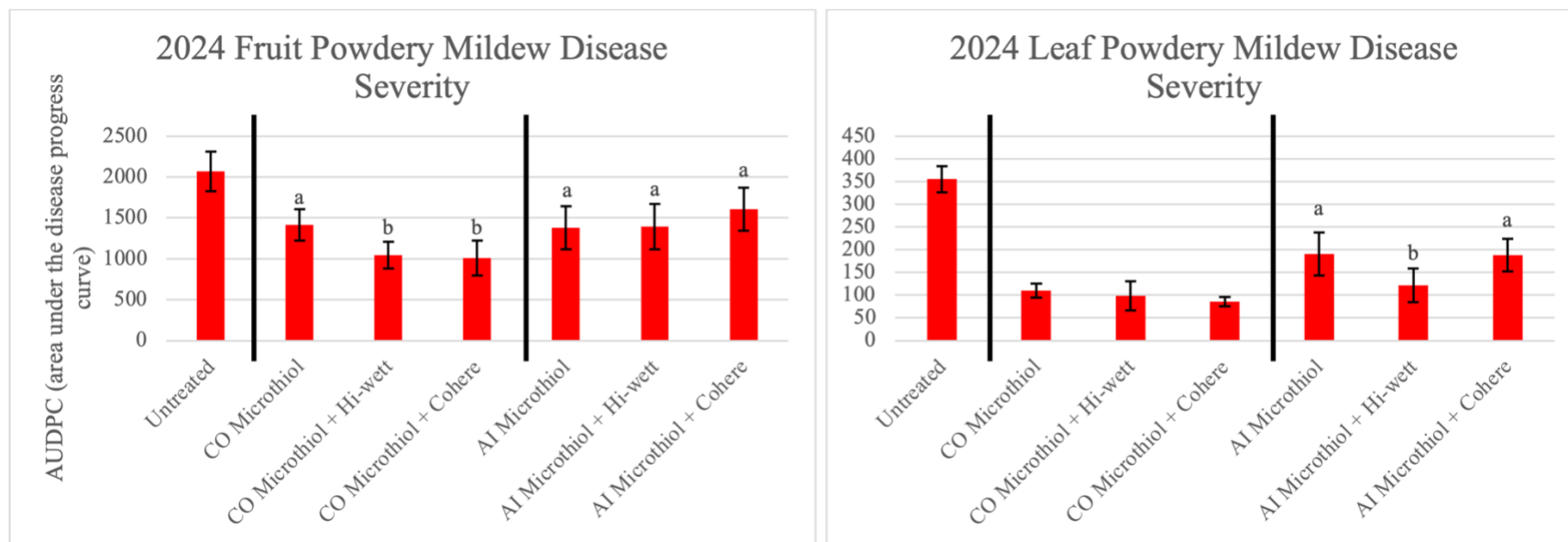


Figure 2.6. Analysis of organosilicone surfactants and nozzle types and their impact on the efficacy of sulfur for control of grapevine powdery mildew as measured through leaf severity (2024). Treatment dates were: 26 April (pre bloom), 13 May (bloom), 28 May (post bloom), 10 Jun (first cover), 25 Jun (second cover), 9 Jul (third cover). Percent fruit area covered by powdery mildew calculated from five clusters per plant. Percent leaf area covered by powdery mildew calculated from 25 leaves per plant. Bars with the same letter are not significantly different when comparing each pair using Fishers protected LSD ($P \leq 0.05$). The untreated control was not utilized in the ANOVA or post-hoc test, but is provided for reference. Statistics not shown due to high variability and insignificant ANOVA F-test. Data shown for reference only. Vertical black lines separate different analyses. Error bars represent standard error.

CHAPTER 3

ASSESSMENT OF SULFUR PHYTOTOXICITY ON *VITIS VINIFERA* GRAPEVINES IN
NORTHERN GEORGIA

Sanders, W., Brewer, M.T., Severns, P.M., Lowder, S.R., and Brannen, P.M. 2024. To be submitted to *Crop Protection*.

ABSTRACT

Grapevine powdery mildew (GPM), caused by the fungal pathogen *Erysiphe necator*, is an economically important disease wherever grapes are grown. Elemental sulfur is a contact fungicide used as a resistance management tool in powdery mildew control programs. However, sulfur has been reported to induce phytotoxicity when hot temperatures and high relative humidities (RH) occur simultaneously. Therefore, grape producers in northern Georgia (U.S.) often avoid application of sulfur during mid- to late summer. In this study, we assessed the risk of phytotoxicity development when Microthiol Disperss (sulfur) was applied at high temperatures in *Vitis vinifera* vineyards in northern Georgia, and for comparison, we also examined the risk of applying sulfur to interspecific hybrid cultivars with American grape heritage. On days that were selected for their predicted high temperatures, the highest label rate of sulfur was applied at 3:00 p.m. or 7:00 p.m. to four *V. vinifera* cultivars located across four commercial vineyards (2023 and 2024) and three hybrid cultivars located at a university research farm (2024 only). One week after application, treated leaves were assessed for differences in phytotoxicity compared to an untreated control. Following sulfur application, significant phytotoxicity (scorch or discoloration) damage was not observed on any *V. vinifera* grapes at any range of temperature (21.7°C-33.1°C) or RH (45%-80%). Though high temperatures (>30°C) occurred at the time of application, high RH (>70%) did not co-occur at a level purported to cause phytotoxicity. Based on a review of historical data over the last 21 years, we can predict that sulfur phytotoxicity is highly unlikely to occur on *V. vinifera* vines in northern Georgia. The known combination of high temperatures × and high RH that reportedly cause phytotoxicity are exceedingly rare and likely short-lived. The only significant phytotoxic response was observed on the *Vitis* hybrid ‘Crimson Cabernet’ when sulfur was applied at 3:00 p.m. (31.7°C, 45% RH),

providing greater scorch damage when compared with untreated controls and sulfur application at 7:00 p.m. (27.8°C, 56% RH) ($P \leq 0.05$). The same pattern was observed with two other hybrid grape cultivars, although the mean phytotoxicity was not significant.

INTRODUCTION

Grapevine powdery mildew (GPM), caused by *Erysiphe necator*, is one of the most economically important diseases of winegrapes worldwide (Gadoury et al., 2012). GPM infects the green parts of vines and can cause reductions in fruit quality even at low levels of disease (Iland et al., 2011). For this reason, commercial winegrape operations require multiple fungicide applications each year to control GPM, as well as other diseases. Powdery mildew fungicide programs often include a combination of systemic and contact products, the most common systemic products being quinone outside inhibitors (QoIs), demethylation inhibitors (DMIs), and succinate dehydrogenase inhibitors (SDHIs). Fungicides with these modes of action can provide high efficacy at low rates but are at risk for the development of fungicide resistance, reducing the effectiveness of sprays over time. Resistance is occurring in both the United States (Miles et al., 2012; Warres, 2021) and abroad (Beresford et al., 2016; Taksonyi et al., 2013), necessitating improved resistance management strategies.

Elemental sulfur is a prevalent contact material commonly used as a fungicide-resistance management tool for GPM populations. It's low mammalian toxicity, relative cost-effectiveness, and multi-site mode of action make it a staple fungicide in vineyard operations (Williams and Cooper, 2004; Cooper and Williams, 2004). However, sulfur applications are not without challenges. Under ideal conditions, sulfur residues have been shown to provide disease reduction

for as long as 21 days after application (Neill et al. 2015), but this residue is easily washed off by rain, making more frequent application necessary for effective control. Additionally, its generally lower efficacy compared to many systemic fungicides forces growers to supplement GPM management programs with fungicides associated with resistance development in *E. necator* populations, especially during critical infection periods. Most significantly, sulfur has been known to cause phytotoxicity when applied during hot and humid weather conditions. Emmett et al. (2003) reported that weather conditions conducive for sulfur phytotoxicity on *V. vinifera* grapes are temperatures exceeding 30°C and relative humidities (RH) greater than 75%. Their report further indicated that phytotoxicity was not a significant concern if the RH did not exceed the 75% threshold, even at temperatures exceeding 40°C. However, within the same report, this relative humidity threshold was also proposed to be 70% (Emmett et al., 2003). To further complicate matters, The Compendium of Grape Diseases (Wilcox et al., 2015) reports the conducive conditions as 32°C and 70% RH. Ultimately, the precise conditions necessary for sulfur phytotoxicity to occur on *V. vinifera* grapes are uncertain, however, it is generally accepted that the heat and humidity associated with mid-summer weather is sufficient to cause concern in many areas of the world. Sulfur phytotoxicity is better defined for interspecific hybrid grape varieties (*V. vinifera* × American grape heritage) which have been shown to have greater susceptibility toward sulfur burn than *V. vinifera* (McManus et al., 2017; Köycü et al., 2017). Interspecific hybrids with *V. aestivalis*, *V. rupestris*, *V. riparia*, and *V. labrusca* lineage have been found to be sensitive to sulfur damage (Duncan, 2016; McManus et al., 2017; Köycü et al., 2017; Beckerman et al., 2022). The temperature and relative humidity thresholds for sulfur phytotoxicity on interspecific hybrids is unclear, however, damage has been reported on hybrids at temperatures as low as 24 degrees celsius (Köycü et al., 2017). Despite these downsides,

sulfur is one of the most widely used fungicides in vineyards, with 30% of fungicide applications in the United States containing sulfur (Oliver et al., 2024).

In Georgia (U.S.), the hot and humid climate requires that growers use aggressive fungicide spray programs to manage GPM. Sulfur application typically begins immediately after bud break in the early spring and continues throughout the season at 7- to 14-day intervals. Sulfur sprays will even continue after harvest to reduce stress on vines and decrease overwintering inoculum (chasmothecia) for the following season. Despite the need for effective GPM control, growers will often withhold sulfur applications during periods of elevated temperature ($>30^{\circ}\text{C}$), due to fear of phytotoxicity. Withholding sulfur application can come at the detriment of fungicide programs, as growers often use resistance-prone fungicides instead. In these cases, sulfur application alone would likely suffice due to the unfavorable temperature for GPM growth (Wilcox et al. 2015).

When reviewing over 20 years of research trials conducted at the Georgia Mountain Experiment Station (Blairsville, GA) on *V. vinifera* vines, application of sulfur has never resulted in phytotoxic symptoms, despite temperatures during application being sufficiently hot to presumptively cause a phytotoxic response (P.M. Brannen, personal observation). Therefore, it is not clear whether phytotoxicity from sulfur applications is a valid concern in this region. If the risk of phytotoxicity is truly minimal, an understanding of the actual risk would allow growers in this region to spray sulfur as needed, regardless of typical summer weather conditions. This would be useful to vineyard managers, because they could increase the use of sulfur throughout the season and use at-risk fungicides primarily during the critical infection window for *E. necator* – pre-bloom, bloom, and post-bloom. If sulfur does cause damage if applied during high

temperatures, there could be opportunities to apply sulfur under cooler conditions (e.g. morning or evening), still allowing for optimal GPM management programs.

Therefore, the primary objective of this study was to determine whether temperatures and RH associated with mid- and late-afternoon spray timings interact with sulfur treatments to cause leaf phytotoxicity in northern Georgia *V. vinifera* vineyards. In addition, a limited number of interspecific grape varieties were evaluated for potential phytotoxic responses in more sulfur-sensitive cultivars.

MATERIALS AND METHODS

Sulfur application trials. Phytotoxic effects of the micronized sulfur product Microthiol Disperss (United Phosphorus, Inc., Cary, NC) was assessed in on-farm and research facility trials across several wine grape cultivars using maximum-rate applications of sulfur during the summer months of 2023 and 2024. For on-farm trials, seven cultivars were tested across four vineyards located in Lumpkin, Oconee, Rabun, Union, and White Counties, which are all located in northern Georgia. Four *V. vinifera* cultivars (‘Cabernet Franc’, ‘Chardonnay’, ‘Merlot’, and ‘Riesling’) unequally distributed across four commercial vineyards were evaluated in 2023 and 2024. Trials conducted on the three *V. vinifera* × native grape hybrids (‘Camminare Noir’, ‘Crimson Cabernet’, and a non-released cultivar, ‘UCD-07370-84’) were conducted at the University of Georgia Horticulture Farm (Vineyard E) (Watkinsville, Georgia) in 2024 only. In each location, a varied grower-standard spray program was administered. All tested sulfur applications were in addition to any other fungicides or other materials applied as part of the grower-standard regimens. Treatments were applied at each location in a completely randomized design, with five replications per treatment in vineyards A (Lumpkin County), B (Oconee

County), and C (Rabun County). Vineyard D (Union County) had three replications for the ‘Cabernet Franc’ and two for the ‘Riesling’. Each plot consisted of a single vine, and untreated vines were left between treatment vines to alleviate the confounding effect of potential spray drift. Treatments consisted of: (1) an untreated control; (2) Microthiol Disperss applied at 3:00 p.m.; and (3) Microthiol Disperss applied at 7:00 p.m. Applications of Microthiol Disperss were administered at a rate of 11.2 kg/ha with either an SR 450 (Stihl, Inc., Waiblingen, Germany) or a Power Backpack Mister 451 (Solo Global, Inc., Newport News, VA) mist blower backpack sprayer, and rates were calculated to correspond to a spray volume of 475 L/ha. Application dates were selected based on local weather forecasts with sunny skies and a predicted maximum temperature for the day $\geq 30^{\circ}\text{C}$, but these temperatures were not always achieved. All sites were sprayed twice during the first year of testing, using the same vines for application if no visual phytotoxicity was detected. For year two, different vines were selected on each site, and applications were made once on sites C and E, and twice on sites A, B, and D.

HOBO MX temperature and humidity data loggers (Onset Computer Corporation, Bourne, MA) were attached to the inside of nearby vines to monitor the temperature and humidity at the time of sulfur application. One week after application, a representative sample of 25 leaves per plot was collected, and each leaf was visually assessed for the level of phytotoxicity observed through a visual assessment scale (Figure 3.3). The incidence (percent of leaves with scorch symptoms) and severity (percent of leaf surface with scorch symptoms) was recorded. Any damage to leaves clearly resembling alternative sources of necrosis or scorch symptoms, such as downy mildew, Pierce’s disease, or physical damage, was disregarded. Data was analyzed using a Fisher’s protected LSD in JMP Pro version 17.0.0 (JMP Statistical Discovery, LLC, Cary, NC).

Historical data analysis. Past temperature and RH data (1 June 2003 – 26 August 2024) was retrieved from the University of Georgia Weather Network (<http://weather.uga.edu/>) for two locations that are representative of the region where the *V. vinifera* trials were conducted (Georgia Mountain Research Station, Blairsville, Georgia and Three Sisters Vineyard and Winery, Dahlonega, Georgia). Using Microsoft Excel (Microsoft, Inc., Redmond, WA), maximum temperatures were charted for days in which the $RH \geq 75\%$ during the months of June, July and August, for the entirety of the 21-year data set. Days with risk for sulfur phytotoxicity were determined by charting days in which a 30°C temperature occurred in conjunction with a $RH \geq 75\%$ and comparing to a 30°C threshold phytotoxicity line based on temperature.

RESULTS

Phytotoxicity of sulfur to *V. vinifera* grapes. Across all trials, no phytotoxicity due to sulfur was observed at any location where sulfur was applied to *V. vinifera* grapes. These observations were confirmed during analysis of collected leaves, as no statistical difference ($P > 0.05$) was found between treatments in any of the replications of the trial from any site (Tables 3.1 and 3.2). Leaf scorch was the only symptom associated with phytotoxicity to assess in these trials, and scorch incidence was highly variable between cultivars and locations, with some treated vines having low incidence ($< 2\%$) and some untreated vines having significant scorch symptoms ($> 60\%$) (Tables 3.1 and 3.2). These symptoms were caused by factors other than sulfur, such as fungicides applied by farm staff, as similar levels of symptom development were recorded on both untreated vines and those treated at 3:00 or 7:00 p.m.. Severity was generally uniformly low across sites and rarely surpassed 1% levels.

Phytotoxicity of sulfur to hybrid grapes. Though this trial was only conducted once, interspecific hybrid vines, as compared to *V. vinifera*, had more obvious scorch symptoms that could be attributed to sulfur applications. ‘UCD-07370-84’ and ‘Camminare Noir’ displayed an increased level of scorch symptoms when sulfur was applied; however, application of sulfur at either time did not result in statistically significant differences in phytotoxicity in comparison with the control (Table 3.2). When sulfur was applied to ‘Crimson Cabernet’, the symptoms of phytotoxicity damage were most pronounced. More damage was observed on leaves collected from the 3:00 p.m. sulfur application than those collected from the untreated control or the 7:00 p.m. application ($P \leq 0.05$) (Table 3.2). Extensive leaf yellowing, at essentially 100%, was observed throughout the vines when sulfur was applied to ‘Crimson Cabernet’, without regard to the time of application (Fig. 3.4).

Historical weather data and likelihood of phytotoxicity with sulfur. Historical temperature and RH data collected from two weather stations in northern Georgia revealed the rarity of weather events with 30°C or greater temperatures and $\geq 75\%$ RH (Figs. 3.1 and 3.2). A review of 21 years of data collected from a weather station in Dahlonega, Georgia revealed that there were only 11 days during this timeframe with the presumptive conditions necessary to cause a phytotoxic response if sulfur were applied (Fig. 3.1). Most of these days occurred either in 2010 or 2011 during July or August, but there was also one day during August of 2005 when conditions exceeded the predicted phytotoxic threshold. However, the timeframes in which the damage could occur (duration of these conditions for these specified days) were less than one hour. Indeed, temperature $\times$ RH conditions proposed as conducive to sulfur phytotoxicity never lasted more than 45 minutes, and never occurred during the morning hours (data not presented). Data collected from the Blairsville, Georgia weather station indicated that one day in June 2022

could have provided conditions that could possibly lead to phytotoxicity with sulfur when applied to *V. vinifera* grapes (Fig. 3.2), but again, the duration of these conditions was < 1 hr.

DISCUSSION

Sulfur causes phytotoxic symptoms when applied to several crops, including cucurbits (Branham et al., 2020), strawberries (Onofre et al., 2021) and grapes (Köycü et al., 2017). The potential for phytotoxicity with sulfur can become an obstacle to the implementation of an optimal fungicide spray program – one that incorporates fungicides in a manner that leads to limited development of fungicide resistance in local *E. necator* populations and maintains plant health and fruit quality. Though caution is warranted in some circumstances, it has been found that sulfur phytotoxicity can vary between grape cultivars, and many cultivars may not have this problem (Perchepped et al., 2004; Köycü et al., 2017). In this study, the phytotoxic effect of sulfur applications on *V. vinifera* cultivars was evaluated to examine the risk of damage to vines in the hot, humid climate observed in northern Georgia during the summer. We found that sulfur, applied at the highest labeled rate, did not cause a significant phytotoxic effect when sprayed on *V. vinifera* vines. No phytotoxicity was observed on *V. vinifera* that could be attributed to sulfur, regardless of the application timing or weather conditions during the sprays (Tables 3.1 and 3.2). Even application of sulfur at temperatures as high as 32°C provided little to no damage (Fig. 3.3; Tables 3.1 and 3.2).

Though the lack of phytotoxicity is somewhat surprising given the anecdotal temperature recommendations for ceasing application of sulfur found in various spray guides, it is consistent with both temperature and relative humidity thresholds for phytotoxicity proposed by Emmet et al. ($\geq 30^{\circ}\text{C}$, $\geq 75\%$) and Wilcox et al. ($\geq 32^{\circ}\text{C}$, $\geq 70\%$) (Emmet et al., 2003; Wilcox et al., 2015).

Additionally, temperatures $> 30^{\circ}\text{C}$ did not cause phytotoxicity if the RH did not reach the 75% or 70% thresholds. Therefore, the findings of our study are likely due to the lack of exceeding the RH threshold during and immediately following sulfur applications, as temperatures of $\geq 30^{\circ}\text{C}$ are routinely reached during Georgia summers, even in northern Georgia, but $\geq 70\%$ RH is rarely achieved while temperatures are $\geq 30^{\circ}\text{C}$.

Historical weather data from the UGA Weather Network (<http://weather.uga.edu/>) collected and analyzed from two sites in northern Georgia confirmed that greater than 75% humidity and 30°C weather conditions are rare. Over the course of 21 years in the Dahlonega area (elevation 440 m), only 11 days experienced predicted phytotoxicity-conducive weather parameters, and the conducive conditions never lasted longer than 45 minutes (Figure 3.1). These conditions are even rarer with increases in latitude and elevation, and data collected from the Blairsville, Georgia weather station (Elevation 540 m) revealed only one day with conducive conditions within the same 21-year timespan (Figure 3.2). The rarity of these environmental conditions helps to explain the lack of a phytotoxic response observed during these trials, as well as during previous trials with sulfur in northern Georgia (citations or personal observation). Additionally, the differences in temperature and RH among vineyards locations highlight the need to examine phytotoxicity risks independently by region. However, a warning is also warranted, as some *V. vinifera* grape cultivars have been reported to respond negatively to sulfur (Wilcox et al., 2015). If significant sulfur damage is observed on any grape cultivar, its application should be ceased immediately.

To confirm that the sulfur applications in the *V. vinifera* trials could actually induce phytotoxicity, we conducted limited testing on *V. vinifera* $\times$ American grape cultivars, as some interspecific hybrid cultivars are often known to have sensitivity to sulfur, resulting in a sulfur-

induced phytotoxic response. As expected, interspecific hybrids showed an increased sensitivity to sulfur, as compared to *V. vinifera*. The greatest and only significant response was observed on ‘Crimson Cabernet’, possibly due to its 50% heritage from the *Vitis aestivalis* hybrid ‘Norton’ (also known as ‘Cynthiana’) (Duncan, 2016). Though not statistically significant, sulfur application to ‘UCD-07370-84’ and ‘Camminare Noir’, both of which contain ~ 3% *V. Arizonica* and 3% *V. rupestris* heritage and are derived from the UC-Davis breeding program for Pierce’s disease resistance, resulted in an increased level of phytotoxicity. The phytotoxic responses for all three were less when applications were made at 7:00 pm (Table 3.2), presumably due to the cooler temperatures, but this response was not statistically significant for the cultivars with 6% American grape heritage. Though not statistically significant, the response observed does warrant further study as to the impact of sulfur on these cultivars and others with variable proportions of American grape species background in their lineages. Additional phytotoxic effects were observed on ‘Crimson Cabernet’, and these included a distinct blackening of inner portions of leaves with yellowing throughout (Figure 3.4). However, the most interesting observation was the extensive necrosis on the tips of leaves. The vast majority of observed phytotoxicity damage was limited marginal necrosis (scorch) on the very tips of fully expanded leaves (Figure 3.4). We hypothesize that this is due to spray solution sliding down the midrib of the leaf and resulting in a greater sulfur concentration on the leaf edge, resulting in a phytotoxic response.

CONCLUSIONS

The findings of this study have implications for growers in northern Georgia concerned with the potential for sulfur burn during the summer months. The absence of phytotoxicity on *V.*

vinifera vines and the rarity of conducive weather conditions demonstrate that sulfur phytotoxicity is likely not a relevant issue in the region where *V. vinifera* grapes are grown in Georgia. Additionally, concerned growers can likely avoid the conditions that may induce sulfur phytotoxicity by making applications that coincide with cooler conditions, such as those observed in the late evening or early morning. In conclusion, sulfur will not likely cause issues on *V. vinifera* grapes grown in northern Georgia, and sulfur application for GPM management should be incorporated throughout the season, as sulfur is a valuable tool for both efficacy against GPM and management of potential fungicide resistance in *E. necator* populations. This type of practical, applied research should be conducted wherever *V. vinifera* grapes are grown, as it can potentially expand the use of sulfur for summer applications, while also identifying regions in which sulfur phytotoxicity could be problematic, thereby limiting its use if needed as well.

LITERATURE CITED

- Beckerman, J., Bessin, R., Welty, C., Athey, K., Wahle, E., Lewis, D., Long, E., Joshi, N., Guedot, C., Meyer, S., Strang, J., and Gaulthier, N., 2022. Midwest Fruit Pest Management Guide 2023-2024. <https://ag.purdue.edu/departments/hla/extension/docs/id-465.pdf>.
- Beresford, R.M., Wright, P.J., Wood, P.N., and Agnew, R.H., 2016. Sensitivity of grapevine powdery mildew (*Erysiphe necator*) to demethylation inhibitor and quinone outside inhibitor fungicides in New Zealand. N. Z. Plant Prot. 69, 1-10.
- Branham, S.E., Daley, J., Levi, A., Hassell, R., and Wechter, W.P., 2020. QTL mapping and marker development for tolerance to sulfur phytotoxicity in melon (*Cucumis melo*). Front. Plant Sci. 11, 1097.
- Cooper, R. M., and Williams, J. S., 2004. Elemental sulphur as an induced antifungal substance in plant defence. J. Exp. Bot. 55, 1947-1953.
- Duncan, L. M., 2016. Quantitative Trait Loci (Qtl) Analysis Of Sulfur Sensitivity In Vitis Aestivalis-Derived 'Norton'. MSU Graduate Theses.
- Emmett, B., Rozario, S., and Hawtin, J., 2003. Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines. Grape Wine Res. Dev. Corp. 98, 220.
- Gadoury, D.M., Cadle-Davidson, L., Wilcox, W.F., Dry, I.B., Seem, R.C., and Milgroom, M. G., 2012. Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Mol. Plant Pathol. 13, 1-16.

- Köycü, N.D., Stenger, J.E., and Hatterman-Valenti, H.M., 2017. Cold climate winegrape cultivar sensitivity to sulfur in the northern great plains region of the United States. *HortTechnology* 27, 235-239.
- McManus, P. S., Kartanos, V., and Stasiak, M., 2017. Sensitivity of cold-climate wine grape cultivars to copper, sulfur, and difenoconazole fungicides. *Crop Protection* 92, 122-130.
- Miles, L.A., Miles, T.D., Kirk, W.W., and Schilder, A.M.C., 2012. Strobilurin (QoI) resistance in populations of *Erysiphe necator* on grapes in Michigan. *Plant Dis.* 96, 1621-1628.
- Neill, T., Livesay, A., Albrecht, A., and Mahaffee, W., 2015. Seeking the right level of sulfur to fight powdery mildew on grapes. OSU Extension Service. <https://extension.oregonstate.edu/crop-production/wine-grapes/seeking-right-level-sulfur-fight-powdery-mildew-grapes#:~:text=Sulfur%20applications%20of%20less%20than,mildew%20on%20leaves%20and%20fruit>.
- Oliver, C., Cooper, M., Ivey, M. L., Brannen, P., Miles, T., Lowder, S., Mahaffee, W., and Moyer, M. M., 2024. Fungicide use patterns in select United States wine grape production regions. *Plant Dis.* 108, 104-112.
- Onofre, R.B., Gadoury, D.M., and Peres, N.A., 2021. High efficacy and low risk of phytotoxicity of sulfur in the suppression of strawberry powdery mildew. *PHP.* 22, 101-107.
- Perchepied, L., Périn, C., Giovino, N., Besombes, D., Dogimont, C., and Pitrat, M., 2004. Susceptibility to sulfur dusting and inheritance in melon. *Progress in Cucurbit Genetics and Breeding Research.* 353-357.
- Taksonyi, P., Kocsis, L., Matyas, K.K., and Taller, J., 2013. The effect of quinone outside inhibitor fungicides on powdery mildew in a grape vineyard in Hungary. *Sci. Hortic.* 161, 233-238.

- Warres, B.E., 2021. Fungicide efficacy and resistance management for *Erysiphe necator* [Doctoral dissertation, University of Georgia].
- Wilcox, W. F., Gubler, W. D., and Uyemoto, J. K., eds., 2015. Compendium of grape diseases, disorders, and pests, 2nd Ed. APS Press, St. Paul, MN.
- Williams, J.S., and Cooper, R.M., 2004. The oldest fungicide and newest phytoalexin—a reappraisal of the fungitoxicity of elemental sulphur. *Plant Pathol.* 53, 263-279.

Table 3.1. Effect of sulfur application in 2023 on phytotoxicity of grapes when applied at different times

Vineyard	Cultivar	First trial ^{ac}						Second trial ^{cb}					
		Incidence			Severity			Incidence			Severity		
		Untreated	3:00 p.m.	7:00 p.m.	Untreated	3:00 p.m.	7:00 p.m.	Untreated	3:00 p.m.	7:00 p.m.	Untreated	3:00 p.m.	7:00 p.m.
A	Cabernet Franc	15.2	5.0	11.2	0.5	0.1	0.9	20.0	18.4	16.0	0.4	0.5	0.3
	Chardonnay	12.0	12.2	14.4	0.1	0.6	0.8	12.0	10.4	12.8	0.3	0.1	0.1
	Merlot	6.4	9.6	7.2	0.2	1.0	0.2	5.6	8.8	8.0	0.2	0.3	0.3
	Temperature/Humidity ^d		31.2/55	26.8/71		31.2/55	26.8/71		32.5/63	29.3/66		32.5/63	29.3/66
B	Cabernet Franc	16.0	22.4	16.0	0.3	0.5	0.3	28.0	38.4	36.0	0.5	0.6	0.7
	Chardonnay	38.4	41.6	36.0	0.6	0.9	0.7	24.0	32.8	35.2	0.5	0.9	0.9
	Merlot	8.0	18.4	21.6	0.2	0.4	0.5	23.2	24.0	34.4	0.4	0.5	0.8
	Temperature/Humidity		31.8/56	27.9/69		31.8/56	27.9/69		29.8/60	27.7/66		29.8/60	27.7/66
C	Cabernet Franc	46.0	52.0	44.0	0.92	1.3	0.86	25.6	28.8	34.4	0.5	0.6	0.7
	Temperature/Humidity		26.3/67	24.4/78		26.3/67	24.4/78		30.9/48	26.4/65		30.9/48	26.4/65
D ^e	Cabernet Franc	6.7	8.0	16.0	0.1	0.1	0.3	61.3	68.0	70.7	3.1	3.1	3.0
	Riesling	2.0	18.0	0.0	0.0	0.46	0.0	52.0	56.0	48.0	1.2	2.1	1.0
	Temperature/Humidity		31.1/57	27.9/69		31.1/57	27.9/69		32.7/50	26.9/80		32.7/50	26.9/80

^aTreatment dates by vineyard for the first trial were as follows: A = 24 July, B = 18 July, C = 16 July, and D = 25 July.

^bTreatment dates by vineyard for the second trial were as follows: A = 23 August, B = 23 August, C = 24 July, and D = 25 July.

^cIncidence (% leaves with phytotoxicity) and severity (% coverage of phytotoxicity) from 25 leaves per replicate.

^dTemperature and RH recorded at the time of application.

^eMean incidence and severity of phytotoxicity for ‘Cabernet Franc’ and ‘Riesling’ were calculated using three and two replicates, respectively, in vineyard D, as opposed to five replicates in vineyards A-C.

Table 3.2. Effect of sulfur application in 2024 on phytotoxicity of grapes when applied at different times

Vineyard	Cultivar	First trial ^{a,c}						Second trial ^{b,c}					
		Incidence			Severity			Incidence			Severity		
		Untreated	3:00 p.m.	7:00 p.m.	Untreated	3:00 p.m.	7:00 p.m.	Untreated	3:00 p.m.	7:00 p.m.	Untreated	3:00 p.m.	7:00 p.m.
A	Cabernet Franc	4.8	1.6	3.2	0.1	0.1	0.0	20.8	15.2	8.0	0.3	0.3	0.2
	Chardonnay	2.4	1.6	5.6	0.1	0.1	0.1	5.6	7.2	16.0	1.6	0.4	1.1
	Merlot	4.0	7.2	4.8	0.1	0.1	0.1	57.6	64.0	54.4	1.2	2.8	1.6
	Temperature/Humidity ^d	31.1/50		28.3/59		31.1/50		28.3/59		33.1/36		27.0/59	
B	Cabernet Franc	32.8	46.4	41.6	0.5	0.9	0.7	-	-	-	-	-	-
	Chardonnay	60.0	67.2	60.8	0.7	1.0	0.9	45.6	60.0	51.2	0.8	0.9	0.8
	Merlot	-	-	-	-	-	-	-	-	-	-	-	-
	Temperature/Humidity	28.4/40		21.7/58		28.4/40		21.7/58		32.4/41		31.6/45	
C	Cabernet Franc	48.0	40.0	42.4	0.7	0.6	0.7						
	Temperature/Humidity	- ^e		-		-		-		-		-	
D ^f	Cabernet Franc	1.3	0.0	8.0	0.0	0.0	0.1	8.0	14.7	20.0	0.1	0.5	0.7
	Riesling	2.0	6.0	0.0	0.0	0.1	0.0	10.0	10.0	22.0	0.3	0.1	0.6
	Temperature/Humidity	30.9/58		27.0/76		30.9/58		27.0/76		31.9/36		25.1/68	
E	Crimson Cabernet	11.2 a ^g	48.8 b	22.4 a	0.1 a	2.1 b	0.6 a	-	-	-	-	-	-
	UCD-07370-84	9.6	24.8	20.8	0.2	1.5	1.8	-	-	-	-	-	-
	Camminare Noir	3.2	14.4	11.2	0.1	0.9	1.3	-	-	-	-	-	-
	Temperature/Humidity	31.7/45		27.8/56		31.7/45		27.8/56					

^aTreatment dates by vineyard for the first trial were as follows: A = 11 July, B = 20 August, C = 28 August, D = 31 July, and E = 19 June.

^bTreatment dates by vineyard for the second trial were as follows: A = 28 August, B = 27, and D = 27 August.

^cIncidence (% leaves with phytotoxicity and severity (% coverage of phytotoxicity) from 25 leaves per replicate.

^dTemperature and RH recorded at the time of application.

^eWeather data not recorded.

^fMean incidence and severity for ‘Cabernet Franc’ and ‘Riesling’ were calculated using three and two replicates, respectively, in vineyard D, as opposed to five replicates in vineyards A-C.

^gMeans followed by the same letter are not significantly different when comparing each pair using Fishers protected LSD ($P \leq 0.05$). Where no letters are shown, there were no statistical differences in the data.

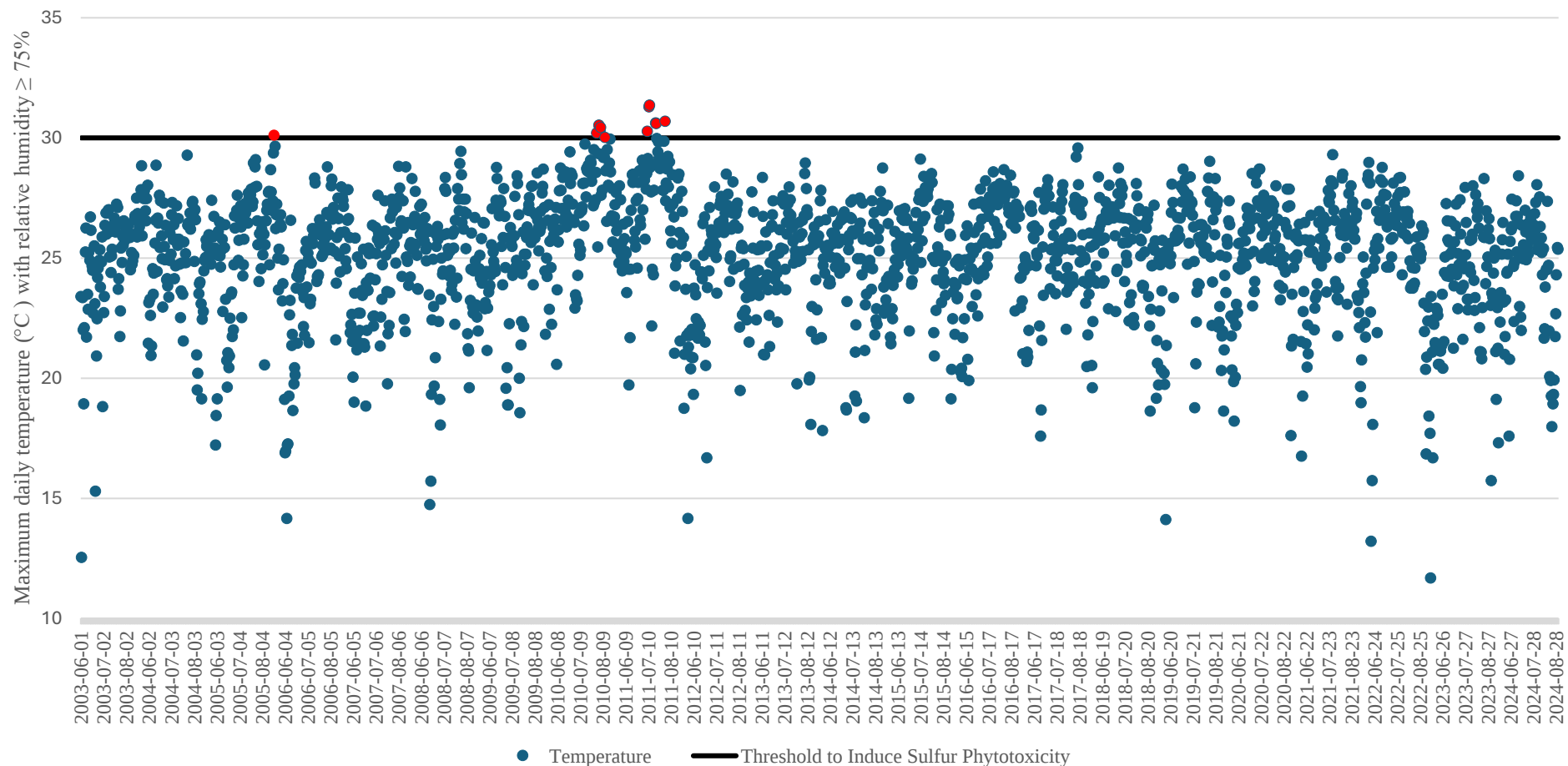


Figure 3.1. Twenty-one year daily maximum temperatures recorded when the maximum daily (same day) relative humidity (RH) $\geq 75\%$ (Three Sisters Vineyard; Dahlonega, Georgia). Any day in which the temperature crosses the black horizontal line (30°C) is displayed in red, and the conditions of RH and temperature could have theoretically resulted in some level of phytotoxicity on *Vitis vinifera* grapes. Only data from the summer months (June, July, and August) are displayed. Only rarely did these conditions occur for this site, and the duration of the simultaneous events was minimal.

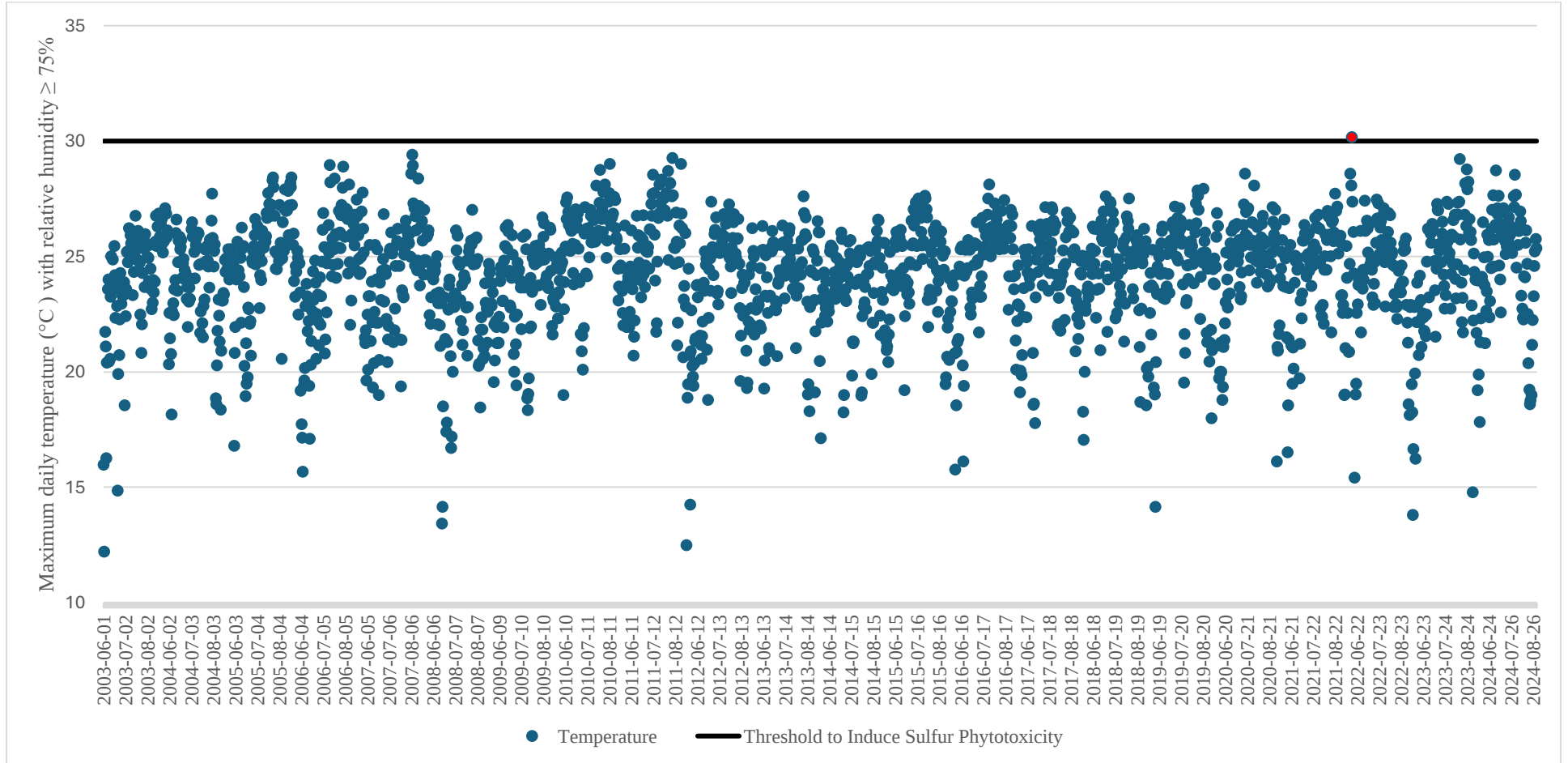


Figure 3.2. Twenty-one year daily maximum temperatures recorded when the maximum daily (same day) relative humidity (RH) $\geq$ 75% (Georgia Mountain Research Station, Blairsville, Georgia). Any day in which the temperature crosses the black horizontal line (30°C) is displayed in red, and the conditions of RH and temperature could have theoretically resulted in some level of phytotoxicity on *Vitis vinifera* grapes. Only data from the summer months (June, July, and August) are displayed. These conditions only occurred once for this site.

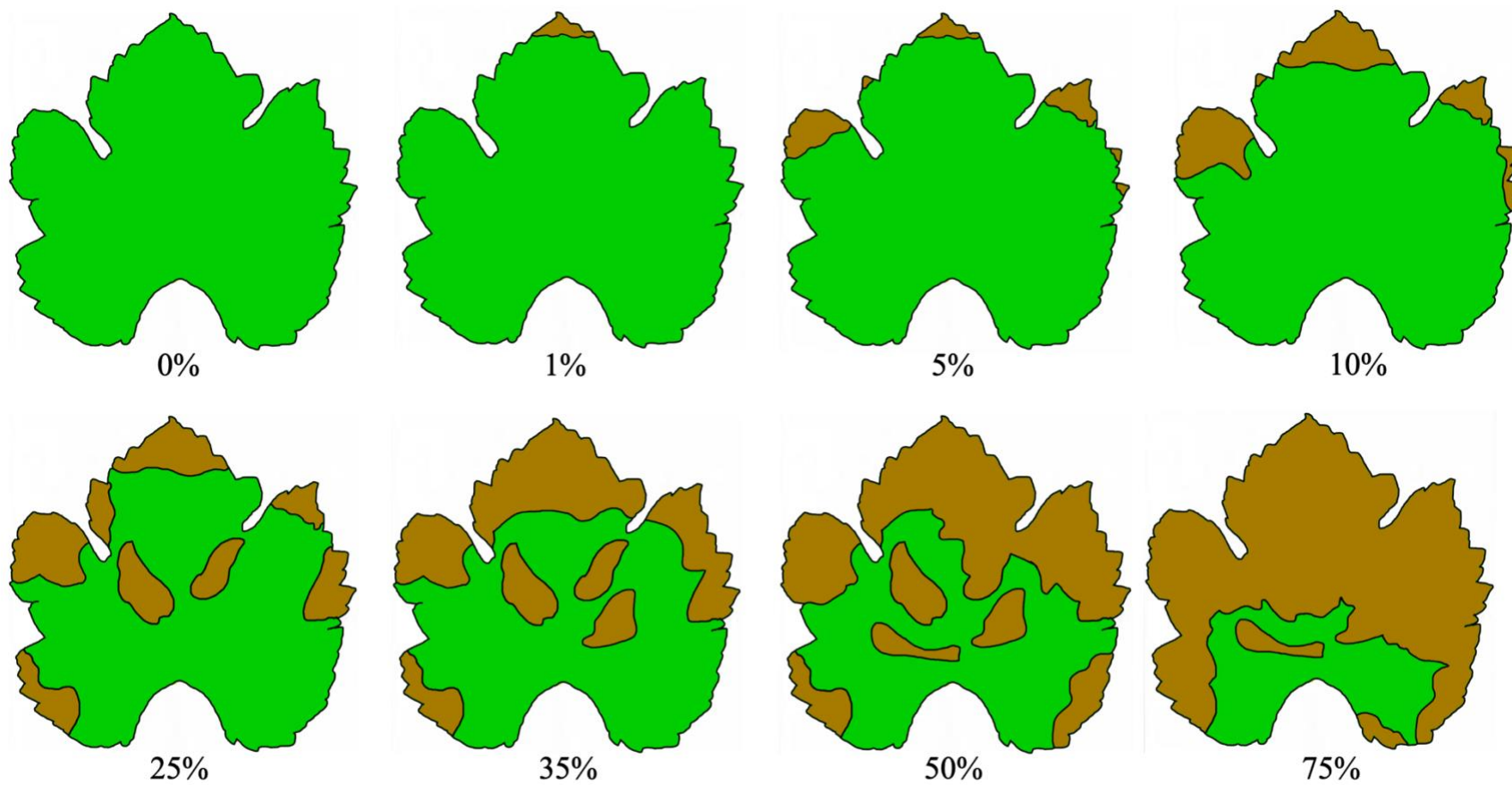


Figure 3.3. Damage scale used to assess phytotoxicity (scorch symptoms) on individual grape leaves. Numbers below leaves correspond to percent scorched (necrotic) tissue area.



Figure 3.4. ‘Cabernet Franc’ vines one week after Microthiol Disperss was applied at 11.2 kg/ha – the maximum single application rate. From left to right: untreated control, 3:00 p.m. application (31.2°C and 55% relative humidity at time of application), and 7:00 p.m. application (26.8°C and 71% relative humidity at time of application). Sulfur applications were conducted on 24 July. Sulfur did not result in phytotoxicity.



Figure 3.5. Various forms of phytotoxicity observed on hybrid grapes (*Vitis vinifera* × American grape species) following application of Microthiol Disperss applied at 11.2 kg/ha – the maximum single application rate. From left to right: yellowing of leaves of ‘Crimson Cabernet’, scorching on leaves of ‘UCD-07370-84’, and mild marginal necrosis or scorch of ‘Crimson Cabernet’.

CHAPTER 4

CONCLUSIONS

Grapevine powdery mildew (GPM), caused by the fungal pathogen *Erysiphe necator*, is a disease of primary concern wherever grapes are grown. Growers use a multitude of systemic fungicides to combat this disease; however, many of them can lead to resistance development within fungal populations, including GPM. Sulfur is the most frequently used contact fungicide for GPM control and is an excellent resistance management tool, despite its efficacy being lower than some systemic products. Given the growing fungicide resistance problems in GPM populations within winegrape operations, research is needed on how to utilize low-risk materials such as sulfur to their fullest extent. In these studies, non-ionic organosilicone surfactants and air induction nozzles were assessed for their abilities to increase the efficacy of sulfur, potentially through improvements in spray coverage. Additionally, the sensitivity of European winegrapes to sulfur phytotoxicity was assessed in northern Georgia during potentially conducive weather conditions.

Three non-ionic organosilicone surfactant products (Hi-Wett, Cohere, and Silwet L-77) were tested in tank mixes with a micronized sulfur product (Microthiol Disperss) on a block of ‘Chardonnay’ vines at the Georgia Mountain Research and Education Center in Blairsville, Georgia (Chapter 2). In 2023, Hi-Wett (Loveland Products, Inc., Loveland, CO), Cohere (Helena Agri-Enterprises, LLC, Collierville, TN), and Silwet L-77 all provided significant improvements in GPM disease control when sprayed both independently and as a tank mix with Microthiol Disperss (United Phosphorus, Inc., Cary, NC). An analysis for synergistic interactions using the

Colby method during this year revealed that each product interacted synergistically with Microthiol Disperss. However, when a second trial was conducted in 2024, the results were inconsistent with the previous year. Though the addition of surfactants resulted in increased disease control, the effect was diminished compared to 2023. In 2024, Hi-Wett improved GPM control on leaves, but not fruits, while Cohere provided no advantage. Silwet L-77 was effective during both years, raising questions about the chemical activity of these products. These inconsistencies were likely due to differences in rainfall between the two years. We can speculate that the consistent rainfall in 2023 could have washed off sulfur residue and necessitated re-application, whereas the drought conditions of 2024 may have caused sulfur to build up – decreasing the utility of the coverage-improving surfactant. Regardless of inconsistencies, surfactants generally resulted in improvements in disease control. This research demonstrated that surfactants do have utility in tank mixes for sulfur application for grapevine powdery mildew control.

Another trial was conducted at the Georgia Mountain Research and Education Center on a block of ‘Merlot’ vines to assess the efficacy of air induction nozzles for the application of sulfur with and without an added surfactant (Chapter 2). One year of data was collected for this trial in 2024. In this trial, air induction (AI) nozzles were generally less efficacious in terms of disease control when compared to standard cone nozzles typically used in vineyard operations in Georgia. Additionally, a positive impact on GPM disease management was found with an added surfactant when using a cone nozzle, but this was not observed when using the AI nozzle. Coverage was assessed using water-sensitive cards and compared between treatments of each nozzle with and without a surfactant. Coverage was similar between nozzle types, and a surfactant provided improved coverage when using either nozzle type. However, the spray

pattern was strikingly different between nozzles, which likely explains the differences in disease control. This research demonstrated that air induction nozzles are not likely to provide an improvement over the current industry standard; however, additional trials are needed to confirm these results.

The sensitivity of European winegrapes to the phytotoxic effect of sulfur is not well-described in the hot and humid climate of northern Georgia. This causes many growers to utilize caution when applying sulfur to vines during the summer months. Sulfur phytotoxicity (sensitivity) was assessed at five separate locations in northern Georgia on four cultivars of European winegrapes, as well as three interspecific hybrid cultivars of winegrapes (Chapter 3). High-rate treatments of Microthiol Disperss were made during the mid- and early evening to compare any phytotoxicity symptoms to an untreated control. We determined that sulfur phytotoxicity on European winegrapes is a rare occurrence in northern Georgia, even during temperatures as high as 33°C. All replications of the experiment using European grapes yielded no differences in phytotoxicity among treatments. However, phytotoxicity was observed when testing interspecific hybrid grape cultivars, the greatest amount of which was found on the cultivar with the greatest percentage of American grape lineage. Climate data from the northern Georgia area revealed that the proposed weather conditions conducive to sulfur phytotoxicity ($\geq 30^{\circ}\text{C}$ and 75% humidity) are rare, explaining the lack of phytotoxicity on European grapes observed during the trial. This research revealed that phytotoxicity is likely not a problem for northern Georgia growers, as the proposed conditions rarely occur.

Grapevine powdery mildew has been a problematic disease for grape producers since its discovery in the mid-1800s. The aggressiveness and fungicide resistance potential of *E. necator* indicate that it will continue to be a priority for current and future disease-management programs

wherever European winegrapes are grown. The information gathered in this study can be applied by winegrape producers to improve their disease management strategies and maximize GPM control, especially in the northern Georgia area. Tank mixing non-ionic organosilicone surfactants with sulfur can provide additional efficacy for GPM control. Additionally, mid-summer sulfur applications in northern Georgia should not be avoided for fear of phytotoxicity, as proposed phytotoxicity-conducive weather is rare.