GRAPE (*Vitis vinifera* 'Pinot noir', 'Pinot gris')
Powdery Mildew; *Erysiphe necator*

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Efficacy of phenologically based fungicide programs using biological products and micronized sulfur while using an Intelligent and airblast sprayer for management of grape powdery mildew, 2022.

Micronized sulfur and biological fungicides were applied during critical periods for powdery mildew management on Pinot noir and Pinot gris vines at the Botany and Plant Pathology Field Laboratory in Corvallis, Oregon. In the past, use of biological products all season long generally resulted in poor powdery mildew control. In these trials, treatments focused on evaluating biological fungicides applied before and after flowering (Figure 1). Sulfur is an effective material for management of powdery mildew and was used in the program during the critical powdery mildew management period of bloom and early berry development (Figure 1). This type of fungicide regime was also evaluated in 2021 and found to be a more realistic way to evaluate the activity of biological fungicides than their application alone. Additionally, each of the biological fungicide treatments were applied using the Intelligent Spray System (ISS) to compare the efficacy of using the system with the same program.

The sprayer used (50 gallon Pak-blast, Rears Mfg., Coburg, OR) was a standard air blast sprayer retrofitted with a Lidar laser sensor, Doppler speed sensor, embedded computer, and individual pulse width modulation (PWM) solenoid valves at each sprayer nozzle. These components make up the ISS that adjusts pesticide application volume in real time to match plant canopy characteristics. In doing so, the sprayer can minimize pesticide use and off target drift while keeping pest management similar to standard sprayers. A spray console wired to the system allowed use of either the ISS components or standard constant-rate operation mode. When the ISS was used it was referred to as "intelligent mode," and when the system was off and standard operation occurred it was referred to as "standard mode." The sprayer was operated using a Kubota M5N-111 tractor and the nozzles in the sprayer were TeeJet ceramic D3 discs and DC25 cores.

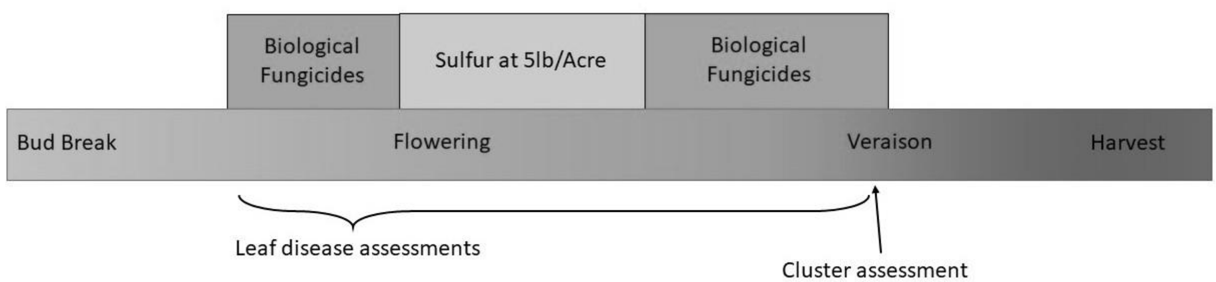

Figure 1. Fungicide application and data acquisition timings in the Pinot noir and Pinot gris trials.

Treatments (Table 1) were arranged in a randomized complete block design in each cultivar. For both the Pinot noir and Pinot gris trials, fungicide programs were initiated just after powdery mildew was located with intensive scouting in late-May. Until 50% bloom, biological fungicides (Table 1) were applied to vines. Upon 50% bloom, 5 lb/A Microthiol Disperss (MD) micronized sulfur was applied to vines (Table 1). Included in the treatments was a 5 lb/A MD control whereby MD was only applied during the bloom period, and outside of this interval no other treatments were applied (Table 1). After 4 weeks post flowering (5 applications) the fungicide program for each cultivar reverted to application of the biological fungicide treatment (Figure 1).

The fungicide schedule used in the study was based on the different stages of a grape powdery mildew (GPM) epidemic. Initially, before grapevines flower, fungicide programs are focused on preventing GPM infections from occurring, primarily on leaves. During flowering and for approximately 3 weeks thereafter, fungicide programs are focused on preventing infection of the grape clusters. Flowering and fruit set are generally seen as the most critical time for management of GPM, as this is when the inflorescences and subsequently the developing berries, are most susceptible to infection. After three weeks post flowering, fungicide programs focus on keeping GPM levels low on both leaves and fruit to keep overall inoculum to a minimum. Fungicide program intensity is usually not as aggressive after bloom and early fruit set as the earlier two sections of the season as both leaves and fruit develop ontogenic resistance to infection as they age. The trial regime allowed testing of whether using biologically-based products to manage the leaf infection and the early epidemic period would lead to effective GPM management, while not overwhelming the biological fungicide products with the high disease pressure of the research vineyard.

The blocks used consisted of 'Pinot noir' and 'Pinot gris' planted in 1998 on *V. rupestris* x *V. riparia* 101-14 rootstock with 7x8 ft spacing. A single buffer rootstock vine was trained between each set of treatment vines and a buffer row of rootstock vines separated each varietal row, which helped minimize plot-plot interference. Vines were trained to a Guyot (vertical shoot position) system and pruned by 24 Feb. Shoot thinning by hand occurred from 9 May to 10 Jun and sucker removal by hand was continuous throughout the season. Shoots were cut above the top wire on 21 Jun and maintained at this height throughout the growing season. Fungicide treatments were applied every 7-10 days. Each treatment was replicated on 4 sets of 5 vines.

Table 1. Biological fungicide treatments applied to Pinot noir or Pinot gris vines.

Pinot noir		**Pinot gris**	
Treatment[x]	Sprayer Mode[y]	Treatment[x]	Sprayer Mode[y]
Non-treated	N/A	Non-treated	N/A
MD (5 lb/A) bloom-early berry development only	Standard	MD (5 lb/A) bloom-early berry development only	Standard
	Standard		Standard
LifeGard (4.5 oz/100gal)	Intelligent high	Theia (3 lb/A)[z]	Intelligent high
	Intelligent low		Intelligent low

[x]Treatments applied at 80psi at approx. 430 PTO rpm and 3 mph. MD = Microthiol Disperss
[y]Intelligent high and low treatments applied at 0.12 fl oz/ft^3 and 0.06 fl oz/ft^3 of grape canopy, respectively.
[z]Treatment mixed at the rate indicated if the sprayer was used in standard mode. Actual rates applied by the ISS were lower due to the lower volume applied in intelligent mode.

Spring weather conditions were very wet resulting in the second wettest spring on record. A frost event on 14 Apr resulted in delayed vine development and injured or killed 20% if the primary buds. Signs of powdery mildew were first found on 23 May on longer shoots (~7in) from primary buds that were not killed in the Apr frost. The 2022 season was generally observed to be a high pressure GPM year, as noted by the high severity observed on non-treated vines and throughout Willamette Valley commercial vineyards, compared to 2021. Bloom took place from approximately 24 Jun to 5 Jul with most caps detaching from 25 to 30 Jun.

Leaf and cluster data were taken on the middle three vines of each experimental plot by randomly examining either 25 clusters or leaves on both the east and west side of the row for a total of 50 units examined per plot. The incidence of powdery mildew on leaves was recorded weekly from 21 Jun through 16 Aug. The severity of powdery mildew on clusters was taken on 12 Aug. Leaf incidence data was analyzed by calculating the area under disease progress curve (AUDPC) which was calculated by multiplying the mean incidence from two observation dates by the number of days between observations ($\Sigma[Y_{i+1} + Y_i)/2][X_{i+1}-X_i]$ where Y_i is incidence of mildew at i*th* observation and X_i is the day of the i*th* observations) and adding together the values. AUDPCs were calculated using the agricolae package and modeled with linear models. Cluster severity percentages were modeled using a generalized linear mixed model with block fitted as a random effect. Cluster severity treatment contrasts were conducted using the emmeans package and model fit was checked with the DHARMa package. Uncertainty was estimated using asymptotic 95% confidence intervals. All data was analyzed in R version 4.0.3.

In the Pinot noir trial the AUDPC value was significantly higher in the non-treated plot than any of the fungicide treated vines, however in the Pinot gris trial the 5 lb/A MD treatment was not significantly different than the non-treated control (Table 2). All fungicide treated plots in the Pinot noir trial had AUDPC values that were not

Table 2. Area under disease progress curve (AUDPC, leaf disease) and percent infected berries from Pinot noir and Pinot gris biological fungicide Intelligent Sprayer trials at the Botany and Plant Pathology field lab in 2022.

Pinot noir			Pinot gris		
Treatment^{xy}	**AUDPC^{z}**	**Percent Infected Berries^{z}**	**Treatment^{xy}**	**AUDPC^{z}**	**Percent Infected Berries^{z}**
Non-treated	2553.5 (2553.49-2553.51) A	99.4 (99.2-99.6) A	Non-treated	2545 (2352-2737) A	98.8 (98.1-99.2) A
MD (5 lb/A) bloom only	2140 (2037-2243) B	77.2 (71.2-82.2) CD	MD (5 lb/A) bloom only	2151 (1959-2343) AB	78.8 (70.4-85.3) B
LifeGard Standard	2160 (1989-2330) B	74.7 (68.4-80.2) D	Theia Standard	1728 (1536-1920) C	67.8 (57.5-76.6) B
LifeGard Intelligent High^{z}	1954 (1575-2334) B	85.8 (81.5-89.2) B	Theia Intelligent High^{z}	1851 (1658-2043) BC	70.8 (60.9-79.1) B
LifeGard Intelligent Low	2194 (2128-2260) B	83.5 (78.7-87.3) BC	Theia Intelligent Low^{z}	2013 (1821-2205) BC	79.8 (71.7-86.0) B

^{y}All treatments were applied at 80psi at approx. 430rpm PTO; intelligent high and low sprayer treatments applied at 0.12 fl oz/ft^{3} and 0.06 fl oz/ft^{3} of grape canopy, respectively.
^{z}Estimates are followed by asymptotic 95% confidence intervals in parentheses. Treatments followed by different letters are significantly different from each other, marginal means contrast ($p<0.05$) with p values adjusted using Tukey method.

significantly different from each other. In the Pinot gris trial, both Theia intelligent modes treatments resulted in AUDPC values that were not significantly different from the 5 lb/A MD at bloom treatment, but also not significantly different from the Theia standard treatment. The Theia standard mode treatment resulted in significantly lower AUDPC value than the 5 lb/A MD at bloom treatment. In both the Pinot noir and Pinot gris trials all fungicide treatments resulted in significantly lower cluster severity than on non-treated vines. In the Pinot noir trial both of the Lifegard intelligent treatments resulted in the highest average cluster severity among fungicide treated plots, however Lifegard intelligent low was not significantly different than the 5 lb/A MD at bloom treatment. The Lifegard standard treatment resulted in the lowest observed average cluster severity in that trial (Table 2). In the Pinot gris trial all fungicide treatments resulted in cluster severity values that were significantly lower than that of non-treated vines but none were significantly different from each other.

Spray volume applied in the Pinot noir and Pinot gris trials were very similar due to using the same settings in both trials. For standard mode treatments the volume applied remained between 60 and 70 gallons per acre (GPA) for the entire season, and were just under 70 GPA during the bloom period (Figure 2A and 2B). Intelligent mode treatments in both trials started the season at around 10 GPA. Intelligent high mode volumes increased quickly to over 40 GPA at the beginning of bloom whereas intelligent low mode applied just under 30 GPA at the beginning of bloom. After bloom, intelligent high and low mode remained at around 50 GPA and 40 GPA for the rest of the season, respectively. (Figure 2A and 2B). In the Pinot noir trial, Lifegard standard mode resulted approximately 3 oz/A at each application throughout the trial, while in intelligent mode a lower than recommended rate of 1 oz/A to 4.5 oz/A was applied for the first two and first four applications in intelligent high and low modes, respectively, but after bloom rates within the suggested range were applied (Figure 3A). In the Pinot gris trial, 3.5 lb/A to 4 lb/A of Theia were applied in standard mode, while in intelligent mode the amount ranged from about 1 lb/A for both modes at the first application to around 2.1 and 2.5 lb/A after the bloom period for intelligent low and intelligent high, respectively (Figure 3B). The suggested product application rate range for Theia is from 1.5 lb/A to 5 lb/A, so most applications made using intelligent high mode were above the lowest rate except for the first three, while most of the pre-bloom applications using intelligent low mode were below the lowest label rate except for the last application (Figure 3B). During the bloom period when Microthiol Disperss was applied to all treated plots in both studies just over 6 lb/A was applied for the duration of the bloom period.

Lifegard and Theia are both biological fungicides with live organisms that colonize the plant tissue in the course of their mode of action. However the two products have different targets in their mode of action. Theia contains live spores of a *Bacillus* bacterium that colonize the plant tissue and directly exclude fungi from infecting the plant, in addition to production of anti-microbial compounds and activating the plant defense response. Lifegard does not have any direct activity on the target plant pathogenic fungi and instead works solely by activating the plant defense response. The differing modes of action of these products could explain the different disease levels observed on leaves and clusters in this study. For example, in the Pinot noir trial, leaf disease levels, as measured by AUDPC showed the application of Lifegard having no difference than waiting until bloom to apply MD. However the Pinot noir cluster severity data showed that the Lifegard standard treatment resulted in the lowest cluster severity in that trial. For the Pinot gris trial where Theia was applied, the opposite in terms of AUDPC and cluster severity was observed. In the cluster severity data there was no significant difference between applying Theia before bloom, or waiting until bloom to apply MD, however the leaf disease data showed that Theia did result in lower AUDPC values than the 5 lb/A MD control. This could indicate that Lifegard was more effective at activating the plant defense response at each application, leading to inflorescences being primed for defense at bloom. Conversely, Theia could have acted by mainly inhibiting foliar GPM infections and perhaps was not as effective at priming the inflorescences for infection defense.

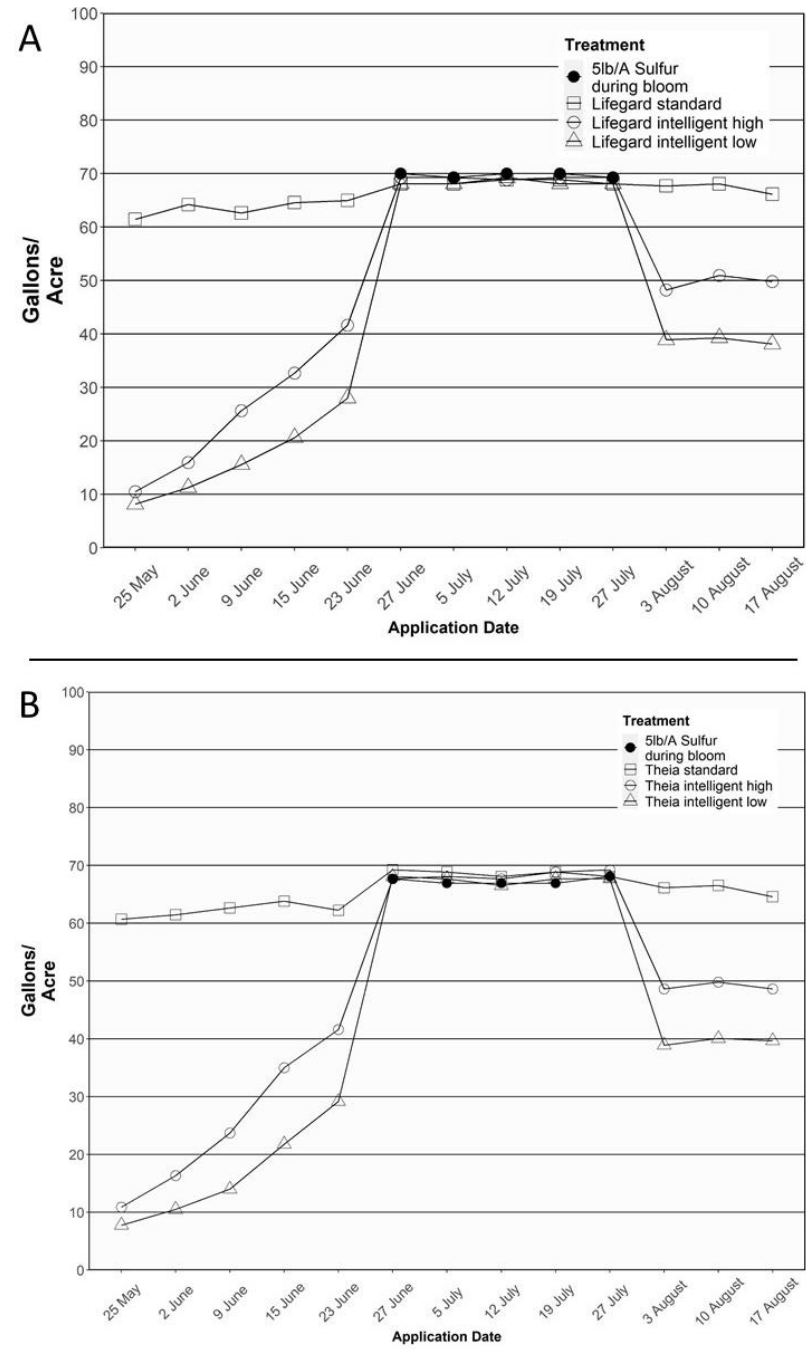

Figure 2. Spray volume applied to Pinot noir (A) and Pinot gris (B) vines.

While applications of Theia before and after the bloom period did not improve control of GPM on clusters compared to application of MD alone, the GPM levels in the Pinot gris trial were all lower for their respective application method than those observed using Lifegard in the Pinot noir trial. Theia may have been more effective at controlling GPM infections on leaves due to its high concentration of the active ingredient bacterium compared to Lifegard. The Theia formulation is composed of 100% of its active ingredient bacterium, *Bacillus subtilis* strain AFS032321, whereas the Lifegard contains 40% of its active ingredient bacterium, *Bacillus 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 mean that there was already a pre-packaged amount of anti-fungal compounds present in the product. This could have made Theia more effective at suppressing GPM infections that were already present at the time of application than Lifegard, which is labeled as having no direct impact on the pathogen.

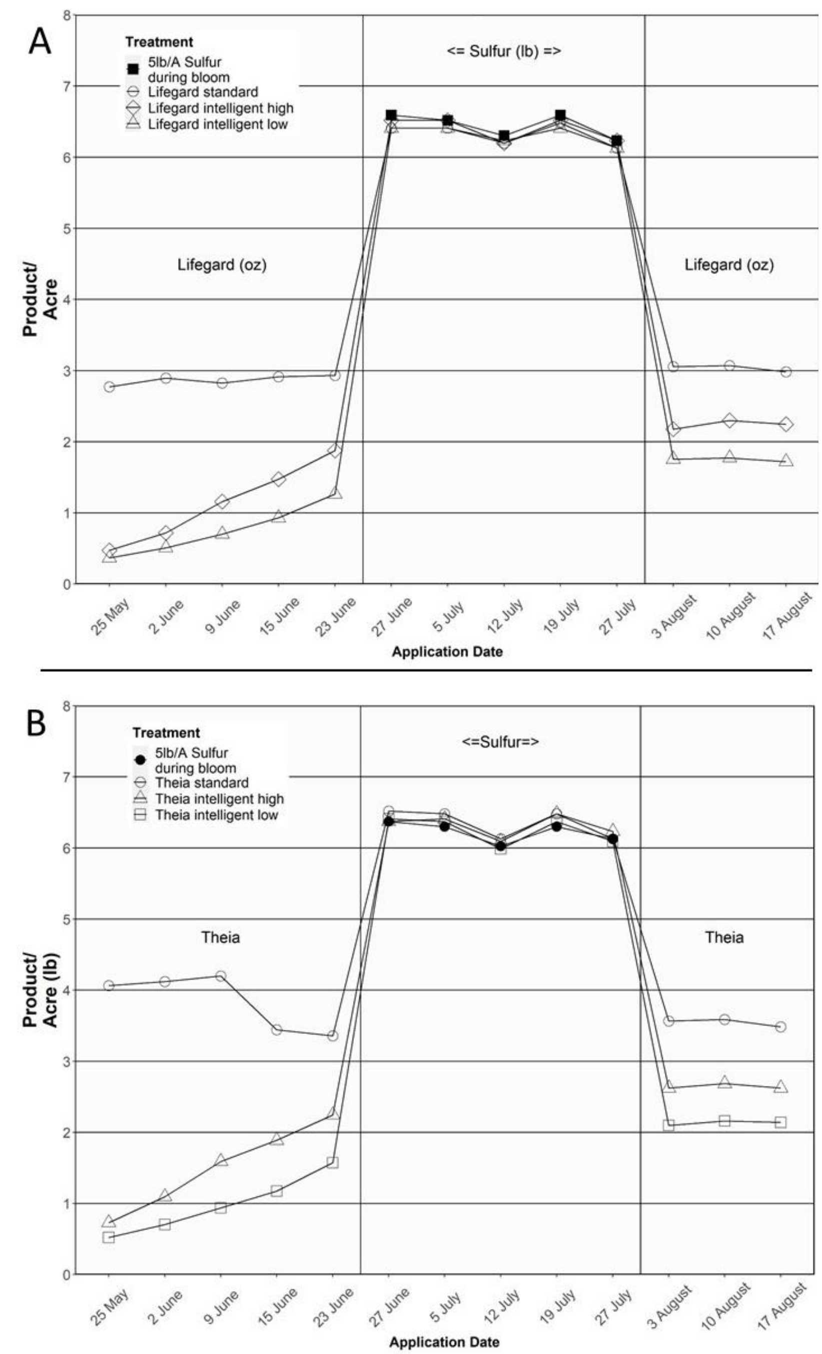

Figure 3. Amount of formulated product applied to Pinot noir (A) and Pinot gris (B) vines.

The sprayer settings may also have impacted the AUDPC and cluster severity disease levels. In the Pinot noir trial both of the intelligent mode treatments resulted in significantly higher cluster severity than the Lifegard treatment applied in standard mode. That could be because in intelligent mode less than the suggested application rate of 1 oz/A to 4.5 oz/A were applied at the beginning of the season. However, after bloom when the Lifegard sprays were resumed the intelligent treatments did result in applied quantities within the recommendation for the remaining applications. In the Pinot gris trial, the Theia treatments applied in intelligent mode resulted in higher AUDPC values than when Theia was applied in standard mode, however the AUDPC values between the two sprayer setting types were not significantly different. Application rates for biological fungicides are not always progressive, meaning that when a larger amount of fungicide material is applied, it does not always result in better disease control. In some cases applying a higher amount of material has been observed to result in higher disease levels. This was observed in a 2021 trial where higher cluster GPM severity was observed when Aviv or Serenade ASO were applied in standard mode than intelligent mode. However the application rate of Theia or Lifegard may be progressive as that same phenomena was observed in 2021.

Future trials could evaluate usage of other products in the same regime to see if similar results were obtained. Additionally testing of biological active ingredient viability before and/or during application could elucidate if there is an effect on the product when applied in intelligent vs standard mode.