GRAPE (*Vitis vinifera* 'Chardonnay')
Powdery Mildew; *Erysiphe necator*

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Efficacy of tank mixing biological fungicides with sulfur while using an Intelligent and airblast sprayer for management of grape powdery mildew, 2021.

Tank mixes of biological fungicides and sulfur were used for grape powdery mildew (GPM) management of Chardonnay vines at the Botany and Plant Pathology Field Laboratory in Corvallis, Oregon. The treatments focused on evaluating whether tank mixes of two different biological fungicides and a low rate of sulfur would provide better control than using the low rate of sulfur alone. Each of the tank mix treatments were applied using the Intelligent Spray System (ISS) either on or off so that the tank mix was applied both in intelligent or standard mode (Table 1).

The sprayer used (50 gallon Pak-blast, Rears Mfg., Coburg, OR) was a standard "off-the-shelf" 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 adjust pesticide application volume in real time to match plant canopy characteristics, with the goal of minimizing 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.

Treatments (Table 1) were arranged in a randomized complete block design. In intelligent mode the quantity of spray released changes each week as the grapevines grow and add canopy; records from previous years were used to approximate the amount of volume that was predicted to be released at each spray date, and thus estimate the quantity of biological fungicide and Microthiol Disperss (MD) needed to maintain a similar amount per acre as standard mode.

The blocks used consisted of 'Chardonnay' 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 15 March. Shoot thinning by hand occurred from 22 April to 15 May and sucker removal by hand was continuous throughout the season. Shoots were cut above the top wire on 10 June 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 Chardonnay vines in 2021.

Treatment^{xy}	Sprayer Mode^{z}
2.5lb MD/A Control	Standard
12oz/A Actinovate + 2.5lb MD/A	Intelligent
12oz/A Actinovate + 2.5lb MD/A	Standard
4qt/A Serenade ASO + 2.5lb MD/A	Intelligent
4qt/A Serenade ASO + 2.5lb MD/A	Standard

^{x}Treatments applied at 80psi at approx. 430 PTO rpm and 3mph. MD = Microthiol Disperss
^{y}Mix rates for intelligent mode treatments were determined by volumes applied in previous years. Different concentrations were required at each application due to different spray volume output due to canopy growth.
^{z}Intelligent mode treatments applied at 0.12fl oz/ft^3 of grape canopy.

Table 2. Area under disease progress curve (AUDPC, leaf disease) and percent infected berries from the Chardonnay biological fungicide Intelligent Sprayer trial at the Botany and Plant Pathology field lab in 2021.

Treatment[v]	**AUDPC**[w]	**Percent Infected Berries**[w]
2.5lb MD/A Alone	1011 (537-1485) A	27.3 (14.7-45.0) A
12oz/A Actinovate Intelligent + 2.5lb MD/A	708 (652-768) A	12.9 (6.3-24.3) ABC
12oz/A Actinovate Standard + 2.5lb MD/A	872 (730-1013) A	20.1 (10.4-35.4) AB
4qt/A Serenade ASO Intelligent + 2.5lb MD/A	588 (300-876) A	10.6 (5.1-20.5) BC
4qt/A Serenade ASO Standard + 2.5lb MD/A	800 (275-1325) A	7.6 (3.6-15.2) C

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

Rainfall for the dormant season (Oct 2020 through March 2021) was close to normal but spring rainfall was the second lowest ever recorded. Spring weather was mild with a few rain events to initiate ascospore release and subsequent primary infection. Signs of powdery mildew were first found on 13 May as a few scattered lesions in a neighboring vineyard block. Bloom took place from approximately 1 to 10 June with most caps detaching from 1 to 4 June.

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 16 June through 18 August. The severity of powdery mildew on clusters was taken on 4 August. 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 a linear model. 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.

AUDPC values were not significantly different among all treatments (Table 2). There was a wide spread between the highest value and the lowest value indicating significant leaf incidence variation within treatments. On 4 August average cluster severity was 27.3% in the 2.5 lb MD/A alone treatment which was not significantly different than the cluster severity observed on vines treated with Actinovate in both standard and intelligent mode (Table 2). Both Serenade ASO treatments resulted in significantly lower cluster severity than the 2.5 lb MD/A control but the Serenade ASO intelligent treatment was not significantly different than either of the Actinovate treatments. The Serenade ASO standard mode treatment resulted in significantly lower cluster severity than both Actinovate treatments and the 2.5 lb MD/A control.

In standard mode treatments, a fairly consistent spray volume was applied over the course of the season from 52.4 to 56.3 gal/acre (Figure 1). In intelligent mode application volume ranged from 13.6 gal/acre early in the season to a maximum of 41.4 gal/acre later in the season when the grapevines had a full canopy. For all tank mixes the amount of MD remained fairly consistent with some larger deviations earlier in the season as the amount the sprayer applied was slightly different than what was expected based on previous records (Figure 2B). For example the amount of

MD applied was as high as 3.5 lb/A (Actinovate intelligent, 28 May) but most of the applications after early canopy development resulted in an amount of MD applied ranging from 2.4 lb/A to 2.8 lb/A across tank mixes (Figure 2B). For the biological fungicides, Serenade ASO intelligent and standard mode treatments stayed fairly consistent with intelligent mode ranging from 3.8 to 5.4 qt/A and standard mode ranging from 4.3 to 5.3 qt/A (Figure 2A). For Actinovate, intelligent mode applications ranged from 12 to 16.8 oz/A while standard mode applications ranged from 12.9 to 15.8 oz/A over the course of the season (Figure 2A).

The 2.5 lb MD/A rate was chosen as the control and tank mix partner for the biological fungicides because it is below the lowest recommended rate on the Microthiol Disperss (MD) label of 3 lb/A. Being below that rate it was hypothesized that GPM control would not be as effective as if MD was applied within the label recommended rate of 3 to 10 lb/A. This was based on experience of previous trials where MD rates were applied below 3 lb/A for all or part of the season resulting in poor GPM control on berries and clusters. Therefore the premise of the trial was to mix a rate of MD that would be ineffective on managing GPM alone, and determine if adding in a biological fungicide would augment control.

The addition of the biological fungicides did not result in significantly lower AUDPC values compared to the 2.5 lb MD/A control. Cluster severity however, was significantly lower on vines treated with Serenade ASO in both standard and intelligent mode but not for Actinovate treated vines. MD rate was kept consistent between the Serenade ASO treatments and the 2.5 lb/A control, so the lower cluster severity among Serenade ASO treatments implies that Serenade ASO augmented the control from that of MD alone. The active ingredient in Serenade ASO is a bacterium, *Bacillus subtilis* QST 713,

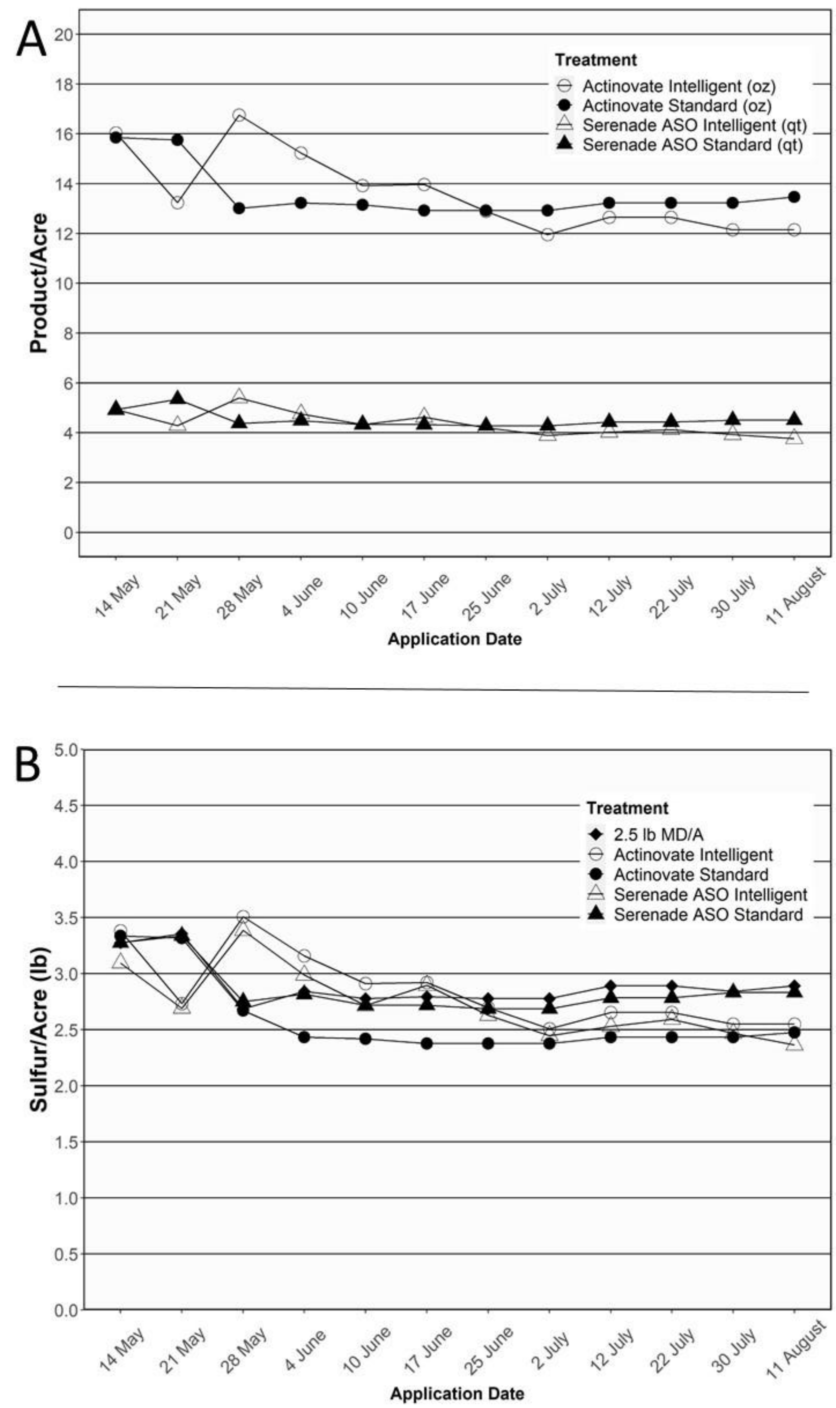

Figure 2. Quantity of biological fungicide (A) and concurrent amount of Mictothiol Disperss (MD) micronized sulfur (B) applied on each application date in the 2021 Chardonnay trial. Units for biological fungicides are indicated in the legend.

while the active ingredient in Actinovate is a different bacterium, *Streptomyces lydicus* WYEC 108. Both products are reputed to act by activating plant defense responses and inhibiting pathogen growth through colonization of plant tissue, thus excluding the pathogen of interest. Actinovate is labelled for GPM suppression or control while Serenade ASO is labelled for GPM control.

Both Actinovate and Serenade ASO were tank mixed with MD (80% micronized sulfur) during their application throughout the duration of this study. On the Serenade ASO technical datasheet it specifically says that tank mixing Serenade ASO with sulfur is compatible, while on the Actinovate technical datasheet it specifically says to not mix Actinovate with products that contain a high percentage of sulfur. Both products claim to contain live bacteria that go on to colonize plant tissues when applied, so tank mixing Actinovate with 80% sulfur may have harmed the viability of the bacteria, thus hindering their ability to effectively colonize and protect plant tissues from GPM protection. Viability studies were not conducted on fungicide mixtures but could have elucidated whether tank mixing was decreasing the amount of live bacteria in the spray mixture. Another factor with long spray programs such as those for GPM is the residues left on plant tissues from previous applications. When applied to leaves that had numerous applications of sulfur, the amount of residue built up may have also played a role in decreasing the effectiveness of the bacteria in Actinovate at colonizing plant tissues.

While the sulfur may have played a role in inhibiting the effectiveness of Actinovate, a trial using Actinovate alone for management of GPM was conducted the previous year (See 2020 Fruit and Ornamental Disease Testing Program booklet). In that trial, using Actinovate alone resulted in GPM levels on leaves and fruit that were not significantly different from vines that were not treated with any fungicide. Over the course of that trial Actinovate treated vines increased in disease levels as quickly as the non-treated vines, implying that Actinovate provided little to no suppression of GPM.

Rates of each biological fungicide and sulfur were attempted to be kept consistent between standard and intelligent mode. Due to variability in plant growth compared to previous years the amount of spray released in the first 4 applications resulted in an amount of sulfur applied per acre that was at or above the 2.5 lb MD/A rate. For some treatments (ex. Serenade ASO standard, 2.5 lb MD/A control) the rate of sulfur applied was above 2.5lb MD/A for the entire season. Part of this was likely due to using small plots for this study and the subsequent volume and pesticide quantity calculations. When extrapolating out spray volumes applied to small plots to the amounts of spray that would have been applied to an acre, even very small variations in spray applied, such as that from turning the sprayer off a second or two later than usual, can have large effects on the calculated spray volume and amount of product applied on a per acre basis. It is unclear whether attempting to keep the rate of pesticide applied to intelligent and standard mode plots consistent had any effect on the disease levels observed between intelligent and standard mode plots.

Future trials should evaluate the viability of the microorganisms in the course of tank mixing and/or application. In addition, a lower rate of sulfur tank mixed with biological fungicide could inhibit the biological fungicides less and perhaps provide for clearer differentiation of whether adding the biological fungicide augmented control of GPM or not.

Note: Despite the application of sulfur on June 25 just prior to a 3 day heat wave with temperatures above 100 F, sulfur phytotoxicity was not observed on any leaves or fruit.