Project Title and UGMVE proposal number

Botrytis Bunch Rot: Who, Where, When, And What to Use

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Summary

From 2020-2022 eleven sites from nine different vineyards in Oregon and four Washington vineyard sites were surveyed for *Botrytis* by sampling grape inflorescence and fruits, vineyard floor debris (prior year rachis) and nuisance blackberry. *Botrytis* on grape inflorescence and fruit varied from site to site and year to year likely due to yearly disease pressure differences and unique site microclimates. Amount of prior year dead grape rachis on the vineyard floor sporulating with *Botrytis* infestations varied from year to year but generally declined as the season progressed. Incidence of *Botrytis* on vineyard floor debris in all but one site in 2021 was over 75% in late April and all sites sampled decreased over time to under 25% by September. Wild nuisance blackberry flowers and fruits adjacent to the vineyard were also found to be potential sources of *Botrytis* inoculum throughout the season, Historical spore trap DNA samples from the Willamette Valley were re-assayed for presence of *Botrytis cinerea* spores and showed that *Botrytis* inoculum is nearly continually present in the vineyard. Increases in inoculum were correlated with the bloom to fruit set period and temperatures around 13-22°C (55-72°F). These results suggest that bunch rot risk can be reduced by focusing fungicide applications to periods before berry touch and reducing the amount of bunch trash. Single spore *Botrytis* isolates collected from vineyard survey samples were assayed for fungicide tolerance to Benomyl (FRAC 1), Iprodione (FRAC 2), Myclobutanil, Tebuconazole, Difenoconazole (FRAC 3), Fluopyram, Boscalid (FRAC 7), Cyprodinil (FRAC 9), Trifloxystrobin, Azoxystrobin (FRAC 11), Fludioxonil (FRAC 12), and Fenhexamid (FRAC 17). In the over one hundred isolates tested so far, there has been fungicide tolerance seen in all FRAC groups mentioned (except Fludioxonil) with multiple fungicide group tolerance seen in 10% of the isolates tested. These results indicate a fungicide group for *Botrytis* management should not be used more than once in season and there is possible carryover of resistant inoculum from the prior year on rachis debris. This year's fungicide use and rotation decisions should consider what fungicide were used the previous year. All the results from 2020-2022 seasons are publicly available at http://gall-id.cgrb.oregonstate.edu:3838/grunwald/wonga4/

Objectives and Experiments Conducted

1. **Characterize fungicide resistance of *Botrytis* isolates from vineyards.**

Commercial vineyards sampled were located throughout the Willamette Valley of Oregon, and the Yakima Valley and Puget Sound of Washington. Sites included organically and conventionally managed vineyards that have had *Botrytis* control issues in the past. To sample *Botrytis* in vineyards, 30 grape inflorescence or clusters were randomly sampled, and incubated for 5 days at 20°C and high humidity in a growth chamber to stimulate the growth and sporulation of *Botrytis*. Samples with *Botrytis* were counted, and the *Botrytis* spores collected by gently touching the surfaces with a cotton swab which was then washed in 0.05% Tween 20 solution, centrifuged to collect the spores, resuspended in 30% glycerol, and stored at -20°C. Single spore isolates were generated by spreading 10-100 µL of the thawed conidia in glycerol suspension onto a 60 mm petri dish of Gelzan gellan gum media, incubating at room temperature for 24 hours, and transferring a single germinated spore to potato dextrose agar amended with 20 µg/mL streptomycin (SPDA). Isolates were grown under a 16-hour photoperiod fluorescent and blacklight for 10-14 days at room temperature to stimulate sporulation. Conidia were washed off the plate with 30% glycerol and stored at -20°C.

Single spore *Botrytis* isolates collected from clusters were screened for fungicide resistance using a microtiter plate assay, adapted from Weber and Hahn (2011), to determine the half maximal dose to inhibit *Botrytis* conidia growth. Briefly, single spore isolates were grown on SPDA under 16-hour photoperiod of florescent and blacklight for 10-14 days at room temperature to stimulate sporulation. Three milliliters of 0.05% Tween 20 were pipetted onto the sporulating *Botrytis* culture and gently rubbed with a sterile loop to dislodge conidia and adjusted to 10,000 conidia per milliliter in 2% malt extract broth amended with 20 µg/mL streptomycin (SMEB), 100 µL of the inoculated SMEB was pipetted into wells of a 96-well microtiter plate with 10 µL of fungicide solutions such that final concentration was 1 to 1,000 µg/mL. Fungicide classes tested were Benomyl (FRAC 1), Iprodione (FRAC 2), Myclobutanil, Tebuconazole, Difenoconazole (FRAC 3), Fluopyram, Boscalid (FRAC 7), Cyprodinil (FRAC 9), Trifloxystrobin, Azoxystrobin (FRAC 11), Fludioxonil (FRAC 12), Fenhexamid (FRAC 17). Treatments of FRAC 11 fungicides were tested at a single discriminatory dose and treated with ~10 µg/mL salicylhydroxamic acid (SHAM) to inhibit alternative oxidase. *Botrytis* growth was measured by optical density at a wavelength of 405 nanometers at the time of inoculation and 48 hours after incubation at room temperature in the dark. The change in growth was corrected to blank uninoculated wells for each treatment and the half maximal inhibitory concentration (IC50) for each fungicide was estimated for each isolate using a two-parameter log-logistic dose response model. Isolates were considered to be tolerant based on previously established thresholds using similar methods (Weber and Hahn 2011; Wang et al. 2020). Isolates that were inhibited by greater than 50% compared to the no Azoxystrobin or Trifloxystrobin (10 ug/mL) or Benomyl (1 ug/mL) treatment control or SHAM control were labeled as sensitive.

Finally, to confirm the fungicide tolerance, isolates of *Botrytis* have begun to be gene sequenced to confirm the species of *Botrytis* as well as the specific mutations (or lack thereof) conferring fungicide tolerance or sensitivity.

2. **Monitor potential sources of inoculum in and around vineyards.**

While sampling grape inflorescence and fruit, samples were collected from wild blackberry (*Rubus armeniacus*) and vineyard floor rachis debris from the prior year by randomly sampling 15-30 pieces of debris or blackberry blossoms or fruit at each location. The debris was disinfested in 10% bleach for 2 minutes and rinsed twice with distilled water before incubation. Incubation, conidia extraction, and isolate generation were performed as described above.

3. **Monitor inoculum levels throughout the growing season.**

Airborne *Botryis* inoculum concentration was monitored by quantitative polymerase chain reaction (qPCR). Historical DNA samples from rotating impaction spore traps from *E. necator* inoculum monitoring. Over 1,200 samples sourced from eight sites over six growing seasons were reanalyzed using a *Botrytis* specific assay (Suarez et al. 2005). Regional weather data was collected from the closest publicly available weather station to correlate environmental conditions that may promote increases in *Botrytis* inoculum.

Summary of Major Research Accomplishments

1. **Characterize fungicide resistance of *Botrytis* isolates from vineyards.**

To date, approximately 45% of isolates are tolerant to a single FRAC groups and 10% of the total isolates collected to date are tolerant to more than one fungicide group with some level of tolerance seen in all fungicide classes (Table 1 and 2). Tolerance to synthetic botryticides were found in both organic and conventional fields indicating that tolerant inoculum may have emerged from fungicide use to control *Botrytis* and/or powdery mildew and has been moving between vineyards. Interestingly, tolerance to Benomyl was found in only 6% of the tested isolates and suggests that while Benomyl resistant populations are still present after decades of not being used in grapes, they are slowly decreasing. Overall, the results show that there is fungicide tolerance in *Botrytis* populations in vineyards to common synthetic grape botryticides and monitoring needs to be continued. These results also indicate that synthetic fungicides should be rotated, and a fungicide class not used more than once per season to minimize the selection of tolerant populations of *Botrytis*. In addition, applications should be targeted during bloom to berry touch especially when conditions are favorable for conidia production and grape tissues are most susceptible to colonization.

Table 1. Fungicide tolerance tested *Botrytis* isolates.

Fungicide common name	FRAC [a] code	IC_{50} Threshold Concentration [b] (µg/mL)	Proportion of tolerant isolates
Benomyl	1	1	7/114
Iprodione	2	10	5/114
Myclobutanil	3	10	43/96
Tebuconazole	3	10	0/114
Difenoconazole	3	10	3/114
Fluopyram	7	10	9/114
Boscalid	7	10	13/114
Inpyrfluxam	7	10	0/114
Cyprodinil	9	1	6/114
Azoxystrobin	11	9.1	21/114
Trifloxystrobin	11	9.1	17/114
Fludioxonil	12	1	0/15
Fenhexamid	17	1	4/114

[a] Fungicide Resistance Action Committee mode of action groups.
[b] Thresholds adapted from Weber and Hahn 2019.

Table 2. Multiple fungicide group tolerance frequency of *Botrytis* isolates.

Tolerance [b] to number of FRAC code(s)	Number of isolates	Percent of total isolates [c]
0	54	47.3
1	49	43.0
2	4	3.5
3	3	2.6
4	3	2.6
5	1	0.9

[a] Fungicide Resistance Action Committee mode of action. A total of seven unique modes of action were assayed against the *Botrytis* isolates.
[b] Tolerance thresholds adapted from Weber and Hahn 2019.
[c] Out of 114 total isolates assayed to all seven FRAC codes

2. Monitor potential sources of inoculum in and around vineyards.

The incidence of *Botrytis* found on grape tissues was lower in the 2021 season than in 2020 and 2022 likely due to a warmer and drier growing season which resulted in a lower disease pressure. However, in 2021, over 75% rachis debris samples on the vineyard floor were infested with viable *Botrytis* in all but one site. The infested rachis tissue decreased steadily over the season and all sites had less than 25% infestation incidence by September. This result suggests that even in low disease pressure years such as 2021, viable *Botrytis* is still present in the vineyard and will produce large quantities of inoculum under favorable conditions such as humid conditions at bloom. If favorable conditions occur at or around bloom time without the appropriate management strategies, inflorescence can become colonized with *Botrytis* which can ultimately lead to a serious epidemic later in the season around harvest. These results also support previous findings that *Botrytis* infections and /or colonization occur earlier in the season on inflorescence and become latent infections until harvest (McClellan and Hewitt 1973). It also confirms that vineyard debris is an important factor in those early season infections (Jaspers et al. 2013). *Botrytis* on feral Himalayan blackberries adjacent to sampled vineyards were found at low levels throughout the season. This suggests that wild Himalayan blackberries could be contributors to grape cluster infections both early and late in the season and harbor fungicide resistant populations form the previous year.

3. Monitor inoculum levels throughout the growing season.

Airborne spore samples from impaction spore traps collected from 2017 to 2022 at seven commercial sites and one research vineyard were assayed by quantitative polymerase chain reaction (qPCR) to estimate the number of *Botrytis* conidia in the air and in the canopy. *Botrytis* was detected in nearly all samples, indicating that *Botrytis* inoculum is a continuous presence the entire growing season. In most years and sites, increases of airborne inoculum were seen around mid to late June (Figure 1), when temperatures ranged from 55-71°F with high humidity.

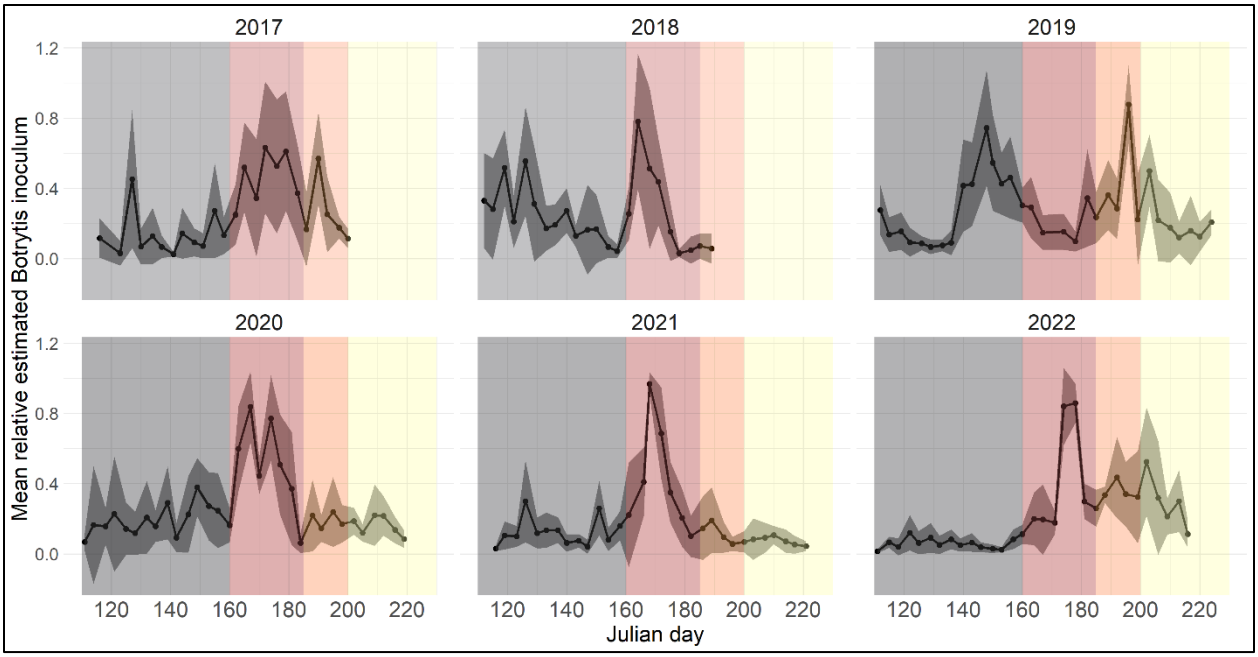

Figure 1. Trends of estimated *Botrytis* inoculum detected by qPCR from DNA samples extracted from rotating impaction spore traps deployed in vineyards within the Willamette Valley, OR from 2017-2022. The estimated *Botrytis* inoculum was normalized to the annual maximum for each site and averaged across sites for each year. Darkened regions represent the standard deviation of the mean. Day of the year is shown as the Julian day for each year sampled. Colored bands are the approximate phenological stages of grapes in the Oregon Willamette Valley: grey = shoot growth (up to June 10), red = bloom (June 10-July 5), orange = berry set (July 5-20), yellow = berry touch (July 20-Aug 14).

Taken together, these results confirm that bloom to berry touch is a critical time for *Botrytis* establishment and thus management. Fungicide and cultural management strategies should be targeted to this period.

Outside Presentations of Research

All results and figures from the all-field seasons are publicly available online at: Willamette Valley Vineyard *Botrytis* Fungicide Tolerance and Inoculum Source

This method of research dissemination provides growers with an easily accessible source of information that will be the most up to date on Botryticide tolerance and will updated as we process more isolates..

Research was presented at the American Phytopathological Society Conference in August 2021 and 2022, the Sustainable Ag Expo in November 2021, the Northwest Small Fruits Conference in December 2021 and 2022, and the Oregon Wine Symposium in 2021, 2022, and 2023.

Research Success Statement

This research provides growers with a status of fungicide resistance of *Botrytis* in vineyards so that effective chemical management options can be made. This research also provides insights into the

sources and changes in *Botrytis* inoculum during the season so that integrative pest management decisions can be made more accurately.

Funds Status

Funds were spent on graduate research assistant stipend, undergraduate assistant wages, benefits and overhead, travel to and from field sites for sample collections purchasing consumable and technical grade fungicides for the incubation, sampling, isolate generation, and fungicide tolerance assays. Funds were also spent on reagents for qPCR assays to test inoculum levels from historical DNA samples, and PCR and DNA sequencing costs for genotyping isolates.