Grant Report Template

1.0 INSTRUCTIONS

Use this grant report template to communicate progress on your project objectives to the Virginia Wine Board and its administrative agents.

This simplified form focuses attention on the intended and achieved results of the project, including how project results are separately shared with their intended beneficiaries. This report is not the place for a detailed technical discussion of research methodology or results.

- During the proposal stage, applicants complete the first (WHITE) sections to summarize the project's objectives, deliverables, and intended impact plus planned communication to stakeholders.
- At the midpoint of the project (December 1, due December 15), Research and Education grantees complete the center (GRAY) sections to note progress as well as expenditures to date.
- Finally, upon project conclusion (May 31, due June 30), all grantees complete the final (BLUE) sections to describe the project's results and communication, as well as the final expenditures.

2.0 GRANTEE INFORMATION

Project Title	Maintain fungicide resistance testing capabilities for grape diseases-FY25			
Organization	Virginia Tech			
Proposal # *(if needed)*	PAJG6HJU		Award # *(if needed)*	
Project Lead		Mailing Address	Research	x
Name	Anton Baudoin	410 Price Hall, 170 Drillfield Dr	Education	
Title	Associate Professor Emeritus	School of Plant and Environmental Sciences	Marketing	
Email	abaudoin@vt.edu	Virginia Tech	Continuing?	◊
Phone	Office: 540-231-5757 Cell: 540-808-6345	Blacksburg, VA 24061-0331	Year	_ of _

3.0 PROJECT OBJECTIVE, PROGRESS, AND IMPACT

3.1 PROPOSAL (February)

Summarize the project objective, the intended deliverable or result, and expected impact. (1-5 sentences or bullets)

1. Testing of grape pathogens for fungicide resistance to determine distribution of known resistance types, and as new issues may arise.
2. Further investigation of variability of phosphite sensitivity among grape downy mildew isolates

Impact: (hopefully) early warning to growers when fungicide resistance problems arise

Summarize the project's workplan (1-5 sentences or bullets)

Visit vineyards and accept samples submitted by grower and Extension personnel (encouraged). Conduct bioassays for fungicide sensitivity.

How will you know your project has been successful? What project indicators will measure progress or success? (1-5 sentences or bullets)

Number of samples tested and found sensitive (ideally) or resistant and grower notified. Detection of any new resistance problems and development of recommendations.

3.2 Mid-Year Report (December)—Research and Education Grants only

New grape pathogen samples that were collected or received since June of 2024 are:

- Powdery mildew samples from 13 vineyards
- Downy mildew samples from 7 vineyards
- Botrytis samples from 6 vineyards

In the summer, I was told, unexpectedly, that my lab and office had to be vacated and operations moved to a different, much smaller location, which took a lot of my time, and which reduced my ability to go out on collecting trips – only one such trip was completed. I did reach out to growers, consultants, and students, and received useful samples from three growers, one consultant, one extension scientist, one graduate student, and one undergraduate student who was taking VT's Viticulture online class.

Because powdery mildew samples that we had bioassayed earlier in 2024 had shown evidence of at least partial boscalid resistance (as reported in my June 2024 report to the Wine Board), assistance was sought from scientists who had been involved with the federally funded FRAME Networks projects in recent years (https://framenetworks.wsu.edu/). At the time, their funding had ended (more recently it was extended), but Dr. I. Stergiopoulos (University of California, Berkeley) and T. Neill (USDA, Corvallis, OR) agreed to analyze a number of our samples. Thirty samples were submitted, and results so far include:

29 of 30 samples contained the G143A mutation that conditions resistance to QoI (strobilurin, FRAC group 11) fungicides. This was expected based on our previous research in Virginia.

28 of 30 samples also contained the Y136F mutation that can condition resistance to demethylation inhibitor fungicides (FRAC group 3, e.g., Rally, Inspire, Cevya, etc.). This gene is often present in the form of multiple copies in a cell, and the mutation was almost always found as a mix with the wild-type, and depending on the proportions present, may lead to various degrees of sensitivity, from almost completely sensitive to fairly high degrees of resistance. This was also not unexpected based on our previous research in Virginia.

As of this writing, 4 of the samples have been analyzed for mutations related to succinate dehydrogenase inhibitor resistance (FRAC-group-7, Endura, Luna, Aprovia, Kenja, etc.); more results are expected later in December or January. Three of the four that had been suspected based on our bioassays indeed had mutations: pH242R and pG169D, both of which have been documented to be associated with reduced sensitivity to boscalid and fluopyram. This IS new information for Virginia and, apparently, we have the same mutations that have been detected recently in the western US and in Michigan. And they appear to be widespread: one isolate came from Albemarle Co, one from the northern Shenandoah Valley, and one from eastern Virginia.

We haven't been able to carry out many additional bioassays due to my lab move and the need to set up our facilities in a new space. When this was completed by early October, grape leaf production had become very slow (as happens every year) and all the usable leaves that can harvest in the greenhouse are needed to maintain our downy, and especially (since we cannot reliably freeze and resurrect those) our powdery mildew cultures, so we'll have to wait until leaf production increases again later in the winter. We would like to conduct bioassays with not only boscalid (Endura), but also Luna, Kenja, Aprovia, as well as Vivando and Quintec.

As in previous years, a downy mildew spray trial focusing on the performance of phosphorous acid (phosphite) fungicide was conducted. Grape plants in 2- to 5-gallon pots were kept outdoors and monitored regularly for the first evidence of downy mildew. The plan was to start fungicide applications at the very first evidence of downy mildew development, allowing a very small population to develop, which would subsequently be subjected to selection pressure by the fungicide.

Disease development did not follow the plan exactly. The weather was dry until mid-July. A period with almost daily rains started on July 16. By July 28, no evidence of downy mildew had appeared on any of the plants, and a few plants that had been inoculated with an isolate collected at this same location the previous year and had developed a few sporulating spots were placed outside among the experimental plants. Clear downy mildew symptoms were detected on experimental plants by August 5, and disease increased quickly, which, in light of the drier weather in the preceding week, was probably due to the rainy period in the second half of July. This was more initial disease than was planned, but spray treatments were initiated on August 7, just a few days before the rains that came with hurricane Debby. Then, rain became sparse again for the remainder of August and the first half of September. Plants were rated for downy mildew and defoliation on September 2 and October 3 (Table 1). By the latter date, control (untreated) plants had lost almost all of their leaves. Downy mildew samples were collected from all treatments (except mancozeb) and stored in the freezer for later bioassay to determine whether any differences in phosphite sensitivity can be detected.

Table 1. Control of grape downy mildew by different phosphite frequencies, 2024.

Treatment, Rate per 100 gal	Average number of leaves remaining per plant		Average defoliation %		Average downy mildew on remaining leaves, %	
	2-Sep	3-Oct	2-Sep	3-Oct	2-Sep	3-Oct
Control 14d, water	45	6	55	95	33	56
Mancozeb 14d, 3 lbs.	83	61	14	41	18	24
Prophyt 7d, 0.5%	65	36	22	67	17	30
Prophyt 10d, 0.5%	76	69	25	54	16	35
Prophyt 14d, 0.5%	76	41	24	59	18	31

3.3 Final Report (June)

Compare the project to the objective, workplan, and project indicators. Provide (as a link or attachment) the project deliverable or result. Describe the realized or expected impact of the project.

Key Takeaways (57 samples tested spanning 23 locations):

- For powdery mildew, expect reduced efficacy of FRAC Group-7 fungicides in most of Virgina in vineyards where they have been used regularly, as seen recently in Michigan and the Western states.
- For downy mildew, Ridomil continues to work in Virginia, despite European reports. Reduced sensitivity to FRAC group P 07 is clear but highly variable and not yet well understood.
- Resistance to FRAC Group 7 in Botrytis is variable: resistance to boscalid is common, resistance to Luna, Kenja, Aprovia, and others is present but much less common, and resistance to pydiflumetofen, one component of Miravis Prime, has not yet been detected in Virginia vineyards.

Powdery mildew

FRAC Group-7 resistance. At the time of the midterm report, only 4 of the powdery mildew swabs (from 3 vineyards, collected in 2023) had been tested for Group-7 resistance mutations. The remainder has now been tested as well. Among the 13 Virginia vineyards where PM samples were collected in 2024, 8 had mutations associated with reduced sensitivity or resistance to at least some Group-7 fungicides.

The H242R mutation was found in 5 of those (plus the 3 locations sampled in 2023)

The G169D mutation in 4 of those (plus the 3 locations sampled in 2023)

The S70P mutation (also known as g.A472G) was present in one vineyard, in multiple samples from different blocks. There is not much information about this mutation and to what degree it may affect sensitivity to Group-7 fungicides. The grower has been contacted in hopes of being able to provide fresh powdery mildew sample in 2025.

It was not possible to conduct bioassays on most 2024 samples, since they were collected too late in the fall and could not be grown on leaf tissue. Only one powdery mildew sample that had the H242R mutation was successfully tested by bioassay, and it was indeed largely resistant to boscalid (Endura and Pristine): a rate of 4.5 oz Endura per 100 gallons still allowed development of 24% (after 7 days) to 73% (10 days) of the number of spore stalks compared to water-treated tissue to develop, while this number should be 0% for sensitive isolates.

These are similar to what our previous tests have shown: the fungicide may still provide *some* inhibition of the pathogen's development, but efficacy in the field is expected to be greatly reduced. Samples with such mutations and resistance in bioassays are not geographically limited; they are from northern, central, eastern, and northwestern parts of Virginia.

Vivando (metrafenone) belongs to a FRAC code group (50) with only one other member: Prolivo (pyriofenone). Powdery mildew from a vineyard in the Shenandoah Valley has shown reduced sensitivity to metrafenone in repeated bioassays, where low label rates that would completely stop development of sensitive isolates, still allowed considerable powdery mildew development of this isolate. Isolates from other locations have sometimes shown some development as well on metrafenone-treated tissue, but in most of those cases, repeat bioassays have not confirmed the result. Since there is no known molecular test for metrafenone resistance, the status of most 2024 samples is unknown; the one isolates tested by bioassay was sensitive.

Downy mildew

We have de-emphasized testing for strobilurin (QoI) resistance because in recent years, almost all downy mildew samples from conventionally treated vineyards have been resistant or, at minimum contained a mix of resistant and sensitive pathogen.

All samples (from 11 vineyards in 2023-2024 collections) tested for mefenoxam (Ridomil) resistance have been sensitive. Resistance to this group had become widespread in Europe but has not yet been detected in North America.

Resistance to mandipropamid (Revus) was detected in 5 vineyards of the 11 mentioned, but in some of those samples, a sensitive subpopulation was present as well. This may happen when different vineyard blocks are subject to different spray schedules, or when the fungicide in question has not been used in a while.

A complex pattern has emerged with respect to sensitivity to the phosphite or phosphonate fungicides (FRAC group P 07) recently. Many samples have shown better growth on phosphonate-treated tissue than isolates that were collected, say, 10 years ago, some of which we have kept stored in a freezer. Historically, we have usually used Prophyt as or test product, but in the past year, we have also started including Phostrol and Fosphite

Numbers in the following express downy mildew development (number of spore stalks, each an average of 10 inoculation sites) on treated grape leaf tissue as a percentage of downy mildew development on untreated (water-treated) tissue. So, 0% would mean excellent control, and 100% would mean no disease control, just as much downy mildew as on untreated grape leaf. Ten years ago, with 0.3% Prophyt, we would get mostly zero but occasional small numbers (very slight downy mildew development). But we also get a fair amount of variation (which is why this is taking so long).

Historically sensitive isolate (%): 0, 0, 0, 7, 18, 0, 21, 0, 10, 2, 60 (the 60 was unusual, perhaps due to contamination? time to pull a new version from the freezer collection)
Example of a recent isolate (%): 81, 0, 5, 58, 37, 65, 2, 41, 83, 7

The Prophyt label lists 0.5% as the maximum concentration, and produced similar "not-close-to zero" results:

Prophyt at 0.5%: 48%, 49%. Prophyt at 0.3%: 72%, 12% of growth on non-treated leaf
Phostrol at 0.5%: 51%, 22%
Fosphite at 0.5%: 33%

Those percentages are also not all close to 100%, so there is still some effect of the fungicide, but it's variable. We also tried 1% Prophyt in an experiment (even though its label says to not go above 0.5% concentration) and got excellent disease control (1%) and no evidence of phytotoxicity. We will repeat this, but with some isolates, even 1% didn't work all that great.

Conclusion: it is fairly clear that "reduced sensitivity" exists and that this is fairly common in Virginia now, but it is hard to quantify because we get so much variability; one test may yield a high number (poor disease control), but the next test may give pretty good control. Using higher rates would probably improve control, but may also increase the chance of some leaf damage.

Botrytis bioassays

Only a very limited number of Botrytis samples was collected or submitted in 2024, presumably due to the generally dry growing season. Among the Groups-7 fungicides:

- boscalid (Endura) resistance was widespread as in the past
- benzovindiflupyr (Aprovia), isofetamid (Kenja), and fluopyram (Luna) resistance were occasionally present
- And the only group-7 fungicide to which resistance has NOT yet been found in Virginia continued to be pydiflumetofen, which is one component of Miravis Prime where it is mixed with fludioxonil, another compound to which resistance has only rarely been detected in Virginia.

Black rot

No samples of the black rot pathogen have been tested, since few were collected and the ones collected were obtained late in the season when the diseased tissue becomes overgrown by faster-growing fungi. Spores are still produced, but do not germinate on our traditional agar media. However, a technique was developed to obtain the black rot pathogen in culture by incubating field-collected diseased material wet for 24 hours to stimulate spore production, and then placing it on young leaves of greenhouse-maintained grape plants and keeping it wet for another 24 hours to allow infection. After about 12 days, fresh black-rot leaf spots tend to develop, and the fungus can be isolated from those with less interference from contaminants. Several isolates are being maintained in pure culture to serve as controls when bioassays from vineyard-collected samples become possible.

4.0 COMMUNICATION WITH STAKEHOLDERS

4.1 PROPOSAL (February)

Summarize how you will share project information or results. For example, will you submit for publication in a peer reviewed journal? Present at a technical conference? Conduct a training? Post on a site? Identify the specific audience/s you will inform. (1-5 sentences or bullets)

Results for individual vineyards will be shared with vineyard managers as they become available. Publications will be prepared when novel results are obtained. Results will be incorporated in Extension pest management recommendations.

4.2 Mid-Year Report (December)—Research and Education Grants Only

I have already communicated with Dr. Mizuho Nita about the boscalid resistance and new information about the mutations, and will communicate with growers and managers of

vineyards where samples originated as soon as the more complete molecular mutation analysis is received this winter.
4.3 Final Report (June)
Describe how the technical or material content of the project was or is planned to be shared with stakeholders or beneficiaries. List title, date, type (article, brochure, presentation, or other), purpose, and estimated audience reached. Provide a copy or link if (when) available for inclusion on the virginiawine.org site. Additional information was shared with Dr. Mizuho Nita for use in his Extension updates, and a brief report is being drafted. Information has been communicated via email to several growers and managers of vineyards where samples were obtained, or consultants who provided samples.
5.0 BUDGET

Budget Summary			**Mid-Year** Research/Education only		**Final**	
Expense Category	**5.1 Requested**	**5.2 Awarded**	**5.3 Spent**	**5.4 Remaining**	**5.5 Spent**	**5.6 Remaining**
Personnel	7,040	7,040	8,248.65	-1,208.65	10,098.93	
Fringe Benefits	460	460	0	460.00		
Travel	500	500	182.00	318.00	182.00	
Equipment (Rental)						
Supplies	800	800	84.90	715.10	84.90	
Contractual	1,800	1,800	544.35	1,255.65	834.17	
Other	600	600	0	600.00		
Total	11,200	11,200	9,059.90	2,140.10	11,200.00	0.00

I. Title: **Maintain fungicide resistance testing capabilities for grape diseases-FY25.**

II. Project Lead: **Anton Baudoin**, Associate Professor Emeritus

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Virginia Tech, Blacksburg, VA 24061

Telephone: 540-231-5757 (office). 540-808-6345 (cell)

III. Organization: Virginia Tech, School of Plant and Environmental Sciences

IV. Amount Requested: $**11,200**

V. Type of Project (Select all that apply):
   (X) Research
   ( ) Education
   ( ) Marketing

VI. Does this project build on or continue prior year funding by the Board? _X_Yes ___No

It is a follow-up of projects funded in recent years on fungicide resistance of grape pathogens, but not previously proposed as a multi-year project.

NARRATIVE

Objectives

1. Testing of grape pathogens for **fungicide resistance** to determine distribution of known resistance types, and as new issues may arise.
2. Further investigation of variability of **phosphite sensitivity** among grape downy mildew isolates

I. Problem Statement

The Baudoin lab has addressed fungicide resistance issues of grape pathogens in Virginia for over 20 years. Discoveries have included the first-in-the-USA detection of widespread resistance in grape powdery and downy mildew to strobilurin fungicides (Abound, Flint, Pristine, etc. since around 2005, still extremely common), widespread as well as limited resistance to several anti-Botrytis fungicides over the years, quinoxyfen resistance of grape powdery mildew which has turned out to be limited in geographic spread and control impact, and, since 2016, first-in-the-USA detection of Revus/Forum resistance in grape downy mildew with documented control failures when present at high frequencies. Our first-in-the-USA detections are *not* due to Virginia having an earlier problem with fungicide resistance (similar problems are found in other states once people start testing), but just due to earlier looking for them.

Revus (and Forum, FRAC group 40) fungicide resistance in grape downy mildew has been identified in almost all regions of Virginia since 2016. More than 15 geographically dispersed locations were detected in previous years. Since these collections were often NOT based on complaints about control failure, this indicates that this type of resistance is geographically widespread, although sometimes at low local frequencies. At one vineyard where all isolates collected in 2016 had this Group-40 resistance, it was not detected in a few isolates collected in 2021. Occasional use of this group of fungicides may still be effective in such situations.

Experiments with control of downy mildew control by Forum and Revus on potted plants under field conditions in 2018-2022 confirmed poor or no control of resistant isolates. If, as appears to be common, only a portion of the downy mildew population has the resistance gene, the first one or perhaps two applications of a season may still provide reasonable or some disease control (as was seen in these experiments), but additional applications would be expected to have little or no effect. Forum appears to be not as commonly used in Virginia as Revus, and it appears less affected by the resistance.

Grower concerns about downy mildew in recent years have often centered on suspicions that phosphites may not provide the expected level of control anymore. We explored this question in 2014-2016 and, at the time, did not find distinct differences in sensitivity. One limitation of this fungicide group is that, at times of high diseases pressure, phosphites may need to be applied as frequently as every 7 days and some vineyard managers may not realize this or the weather may prevent timely application. However, some of the downy mildew isolates collected in 2021 showed surprisingly strong growth on phosphite-treated leaf tissue, although sometimes inconsistently so from experiment to experiment, and warrant additional testing.

Since the last extensive Virginia wine grape collection effort for Botrytis occurred in 2015 and new chemistries have come into use in recent years, this aspect will be emphasized in the upcoming season if conditions are favorable for Botrytis development. Kenja and Luna are examples that have a mode of action similar to that of boscalid (Endura and Pristine) but a very different resistance pattern. In 2022-23, we identified Botrytis resistance to Luna, Aprovia, and Kenja as well as the fluxapyroxad component of Merivon (the other component had already failed) in two Virginia vineyards (about 30 miles distant from each other) with unexpected Botrytis bunch rot outbreaks.

For the management of black rot, besides the multi-site fungicides such as mancozeb, two groups of single-site fungicides (with inherent higher resistance risk) have been very effective: the sterol inhibitors (FRAC group 3) and the strobilurins (group 11). However, both have been in common use now for several decades, and concern has grown recently that Group 3 applications have not been as effective as expected in some locations.

II. Impact on Industry

Fungicide resistance has two serious economic consequences: continued application of ineffective fungicides causing unnecessary expense and environmental impact, and, even more damaging, unexpected fungicide failures leading to disease outbreaks. Some samples are submitted by growers, consultants, or extension personnel with suspicions of fungicide resistance (sometimes borne out by tests, but also sometimes *not* confirmed) but, much more commonly, resistant samples are obtained from vineyards where growers were not aware of its presence, or samples may have been submitted to be tested against a completely different fungicide.

III. Procedures / Project Plan

Fungicide resistance. Besides continued survey for Revus/Forum resistance, additional fungicide modes of action that require vigilance are Vivando (resistance documented in Italy), Quintec (resistance in one Virginia location as well as in Europe), Torino, and Endura, Pristine, Luna, Aprovia, and Miravis (which have related modes of action) for powdery mildew, and Ridomil and Zampro (in addition to phosphites, below) for downy mildew as well as several anti-Botrytis fungicides.

Several statewide collection trips will be conducted in the summer and fall of 2024, timing and locations depending on weather patterns; more fall time for this will be available than before since Anton Baudoin has now retired from teaching duties (but has been promised continued access to office, lab, and greenhouse space). Sample submission by growers with concerns will also be encouraged. How frequently specific fungicides or groups have been applied in recent seasons would be extremely helpful information in deciding what to test for, but this information is often not made available.

Phosphite sensitivity. A concern about the efficacy of phosphite fungicides has reemerged in recent years. We routinely use 0.3% Prophyt as a test concentration in laboratory leaf bioassays (0.5% is the maximum application concentration permitted by the label), and historical isolates have shown only slight or no growth on grape leaf tissue treated with this rate. However, many 2021 isolates have shown fairly good, growth on such treated leaf tissue. We obtained fresh fungicide sample to rule out deteriorated product, and started including in tests a 2006 isolate that had been stored frozen, but the pattern persisted while the 2006 isolate was well-controlled. Downy mildew isolates with suspiciously strong growth will be subject to increased lab testing with different concentrations and phosphite samples as greenhouse-grown leaf tissue becomes available again in February and March. Also included will be downy mildew samples collected from a 2022 potted-plant trial of phosphite application frequencies, separately from non-sprayed plants early in the season and from plants which had received weekly phosphite applications. In addition, downy mildew isolates with suspiciously strong growth on treated leaf pieces will be subject to whole-plant testing with different concentrations and phosphite samples.

The potted-plant trial under field conditions will be repeated in 2024, with focus on phosphite concentration and application frequency as it relates to disease pressure which will be monitored using an on-site weather station. Isolates collected from non-treated control plants early in the season will be compared with those collected late in the season from the most intensively phosphite-treated plants to look for potential sensitivity differences (repeat of 2022 experiment, which was initiated but turned out unsuccessful in 2023)

IV. Technology Transfer

As samples are tested, growers are notified as to the results. Results are also disseminated via extension and industry meetings (for example, by Dr. Mizuho Nita)

V. Outcomes and Benefits Expected: Economic, environmental, etc.

See also Impacts on Industry section. Undetected fungicide resistance leads to continued application of ineffective fungicides causing unnecessary expense and environmental impact, and, even more damaging, unexpected fungicide failures leading to damaging disease outbreaks. On the other hand, repeated failure to detect fungicide resistance to a specific material increases confidence that those treatments will be effective. Growers who follow recommended practices of mixing and rotating different fungicide modes of action often have difficulty determining which are still effective and which are not.

BUDGET

Category	Requested $
Personnel - Hourly wage (undergraduate students)	7,040
Fringe Benefits on student summer wage	460
Travel	500
Equipment (Rental)	--
Supplies	800
Contractual (greenhouse user fees, equipment maintenance and repair)	1,800
Other (publication charges, conference registration, etc.)	600
Total	**11,200**

Budget Narrative

Personnel:

Hourly wage, undergraduate student(s), ~24 hours per week during summer (~11 weeks) and ~8 hours per week during the academic year, averaging ~$15 per hour.

Fringe Benefits:

Summer wage: fringe benefits 6.34% until 7/1/2024; 6.65% thereafter

Contractual Services:

Greenhouse space use charge ($0.01 per day per square foot) approx. $1,500 per year, plus $300 for equipment maintenance and repair.

Travel:

In-state sample collecting trips, state vehicle, occasional overnight lodging.

Attending occasional domestic meetings (regional plant pathologists, grape and wine industry meetings)

Materials and Supplies:

Greenhouse supplies (pots, potting mix, fertilizer, light bulbs, pesticides), laboratory supplies (glass and plasticware, culture media, reagents, microscope slides)

Equipment: - none planned

Other:

Publication costs for scientific publications