Title of Project: How different is the epidemiology of grape downy mildew in the Deep South compared with what we think we know from temperate and Mediterranean regions?

Final Report, Research Proposal

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Objective:
Elucidate the cause(s) of delayed onset of grape downy mildew observed in Georgia vineyards to build predictive capability for improved disease management.

Justification:
Downy mildew, caused by the oomycete pathogen *Plasmopara viticola*, is one of the most important grape diseases worldwide and can cause significant yield and quality losses where it occurs. The disease is prevalent and severe in the southern grapevine growing regions. Producers apply regular fungicide treatments to manage downy mildew, generally starting at bud-break or new shoot development in the spring (Nita 2014); however, there is limited information about the epidemiology of the disease in hot and humid environments that could aid in better timing of these applications. This includes knowledge gaps regarding the mode of overwintering of the pathogen, the source and timing of initial inoculum, the roles of sexual vs. asexual inoculum in early and later stages of disease progression, and the characterization of periods during the growing season and after grape harvest that are favorable or not favorable for disease development. It is the overall aim of this project to provide a foundation for more rational management of grape downy mildew based on a better understanding of the disease cycle under the specific environmental conditions of the Southeast through a combination of field epidemiology, computer simulation, and population genetics.

Downy mildew arguably is one of the best-studied diseases of grape, and there is a wealth of published information on its epidemiology and management (Gessler et al. 2011). However, virtually all of this information has been derived in temperate and Mediterranean climates in the US and Europe. The Southeast provides an extreme environment for growing wine grapes, and may provide an extreme environment for *P. viticola* as well, necessitating adaptations in its life cycle. In 2015, detailed disease monitoring in an unsprayed experimental vineyard (cvs. Merlot and Chardonnay) at the Georgia Mountain Research and Education Center (GMREC) in Blairsville showed that the disease appeared much later than expected based on environmental conditions known to be favorable for the disease: the earliest symptoms were observed on 15 June, whereas the comprehensively validated UCSC downy mildew simulation model (Caffi et al. 2009, 2011; Rossi et al. 2009) predicted the first appearance of symptoms on 22 April based on the environmental conditions at the site. Additional research over multiple years is needed to confirm the phenomenon of significantly delayed onset of grape downy mildew in Georgia vineyards and to elucidate its potential cause(s). This will be critical for building predictive capability for improved disease management.

In this project we continued detailed monitoring of downy mildew development on both Merlot and Chardonnay at the GMREC experimental vineyard, and compared the timing of disease onset with that

predicted by the UCSC downy mildew model. In addition, we conducted field and laboratory experiments to explore three potential mechanisms that could result in delayed or variable onset of downy mildew epidemics in the southeastern environment:

- low levels of primary inoculum due to unfavorable autumn conditions for oospore formation;
- delayed oospore germination in the spring in the southeastern vineyard environment;
- presence of cryptic species (clades) of *P. viticola* with different environmental optima.

Methodology:

Epidemic progress and model predictions

We utilized the UCSC model (Caffi et al. 2009, 2011; Rossi et al. 2009) to simulate development of downy mildew at the GMREC in Blairsville during the 2016 growing season. Specifically, the timing of oospore germination, release of zoospores, infection, and appearance of the first visible symptoms were simulated using inputs of hourly weather data collected locally. The model output of the time of the appearance of the first downy mildew symptoms was compared with the time of disease onset in field surveys at the GMREC in an experimental vineyard planted to Merlot and Chardonnay. Vines in two treatments were evaluated, unsprayed (no fungicide) and "allow downy mildew" (with fungicide application against other diseases but avoiding active ingredients effective against *P. viticola*). Disease monitoring commenced on 30 April and ended on 10 August 2016.

Oospore formation in the autumn

Merlot and Chardonnay leaves with downy mildew symptoms were collected in the GMREC vineyard on 8 September and 6 October 2016, stored at 4^{o}C, and checked microscopically for the presence of oospores as described below. Also during the autumn we exposed two sets of containerized 5-gal trap plants of cv. Chardonnay to natural *P. viticola* inoculum in the GMREC vineyard to quantify the formation of oospores during the late season. The first set of 10 plants was exposed on 11 August and retrieved on 8 September. The second set of 5 plants was exposed on 8 September and retrieved on 6 October. Leaves with downy mildew symptoms were cleared, stained with acid fuchsin, and assessed microscopically (200x) for oospores.

Oospore germination in the spring

Merlot and Chardonnay leaves with downy mildew symptoms were collected in the GMREC vineyard in mid-October 2015. Following drying in paper bags at ambient temperature, leaves were enclosed in nylon mesh in a plastic pot and deployed on the vineyard floor at Blairsville on 4 December 2015 for subsequent sampling during the spring to determine the timing of oospore germination. There was 25 g of leaf litter per pot and 20 pots in the experiment. Two pots with leaf litter (10 g/pot subsamples) were retrieved to the lab at biweekly intervals between 13 April and 10 August 2016. To determine oospore germination, we used the method described by Rossi et al. (2008). Retrieved leaf litter samples were covered with cheese cloth, placed in a glass dish, and flooded with 50 ml distilled water. Fifteen Chardonnay leaf disks (12 mm diameter) were floated on top of each leaf litter suspension and incubated at 20^{o}C. After 24 h, the leaf disks were removed and replaced with 15 new leaf disks. Removed leaf disks were placed in a moist chamber with the abaxial surface facing up, incubated at 20^{o}C and observed for sporulation of *P. viticola* for 14 days.

Presence of different cryptic species of *P. viticola*

During 2014-2015 we already collected a total of 162 single-lesion or single-sporangium isolates of *P. viticola* in northern and western Georgia. Among those, 109 isolates were genotyped to cryptic species level using the cleaved amplified polymorphic sequence (CAPS) method described by Rouxel et al. (2014). Based on the results of CAPS analysis, all of these isolates were identified as *P. viticola* clade *aestivalis*. Further confirmation of CAPS result was obtained by sequencing the ITS1 region of 45 isolates. In 2016, we continued to collect *P. viticola* isolates from northern, western, and southern Georgia (Table 1). These isolates were subject to ITS PCR and CAPS analysis to identify cryptic species status.

Table 1. Single-lesion isolates of *Plasmopara viticola* from which DNA was extracted in 2016 for determination of cryptic species and population structure analyses.

County	Host cultivar	Number of isolates
Union (N)	Chardonnay	161
	Merlot	238
Haralson (W)	Blanc du Bois	34
	Lenoir	3
	Lomanto	5
Colquitt (S)	Blanc du Bois	47
	Lenoir	1
Mitchell (S)	Blanc du Bois	4
Total		493

N = northern GA, W = western GA, S = southern GA.

Results:

Epidemic progress and model predictions

Downy mildew epidemics started earlier in 2016 than in 2015. The first symptoms were observed on 11 May and 24 May 2016 on cultivars Chardonnay and Merlot, respectively; these dates are 34 to 44 days earlier than in 2015. Infection of berries, an unusual occurrence in north Georgia vineyards, was first observed on 30 May 2016 (Fig. 1), most likely associated with the early onset of the epidemic before berries reached a stage of ontogenetic resistance. Disease incidence on leaves approached 100% by early August, about 1.5 months earlier than in 2015 (Fig. 2). For model simulation, the UCSC model predicted disease onset on 7 May, very close to the observed disease onset of 11 May (Fig. 3).

Fig. 1. Downy mildew-infected Chardonnay berries at the GMREC, Blairsville, June 2016.

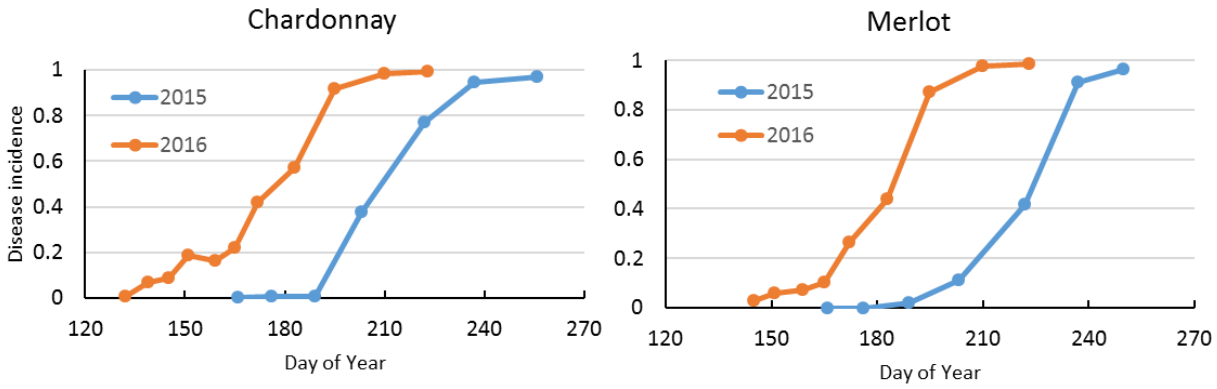

Fig. 2. Comparative epidemic development of downy mildew on Chardonnay and Merlot at the GMREC, Blairsville, in 2015 and 2016.

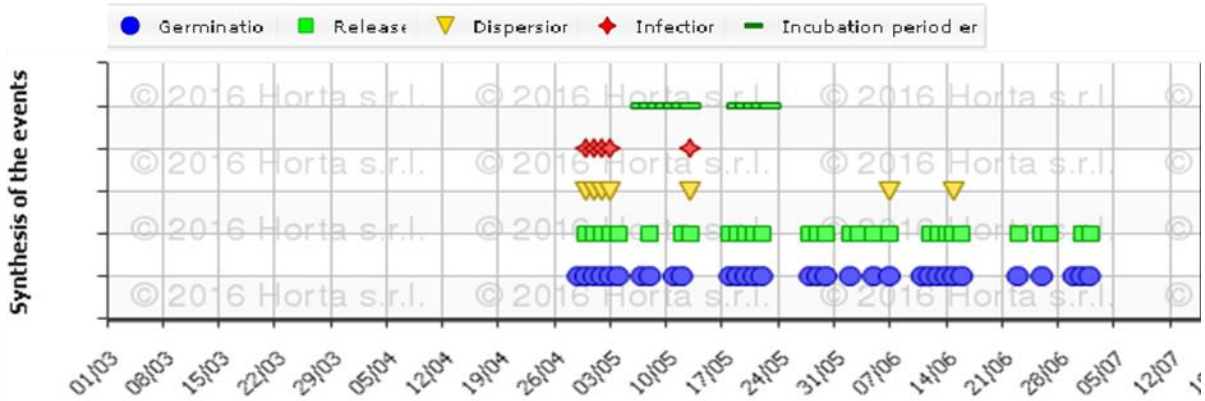

Fig. 3. UCSC model prediction of downy mildew primary infection using hourly weather data from the GMREC, Blairsville, during the 2016 growing season. The model predicted disease onset on 7 May, whereas the first leaf symptoms in the field were observed on 11 May (Fig. 2). Blue circles: germination of oospore cohorts; green squares: release of zoospores; yellow triangles: dispersion of zoospores; red diamonds: infection; green bars: appearance of symptoms. Note: dates indicated in European notation, i.e., 01/03 = 1 March.

Oospore formation in the autumn

Microscopic examination for oospores was conducted during the autumn of 2016 from naturally infected leaves and from leaves of trap plants where the approximate age of the infections was known based on their exposure windows. Although preliminary sampling in 2015 showed oospores to be present at the GMREC (albeit in low numbers), no oospores were found in the 2016 leaf samples, neither the naturally infected field samples nor the trap plant samples.

Oospore germination in the spring

Leaf samples collected at the GMREC in 2015 and used in the spring 2016 germination experiment contained oospores based on preliminary microscopic examination. In the germination experiments, a total of 3,930 leaf disks were used in an attempt to detect oospore germination on 10 sampling dates between 13 April and 10 August 2016 (Fig. 4). However, no infections (indicative of successful oospore germination) were detected in these assays from any of the sampling dates.

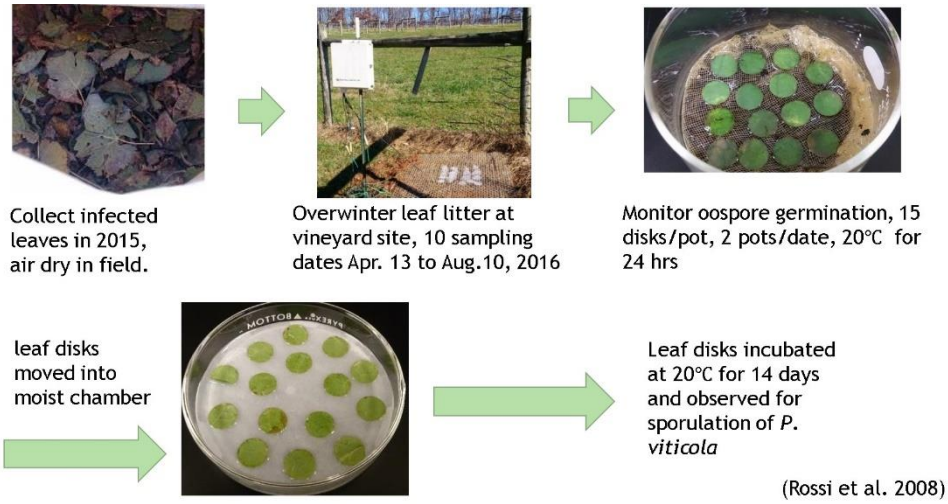

Fig. 4. Floating leaf disk assay to test for oospore germination in leaf litter during spring 2016 at the GMREC, Blairsville.

Presence of different cryptic species of *P. viticola*

A total of 109 isolates collected from 10 vineyards in 7 counties covering 17 cultivars of *V. vinifera* and interspecific hybrids in 2014-2015 were analyzed for cryptic species in 2016. The resulting phylogenetic tree showed that all isolates from western and northern Georgia clustered with *P. viticola* clade *aestivalis* (Fig. 5A), a cryptic species that is common in grape production regions in the northern and mid-Atlantic US (Rouxel et al. 2014).

By the end of 2016, DNA from a total of 493 isolates had been extracted (Table 1). Based on the results obtained so far, we only found *P. viticola* clade *aestivalis* in northern and western Georgia. However, in a sample of 50 isolates collected in southern Georgia in 2016, 43 isolates (86.0%) showed the same pattern as a reference isolate for another cryptic species, *P. viticola* clade *vinifera* (Fig. 5C and D). Some of these isolates were recently submitted for sequencing and further analyses are ongoing.

Conclusions:

The time of downy mildew onset in the field was considerably earlier in 2016 than in 2015, and we even observed early-season berry infections in 2016 (an unusual occurrence since disease at the GMREC typically seems to start after berries have become ontogenetically resistant). The UCSC downy mildew model predicted the timing of epidemic onset in 2016 much more accurately than in 2015.

Targeted research was conducted to better understand the reason(s) for the variability in disease onset and model performance. We attempted to monitor oospore germination using the method described by Rossi et al. (2008), but no oospore germination was observed during 10 sampling dates. We hypothesize that oospore production is limited in the hot environment in Georgia, and this seems to be supported by our observations on infected leaves collected in the autumn and observed for presence of oospores. Although we previously demonstrated that both mating types of *P. viticola* are present in northern Georgia, we have limited knowledge about the mating behavior of this pathogen and a limited ability to detect mating types efficiently (i.e., molecular markers for mating types have not been found for any downy mildew species). Further investigation is needed to elucidate the factors affecting the formation of oospores and why we cannot detect them in downy mildew-infected leaves.

With regard to cryptic species of the pathogen, a total of 195 isolates collected in 2014-2016 were analyzed using CAPS analysis. Among these, 152 isolates (77.9%) were *P. viticola* clade *aestivalis* whereas 43 isolates (22.1%), all from southern Georgia, appear to be *P. viticola* clade *vinifera*. Although ITS sequencing is still ongoing, this result not only reveals mixed populations of cryptic species in southern Georgia vineyards, but also suggests that further genotyping and phenotyping is needed to elucidate the genetic diversity of the pathogen and whether different cryptic species have different environmental preferences.

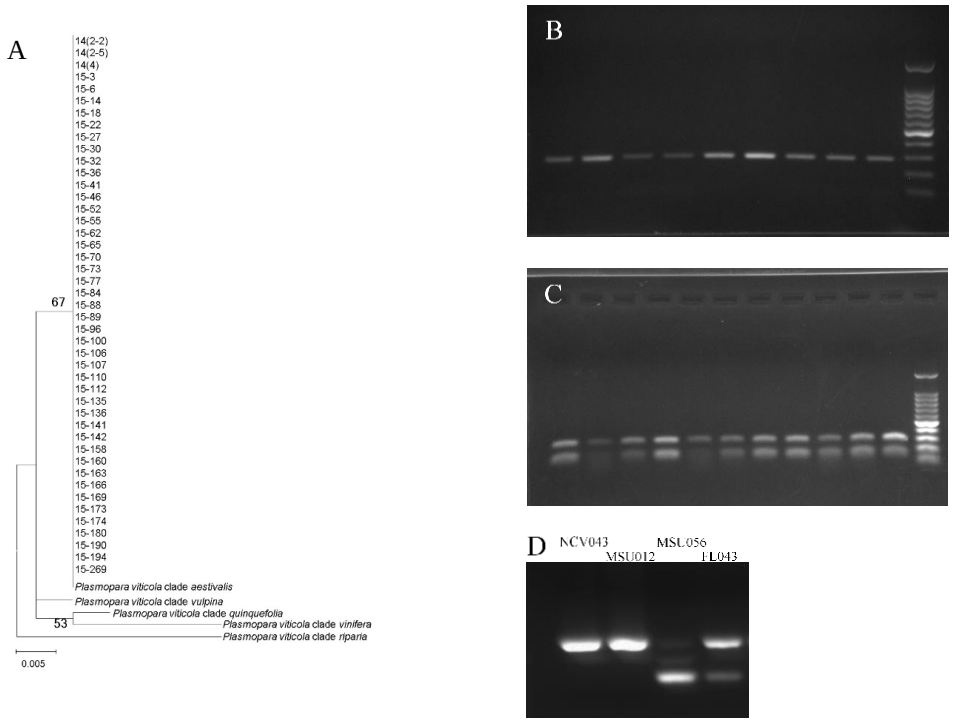

Fig. 5. (A) Phylogenetic tree of 45 isolates collected in 2014-2015 and five cryptic species of *P. viticola* based on ITS1 sequence. Downy mildew isolates collected from Haralson County in western Georgia (B) and Colquitt County in southern Georgia (C) showing different banding patterns after CAPS analysis. (D) CAPS results of reference isolates of *P. viticola* clade *aestivalis* (NCV043 & MSU012) and clade *vinifera* (MSU056 & FL043).

Literature Cited:

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Impact Statement:

Situation

Downy mildew is one of the most common and destructive diseases of bunch grapes in the Southeast. Fungicides are applied routinely by grape producers to combat this disease, but there is currently limited guidance as to how to best time these applications based on pathogen biology and environmental conditions. The goal of this study was to provide a foundation for more rational management of grape downy mildew based on a better understanding of the disease cycle under the specific environmental conditions of the Southeast through a combination of field epidemiology, computer simulation, and population genetics. We were especially interested in identifying factors that could explain epidemic variability among years, such as formation and germination of oospores (overwintering inoculum) or the presence of different cryptic species (clades) of the pathogen.

Response

Disease onset and progression were monitored closely in unsprayed and minimally sprayed grapevine plots at the Georgia Mountain Research and Education Center (GMREC) in Blairsville. Disease onset data were compared with those predicted by the widely validated UCSC downy mildew model out of Italy. In the autumn of 2016, naturally infected leaves and leaves from potted trap plants exposed during monthly periods were observed microscopically for the presence of oospores. Leaf litter with oospores collected in the previous year was exposed at the GMREC, and samples were taken periodically between April and August to determine presence of oospore germination via a leaf disk infection assay. Additional isolates of the pathogen were collected for cryptic species analysis, with a focus on locations in western and southern Georgia in 2016.

Impact

By the end of 2016, DNA from close to 500 isolates had been isolated for population and cryptic species analysis. All isolates from northern and western Georgia tested so far belong to *Plasmopara viticola* clade *aestivalis*, whereas almost 90% of isolates from southern Georgia appear to belong to clade *vinifera*. This result reveals mixed populations of cryptic species in the state, which could be one cause of the observed epidemic variability among years and locations. Based on negative results obtained in the spring oospore germination study it appears that oospore production is limited in the hot environment in Georgia, and this is supported by our direct observations on infected leaves collected in the autumn and observed for presence of oospores. Although the UCSC model predicted disease onset accurately at the GMREC in 2016, the failure of this model in 2015 suggests that additional validation and refinement are needed.