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Application Summary

Competition Details

Competition Title:	2021 Request for Applications: NCSFR
Category:	Open Funding Opportunities
Cycle:	2021-2022
Submission Deadline:	04/1/2021 10:00 PM

Application Information

Submitted By:	Walter Mahaffee
Application ID:	24
Application Title:	BOTRYTIS BUNCH ROT: WHERE, WHEN, AND WHAT TO USE
Date Submitted:	04/8/2021 8:29 AM

Personal Details

Applicant First Name:	Walter
Applicant Last Name:	Mahaffee
Email Address:	walt.mahaffee@usda.gov
Phone Number:	(541) 738-4036
Principle Investigator (PI):	Walter Mahaffee
University/Institution:	USDA-ARS Horticulture Crops Research Unit
Department:	Horticulture Crops Research Unit
Is a USDA-ARS PI or Cooperator(s) part of this project?:	Yes

If USDA-ARS PI and other cooperator(s), list names and organization.

Walt Mahaffee, USDA-ARS-HCRL

Virginia Stockwell, USDA-ARS-HCRL

Rachel Naegele, USDA-ARS-SJVASC

Suggest first potential scientist review panelist based on relevant discipline or working group your project falls under:	Akif Eskalen; aeskalen@ucdavis.edu; Plant Pathologist; University of California-Davis
Suggest second potential scientist review panelist based on relevant discipline or working group your project falls under:	Jay Pscheidt; pscheidj@science.oregonstate.edu; Plant Pathologist; Oregon State University

Suggest first industry review panelist based on relevant discipline or working group your project falls under:	Melissa Hansen; mhansen@washingtonwine.org; Research Program Director; Washington State Wine
Suggest second industry review panelist based on relevant discipline or working group your project falls under:	Dia Crisp; Daic@lumoswine.com; Vineyard Manager; Lumos Wine Co.

Application Details

Proposal Title

BOTRYTIS BUNCH ROT: WHERE, WHEN, AND WHAT TO USE

Budget Requested (AMOUNT MUST BE BELOW $50 000)

50,000

Are you submitting a new proposal?

No

Are you submitting a continuing project proposal?

Yes

Which year of funding are you requesting for this project (if continuing project)?

2

Project length in years from start to finish

3 year project

Are you applying to other groups for funding this project?

Yes

If you are applying for funding from other groups, please list how much money for each request.

$72,664

Technical Working Group

Pest Management

Commodity Group

Grape Viticulture

What are the primary and secondary NCSFR Research Priorities being addressed?

Wine & Juice Grape Viticulture Research Priorities 1D) Biology and management of botrytis and powdery mildew.

Project Summary (1-page)

Bunch rot in grapes is a significant threat to wine and juice grape production and is mainly controlled using fungicides. However, the emergence of resistance to several of the commonly used fungicides in berry crops suggests that fungicide resistant populations could also be accumulating in vineyards. There is also concern about where and when the Botrytis epidemics start, as the increased disease leads to a higher probability of resistant populations. There is a need to characterize fungicide resistance in vineyards, study how the epidemic starts, and predict when the optimal time is to apply fungicides for bunch rot to mitigate or delay resistant Botrytis populations.

Fungicide resistance of bunch rot in grapes is not as well understood as in berry crops. Botrytis in other small fruits has been shown to be resistant to multiple fungicide classes. Testing the main fungicide classes that are registered for use against bunch rot in grapes will require inexpensive and high throughput methods. Resistance in Botrytis can be evaluated more efficiently with the development of new microbiological techniques. Also, the cost of DNA sequencing technology continues to decrease, allowing more isolates to be sequenced to determine the mutation(s) that confer resistance.

Botrytis survives winter in infested grapevine tissues that are either retained after pruning or drop to the vineyard floor, which will produce new spores in the spring. Recently, genetic markers have been described that can identify specific isolates of Botrytis and track epidemics that are caused by a single isolate, potentially pinpointing the source, and spread of the epidemic. Managing grapevine detritus would reduce the number of spores produced the following spring and thus, reduce the overall amount of disease.

Bunch rot symptoms are mostly seen near harvest as the clusters begin to ripen. Botrytis can colonize a cluster early in the season and become less active until later in the season, so fungicide applications are typically made at bloom to control bunch rot. By studying when fungicide applications are the most effective, application timing can be targeted to when grape clusters are the most susceptible or when Botrytis is most vulnerable. Reducing the number of fungicide applications will reduce the probability of selecting for resistant populations.

Our objectives are: (1) Collect and identify Botrytis isolates from vineyards and characterize the resistance to a variety of fungicide classes. (2) Monitor bunch rot disease in vineyards to evaluate where and how Botrytis populations originate and change throughout the growing season. (3) Investigate when fungicide applications are the most effective for the control of bunch rot.

We hope to provide the grape-growing community with information on where bunch rot outbreaks originate and the practices that would be most effective to control them. Our study will evaluate the degree of fungicide resistance and epidemiological spread of the pathogen in vineyards and how that impacts disease management practices. The results will be used to develop new integrative pest management programs to reduce and manage the amount of Botrytis and in turn, delay the development of fungicide-resistant populations.

Literature References

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Cooperators: Provide details on the contribution each Cooperator will make to your proposed project.

Cooperator Contributions

Each of the cooperators for the project has many years of experience in Botrytis Bunch Rot and has connections to both industry and academic resources. Virginia Stockwell has years of experience working with Botrytis and has expertise in fungicide resistance phenotype and genotype testing for isolates in small fruits in the Pacific Northwest. Rachel Naegele's group developed the Botrytis SSR genetic markers that will be used for inoculum source identification. Dr. Naegele's expertise in population genetics and molecular biology in Botrytis and grape powdery mildew will highly valuable to epidemiological studies. Michelle Moyer has years of experience working with Botrytis and Washington state vineyard growers. Dr. Moyer has written extension publications on Botrytis, has connections to Washington state growers, and access to the Washington State University Prosser IAREC which has experimental vineyard blocks that can be used for experiments.

Progress Report

2021 Request for Applications: NCSFR

Progress Report Title:	Final Report
Applicant Name:	Walter Mahaffee
Application Title:	BOTRYTIS BUNCH ROT: WHERE, WHEN, AND WHAT TO USE
Application ID:	24
Review Deadline:	10/27/2023 4:00 PM

Final Report

Please fill out the following information by inserting text directly onto the space provided. Please do not write your report on a separate sheet. There are files that need to be filled out and uploaded in the last sections (this includes the 2023 Conference proceedings form). NOTE: Material from this final report will be used to create a NCSFR Factsheet circulated to the PNW industry.

Please email info@nwberries.org if you have received this final reporting request in error. Provide proof of No Cost Extension or other timeline in your email.

Deadline for submission is Oct. 27, 2023 at 4pm PST.

Oral presentations of findings will take place during the NCSFR Confrerence on Nov. 13-15, 2023 in Corvallis, OR. You will be receiving a mailing with more conference information shortly.

Please note: Failure to submit final project reports and research impact statements, as well as to present a final oral presentation at the NCSFR conference, may result in disqualification for future proposals considered for funding through this program. PI is required to present in person during the conference.

Research Reporting

Project Title:	BOTRYTIS BUNCH ROT: WHERE, WHEN, AND WHAT TO USE	
Principle Investigator & Cooperator(s): :	Walt Mahaffee, Alexander Wong, Virginia Stockwell, Rachel Naegele, Michelle Moyer	
Full Project Reporting Period Covered (M/D/Y-M/D/Y):	Start Date 06/1/2020	End Date 10/20/2023
Is this a final report? (Y/N):	Yes	

Abstract:

From 2020-2022, eleven sites from nine different vineyards in Oregon, and four in Washington were surveyed for Botrytis by sampling grape inflorescence and developing fruits, vineyard floor debris (prior year grape rachis), and nuisance blackberries. Botrytis on grape inflorescence and fruits varied from site to site and year to year likely due to yearly disease pressure differences and unique site microclimates. Prior year dead grape rachis on the vineyard floor with sporulating Botrytis infestations varied from year to year as well, 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. Inoculum estimation from historical spore trap DNA samples originally taken to monitor powdery mildew in the Willamette Valley were re-assayed for Botrytis inoculum. These spore trapping results showed that inoculum is nearly continually present in the vineyard and increases in inoculum tended to align with bloom to fruit set, and temperatures around 13-22°C (55-72°F). This result suggests that the most critical time to reduce bunch rot risk is protecting the cluster before bunch closure by applying fungicides or reducing the detritus flower parts that may not have detached. 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), and fenhexamid (FRAC 17). In the over one hundred isolates tested so far, there has been fungicide tolerance seen in all FRAC groups mentioned 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 the possible carryover of inoculum from the prior year on rachis debris suggest that care should also be taken in selecting fungicides across years as well. All the results from 2020-2022 seasons are publicly available online.

Objectives:

The goal is to improve integrated pest management of Botrytis Bunch Rot (BBR) by improving our understanding of when and where Botrytis inoculum is produced and the frequency of fungicide resistance in the populations. This research will survey the current distribution and frequency of Botrytis fungicide resistance in vineyards, examine where inoculum distributes throughout a vineyard, and when botryticide applications should be used by utilizing high throughput biological assays and modern molecular techniques.

The specific objectives of the proposal are:

1. Characterize fungicide resistance of Botrytis isolates from vineyards

2. Investigate Botrytis inoculum source in vineyards

3. Evaluate botryticide application efficacy at different grape phenological stages

Accomplishments:

Objective 1: Characterization of fungicide resistance of Botrytis isolates from vineyards.

Of the total isolates collected and phenotyped to date, around 10% were found to be 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 or powdery mildew and has been moving among vineyards. Interestingly, tolerance to benomyl was found in only 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. Of the FRAC 7 fungicides, 11 of the 16 isolates tolerant to boscalid or fluopyram came from vineyards in the Yakima Valley of Washington, with six of those isolates being tolerant to both SDHIs. 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.

Objective 2: Monitoring Botrytis 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 or drier growing season which resulted in a lower disease pressure (Table 3). However, in 2021, over 75% rachis debris samples on the vineyard floor were infested with viable Botrytis in all but one site (Figure 1). 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 cool and 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. In addition, the carryover of inoculum from season to season also indicates that fungicide tolerant populations could also be carried from season to season as well. This means that growers should take care in selecting fungicides for BBR within as well as across seasons as to not select for fungicide tolerant populations of Botrytis.

These results also support previous findings that Botrytis infections and /or colonization occur earlier in the season on inflorescence and become latent infections until closer to harvest (McClellan and Hewitt 1973) and vineyard debris is an important factor in those early season infections (Jaspers et al. 2013). Botrytis on wild Himalayan blackberries adjacent to sampled vineyards were found at low levels throughout the season (Table 4). This suggests that wild Himalayan blackberries could be contributors to grape cluster infections both early and late in the season.

Objective 3: Evaluate botryticide application efficacy at different grape phenological stages.

Airborne spore samples from impaction spore traps collected from 2017 to 2022 at seven commercial sites and one research vineyard in the Willamette Valley 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 (99.5% of 1,273 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 2 and Table 5), when temperatures ranged from 55-71°F with high humidity (Figure 3).

All results and figures from the 2020 and 2022 field seasons are publicly available online at: Willamette Valley Vineyard Botrytis Fungicide Tolerance and Inoculum Source and will be continually updated as results become available. This method of research dissemination will provide growers with an easily accessible source of information that will be the most up to date on botryticide tolerance.

The research was presented at the American Phytopathological Society meeting in August 2021 and 2022, the Sustainable Ag Expo in November 2021, the Northwest Small Fruits Conference in December 2021, and the Oregon Wine Symposium in February 2022 and 2023. Results were also presented at Alex's PhD defense as a part of his dissertation research work. Results of resistance to botryticides will continue to be shared directly with growers online, at virtual workshops, and through direct communications.

Reasons why goals and objectives were not met (when applicable): :

The entirety of the objective 3 goals was not met due to lab occupancy limitations and hiring restrictions due to the COVID-19 pandemic. However, the results from the project indicate that applications would be most efficacious at the time of bloom to berry touch due to the presence of inoculum in environmentally favorable conditions when the grape flowers are at the most susceptible to Botrytis colonization.

Industry Significance:

This research indicates that fungicide-tolerant Botrytis populations are present in Oregon and Washington vineyards, and that many isolates are tolerant to at least one fungicide class. These data indicate that growers should not use a fungicide class more than once a season for Botrytis management. In addition, management would likely benefit by targeting fungicide applications to when the fruit are the most susceptible (bloom to bunch closure) and inoculum levels are also likely to be high.

Changes to standard production practices :

1. Botryticide applications should be more focused on bloom to pre-bunch closure to manage Botrytis colonization that will lead to future infection.
2. Fungicide FRAC groups for Botrytis should not be used more than once a season to reduce the potential of losses associated with fungicide resistance.
3. Vineyard debris should be managed before bloom to reduce inoculum availability and potential bunch rot.
4. Alternative hosts such as wild blackberry are a source of inoculum, and their management may reduce Botrytis inoculum in vineyards.
5. Weather should be monitored to predict when high amounts of Botrytis inoculum may be produced to focus fungicide applications.

New grower recommendations:

1. To manage vineyard debris, all clusters should be harvested from the vine for removal to prevent the establishment of diseased clusters remaining in the vineyard.
2. The following spring, as much of the remaining debris possible should be managed by tilling the soil to bury debris or mowing to mulch the debris into smaller pieces.
3. Botrytis management fungicide applications should be targeted around bloom to before berry touch, especially with wet and cool weather to reduce the risk of colonization of the developing clusters.
4. Fungicide FRAC codes should not be made more than once per season. Careful considerations in FRAC codes used across years should also be taken as overwintering Botrytis from debris could carry fungicide tolerant populations across seasons as well.

Provide a list of all scientific citations and papers that have been published because of the funding you received from NCSFR :

Abstracts:

Wong, A. and Mahaffee, W., A survey of Botrytis inoculum source and fungicide resistance in Willamette Valley, Oregon vineyards., American Phytopathological Society National meeting, August 2021, Virtual meeting.

Wong, A. and Mahaffee, W., Trends of airborne Botrytis inoculum in vineyards of the Willamette Valley, Oregon., American Phytopathological Society National meeting, August 2022, Pittsburgh, PA.

Extension publications:

Wong, A. and Mahaffee, W., The Where, When, and What to Use for Botrytis Bunch Rot Prevention, OWRI Vine to Wine, July, 2023.

All Funding Sources:

The Oregon Wine Board also funded this project which allowed for expansion of the viticulture regions and number of vineyards sampled, the number of isolates and fungicides examined, and the historical analysis of DNA from vineyard air samples to assess inoculum availability over an extended number of seasons.

Project Keywords:

Grapes, Botrytis Bunch Rot, Fungicide resistance, Inoculum monitoring

Please list the individual number of Post Docs, Docs, Masters Students supported by this research project funding:

Alexander Wong (PhD student/Post-doc)

Figures, and Tables

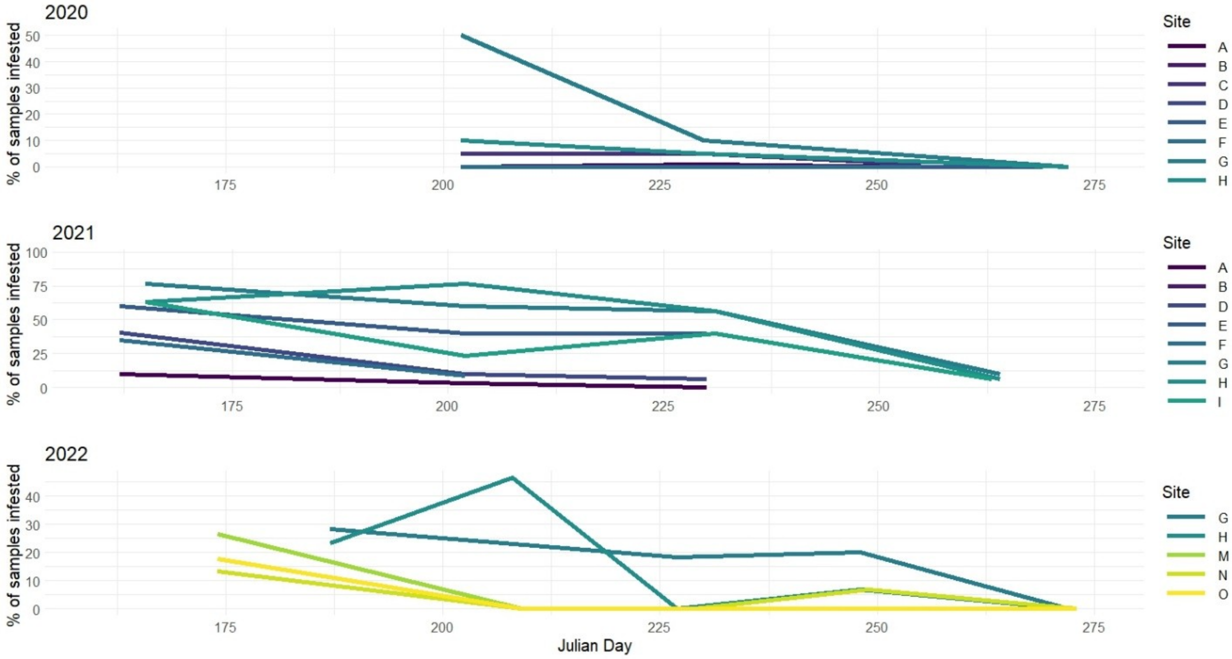

Figure 1. Trends of *Botrytis* infested prior year dead grape debris collected from the vineyard floor between 2020 and 2022. Each vineyard site with vineyard floor debris had up to 30 pieces of prior year rachis debris sampled, surface disinfested, incubated, and visually rated for *Botrytis* at each sampling time point. In 2020, debris sampling included a variety of dead grape tissues including canes, leaves, and rachis. In 2021 and 2022, only dead rachis was sampled. Sampling in 2020 started late due to late notice of funding, and in 2022 due to a prolonged spring which delayed growth. Sites A-K are in Oregon, Sites L-O are in Washington. Sites J, K, L did not have high enough amounts of rachis debris to sample.

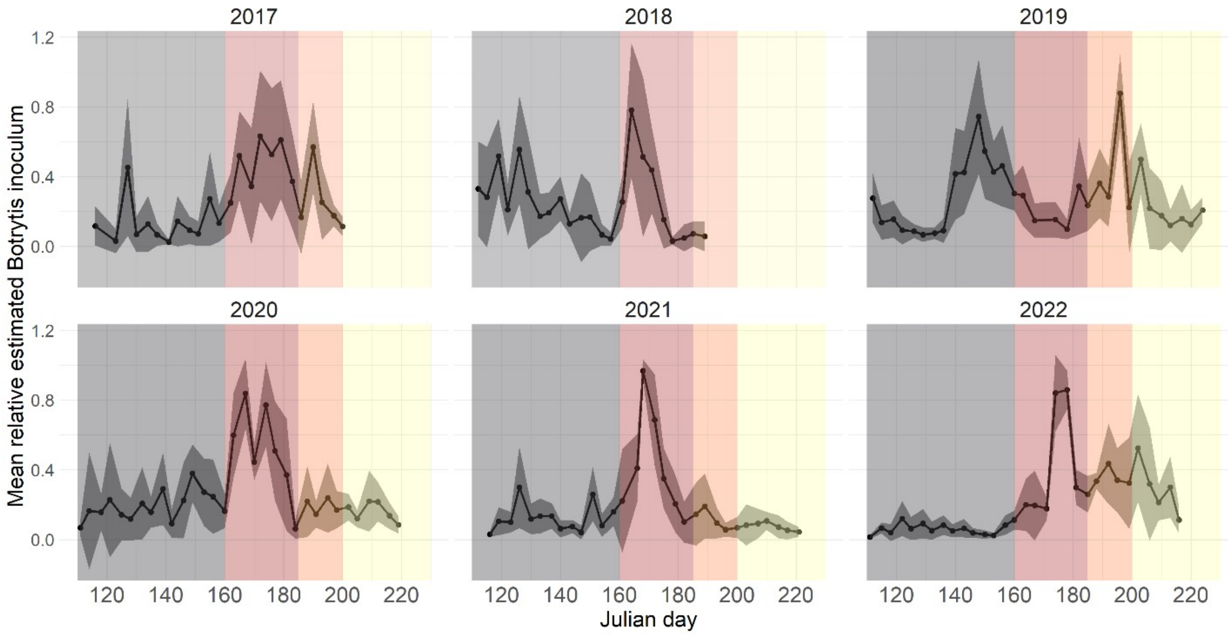

Figure 2. Trends of estimated *Botrytis* inoculum detected by qPCR (Suarez et al. 2005) from DNA samples extracted from rotating impaction spore traps deployed in eight vineyards within the Willamette Valley, OR, from 2017-2022. The estimated *Botrytis* inoculum was normalized to the annual maximum for 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).

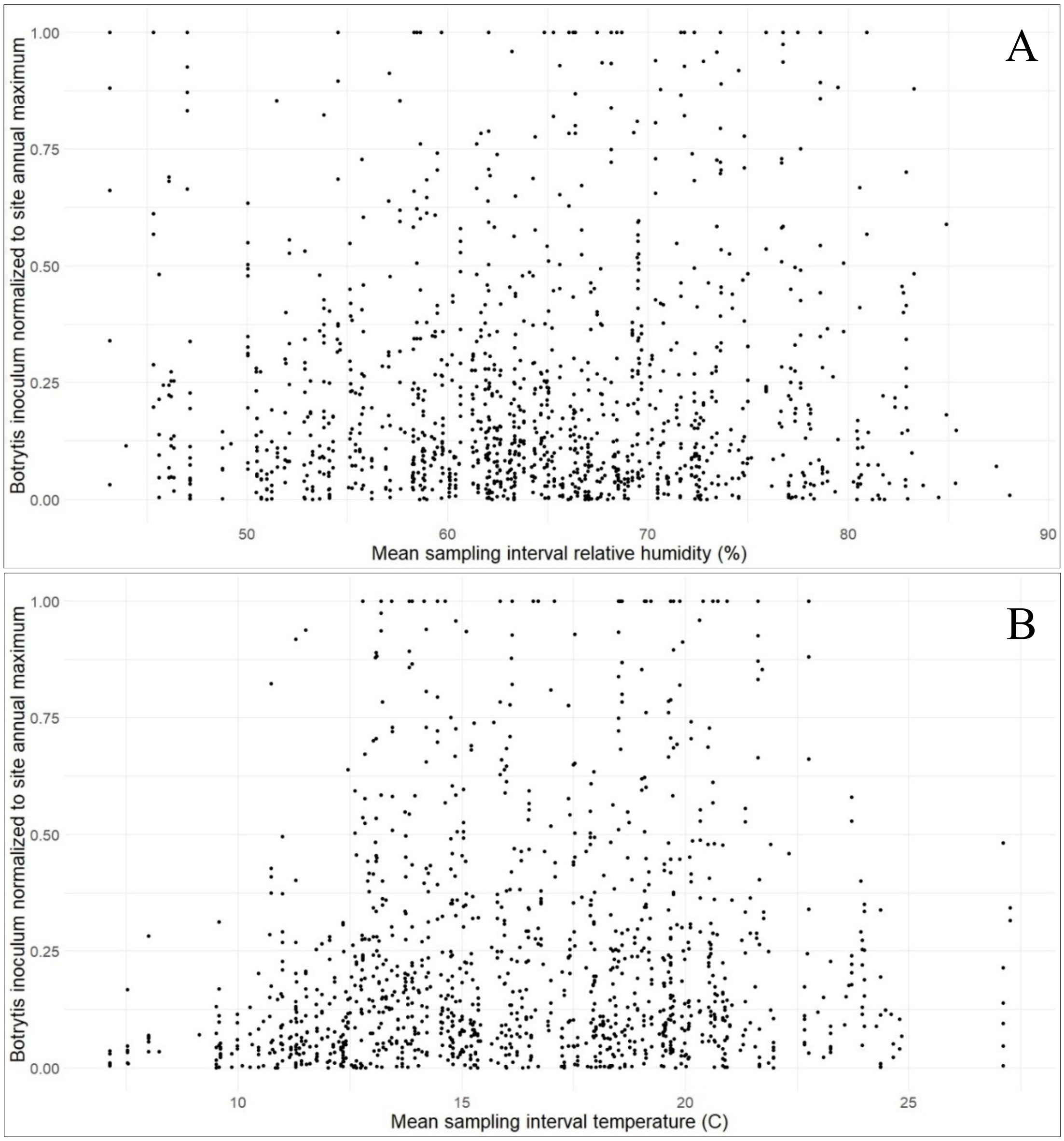

Figure 3. Regional weather and airborne *Botrytis* inoculum concentration from eight sites over six years normalized to each site's annual maximum inoculum concentration. The hourly average of A) relative humidity and B) temperature were averaged over the sampling period (3-4 days). Weather data was derived from the closest weather station from uspest.org/wea.

Table 1. Fungicide tolerance phenotype frequency of *Botrytis* isolates collected from Oregon and Washington vineyards.

Fungicide common name	**FRAC [y] group code**	**EC_{50} Threshold Concentration [z] (µg/mL)**	**Number of tolerant isolates / total isolates tested**
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
Fenhexamid	17	1	4/114

[y] Fungicide Resistance Action Committee mode of action groups.
[z] Thresholds adapted from (Weber and Hahn 2019).

Table 2. The frequency of multi-tolerant *Botrytis* isolates.

Tolerance [x] to number of FRAC [y] code(s)	**Number of isolates**	**Percent of total isolates [z]**
0	54	47.3
1	49	43.0
2	4	3.5
3	3	2.6
>3	4	3.5

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

Table 3. Season average of the percent grape inflorescence and clusters infested with *Botrytis* from vineyards in Oregon and Washington.

		Percent (%) *Botrytis* incidence [y]		
		Year		
Site	**State** [z]	**2020**	**2021**	**2022**
A	OR	3.2	0.8	8.0
B	OR	7.4	0.8	1.3
C	OR	13.9	4.2	2.0
D	OR	5.6	4.2	4.7
E	OR	30.4	5.8	3.3
F	OR	5.6	0.8	2.0
G	OR	29.0	6.7	3.3
H	OR	19.9	3.3	6.0
I*	OR	34.8	11.1	47.5
J	OR		0.8	6.0
K	OR		4.2	4.1
L	WA			12.6
M	WA			6.1
N	WA			2.7
O	WA			2.0

[y] Thirty inflorescence or clusters sampled at each time point for each site. Collections started around bloom and ended shortly before harvest.

[z] Sites in Oregon were all within the Willamette Valley spanning a 165 km range from north to south. Three Washington sites were in the Yakima Valley, and one was in the Puget Sound area. Sites A-K are in Oregon, Sites L-O are in Washington.

* Research vineyard in Corvallis, OR

Table 4. Season average of the percent Blackberry (*Rubus* spp.) flowers and fruits with *Botrytis* infestation that were adjacent to the sampled vineyard sites.

		Percent (%) *Botrytis* incidence [y]		
		Year		
Site	**State** [z]	**2020**	**2021**	**2022**
C	OR	37.8	6.7	4.7
E	OR	11.1	6.0	0.7
F	OR	38.3		
H	OR			11.3
J	OR		2.2	7.5

[y] Up to 30 flowers or blackberry fruits sampled at each time point for each site. Collections started around bloom and ended shortly before harvest.

[z] Sites in Oregon were all within the Willamette Valley.

Table 5. Trends of the airborne *Botrytis* inoculum detected by qPCR from DNA samples extracted from rotating impaction spore traps deployed in vineyards in the Willamette Valley of Oregon from 2017 to 2022.

Year	**Site** [t]	**Mean inoculum** [u]	**Median inoculum** [v]	**Max inoculum** [w]	**Max collection date** [x]	**Mean**[y] **temperature (°C)**	**Mean %** [z] **relative humidity**
2017	S	3,789	1,757	16,713	25-Jun	22.8	43.1
2017	T	2,600	2,086	7,062	28-Jun	20.6	45.3
2017	U	8,962	1,683	43,571	28-Jun	20.9	58.3
2017	V	2,631	1,624	15,132	21-Jun	18.6	66.4
2017	X	2,978	1,597	11,406	9-Jul	19.1	58.5
2017	Z	1,913	1,520	5,313	7-May	13.9	71.6
2018	S	1,421	1,052	5,161	13-Jun	14.2	67.5
2018	T	2,873	1,506	11,972	13-Jun	14.2	67.5
2018	U	7,016	3,908	53,825	17-Jun	16.6	66.3
2018	V	2,753	2,038	11,673	13-Jun	14.2	67.5
2018	W	6,214	2,799	33,353	17-Jun	16.7	59.7
2018	X	3,343	2,114	9,785	13-Jun	14.2	67.5
2018	Z	3,027	1,832	8,636	13-Jun	14.2	67.5
2019	S	2,910	2,386	10,723	15-Jul	19.9	65.3
2019	T	3,719	2,801	11,407	28-May	13.2	76.7
2019	U	11,625	8,614	40,324	15-Jul	20.4	68.7
2019	V	3,641	2,722	27,241	15-Jul	19.9	65.3
2019	W	3,372	1,379	15,124	20-May	12.8	75.9
2019	X	4,122	3,541	14,877	15-Jul	19.9	65.3
2019	Y	3,254	2,201	11,838	19-Aug	19.2	66.3
2019	Z	4,569	4,371	11,558	28-May	13.2	76.7
2020	S	5,409	3735	20,310	15-Jun	13.8	78.6
2020	T	9,483	6,983	31,633	11-Jun	14.4	73.6
2020	U	16,487	7,896	98,846	15-Jun	14.6	80.9
2020	V	14,619	10,858	66,624	15-Jun	13.8	78.6
2020	W	3,253	2,267	12,666	25-Jun	19.7	62.1
2020	X	7,161	4,416	24,614	30-Apr	13.4	68.5
2020	Y	7,761	5,739	39,228	22-Jun	18.5	72.3
2020	Z	7,741	6,287	20,878	22-Jun	18.5	68.2
2021	S	1,539	603	11,547	17-Jun	16.1	71.8
2021	T	2,145	906	9,861	17-Jun	16.1	71.8
2021	U	6,681	2,615	45,769	17-Jun	17.1	77.5
2021	V	2,679	1,192	16,573	21-Jun	19.7	54.5
2021	W	1,471	1,007	6,372	17-Jun	16.1	71.8
2021	X	2,274	1,598	11,031	21-Jun	19.7	54.5
2021	Y	5,274	2,397	36,626	17-Jun	17.1	77.5
2021	Z	2,182	1,287	10,613	17-Jun	16.1	71.8
2022	S	5,587	2,705	21,690	23-Jun	15.9	66.1
2022	T	6,227	3,791	34,733	23-Jun	15.9	66.1
2022	V	10,860	8,505	50,972	21-Jul	19.1	58.6
2022	W	7,385	3,799	38,774	27-Jun	21.6	47.0
2022	X	12,000	5,503	63,971	23-Jun	15.9	66.1
2022	Y	19,402	10,870	92,542	25-Jul	20.7	64.8
2022	Z	14,454	11,182	57,268	23-Jun	15.9	66.1

[t] All sites located in the Willamette Valley of Oregon. Not all sites participated in spore trapping in all years assessed.
[u] Mean *Botrytis* inoculum for each 3– to 4-day sampling interval averaged over the entire season of sampling.
[v] Median *Botrytis* inoculum for each 3– to 4-day sampling interval over the entire season of sampling.
[w] Maximum *Botrytis* inoculum detected at a single sampling interval.
[x] Sampling interval collection date from which the maximum *Botrytis* inoculum concentration was detected.
[y] Mean air temperature during the sampling interval with the observed maximum inoculum. Weather data was derived from the closest weather station from uspest.org/wea.
[z] Mean relative humidity during the sampling interval with the observed maximum inoculum. Weather data was derived from the closest weather station from uspest.org/wea.

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Research Impact Statement

1. **Project Title**: BOTRYTIS BUNCH ROT: WHERE, WHEN, AND WHAT TO USE
2. **Principle Investigator & Cooperator(s)**:
   Walt Mahaffee, Alexander Wong, Virginia Stockwell, Rachel Naegele, Michelle Moyer
3. **Research Objectives & Procedures**:
   The goal is to improve integrated pest management of Botrytis Bunch Rot (BBR). This research surveyed the current distribution and frequency of Botrytis fungicide resistance in vineyards, examined where spores can originate from, how the number of airborne spores fluctuate throughout the season, and when and which botryticide applications are most effective. Commercial organic and conventional vineyards were sampled several times during the growing season from 2020 to 2022. Clusters and other plant material with Botrytis were counted, and Botrytis isolates collected and screened fungicide resistance to multiple fungicide classes. Air samples were also collected using spore traps and the number of Botrytis spores across seasons was estimated by molecular techniques.
4. **Total $ Funding through NCSFR**: $144,000
5. **Economic Impact and Benefits**. Understanding when and where Botrytis spores are dispersing and what fungicides are effective for management will allow for the strategic application of fungicides to periods most likely to improve disease management. This will reduce management costs associated with using ineffective fungicides and the subsequent rescue applications or applying fungicides at ineffective times. The identification of what types of plant materials spores come from will allow growers to focus on cultural practices that reduce these sources and reduce the need for fungicide applications. This integrated approach reduces labor, equipment and fungicide costs while still achieving control, therefore potentially increasing the profitability of the final crop.
6. **Environmental Impact and Benefits**. The project will reduce the use of fungicides that are no longer effective in managing Botrytis, thus improving disease control, and reducing the need for rescue fungicide applications when disease levels are not adequately controlled. Further by identifying the times Botrytis inoculum is present and its source allows growers to focus management practices and reduce unneeded fungicide applications and their associated environmental costs and to reduce the carbon footprint associated with tractor use and fungicide application.
7. **Describe Other Impact and Benefits**. Trained one PhD student and 3 undergrad students.
8. **Concluding statement.** Describe *why* your research is valuable.
   This research provides growers with a status of fungicide resistance in the Willamette Valley vineyards so that effective management decisions can be made that improve disease management. This research also provides insights into the sources and changes in Botrytis availability during the season so that integrative pest management decisions can be made more accurately.

NCSFR Conference Proceedings Form

This form is used to gather project information to complete the conference proceedings document. Please do not change any of the formatting.

Project Title:

BOTRYTIS BUNCH ROT: WHERE, WHEN, AND WHAT TO USE

Authors:

Walt Mahaffee[1], Alexander Wong[2], Virginia Stockwell[1], Rachel Naegele[3], Michelle Moyer[4]

Institution:

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Abstract/ Summary:

Botrytis Bunch Rot (BBR) caused by *Botrytis spp.* can periodically be a serious disease in vineyards, particularly in years of high disease pressure. It can typically be managed using fungicides that prevent infection of grape inflorescences or clusters. The objective of our study was to examine when and where *Botrytis* inoculum may be arising in vineyards, as well as the fungicide tolerance profile of *Botrytis* isolates collected. A vineyard sampling survey of 7 to 14 commercial vineyard sites with a history of bunch rot issues was performed from 2020 to 2022 in Oregon's Willamette Valley and in Washington (in 2022). Grape inflorescences and developing clusters from bloom to harvest were collected to quantify incidence of BBR. In addition, we sampled dead grape rachis debris from the vineyard floor that remained from the prior crop year and nearby blackberries (if applicable). The incidence of *Botrytis* on inflorescences/clusters throughout the field season varied across sites, years, and management practices. *Botrytis* inoculum from rachis debris on the vineyard floor and adjacent blackberries varied but was found to occur throughout the growing season. In 2021, *Botrytis* incidence on rachis debris decreased as the season progressed from upwards of 75% incidence at bloom-time to less than 25% by harvest. This result indicated that inoculum could be generated during the season from vineyard floor rachis debris from crop thinning, or dropping fruit at harvest, and serve as reservoir of fungicide tolerant isolates from the previous season. From each of these sample types, *Botrytis* isolates were collected and screened for tolerance to fungicides. Tolerance of *Botrytis* isolates to benomyl (FRAC 1), iprodione (FRAC 2), myclobutanil, tebuconazole, difenoconazole (FRAC 3), fluopyram, boscalid (FRAC 7), cyprodinil (FRAC 9), trifloxystrobin, azoxystrobin (FRAC 11), and fenhexamid (FRAC 17), was observed. Approximately 10% of the isolates tested were resistant to more than one fungicide mode of action. These results suggest that fungicide resistance exists in vineyard *Botrytis* populations, and the levels are concerning. Therefore, growers should not use a fungicide with an active

ingredient from a single FRAC group (mode of action) more than once each season to reduce selection of tolerant populations. Airborne spore samples from impaction spore traps collected from 2017 to 2022 at seven commercial vineyards and one research vineyard were assayed by quantitative polymerase chain reaction (qPCR) to estimate the number of *Botrytis* conidia in the air at the vine canopy. *Botrytis* was detected in nearly all samples, indicating that *Botrytis* inoculum is present the entire growing season. In most years and vineyards sampled, increases of airborne inoculum occurred during mid- to late- June, when temperatures ranged from 55-71°F. Taken together, these results confirm that bloom to bunch closure (e.g., berry touch) is a critical time for *Botrytis* establishment and thus management to avoid infections. Fungicide applications and cultural management strategies, such as reducing canopy density (e.g., cluster zone leaf removal) should be targeted to this period to reduce the risk of disease development. Results published online here:
http://gall-id.cgrb.oregonstate.edu:3838/grunwald/wonga4/