AJEV | American Journal of Enology and Viticulture

Research Report
DOI: 10.5344/ajev.2026.26008

Phenology-Based Applications of Biofungicides and Sulfur with an Intelligent Sprayer for Management of Grape Powdery Mildew

Brent W. Warneke [1,2*] Lloyd L. Nackley [2] and Jay W. Pscheidt [1]

Abstract

Background and goals

Though biological fungicides are often organic compliant, they can be expensive and have variable efficacy against grape diseases. In research fields, these products have not adequately managed grape powdery mildew (*Erysiphe necator*; GPM) alone. Phenology-based applications of biological fungicides were hypothesized to provide a more effective evaluation strategy for biological fungicide efficacy than traditional season-long regimes, due to their mode of action.

Methods and key findings

In a 2021 experiment, biological fungicides were applied only during bloom and ~4 wk after, with sulfur applied before and after that period (S/B/S treatments). In 2021 to 2023, sulfur was applied only during bloom and 4 wk after, while biological fungicides were applied before and after bloom (B/S/B treatments). Additionally, regimes were applied using a variable-rate intelligent sprayer to determine the effect of variable-rate applications on GPM control efficacy. In the B/S/B regime, the largest applied quantity of LifeGard resulted in the lowest cluster severity in 2022 and 2023, which was significantly lower than phenological sulfur applied alone in 2023. In the B/S/B regime, Theia applied in standard or intelligent modes showed no differences in cluster severity in 2022 and 2023.

Conclusions and significance

When using variable rate sprayers and biological fungicides, some products (such as Theia) may be more robust to application across a wider range of rates for GPM management than others (such as LifeGard). Biological fungicides and phenology-based application regimes could provide a more effective evaluation regime for biological fungicide efficacy research, as well as alternatives to calendar applications or synthetic fungicides for GPM management.

Key words: *Bacillus*, biofungicide, sensors, sustainability, variable rate, vineyard

Introduction

Winegrapes are an important agricultural commodity in Oregon, with a production value of $330 million in 2024 (as reported at https://www.oregon.gov/oda/Documents/Publications/Administration/ORAgFactsFigures.pdf). Additionally, the Oregon winegrape industry is an agritourism draw that contributes $8.1 billion in overall value to the Oregon economy and supports an estimated 39,400 jobs (as reported at https://industry.oregonwine.org/press-releases/2022-economic-impact-of-the-wine-and-wine-grape-industries-on-the-oregon-economy/). Winegrapes (*Vitis vinifera* L.) are European in origin and lack resistance to plant pathogens that originated in North America such as grape powdery mildew (GPM, *Erysiphe necator*) (Kunova et al. 2021).

Due to a lack of constitutive host resistance and low thresholds of infection for winery acceptance, winegrape production relies heavily on fungicide applications for management of GPM. For example, an average of 19.5 kg fungicide/ha is applied annually throughout the European Union to combat this pathogen (Gianessi and Williams 2011). Due to years of intensive fungicide use, resistance to widely used synthetic fungicide groups has been documented in West Coast winegrape growing regions (Miles et al. 2021). Widespread occurrence of fungicide resistance to commonly-used fungicide groups in Oregon has resulted in viticulturists increasingly turning to non-synthetic fungicides with low resistance risk (such as sulfur and biological fungicides) to manage GPM (Williams and Cooper 2004, Oliver and Beckerman 2022).

The increasing demand for non-synthetic fungicides among viticulturists has also coincided with changes in consumer preferences. In recent years, consumer scrutiny has popularized wine made from grapes grown with programs perceived to use less toxic pesticides, such as USDA Organic, Salmon Safe, and similar marketing

[1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331; and [2]Department of Horticulture, Oregon State University, Corvallis, OR, 97331.
*Corresponding author (brent.warneke@OregonState.edu)

Manuscript submitted Feb 2025, accepted April 2026, published July 2026

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programs (Tait et al. 2019). Among consumers, such pressure has resulted in perceived increased value and willingness-to-pay for sustainably produced wine (Tait et al. 2019). Sulfur has long been the industry's favorite non-synthetic fungicide for GPM control due to its reliable efficacy and low cost (Griffith et al. 2015). However, sulfur applications are often curtailed at veraison, or before, to minimize residues on harvested clusters that can result in off-flavors in the resulting wine (Kwasniewski et al. 2014). Therefore, there is demand for other non-synthetic fungicides to manage GPM.

These factors have increased interest in, and the use of, biological fungicides for management of GPM. The main benefits of biological fungicides are their low chances of developing resistance, usually organic certification, and low environmental and worker impact (Oliver and Beckerman 2022). Biological fungicides are products that inhibit pathogen growth through the action or exudates of an organism, or by the physical action of a natural product such as a plant extract or plant-based oil. These products are often considered to have multiple modes of action that are thought to be compatible with resistance management programs, and have favorable use characteristics such as short preharvest and re-entry intervals (Beckerman et al. 2023). While there are many forces encouraging development and use of biologicals, their efficacy is not as reliable for GPM management as synthetic fungicides, and careful consideration of the pathosystem within an Integrated Pest Management (IPM) practice is often required (Beckerman et al. 2023).

Bloom and fruit set (BBCH 55 to 71) are the most critical times of grape development at which to protect clusters from GPM, since the newly forming berry tissue is highly susceptible to infection (Meier 2001). Correspondingly, the most intensive GPM management efforts are focused on this period (Gadoury et al. 2003, Warneke et al. 2020b, Nackley et al. 2021). After berries reach 3 to 4 mm in diameter (approx. BBCH 75), they develop ontogenic resistance and become nearly immune to infection (Gadoury et al. 2003). After BBCH 75, fungicide programs are less intensive and usually stop completely around veraison (BBCH 83) (Meier 2001, Skinkis et al. 2024).

Applications of biological fungicides targeted to different grapevine phenological growth stages may support their mode of action and elucidate an effective approach to evaluate their efficacy for GPM management. In previous studies evaluating season-long applications of single biological fungicides in research vineyards, poor GPM control was often observed (Pscheidt and Kroese 2022, 2023). Targeting biological fungicide applications to early and late parts of the growing season (when disease levels are low and fungicide resistance is a concern, respectively) may more effectively harness the mode of action of a biological fungicide than traditional season-long research regimes. To fill out the program, applying sulfur during bloom and early berry development would minimally impact action of the biological fungicides, in addition to following recommendations to use the most effective fungicides available during this period (Skinkis et al. 2024). However, biological fungicides, and GPM spray programs in general, are expensive and growers frequently aim to streamline application efficiency to save money.

Improving spray application efficiency can provide large savings for grapegrowers. Western Oregon viticulturists usually apply 6 to 16 fungicide applications targeting GPM, depending on the viticulture philosophy of the vineyard, with organic programs typically at the higher end of that range (Thiessen et al. 2017). Such GPM management tactics are costly and can account for as much as 30% of gross production costs (Sambucci et al. 2014). There have been many recent innovations to increase spray application efficiency and thus decrease spray costs (Giles et al. 2011, Warneke et al. 2019). Sensor-controlled sprayers are a rapidly expanding sector that can lead to large savings on spray-related expenses (Warneke et al. 2019, 2020a). Sensor sprayers can save 15 to 73% of applied pesticide volume compared to standard air blast sprayers (Warneke et al. 2022). These reductions in applied pesticide volume result in a cascade of benefits to the farm, such as reductions in the cost of labor (due to sprays being completed more quickly), the amount of water necessary for mixing, and the required amount of fuel (due to fewer hours driven by tractors) (Warneke et al. 2019). The Intelligent Sprayer System (ISS) is the only commercially available light detection and ranging (LiDAR)-controlled variable-rate sensor sprayer and it has been shown to reduce applied spray volume by 30 to 60% compared to standard sprayers, with equivalent disease and pest control (Chen et al. 2019, 2020). However, the ISS has not yet been tested with biological fungicides for GPM management.

The goal of this project was to evaluate ISS variable rate application of biological fungicides, alternated with sulfur, in phenological regimes on GPM control. In doing so, we investigated a new method to evaluate biological fungicides in a research context which is also practically applicable to commercial viticulture fungicide application regimes.

Materials and Methods

An airblast sprayer (Pak-blast 189 L [50 gal], Rears Mfg.) with seven TeeJet ceramic D3 disc and DC25 core nozzles on each side and a 61-cm fan was used for all applications. This sprayer was retrofitted with the ISS, which adds a LiDAR laser sensor, Doppler speed sensor, embedded computer, and individual pulse width modulation (PWM) solenoid valves at each sprayer nozzle. These components adjust pesticide application volume in real time to match plant canopy characteristics. A spray console wired to the system allowed use of either real-time adjustment of application volume (intelligent mode) or a constant application volume (standard mode). In intelligent mode, the spray volume applied per canopy volume was adjusted by changing the "spray rate" parameter. Two spray rates were tested, 62.5 and 125 mL/m^3, hereafter referred to as Intelligent Low and Intelligent High, respectively (Tables 1 to 3). Intelligent Low and High modes consistently applied their respective spray rate to canopy foliage.

Correspondingly, as the crop canopy increased in density due to natural growth and lateral shoot proliferation from hedging, total applied spray volume increased until a plateau was reached when shoot development halted.

Biological fungicides were *Bacillus* based, each with different activity profiles. LifeGard (40% active ingredient [AI], *Bacillus mycoides* isolate J) is a wettable granule marketed as a plant systemic acquired resistance (SAR) activator with no direct effect on the target pathogen. Theia is a dry flowable (100% AI, *Bacillus subtilis* strain AFS032321), Serenade ASO is a liquid suspension (1.34% AI, *B. subtilis* strain QST 713), and Aviv is a soluble liquid (0.08% AI, *B. subtilis* strain IAB/BS03) (Summit Agro). Theia, Serenade ASO, and Aviv are marketed as SAR activators, antimicrobials, and niche competitors.

For all trials, fungicide programs (Figure 1, Tables 1 to 3) were initiated just after the first powdery mildew infections were located through a standard scouting program. For the biological fungicide–sulfur–biological fungicide experiments (hereafter referred to as B/S/B), biological fungicides (Tables 1 to 3) were applied until 50% bloom, then 5.6 kg/ha micronized sulfur (Microthiol Disperss, UPL) was applied until ~4 wk postbloom, when the fungicide program for each cultivar reverted back to what was applied before bloom (Figure 1). For the sulfur–biological fungicide–sulfur experiments (hereafter referred to as S/B/S), the biological

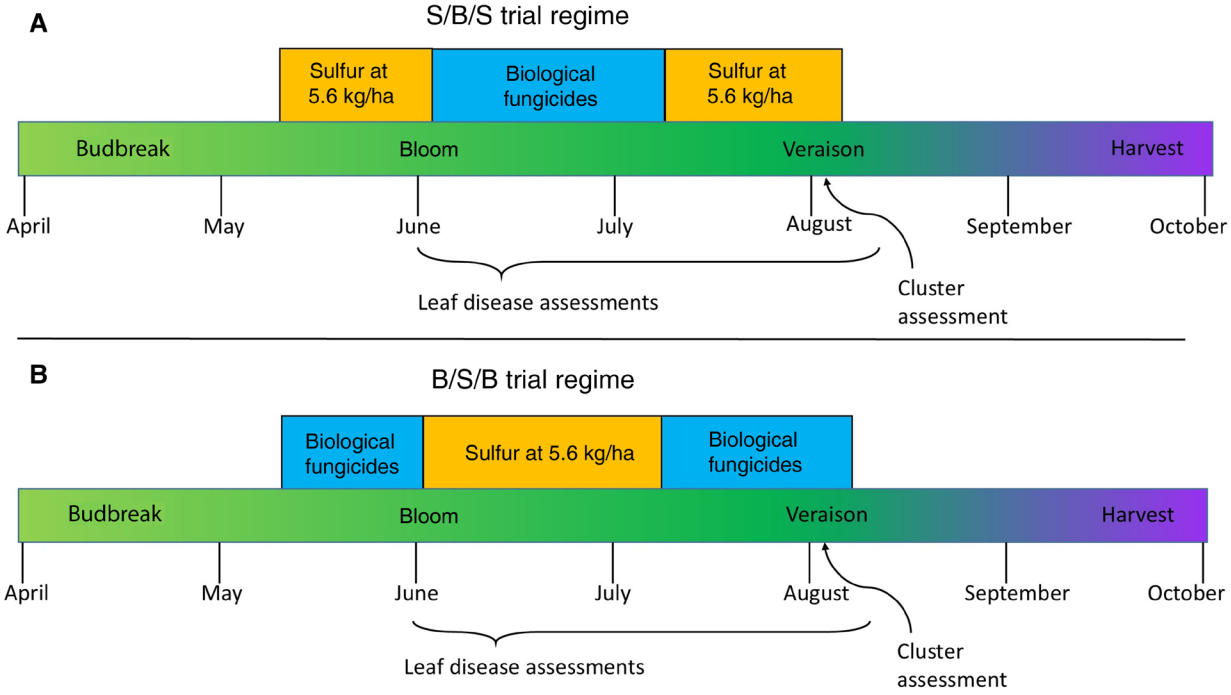

Figure 1 Schematic of fungicide applications (colored boxes), general phenology (within timeline), and data acquisition timing (below timeline) during the (**A**) 2021 sulfur–biological fungicide–sulfur (S/B/S) study and (**B**) 2021 to 2023 biological fungicide–sulfur–biological fungicide (B/S/B) studies. In the S/B/S study, micronized sulfur was applied from BBCH 17 to BBCH 64, biological fungicides were applied from BBCH 65 to BBCH 75, then micronized sulfur applications were resumed until BBCH 81; in the B/S/B studies, biological fungicides were applied from BBCH 17 to BBCH 64, micronized sulfur was applied from BBCH 65 to BBCH 75, then biological fungicide applications were resumed until BBCH 81.

Table 1 Area under disease progress curve (AUDPC, leaf disease) and percent infected berries from the sulfur–biological fungicide–sulfur (S/B/S)[a] intelligent sprayer trial in 2021.

Treatment[b]	Rate[c]	AUDPC[d]	Percent infected berries
Untreated	Untreated	2964 (2916-3012) A	74.0 (68.0-79.2) A
Aviv intelligent	887 mL/379 L	2266 (2081-2451) B	13.8 (10.7-17.7) B
Aviv standard	887 mL/379 L	2231 (1982-2480) B	16.1 (12.5-20.4) B
Serenade ASO intelligent	9.3 L/ha	1974 (1552-2396) B	14.6 (11.3-18.7) B
Serenade ASO standard	9.3 L/ha	2236 (2004-2467) B	18.2 (14.2-23.0) B

[a]5.6 kg/ha micronized sulfur applied from BBCH 17 to BBCH 64, biological fungicides then applied from BBCH 65 to BBCH 75, then 5.6 kg/ha micronized sulfur applications resumed until veraison (BBCH 81).
[b]Aviv contains *Bacillus subtilis* strain IAB/BS03 (0.08%) as the active ingredient (AI) and Serenade ASO contains *B. subtilis* strain QST 713 (1.34%) as the AI.
[c]All treatments applied at 552 kPa; intelligent sprayer treatments applied at 125 mL/m^3 of grape canopy.
[d]Estimates are followed by asymptotic 95% confidence intervals in parentheses. Treatments followed by different letters are statistically different from each other, marginal means contrast ($p \leq 0.05$) with p values adjusted using Tukey's method.

fungicide and sulfur applications were the opposite of the B/S/B regime. Initially, 5.6 kg/ha sulfur was applied until 50% bloom, after which biological fungicides (Table 1) were applied until ~4 wk postbloom. Sulfur applications were then resumed and continued until veraison.

The vines used were Pinot noir and Pinot gris planted in 1998 on *Vitis rupestris* × *Vitis riparia* 101-14 rootstock with 2.1 m × 2.4 m (7 ft × 8 ft) spacing. The non-irrigated vineyard was located at the Oregon State University Botany and Plant Pathology farm (45°33' N; 123°14' W) located in the Willamette Valley American Viticultural Area on level ground at 78-m elevation. All *V. vinifera* vines were cane-pruned and trained to a Guyot trellis system with vertical shoot-positioning. To reduce row-to-row interference, every other row was planted with 101-14 vines and trained to a Guyot trellis with spurs and vertical shoot-positioning. Buffer rows were not sprayed, as only a single side of the airblast sprayer was used. In addition, to reduce plot-to-plot interference, between every five *V. vinifera* vines, a single 101-14 vine was planted and trained as described above. There were no missing vines in the vineyard. All vines were pruned by mid-March each year. For *V. vinifera*, leaves were removed from the east side of the N-S oriented vine rows when berries were grout-sized (BBCH 73). Canopy management tasks such as hedging, shoot tucking, and suckering were conducted as needed throughout the season according to local best practices. Fungicide treatments were applied every 7 to 10 days. Each treatment was replicated on four sets of five vines. Treatments (Tables 1 to 3) were arranged in a randomized complete block design in each experiment, with treatments re-randomized in each block at the beginning of each season.

Leaves and clusters were evaluated for GPM incidence (weekly from mid-June to veraison) and severity (once just prior to veraison), respectively. The middle three vines of each plot were examined for GPM by arbitrarily selecting either 25 clusters or leaves on both the east and west side of the row, for a total of 50 units examined per plot. Weekly leaf incidence levels were used to calculate absolute area under disease progress curves (AUDPC), using the 'agricolae' package ver. 1.3-7 (de Mendiburu 2020, R Core Team 2020).

In 2021, spring weather was mild, rainfall was well below average, and an unusual climate change-related heat dome (heat wave) occurred for 3 days in late June, with temperatures at or above 38°C. This resulted in the second-driest and second-hottest growing season ever recorded at the research site. In 2022, spring weather conditions were very wet, resulting in the second-wettest spring on record. A frost event on 14 April 2022 resulted in delayed vine development

Table 2 Area under disease progress curve (AUDPC, leaf disease) and percent infected berries from the biological fungicide–sulfur–biological fungicide (B/S/B)[a] intelligent sprayer trial in 2021.

Treatment[b]	Rate[c]	AUDPC[d]	Percent infected berries
Untreated	Untreated	2969 (2753-3184) A	75.2 (69.5-80.2) A
LifeGard intelligent	128 g/379 L	741 (526-957) B	9.8 (7.5-12.7) B
LifeGard standard	128 g/379 L	706 (491-922) B	9.1 (7.0-11.8) BC
Theia intelligent	3.4 kg/ha	661 (445-876) B	7.5 (5.7-9.7) BC
Theia standard	3.4 kg/ha	417 (202-633) B	5.5 (4.2-7.3) C

[a]Biological fungicides applied from BBCH 17 to BBCH 64, 5.6 kg/ha micronized sulfur then applied from BBCH 65 to BBCH 75, then biological fungicide applications resumed until veraison (BBCH 81).
[b]LifeGard contains *Bacillus mycoides* isolate J (40%) as the active ingredient (AI) and Theia contains *Bacillus subtilis* strain AFS032321 (100%) as the AI.
[c]All treatments applied at 552 kPa; intelligent sprayer treatments applied at 125 mL/m^3 of grape canopy.
[d]Estimates are followed by asymptotic 95% confidence intervals in parentheses. Treatments followed by different letters are statistically different from each other, marginal means contrast ($p \leq 0.05$) with p values adjusted using Tukey's method.

Table 3 Area under disease progress curve (AUDPC, leaf disease) and percent infected berries from LifeGard biological fungicide intelligent sprayer trials in 2022 and 2023.

Treatment[a]	Rate[b]	2022		2023	
		AUDPC[c]	Percent infected berries	AUDPC	Percent infected berries
Untreated	Untreated	2658 (2470-2846) A	99.5 (99.2-99.7) A	2351 (2164-2539) A	67.2 (58.5-74.9) A
Sulfur bloom only (all plots)	Sulfur 5.6 kg/ha	2162 (1974-2350) B	77.2 (69.9-83.2) BC	1856 (1668-2044) B	44.4 (35.5-53.8) B
LifeGard standard	128 g/379 L	2122 (1934-2310) B	74.7 (67.0-81.2) C	1816 (1628-2004) B	27.1 (20.3-35.1) C
LifeGard Intelligent High	128 g/379 L	1980 (1792-2168) B	85.8 (80.6-89.8) B	1674 (1486-1861) B	43.7 (34.8-53.1) B
LifeGard Intelligent Low	128 g/379 L	2079 (1891-2267) B	83.5 (77.6-88.0) BC	1773 (1585-1961) B	32.2 (24.5-40.8) BC

[a]LifeGard contains *Bacillus mycoides* isolate J (40%) as the active ingredient.
[b]All treatments applied at 552 kPa; Intelligent High and Low sprayer treatments applied at 125 and 62.5 mL/m^3 of grape canopy, respectively. Treatments were mixed at the rate indicated if the sprayer was used in standard mode. Actual rates applied by the intelligent sprayer system were lower due to the lower volume applied in intelligent mode.
[c]Estimates are followed by asymptotic 95% confidence intervals in parentheses. Treatments followed by different letters are significantly different from each other, marginal means contrast ($p \leq 0.05$) with p values adjusted using Tukey's method.

and injured or killed ~20% of the primary buds. In 2023, spring weather conditions were normal-to-dry in April and the first week of May but then became very dry, with little rainfall for the remainder of the season. In 2021 and 2022, the Gubler-Thomas powdery mildew index was at low-to-moderate infection risk from mid-April until mid-June, with high infection risk for most of the remaining growing season (Thomas et al. 1994). In 2023, from late-May until September, the model mostly showed a high risk of infection.

The leaf incidence AUDPC data were analyzed using a generalized least squares model to accommodate non-homogenous variance between treatments. The 2021 cluster severity was used as a binomially distributed probability of berry infection and was modeled using a generalized linear mixed model (GLMM) in the 'lme4' package ver. 1.1-37, with block fitted as a random effect (Bates et al. 2015). Leaf incidence AUDPC data from the 2022 to 2023 LifeGard and Theia B/S/B trials were respectively combined to improve statistical power and analyzed with a generalized least squares model to accommodate non-homogenous variance between treatments. Cluster severity data from the 2022 to 2023 Pinot noir and Pinot gris trials were each combined and analyzed using a GLMM in the 'lme4' package, with block fitted as a random effect (Bates et al. 2015). Treatments in both cluster severity and AUDPC analyses were contrasted using estimated marginal means ('emmeans' package ver. 1.11.0) and the fit of all GLMMs was checked with an *overdispersion* function and the 'DHARMa' package ver. 0.4.7 (Bolker et al. 2009, Hartig 2020, Lenth 2020). Any overdispersion caused by extrabinomial variation in the cluster severity GLMMs was corrected for using an observational level random effect (Harrison 2014). Uncertainty was estimated using asymptotic 95% confidence intervals. All data were analyzed in R ver. 4.5.0 (R Core Team 2020).

Results

S/B/S experiment

AUDPC values were significantly higher in the untreated plots than in all fungicide-treated plots ($p < 0.01$), while all fungicide-treated plots had AUDPC values that were not significantly different from each other (Table 1). Aviv and Serenade ASO treatments in both intelligent and standard modes resulted in average cluster severities between 13.8 and 18.2%, none of which were significantly different from each other ($p > 0.05$; Table 1).

Spray volumes applied in the 2021 S/B/S trial remained at ~514 L/ha for the season in standard mode (Figure 2A). In the bloom period, intelligent mode treatments resulted in applied volumes between 337 and 374 L/ha. In intelligent mode treatments, ~30% less total spray volume and product was applied than in standard mode treatments. For Aviv, less than the recommended rate of 1096 to 1826 mL/ha was applied in intelligent mode (Figure 2B).

B/S/B experiments

2021 LifeGard and Theia trial

AUDPC values were significantly higher in the untreated plots than in all fungicide-treated plots; however, all fungicide treatments resulted in AUDPC values that were not significantly different from each other (Table 2). Average cluster severity of the untreated control was 75.2%, which was significantly higher than all other treatments. All fungicide treatments resulted in an average cluster severity of less than 10%, with LifeGard and Theia intelligent modes resulting in cluster severities that were not significantly different than when each product was applied in standard mode (Table 2).

Spray volumes averaged ~530 L/ha for both standard mode treatments for the whole season (Figure 3A). Intelligent mode

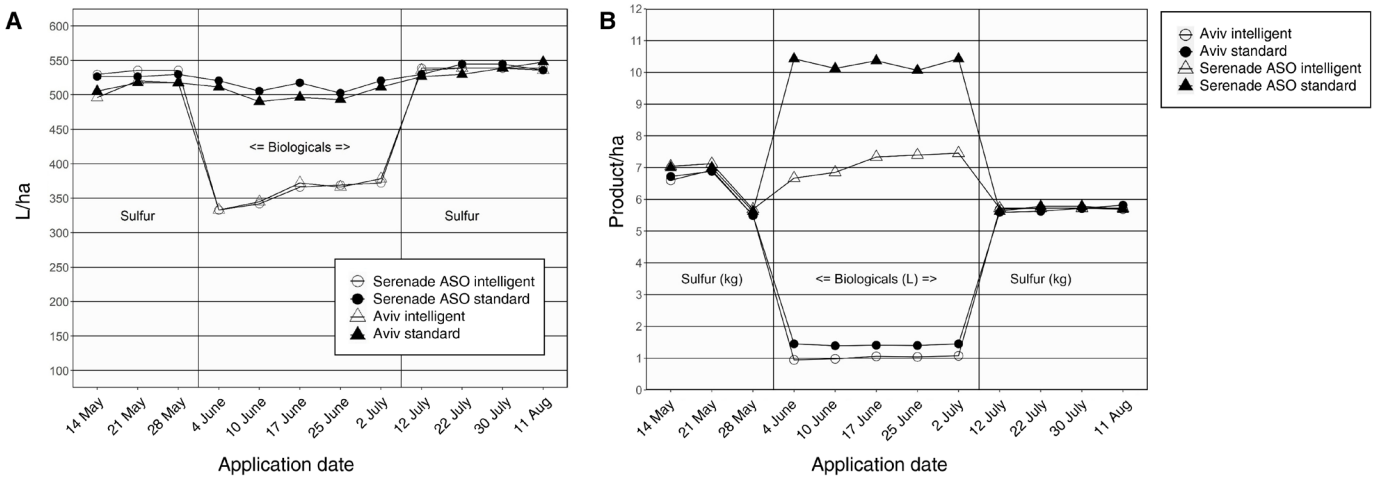

Figure 2 Spray volume (**A**) and pesticide quantity applied (**B**) on each application date in the 2021 sulfur–biological fungicide–sulfur (S/B/S) trial (5.6 kg/ha micronized sulfur applied from BBCH 17 to BBCH 64, biological fungicides then applied from BBCH 65 to BBCH 75, then 5.6 kg/ha micronized sulfur applications resumed until veraison [BBCH 81]). Vertical lines indicate the cutoff when transitioning between biological fungicides and sulfur in the program, and floating labels indicate the products used in the respective panels. Intelligent treatments were applied at 125 mL/m^3 of grape canopy. Aviv contains *Bacillus subtilis* strain IAB/BS03 (0.08%) as the active ingredient (AI) and Serenade ASO contains *B. subtilis* strain QST 713 (1.34%) as the AI.

treatments resulted in 121.5, 252, and 374 L/ha for the first application, before bloom, and after bloom sprays, respectively (Figure 3A). LifeGard applied in intelligent mode resulted in lower than the stated label rate before bloom, but after bloom, the amount applied was within the label rate range (70 to 315 g/ha, Figure 3B). In intelligent mode treatments, ~40% less total spray volume and product was applied than for treatments applied in standard mode.

2022 and 2023 LifeGard trials

AUDPC and cluster severity were significantly higher in the untreated plot than in any fungicide-treated vines in both 2022 and 2023 (Table 3). In general, disease pressure was higher in 2022 than in 2023. In both years, all fungicide-treated plots resulted in AUDPC values that were not significantly different from each other. In 2023, LifeGard applied in standard mode resulted in a 27.1% cluster severity that was significantly lower than sulfur alone, but not significantly different than that applied in Intelligent Low mode treatment at 32.2% (Table 3).

In the 2022 and 2023 trials, spray volume applied for standard mode treatments remained at ~625 and 560 L/ha for the entire season (Figures 4A and 5A). Intelligent Low and High mode treatments started the season at ~100 and 150 L/ha, respectively, in both years. After bloom, Intelligent Low and High modes remained at ~350 and 450 L/ha for the rest of the season, respectively (Figures 4A and 5A). In 2022 and 2023, Intelligent Low mode resulted in 62% and 38% lower total spray volume and product applied than standard mode applications, respectively. In 2022 and 2023, Intelligent High mode resulted in 47% and 28% lower total spray volume and product applied than standard mode applications, respectively. Lower product and spray savings were observed in 2023 due to one fewer application before bloom than in 2022. In intelligent mode, a lower-than-label rate (70 to 315 g/ha) was applied for the first two and first four applications in Intelligent High and Low modes, respectively, in 2022 (Figure 4B). In 2023, a lower-than-label rate was applied for the first application in Intelligent High and Low modes, but in both years, after-bloom rates within the suggested range were applied (Figure 5B).

2022 and 2023 Theia trials

In both years, all fungicide treatments resulted in significantly lower cluster severity than on untreated vines. The sulfur alone treatment resulted in a significantly lower AUDPC and cluster severity than the untreated control in 2022; in 2023, only AUDPC was significantly lower (Table 4). In both years, the Theia Intelligent High, Theia Intelligent Low, and sulfur-only treatments resulted in AUDPC values that were not significantly different from each other. Additionally, in both years, the Theia standard mode treatment resulted in significantly less AUDPC than the 5.6 kg/ha sulfur at bloom treatment. In 2022, all fungicide treatments resulted in cluster severities that were significantly less than untreated vines. Cluster severities among fungicide-treated vines ranged from 67.8 to 79.8%, but none were significantly different from each other (Table 4). However, in 2023 all Theia treatments resulted in cluster severity values that ranged from 20.8% (standard treatment vines) to 27.9% (Intelligent High treated vines); these values were significantly lower than the sulfur-only control, but not significantly different from each other.

In the 2022 and 2023 Theia trials, spray volume applied for standard mode treatments remained at ~608 and 560 L/ha, respectively, throughout the season (Figures 6A and 7A). Intelligent mode treatments in both trials started the season at ~100 (2022) and 150 (2023) L/ha, with lower values in 2022 due to less foliage resulting from a late season frost event. After bloom in both years, Intelligent High and Low modes remained between 410 and 460 L/ha and between 350 and 370 L/ha for the rest of the season, respectively (Figures 6A

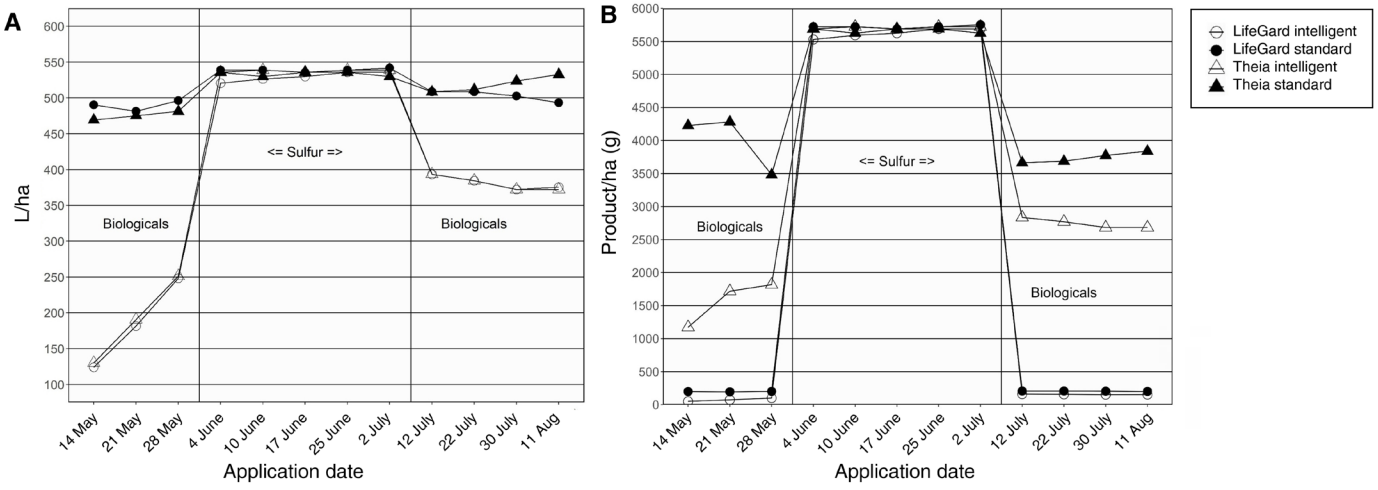

Figure 3 Spray volume (**A**) and pesticide quantity applied (**B**) on each application date in the 2021 biological fungicide–sulfur–biological fungicide (B/S/B) trial (biological fungicides applied from BBCH 17 to BBCH 64, 5.6 kg/ha micronized sulfur then applied from BBCH 65 to BBCH 75, then biological fungicide applications resumed until veraison [BBCH 81]). Vertical lines indicate the cutoff when transitioning between biological fungicides and sulfur in the program, and floating labels indicate the products used in the respective panels. Intelligent treatments were applied at 125 mL/m^3 of grape canopy. LifeGard contains *Bacillus mycoides* isolate J (40%) as the active ingredient (AI) and Theia contains *Bacillus subtilis* strain AFS032321 (100%) as the AI.

and 7A). In 2022 and 2023, Intelligent Low mode resulted in 60% and 39% lower total spray volume and product applied than standard mode applications, respectively. In 2022 and 2023, Intelligent High mode resulted in 45% and 30% lower total spray volume and product applied than standard mode applications, respectively. Lower product and spray savings were observed in 2023 due to one fewer application before bloom than in 2022. The suggested product application rate range for Theia is from 1.7 to 5.6 kg/ha, so all applications made using Intelligent High mode were above the lowest rate except for the first three applications in 2022 and the first application in 2023. All prebloom applications using Intelligent Low mode were below the lowest label rate except for the last application in 2022 and the first two applications in 2023 (Figures 6B and 7B).

Discussion

These studies have shown that some biological fungicides can improve GPM control over phenological application of sulfur alone and that there can be differences in the efficacy of biological fungicides, depending on the rate of application. Additionally, intelligent or standard mode resulted in similar leaf incidence AUDPC and cluster severity for some products and regimes. The products used in both trials were mixed at a rate assumed to be applied in standard mode and as a result, when the sprayer was used in intelligent mode a lower quantity of these products per area was applied. When Aviv or LifeGard were used in intelligent mode during bloom or prior to bloom, respectively, that resulted in an amount of each product applied per area that was near or below the

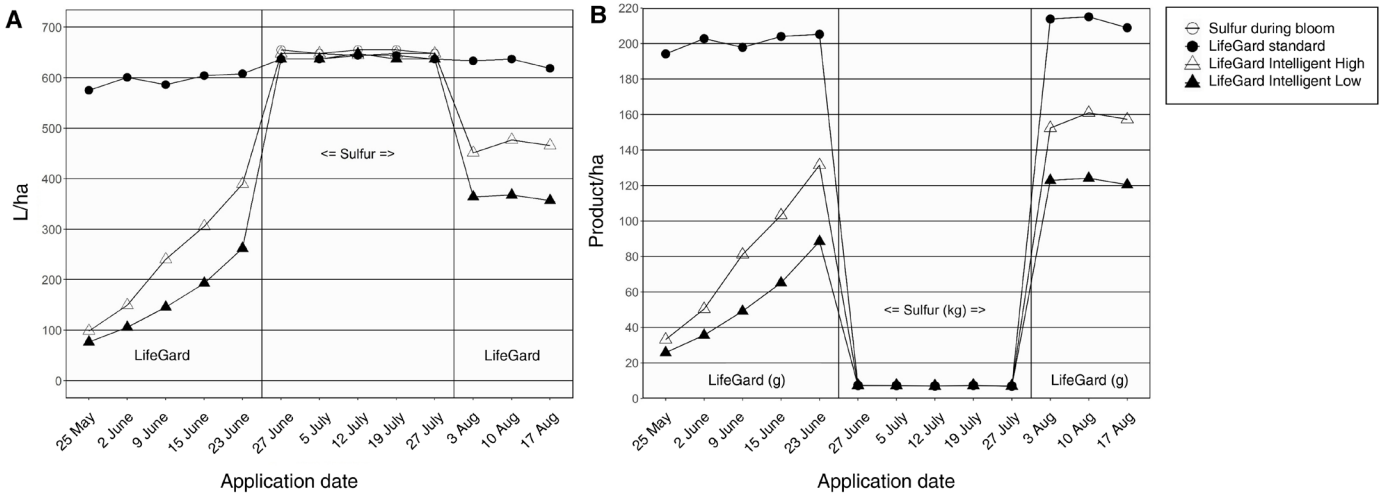

Figure 4 Spray volume (**A**) and pesticide quantity applied (**B**) on each application date in the 2022 LifeGard biological fungicide–sulfur–biological fungicide (B/S/B) trial (biological fungicides applied from BBCH 17 to BBCH 64, 5.6 kg/ha micronized sulfur then applied from BBCH 65 to BBCH 75, then biological fungicide applications resumed until veraison [BBCH 81]). Vertical lines indicate the cutoff when transitioning between biological fungicides and sulfur in the program, and floating labels indicate the products used in the respective panels. Intelligent High and Low sprayer treatments were applied at 125 and 62.5 mL/m^3 of grape canopy, respectively. LifeGard contains *Bacillus mycoides* isolate J (40%) as the active ingredient.

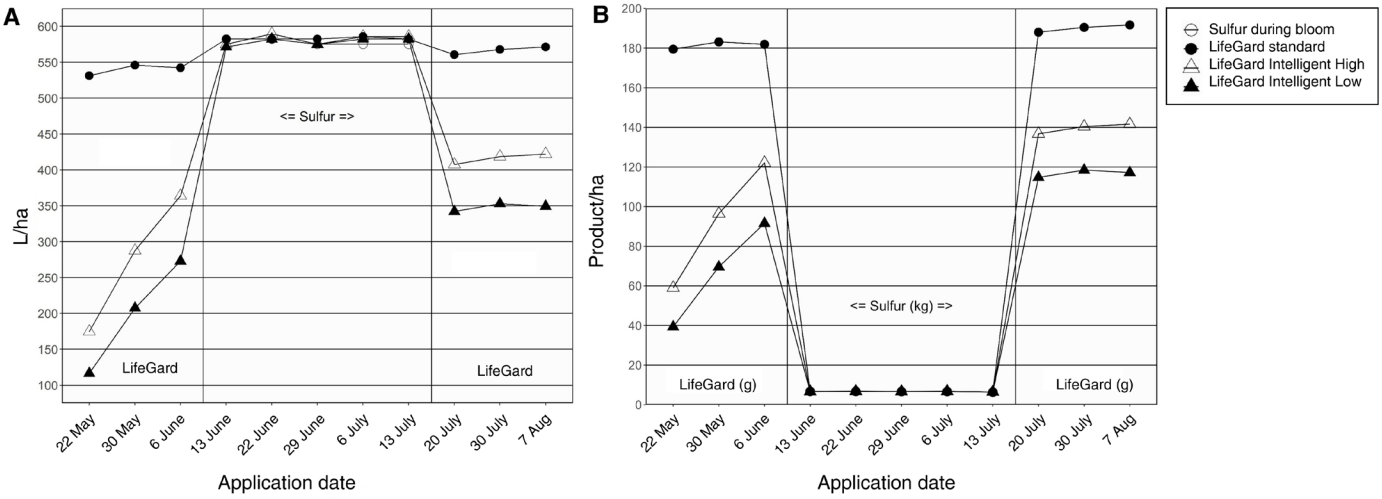

Figure 5 Spray volume (**A**) and pesticide quantity applied (**B**) on each application date in the 2023 LifeGard biological fungicide–sulfur–biological fungicide (B/S/B) trial (biological fungicides applied from BBCH 17 to BBCH 64, 5.6 kg/ha micronized sulfur then applied from BBCH 65 to BBCH 75, then biological fungicide applications resumed until veraison [BBCH 81]). Vertical lines indicate the cutoff when transitioning between biological fungicides and sulfur in the program, and floating labels indicate the products used in the respective panels. Intelligent High and Low sprayer treatments were applied at 125 and 62.5 mL/m^3 of grape canopy, respectively. LifeGard contains *Bacillus mycoides* isolate J (40%) as the active ingredient.

label recommended rate. For these products, it could be that the amount of each product that was applied was sufficient for each to effectively express their mode of action to a similar extent as the label rate range. The equivalency of disease control between intelligent and standard modes could in part be due to the SAR aspect of these products, whereby they activate the defense responses of the plants (Hirozawa et al. 2023). When purely contact-based fungicide materials are applied with the ISS, lower volumes often result in less effective disease control; however, when systemic materials are used, their ability to translocate in plant tissues can make up for the typically lower coverage seen in low volume applications (Warneke et al. 2022, 2023). A similar phenomenon may be seen here, with biological fungicides that primarily activate the SAR response being less sensitive to application rate than purely contact materials.

In 2022 and 2023, use of LifeGard or Theia both resulted in less GPM compared with the application of only micronized sulfur during the bloom period. According to their manufacturer, LifeGard and Theia are both biological fungicides with live organisms that colonize the plant tissue in the course of their mode of action. Theia contains live spores of a *Bacillus* bacterium that colonizes the plant tissue and directly excludes fungi from infecting the plant, in addition to producing antimicrobial compounds and activating the plant defense response. LifeGard also contains live bacterial spores but putatively does not have direct activity on the target plant pathogenic fungi, instead working solely by activating the plant defense response. While applications of Theia before and after the bloom period did not result in less GPM on clusters compared to application of sulfur alone, the GPM levels in that trial were all lower for their respective application method than those observed using LifeGard. Theia may have been more effective at controlling GPM infections on leaves due to its higher concentration of the AI bacterium. The Theia formulation is composed of 100% of its AI bacterium, *B. subtilis* strain AFS032321, while LifeGard contains 40% of its AI bacterium, *B. mycoides* isolate J, with the rest of the formulation made up of inert ingredients. The high concentration of *B. subtilis* in the Theia formulation may signify that there were already pre-packaged antifungal compounds present in the product (Silva et al. 2023). This could have made Theia more effective at managing GPM infections that were already present at the time of application than LifeGard, which is labeled as having no direct effect on the pathogen.

While Theia appeared to be more effective at managing GPM infections than LifeGard, cluster infection was lowest among the LifeGard standard mode plots. In contrast, application of Theia in any ISS mode resulted in GPM cluster severities that were not significantly different from each other. These data indicate that the quantity of LifeGard applied was important to its efficacy. *Bacillus* biological fungicides generally have a few similar modes of action which play into their efficacy in the field (Hirozawa et al. 2023). Some products contain live microorganisms that grow and proliferate when sprayed onto plant surfaces, thus excluding pathogens because of lack of free space on the plant surfaces and/or production of natural antimicrobial compounds (Hirozawa et al. 2023). Other products contain compounds derived from the active bacterium that inhibit the pathogen and/or activate plant defense responses so that infection attempts by the pathogen are less successful (Hirozawa et al. 2023). Lipoproteins are biosurfactants produced by *Bacillus* biofungicides that can have direct antagonistic effects on pathogens through disruption of cell membranes, but some can also induce plant resistance (Hirozawa et al. 2023). Lipoproteins are directly active on target fungi and work by destabilizing membranes; however, they can also activate the SAR response in plants to prime their defense against infection (Hirozawa et al. 2023). If the active agent in LifeGard is a lipoprotein, as the quantity of LifeGard applied increased, so too did the efficacy of the product. Additionally, stabilizing agents are often added to biofungicide formulations to increase their longevity, protect against abiotic factors, and improve their efficacy (Silva et al. 2023). These adjuvants in

Table 4 Area under disease progress curve (AUDPC, leaf disease) and percent infected berries from Theia biological fungicide intelligent sprayer trials in 2022 and 2023.

Treatment[a]	Rate[b]	2022		2023	
		AUDPC[c]	Percent infected berries	AUDPC	Percent infected berries
Untreated	Untreated	2630 (2343-2916) A	98.8 (98.1-99.3) A	2284 (1998-2571) A	49.7 (38.3-61.1) A
Sulfur bloom only (all plots)	Sulfur 5.6 kg/ha	2204 (1917-2490) B	78.8 (70.0-85.5) B	1858 (1572-2145) B	46.5 (35.3-58.0) A
Theia standard	3.4 kg/ha + sulfur 5.6 kg/ha	1599 (1313-1885) D	67.8 (56.9-77.0) B	1254 (967-1540) D	20.8 (14.1-29.5) B
Theia Intelligent High	3.4 kg/ha + sulfur 5.6 kg/ha	1954 (1667-2240) BC	70.8 (60.4-79.5) B	1608 (1322-1895) BC	27.9 (19.5-38.1) B
Theia Intelligent Low	3.4 kg/ha + sulfur 5.6 kg/ha	1901 (1615-2187) C	79.8 (71.3-86.3) B	1556 (1269-1842) C	21.2 (14.5-30.0) B

[a]Theia contains *Bacillus subtilis* strain AFS032321(100%) as the active ingredient.

[b]All treatments applied at 552 kPa; Intelligent High and Low sprayer treatments applied at 125 and 62.5 mL/m^3 of grape canopy, respectively. Treatments were mixed at the rate indicated if the sprayer was used in standard mode. Actual rates applied by the intelligent sprayer system were lower due to the lower volume applied in intelligent mode.

[c]Estimates are followed by asymptotic 95% confidence intervals in parentheses. Treatments followed by different letters are significantly different from each other, marginal means contrast ($p \leq 0.05$) with p values adjusted using Tukey's method.

the biofungicide formulation could have fungicidal activity against the target pathogen and thus as higher quantities were applied, better efficacy was observed. Theia contained only its active organism and no inert ingredients, so there may not have been any incremental increase in disease control efficacy due to the quantity applied over a low threshold dose, making it more difficult to see an application rate effect.

The use of the variable rate intelligent sprayer and the unique setting of the research vineyard resulted in higher GPM infection than is commercially acceptable as a baseline. The intelligent sprayer applies a spray volume that is proportionate to the foliage density present (Chen et al. 2012). During early season growth, this results in an applied spray volume that is much lower than conventional sprayers, and can result in ineffective GPM management when contact mode-of-action fungicides are used (Nackley et al. 2021, Warneke et al. 2022). Coinciding with use of the intelligent sprayer, higher than commercially acceptable mildew levels were observed, largely due to the high disease pressure of the research vineyard where these experiments were conducted. The high GPM pressure is largely due to experiments with untreated controls and ineffective treatments, but also to the generally mild climate of the Willamette Valley that favors GPM infection. This results in GPM incidence and severity on treated vines in the research vineyard that is commercially unacceptable. However, conducting GPM management research in commercial fields can often result in too

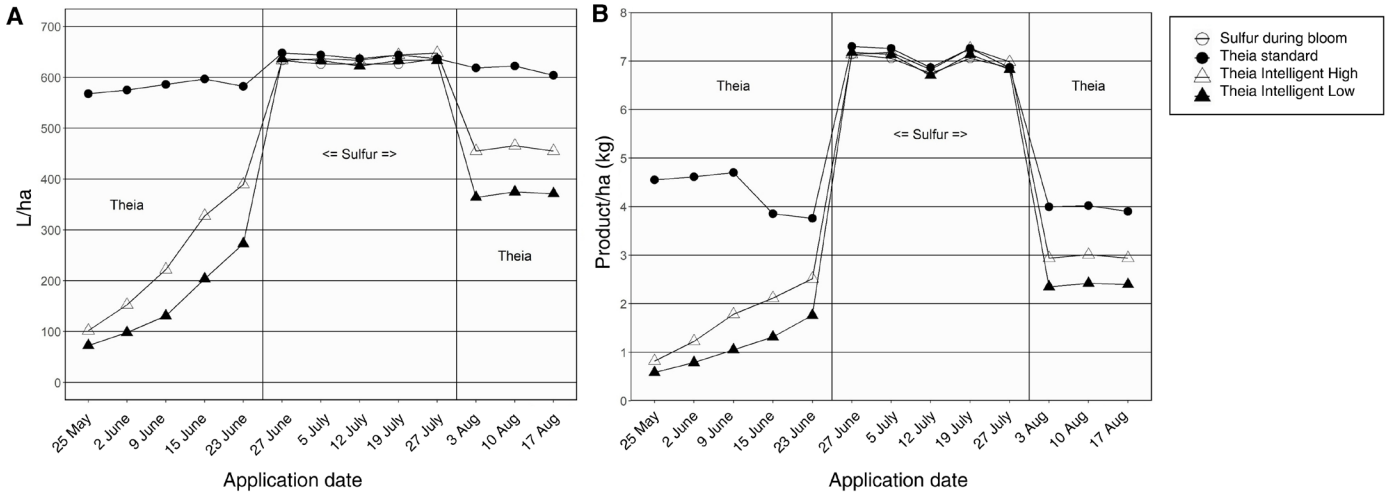

Figure 6 Spray volume (**A**) and pesticide quantity applied (**B**) on each application date in the 2022 Theia biological fungicide–sulfur–biological fungicide (B/S/B) trial (biological fungicides applied from BBCH 17 to BBCH 64, 5.6 kg/ha micronized sulfur then applied from BBCH 65 to BBCH 75, then biological fungicide applications resumed until veraison [BBCH 81]). Vertical lines indicate the cutoff when transitioning between biological fungicides and sulfur in the program, and floating labels indicate the products used in the respective panels. Intelligent High and Low sprayer treatments were applied at 125 and 62.5 mL/m^3 of grape canopy, respectively. Theia contains *Bacillus subtilis* strain AFS032321 (100%) as the active ingredient.

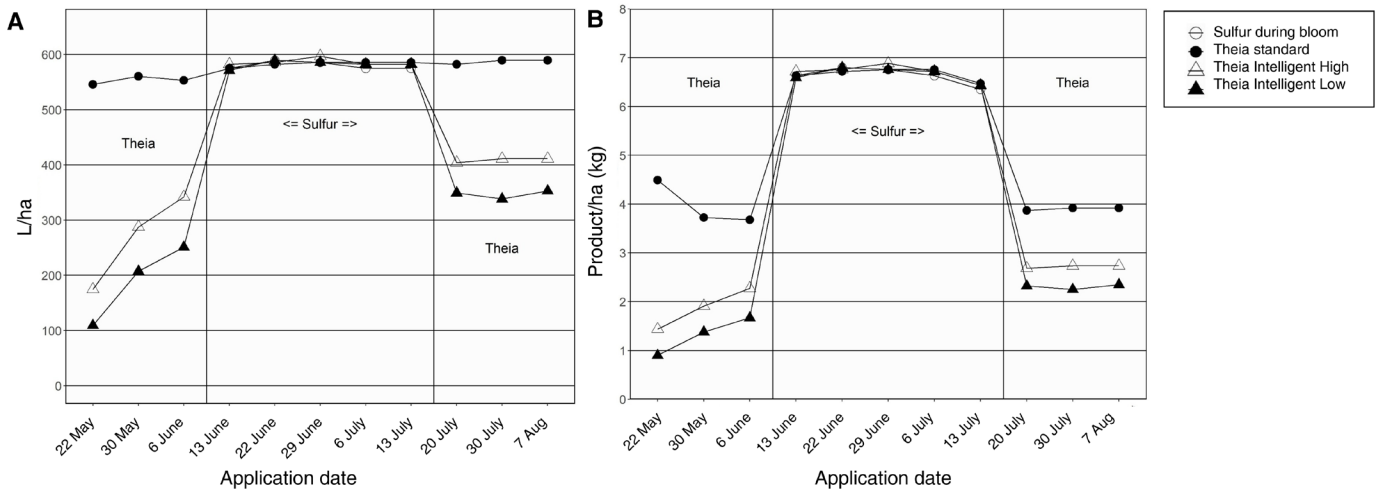

Figure 7 Spray volume (**A**) and pesticide quantity applied (**B**) on each application date in the 2023 Theia biological fungicide–sulfur–biological fungicide (B/S/B) trial (biological fungicides applied from BBCH 17 to BBCH 64, 5.6 kg/ha micronized sulfur then applied from BBCH 65 to BBCH 75, then biological fungicide applications resumed until veraison [BBCH 81]). Vertical lines indicate the cutoff when transitioning between biological fungicides and sulfur in the program, and floating labels indicate the products used in the respective panels. Intelligent High and Low sprayer treatments were applied at 125 and 62.5 mL/m^3 of grape canopy, respectively. Theia contains *Bacillus subtilis* strain AFS032321 (100%) as the active ingredient.

little disease pressure to elucidate the efficacy of fungicide treatments. Testing new products and technologies in high-disease pressure research vineyards can yield results that are not directly comparable to commercial contexts, but can illustrate which products or technologies are effective for GPM management.

If growers use the intelligent sprayer to apply biological fungicides at lower volumes while achieving disease management similar to standard application practices, this could lead to sizable season-long monetary savings. When intelligent sprayers are used, applied spray volume is typically 30 to 60% lower than standard sprayers (Chen et al. 2019, 2020). Such spray savings translate into large monetary savings, as biological fungicides are typically more expensive products than generic synthetic fungicides. Besides the monetary savings on pesticide products, applying lower volumes of fungicides has a positive cascading effect on the productivity of farming operations (Warneke et al. 2019). Tractors are in the field for less time, which translates into lower labor requirements and fuel costs (Manandhar et al. 2020). Additionally, there is less water used, less soil compaction, and less drift/wasted spray released into the environment (Chen et al. 2013, Warneke et al. 2019, 2022). While there are clear monetary benefits to using variable rate sprayer technology, the size of a farm and intensity of its spray program dictate the profitability of adopting the technology (Tona et al. 2018, Manandhar et al. 2020). For example, one study found that for vineyard applications, it only made economic sense to adopt variable rate sprayer technology if the vineyard was 100 ha or larger (Tona et al. 2018). The economic incentives to adopt variable rate sprayer technology may change over time and should be reevaluated as variable and fixed application costs change.

The use of variable rate spray technology presents the opportunities of lower environmental impact and economic benefits to growers; however, pesticide labels and conventional disease control paradigms must be updated to facilitate adoption of this technology (Warneke et al. 2020a). Currently, most pesticide products are applied according to per-area rates (e.g., kg/ha or L/ha), which favors use of constant-rate sprayers. Pesticide companies have little incentive to update their guidance on application rates to concentration-based rates (e.g., mg AI/L) that would facilitate use with variable-rate sprayers (Warneke et al. 2020a). The lack of guidance on the threshold quantity of AI needed per crop area, or more specifically per plant surface area, necessitates research such as that conducted here. This research is needed to both give guidance to end users of the technology, and to nudge chemical companies and policymakers to update regulations to facilitate technology adoption.

Conclusion

These studies demonstrated that certain biological fungicides can more effectively manage GPM than phenological applications of sulfur alone and that the efficacy of some can vary based on application rate while others can result in similar efficacy across a wider rate range. The testing regimes employed in these studies were chosen to moderate the high GPM pressure of a research vineyard to more effectively evaluate the action of the biological fungicides. Farmers using biological fungicides and variable rate sprayers may be able to use lower application rates with some products and achieve similar disease management as the same biological fungicides applied with standard sprayers. Other products may necessitate adjustment of the settings of variable rate sprayers to be applied at rates similar to standard sprayers for efficacy. Experiments such as these should be ongoing as more biological fungicide products become available on the market and as spray technologies and regulations change.

Acknowledgments

We thank Heping Zhu, Kelly O'neil, Kelsey Britton, Jack Hubner, and James Whitney for their assistance with this project. This work was funded, in part, by USDA ARS *Integration of Intelligent Spray Technology into IPM Programs in Specialty Crop Production* (USDA-ARS *Project Number* 58-5082-2-010) *and U.S. Department of Agriculture, Agricultural Research Service, under Cooperative Agreement* No. 59-5082-5-006. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or Oregon State University. USDA is an equal opportunity provider and employer.

CRediT Authorship Contributions

BW: Data Curation, Formal Analysis, Visualization; BW, LN, JP: Conceptualization, Funding Acquisition, Writing – Original Draft, Writing – Review & Editing; BW, JP: Investigation, Methodology; LN, JP: Project Administration, Supervision

ORCID

Brent W. Warneke 0000-0002-2095-8422
Lloyd L. Nackley 0000-0001-7571-3046
Jay W. Pscheidt 0000-0001-9164-8088

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Data Availability

The data underlying this study are available on request from the corresponding author.

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