

TITLE: Survey of grape powdery and downy mildew sensitivity to commonly used fungicides

DATE OF INITIATION: July 1, 2006 (July 1, 2007 for current budget request)

DURATION: 2 years, through June 2008

OBJECTIVES:

1. Evaluate Virginia grape powdery (PM) and downy mildew (DM) populations for resistance to fungicides, with emphasis on the ergosterol biosynthesis inhibiting fungicides (PM) and the QoI fungicides or strobilurins (PM and DM).
2. Develop a test for routine assay of fungicide resistance.

JUSTIFICATION/PRACTICAL IMPORTANCE

Powdery mildew [PM, *Erysiphe (Uncinula) necator*] and downy mildew (DM, *Plasmopara viticola*) are destructive diseases of vinifera and hybrid grapes in the eastern USA. In a survey of Virginia wine industry research and education issues, “identifying/controlling diseases,” “selecting appropriate pesticides/mixing agents,” and “understanding, and reducing effects of PM on fruit/wine quality” were high priorities. Since vinifera and many hybrid grape varieties are highly susceptible to PM and DM, control of these diseases is primarily accomplished through a program of fungicide applications.

Ergosterol biosynthesis inhibitors (**EBI**, common and trade names are shown in Appendix 2) are currently very important for control of PM and black rot, and **QoI** compounds, also known as strobilurins, are very important for both PM and DM, as well as black rot.

Despite the intensive use of fungicides in vinifera and susceptible hybrid grapes, unexpected large crop losses can, and have, occurred in Virginia and other eastern states due to the speed with which these pathogens can develop to epidemic proportions. Possible reasons include poor spray coverage due to insufficient spray volume and/or excessive canopy, excessively long spray intervals, inappropriate fungicide selection, or pathogen resistance to the fungicide chosen. Once significant levels of PM or DM infection have developed, it becomes very difficult to bring the epidemic back under control.

Awareness of actual prevalence of fungicide-resistant strains of powdery or downy mildew will inform fungicide selection by growers, and hence avoid application of ineffective chemicals, cutting production costs, and avoiding crop losses from control failures. Alternatives, although not without drawbacks, are available, including Endura and Quintec (although they are subject to resistance risks as well), a heavy reliance on sulfur, or a judicious program with horticultural oils for PM. In the eastern United States, it is now recommended that growers tank-mix QoI fungicides with sulfur in order to avoid catastrophic losses

if resistance is present (Weigle & Muza 2005, Wilcox 2005), but this makes it difficult to discern erosion of effectiveness. With fungicide prices (Virginia, 2005) such as \$21-29 per acre for Abound, and \$19-38 per acre for Sovran, it is desirable that efficacy is known rather than just a guess. In areas with DMI or QoI resistance, these fungicides may still be used against other diseases, but higher rates (EBI) or alternative materials can be chosen to suppress PM or DM. QoI and EBI fungicides can probably still provide benefits in areas where resistant strains are present, if application can be guided by information on the frequency of resistance (e.g., Genet et al., 2006)

BACKGROUND

Both powdery and downy mildews tend to develop resistance to fungicides with single-site modes of action rather easily. Benzimidazole resistance of grape PM developed in the 1970s in New York and elsewhere, but as late as the early 1990s was present in California only at very low frequency. Mefenoxam (e.g., Ridomil[®]) resistance in DM has been found in Europe but, presumably due to limited use, not yet in the USA. EBI resistance was documented in the 1980s and 1990s in California and New York and was responsible for poor PM control in some vineyards. QoI compounds were introduced in 1997, and practical resistance developed fairly quickly in some pathogens, such as cucurbit and cereal powdery mildew, and grape downy mildew in Europe. Wilcox et al. (2003) reported QoI resistance in New York PM in 2002 and 2003, with complete control failures in some cases.

The sensitivity status of grape powdery and downy mildew to various fungicide groups in the mid-Atlantic states was poorly known, and no surveys had been conducted in Virginia before our current efforts, only occasional samples analyzed. In the absence of a body of data, the assumption has been that the situation will be similar to that in New York, since cultivars, disease pressure, and spray programs are somewhat comparable. However, in Europe, significant differences in prevalence of resistance exist for some pathogens between regions separated by similar distances (e.g., wheat powdery mildew, grape downy mildew, FRAC website), and in California, grape PM control by triadimefon failed in the cooler coastal areas well before it failed in warmer regions. Since resistance management has been emphasized in recent years, many conscientious growers rotate or tank-mix at-risk fungicides, which reduces the risk of control failure, but at the same time makes it more difficult to discern whether the performance of an at-risk fungicide has eroded.

Two new chemistries have entered the market of powdery mildew control in 2004, boscalid (Endura) and quinoxyfen (Quintec), and both are estimated to have a medium risk of resistance development (FRAC

2005), and should not be over-used. After several years of use in Europe, quinoxyfen resistance in cereal powdery mildew has become established in northern Germany, reducing the duration of mildew control in field situations (FRAC 2005). In order to reduce the pressure on these new compounds, it is important to retain older chemistries as options where they remain effective. Inclusion of boscalid and quinoxyfen in our assays will provide early warning if problems develop.

In 2005, over 20 isolates of both PM and DM isolates were obtained. We obtained QoI resistant isolates of both downy and powdery mildew from 4 out of 5 vineyards. This was the first report of this type of resistance for grape downy mildew in North America. In three of the four vineyards, the growers reported struggling with downy mildew control. We have clear evidence of substantial PM resistance to EBI fungicides at several locations as well. In 2006, we obtained an additional 118 downy mildew isolates from 19 locations, and 71 powdery mildew isolates from 17 locations; testing is underway. A graduate student has started working on this project in August 2006. Virginia growers have been informed by direct contact with those whose vineyards were sampled, by an article in *Viticulture Notes* (Baudoin 2006), and by presentations at the VVA annual meetings in February of 2006 and 2007.

PROCEDURES

We will continue to collect PM and DM isolates in various grape-growing areas of Virginia. The focus will be on vineyards with histories of severe PM or DM disease pressure and intensive use of at-risk fungicides. Since both pathogens are easily dispersed by wind, considerable inoculum exchange is expected among vineyards in close proximity, but a few locations with histories of little use of at-risk fungicides will be included in the sampling to gain an idea of local spread of resistant isolates. Within a vineyard, separate samples will be collected from a number of locations to ensure a cross-section of the pathogen population. Grower spray records from recent years will be requested in order to relate fungicide sensitivity to spray history.

Precautions will be taken to prevent cross-contamination of samples: leaf and cluster samples will be sealed in containers during transport to the laboratory, and opened in a laminar flow hood to transfer the pathogen to grape seedlings or leaf discs. In general, the methods described by Ypema et al. 1997, Erickson & Wilcox 1997, Wong & Wilcox 2000, and Miller & Gubler 2004 will be used. PM-free leaf material will be obtained from potted grape plants grown in a greenhouse at 100-105 °F or in transparent isolation tubes to minimize interference from preexisting PM infection (DM infection is not normally a problem in the greenhouse), and only young leaves will be used. Single-spore isolates will be tested for

complete characterization of fungicide resistance of PM and DM populations from selected vineyards or regions. Single spores or spore chains will be removed from field-collected material under a dissecting microscope and transferred to leaf disks in Petri dishes. They will be transferred to fresh leaf disks or to grape seedlings in isolation chambers every 2-3 weeks in order to maintain the isolate. We are still attempting to develop a reliable method of frozen storage (promising for DM, but not yet effective for PM).

Sensitivity assays will be conducted with leaf disks that have been soaked in a dilution series of the fungicide in question. The fungicides tested will include QoI (see Appendix 2) and EBI compounds, thiophanate methyl, boscalid, and quinoxyfen for PM and QoI compounds and mefenoxam for DM. Sensitivity data from the aforementioned studies will be used to guide the selection of dosages. Choices will have to be made in order to keep the amount of labor and space required manageable, but PM cross-resistance to EBI fungicides is not uniform (Erickson & Wilcox 1997) so inclusion of several compounds from a group is indicated, with 3-6 dosages for each compound. After soaking in fungicide, the surface of the leaf disks will be allowed to dry, and disks will then be placed in Petri dishes and inoculated with either PM or DM. The dishes will be incubated under lights for 7-10 days until evaluation. Growth of the pathogen will be evaluated under a dissecting microscope, and both incidence (presence or absence of growth) and severity (percent of leaf disk covered) will be recorded. The fungicide concentration giving 50% growth inhibition will be calculated.

Bioassays will be used because quantitative resistance is expected for EBI fungicides, perhaps also for QoI compounds, and no information on resistance mutations to quinoxyfen and boscalid is available for grape pathogens. However, we will also attempt to identify specific mutations in common resistant strains, and use or develop real-time PCR (polymerase chain reaction) detection techniques following protocols such as those described by Sirven and Beffa (2003) to be able to more quickly test larger numbers of isolates. The Virginia Tech PPWS department has PCR equipment that can be used for this purpose and we are happy that Dr. David Schmale has agreed to collaborate on these aspects. Two specific mutations for QoI resistance have been described, and PCR provides a much quicker test of the presence of these mutations than does bioassay.

Determining population sensitivity through testing of single-spore isolates is time-consuming, so data will be supplemented with seedling bioassays using discriminatory fungicide dose levels as described by Wong and Wilcox (2002) to determine degree of control of bulk populations from different vineyards. The third objective was added to attempt to develop a simple bioassay for fungicide resistance that upon

the conclusion of this project could be maintained for routine use or taken over by a private consultant. A steady supply of mildew-free grape seedlings or tissue-cultured (cloned) plantlets will be produced. Upon demand, plantlets will be treated with discriminatory doses of fungicides in question and either shipped to growers for exposure in the field (PM), or growers will be asked to ship diseased leaves for assay. Inoculum can be applied to plants by gently rubbing onto treated leaves as well as appropriate control leaves, and detailed instructions for procedures and evaluation will be developed.

PERSONNEL AND FACILITIES

Principal investigator:

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Laboratory and greenhouse research will be conducted in facilities of the Dept. of Plant Pathology, Physiology and Weed Science, Virginia Tech. Much of the day-to-day conduct of the survey and assays will be performed by Ms. Jenevlyn Colcol, a MS graduate student who started working on this project in August of 2006, and is supervised primarily by A. Baudoin. Dr. T. K. Wolf will provide advice on locations of disease outbreaks, and will assist in contacts with growers and field collection of samples.

BUDGET (revised June 13, 2007)

Item	Requested from Virginia Wine Board Jul 2007-Jun 2008
Graduate student stipend, step 11, one semester plus part of summer	11,497
Graduate student fringe benefits, 11%	1,215
Graduate student tuition & academic fees (1 semester)	3,856
Partial summer salary PI	4,798
Wage support	1,600
Fringe benefits, salary and wage, 8.5%	544
Supplies and materials	2,000
Travel	990
Contractual Services (Statistical advice and molecular sequencing)	1,000
Total requested	27,500

- This is a 2-year project from July 1, 2006 until June 30, 2008. **SOURCE OF OTHER FUNDS (revised June 13, 2007):** Virginia Agriculture Council provided \$4000 for 2007-08. Viticulture Consortium: East has been discontinued, but funds remaining from 2006-07 will be carried over into 2007-08.
- Graduate student stipend: A major portion of the budget consists of a Master of Science graduate stipend Step 11 plus associated tuition and academic fees. Student has primary responsibility for fungus isolate collection and all aspects of bioassay and PCR, which are very time-consuming.
- Hourly wage assistance, for routine, labor-intensive procedures.
- Supplies and materials: greenhouse supplies, plasticware, PCR primers and probes, chemicals, and general laboratory supplies.
- Travel: for collection of PM isolates and for field work, approximately \$50 per day (state car \$30 per day, plus fuel) plus meals and lodging on overnight trips, one 3-day trip will cost about \$300.
- Statistical advice: faculty consulting rate is \$84/hour, graduate student rate is \$40/hour. Sequencing of PCR products at VBI or other vendor, about \$8 per base

Appendix 1

Literature cited

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- Erickson, E. O., and Wilcox, W. F. 1997. Distributions of sensitivities to three sterol demethylation inhibitor fungicides among populations of *Uncinula necator* sensitive and resistant to triadimefon. *Phytopathology* 87: 784-791.
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- Wilcox, Wayne, 2005. Grape disease control, 2005. <http://flg.cce.cornell.edu/Grape%20Disease%20Control%202005.pdf>. Accessed January 20, 2006.
- Wilcox, W. F., J. A. Burr, D. G. Riegel, and F. P. Wong, 2003. Practical resistance to QoI fungicides in New York populations of *Uncinula necator* associated with quantitative shifts in pathogen sensitivities. (Abstr.) *Phytopathology* 93: S90.
- Wong, F. P., and W. F. Wilcox, 2000. Distribution of baseline sensitivities to azoxystrobin among isolates of *Plasmopara viticola*. *Plant Disease* 84:275-281.
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- Ypema, H. L., Ypema, M., and Gubler W. D. 1997. Sensitivity of *Uncinula necator* to benomyl, triadimefon, myclobutanil, and fenarimol in California. *Plant Disease* 81: 293-297.

Appendix 2. Fungicides Available for Use against Selected Grape Diseases

Common Name	Trade Name(s)	Resistance risk ¹	Black rot ²	Botrytis bunch rot ²	Powdery mildew ²	Downy mildew ²
EBI compounds						
Fenarimol	Rubigan	M	+		+	
Triadimefon	Bayleton	M	+		+	
Myclobutanil	Nova	M	+		+	
Triflumizole	Procure	M	+		+	
Tebuconazole	Elite	M	+		+	
Fenhexamid	Elevate	L-M		+		
QoI compounds						
Azoxystrobin	Abound	H	+	+	+	+
Kresoxim methyl	Sovran	H	+	+	+	+
Trifloxystrobin	Flint	H	+	+	+	+/-
Pyraclostrobin	Component of Pristine	H	+	+	+	+
Other						
Boscalid	Endura (also a component of Pristine)	M		+	+	
Quinoxifen	Quintec	M			+	
Thiophanate methyl (benzimidazole)	Topsin M	H		+	+	
Iprodione	Rovral	M-H		+		
Cyprodinil	Vangard	M		+		
Pyrimethanil	Scala	M		+		
Metalaxyl or mefenoxam	Ridomil Gold MZ (EBDC), Ridomil Gold Copper	H	(+)	(+)	(+)	+
Fosetyl Al	Aliette	L?				+
Phosphonate	Prophyte, Phostrol, Agri-Fos	L?				+
Bordeaux mix		L	+	+	+	+
Copper, fixed	Various	L	+	+	+	+
Captan	Captan, Captec	L	+	+		+
Mancozeb (EBDC)	Dithane DF, Penncozeb, etc	L	+			+
Ferbam	Ferbam	L	+			+
Ziram	Ziram 76, Ziram Granuflo	L	+			+
Sulfur	Microthiol, etc.	L			+	
Oil	Sun Ultra-Fine Oil JMS Stylet-Oil, etc.	L		+	+	
K bicarbonate	Armcarb 100	L			+	
Mono K phosphate	Nutrol	L			+	

Footnotes

1. RR=Resistance risk as estimated by FRAC, that is, risk that fungicide-resistant strains of various pathogens will develop if material is overused. H = documented or expected high risk; M = moderate risk; L = low risk.
2. + = labeled and/or usually moderately or highly effective. (+) = effective only due to mixing partner.

Appendix 3. Biographical Sketch: Anton Baudoin

EDUCATION

Ph.D. Plant Pathology, 1980, University of California, Riverside. Major: Plant Pathology.
Ingenieur (M.Sc.) Agriculture, 1975, Wageningen University, The Netherlands.

PROFESSIONAL EXPERIENCE

1976-1980: Research assistant, Dept. of Plant Pathology, University of California, Riverside.
1980-1981: Postdoctoral plant pathologist, Air Pollution Research Center, UC Riverside.
1981-present: Assistant and associate professor, Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University.

CURRENT TEACHING RESPONSIBILITIES

Pest Control (sections on plant diseases and weeds) for students in 2-year program
Plant Pathology, introductory course for juniors
Principles of Plant Disease Management, a graduate course
Diseases of Crop Plants, a graduate course
Epidemiology of Plant Diseases, an advanced graduate course

RESEARCH AREAS (last 5 years)

Control of fungal diseases of grape (black rot, Botrytis, Phomopsis, downy mildew, powdery mildew)

SELECTED PUBLICATIONS

Grape related

- Pfeiffer, D.G., A. Baudoin, C. Bergh, Updated annually. Diseases and Insects in Vineyards. In: Pest Management Guide, Horticultural & Forest Crops. Virginia Coop. Extension Service.
- Wolf, T. K., Baudoin, A. B. A. M., and Martinez-Ochoa, N. 1997. Effect of floral debris removal from fruit clusters on Botrytis bunch rot of Chardonnay grapes. *Vitis* 36: 27-33.
- Baudoin, A. 1998. Vangard, a new fungicide against Botrytis. *Viticulture Notes* 13 (3): 5-6.
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