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?

Progress Report, Research Proposal

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Objectives:
1) Determine the role of sexual vs. asexual pathogen reproduction in overwintering and in initiating successive disease cycles during the growing season.
2) Conduct computer simulations with a "gold standard" downy mildew model and compare with actual disease development in the vineyard to reveal gaps and discrepancies in our current knowledge.

Justification:
Downy mildew, caused by the oomycete *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 southeastern grapevine growing regions. Producers apply regular fungicide treatments to manage downy mildew, but there is limited information about the epidemiology of the disease in hot and humid environments that could aid in better timing these applications. 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.

Methodologies:
Objective 1
Downy mildew-infected leaf samples were collected from three vineyards in Blairsville and Dahlonega in September and October 2014, returned to the laboratory, and examined microscopically (200x) for oospores after leaf clearing using the method described by Ngugi et al. (2002). In order to confirm the presence in northern Georgia of both mating types of the pathogen (a prerequisite for sexual reproduction and formation of oospores), *P. viticola* isolates obtained from these leaf samples were multiplied on *Vitis vinifera* 'Merlot' leaf disks (12 mm diam.) on RAP-water agar (Wong et al. 2001) at 22^{o}C. Single sporangium isolation was performed, and 11 single sporangium isolates (Table 1) were paired on leaf disks using the method of Wong et al. (2001). Leaf disks were cleared after 3 to 4 weeks and observed microscopically for oospores, the presence of which would indicate that the two paired isolates belong to different (compatible) mating types.

For the population structure analysis, a total of 259 single lesions were collected during the 2015 growing season from 20 grape varieties (*V. vinifera* and French-American hybrids) in 10 vineyards in northern, western, and southern Georgia. Single lesions with vigorous colonies were washed in 10 to 30 µl of sterile distilled water. The resulting sporangia suspension was used to inoculate fresh 'Merlot' leaf

disks on RAP-water agar plates, one isolate/plate. The plates were incubated in the dark for 16 to 24 h at 22°C followed by a 12-h photoperiod for 7 days. Leaf disks with each isolate were placed in long-term storage at 4°C according to the method of Laviola et al. (2006). To date, 66 isolates have been subjected to preliminary molecular analysis to determine which of the recently discovered cryptic species of *P. viticola* they belong to (Rouxel et al. 2014). Total genomic DNA was extracted from colonized leaf disks using the Qiagen DNeasy Plant mini kit (Qiagen, Valencia, CA), the *ITS1* region was amplified by PCR using primers ITS1-O and ITS2, and the amplicon was subjected to restriction analysis with enzyme AseI to distinguish between *P. viticola* clades *aestivalis* and *vinifera*, the two most likely clades of the pathogen in the southeastern U.S. (Rouxel et al. 2014).

Objective 2

We utilized the Università Cattolica del Sacro Cuore (UCSC) model to simulate development of downy mildew at the Georgia Mountain Research and Education Center (GMREC) in Blairsville. This mechanistic, process-based model was developed in Italy (Caffi et al. 2009; Rossi et al. 2008, 2009) and validated successfully across numerous locations in Italy as well as in Quebec, Canada. In the model, 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 at the GMREC. 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 (Table 2), "unsprayed" (no fungicide) and "allow downy mildew" (with fungicide application against other diseases but avoiding active ingredients effective against *P. viticola*). A total of 1,728 leaves were inspected in detail for presence of downy mildew during each survey bout.

Results:

Objective 1

Oospores were detected microscopically in naturally infected leaves from all three vineyards sampled in the fall of 2014, although their density was lower than reported previously in other production regions (Gessler et al. 2011). The mating tests with 11 single-sporangium isolates from these vineyards demonstrated the presence of both mating types in northern Georgia (Table 1, Fig. 1). Out of 10 pairings with these 11 isolates, 9 were conclusive, of which 4 (44.4%) resulted in the formation of oospores. Although based on a small sample, these preliminary results are consistent with the hypothesis of the two mating types being present in a roughly 1:1 ratio in the sampled vineyards.

From the 259 single lesions collected across the state in 2015, a total of 151 *P. viticola* isolates have been recovered to date and placed in long-term storage. Furthermore, DNA from 66 isolates has been extracted and preserved at -20°C for population structure analysis. In the meantime, these same 66 DNA samples were subjected to ITS1 polymorphism analysis to identify cryptic species; based on this sample, only *P. viticola* clade *aestivalis* has been identified from Georgia so far.

Objective 2

Based on simulations with the UCSC model for the Blairsville location, several cohorts of oospore germinated between April 15 and June 15, with the first infection predicted on April 15 and the first visible symptom on April 22 (Fig. 2). However, intensive surveys in the Blairsville vineyard during that period did not reveal the first symptom until June 15 (one 'Chardonnay' leaf with early infection out of 1,728 leaves surveyed; Figs. 3 and 4). Thus, there was an almost 2-month delay between disease onset based on the weather-based model for Blairsville and the actual appearance of the disease in the vineyard. The unexpectedly late disease onset, relative to what was anticipated based on local weather

and the known biology of *P. viticola*, is similar to a previous report from Georgia (Brannen et al. 2011). Thus, follow-up experiments will focus on monitoring the germination of oospores in vineyards to reveal the cause(s) for delayed disease onset and means to improve its predictability.

Conclusions:
Both direct microscopic observation of naturally infected leaves as well as laboratory pairing tests with single-sporangium isolates confirmed that both mating types of *P. viticola* are present and that oospores are being produced, pointing toward a role of the sexual stage of the pathogen in overwintering and primary infection in northern Georgia vineyards. A total of 151 single-lesion isolates of the pathogens have been obtained and stored to date from vineyards in northern, western, and southern Georgia, and these isolates will be utilized for determining within- and across-vineyard population structure of the pathogen. Of 66 isolates tested to date for cryptic species membership, all belong to *P. viticola* clade *aestivalis*. Simulation with the process-based UCSC model for the Blairsville vineyard resulted in a mismatch between predicted and observed symptom appearance of nearly 2 months. The cause(s) for the unexpectedly late symptom appearance in the field will be investigated in year 2 of the project.

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 develop initial information on how the pathogen overwinters in the specific environment of Georgia, when and how initial infection occurs, and whether initial infection can be predicted with an existing, widely validated model for downy mildew progression.

Response
Microscopic examination of naturally infected leaves and laboratory-scale mating type tests with the pathogen were conducted to determine whether oospores, the long-lived overwintering structures of the pathogen are formed readily in northern Georgia. One-hundred fifty-one single-lesion isolates of the pathogen were recovered and stored to conduct genetic analyses to determine the relative importance of sexual and asexual infection cycles of the pathogen. Disease onset and progression were monitored closely in unsprayed and minimally sprayed grapevine plots at the Georgia Mountain Research and Education Center in Blairsville. Disease onset data were compared with those predicted by the widely validated UCSC downy mildew prediction model out of Italy.

Results
Both microscopic examination and mating type tests documented that oospores of the pathogen can be produced in vineyards of northern Georgia. Intensive disease monitoring in the vineyard showed that the first downy mildew symptoms occurred about 2 months later than predicted by the weather-based disease model, suggesting some basic difference(s) in pathogen biology compared with what is known from other production regions of the world. Thus, model-based disease prediction is currently not feasible for grape producers in northern Georgia. Determining the cause(s) for the delayed disease onset in this environment will be critical for improving predictive capacity for managing downy mildew.

References:

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Citations for any publications arising from the project:

None to date.

Table 1. Result of mating type tests after pairing of *Plasmopara viticola* isolate 14-BV-C-2-2 (from 'Chardonnay' in Blairsville, GA) with other single-sporangium tester isolates from Blairsville and two commercial vineyards in Dahlonega, GA.

Tester isolate[a]	Oospores formed (yes/ no)
14-BV-C-2-5	N
14-BV-C-4	N
14-BS-TN-1-1	Y
14-BS-TN-1-2	Y
14-BS-TN-9-1	Y
14-BS-TN-9-2	N
14-CC-CS-4	N
14-CC-CS-6-2	?[b]
14-CC-CS-7	N
14-CC-PM-7-1	Y

[a] All isolates obtained in fall 2014. Locations: BV = Georgia Mountain Research and Education Center in Blairsville; BS = commercial vineyard 1 in Dahlonega; CC = commercial vineyard 2 in Dahlonega. Cultivars: C = Chardonnay, TN = Touriga Nacional, CS = Cabernet Sauvignon, PM = Petit Manseng.
[b] Inconclusive result.

Table 2. Fungicide treatment regimen in experimental plots at the GMREC in Blairsville, GA. The "allow downy mildew" schedule was included based on the expectation that other diseases such as powdery mildew would cause severe disease on totally unsprayed vines, thereby masking downy mildew development.

Treatment	Dormant	Budbreak	New shoot Sprays	Pre-bloom	Bloom	Post-bloom	First cover	Closing	Second and subsequent cover sprays	Veraison	Pre-harvest	Post-harvest
1. Un-sprayed	No treatment											
2. Allow downy mildew	No trtmt.	Manzate Pro-Stick @ 3 lb/A + Microthiol Disperss @ 10 lb/A (pho-mopsis applic.)	Microthiol Disperss @ 5 lb/A + Topsin M 70WDG @ 1.5 lb/A + Rally 40W @ 5 oz/A	Micro-thiol Dis-perss @ 5 lb/A + Topsin M 70WD G @ 1.5 lb/A + Rally 40W @ 5 oz/A	Ele-vate 50 WDG @ 1 lb/A + Micro-thiol Dis-perss @ 5 lb/A + Rally 40W @ 5 oz/A	Micro-thiol Disperss @ 5 lb/A + Topsin M 70 WDG @ 1.5 lb/A + Rally 40W @ 5 oz/A	Micro-thiol Disperss @ 5 lb/A + Topsin M 70 WDG @ 1.5 lb/A + Rally 40W @ 5 oz/A	Elevate 50WDG @ 1 lb/A + Micro-thiol Disperss @ 5 lb/A + Rally 40W @ 5 oz/A	Microthiol Disperss @ 5 lb/A + Topsin M 70WDG @ 1.5 lb/A + Rally 40W @ 5 oz/A	Elevate 50WDG @ 1 lb/A + Micro-thiol Disperss @ 5 lb/A + Rally 40W @ 5 oz/A	Micro-thiol Disperss @ 5 lb/A + Topsin M 70 WDG @ 1.5 lb/A + Elevate 50WDG @ 1 lb/A + Rally 40W @ 5 oz/A	Micro-thiol Disperss @ 10 lb/A

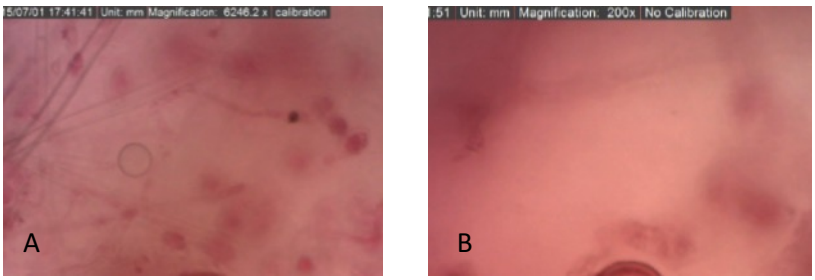

Fig. 1. Oospores produced after pairing single-sporangium isolate 14-BV-C-2-2 of *Plasmopara viticola* (Blairsville, Chardonnay) with tester isolates (A) 14-BS-TN-9-1 (Dahlonega, Touriga Nacional) and (B) 14-CC-PM-7-1 (Dahlonega, Petit Manseng). Magnification = 200x.

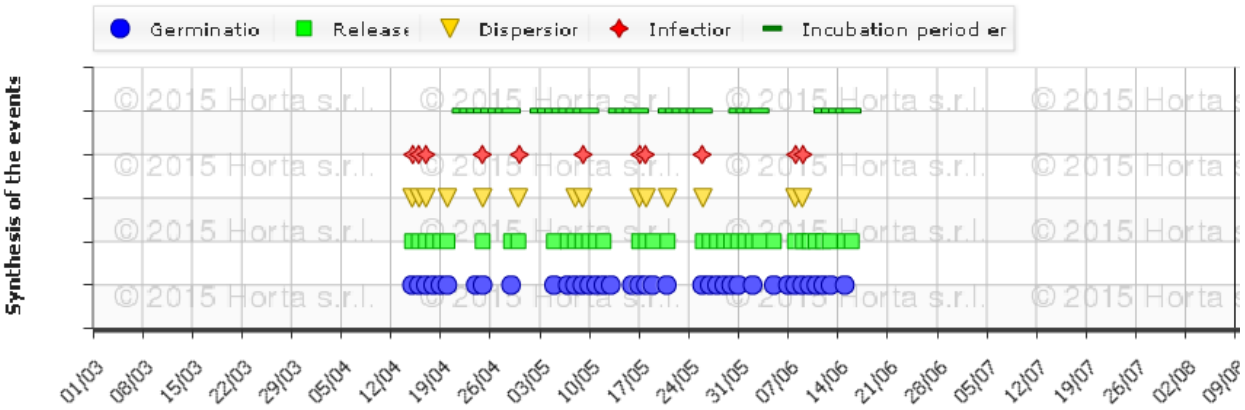

Fig. 2. UCSC model simulation of downy mildew primary infection using hourly weather data from GMREC, Blairsville during the 2015 growing season. Blue circles: germination of oospore cohorts; green squares: release of zoospores; yellow triangles: dispersion of zoospores; red diamonds: infection; green bars: appearance of symptoms. Note: dates indicated in European notation, i.e., 01/03 = 1 March.

Fig. 3. Earliest, faint symptom of grape downy mildew on Chardonnay grape at Blairsville on 15 June 2015. Only one symptomatic leaf was detected following detailed inspection of 1,728 leaves during this survey, indicating that we captured the very early onset of the disease. Sporulation of *P. viticola* was observed on this lesion during subsequent assessments.

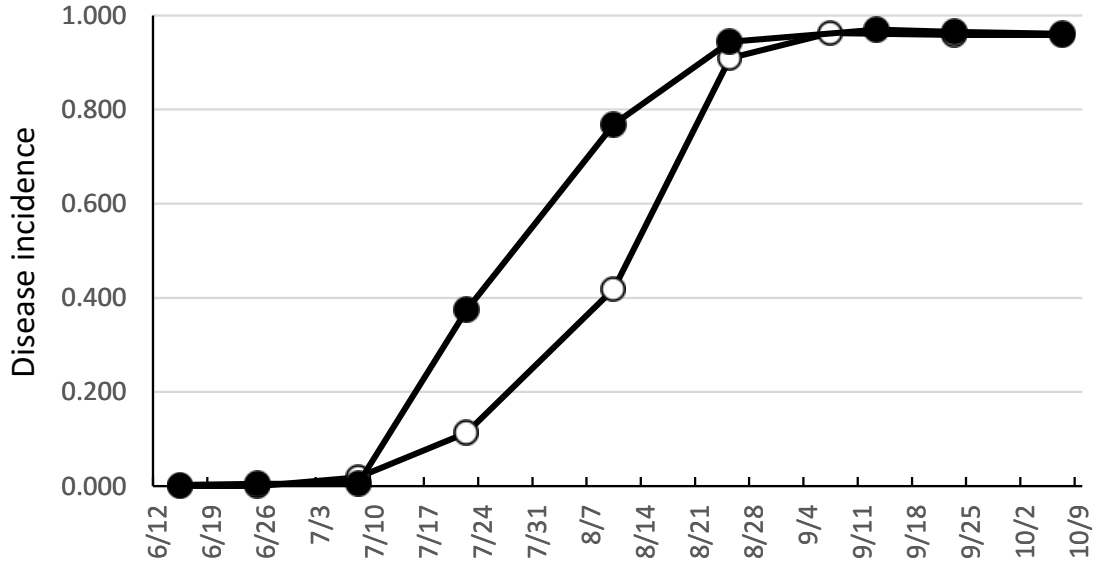

Fig. 4. Disease progress curve of downy mildew disease incidence on vines treated according to the "allow downy mildew" schedule (Table 2) in the GMREC vineyard in Blairsville, GA. Closed circles: Chardonnay, open circles: Merlot.