2007 Research Report to the Michigan Grape & Wine Industry Council

Proposal Title:
Understanding host specificity of *Plasmopara viticola* on grapes.

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Introduction; Priority Addressed:
Downy mildew, caused by the Oomycete pathogen *Plasmopara viticola* is a serious disease of grapes worldwide. The disease has been found on cultivated and wild grapes as well as on related plants in the family Vitaceae. Michigan has various species of cultivated grapes: *Vitis labrusca* L. (fox grape), *V. vinifera* L. (European wine grape), as well as wild grapes such as *V. riparia* Michx. (river bank grape), and *V. aestivalis* Michx. (summer grape). All of these are known to harbor downy mildew. Virginia creeper [*Parthenocissus quinquefolia* (L.) Planch.], a common perennial weed, has also been reported as a host to *P. viticola*. Although a few studies have mentioned variation in pathogenicity between isolates of *P. viticola* on different grape cultivars, races or pathotypes have not yet been identified. It is also not clear whether wild grapes and Virginia creeper, which are common around vineyards in Michigan, can be a source of inoculum for downy mildew epidemics. Recent inoculation experiments on grape leaf disks in Michigan have indicated a degree of host specialization among isolates from different types of grapes. For instance, 'Niagara' vines could only be infected with isolates originating from 'Niagara' vines. Preliminary DNA sequencing of part of the ITS 1 region of ribosomal DNA of the fungus

suggests that isolates fall into fairly distinct groups based on host species. However, this has to be confirmed by sequencing of additional genes. An increased understanding of host specificity of *P. viticola* isolates is necessary to determine if we are dealing with separate populations (and possibly different species) of downy mildew in the state. These populations may differ in their biology as well. This is the first study of its kind that looks at host specialization in downy mildew at the species level, which potentially has far-reaching implications for disease-resistance breeding programs in grapes and studies of the biology of the pathogen.

Original goals and objectives of the project:
The goal of the project was to increase our understanding of the host-pathogen relationship of downy mildew isolates with their hosts in the grape family. The specific objectives of this proposal were to:

1) Determine genetic diversity among *P. viticola* isolates from different grape hosts
2) Assess morphological diversity among *P. viticola* isolates from different grape hosts

Project Period: October 1, 2006 to September 30, 2007.

Work accomplished during the period, including methods

1) Determine genetic diversity among P. viticola isolates from different grape hosts

Downy mildew samples (144 total) were collected throughout Michigan from naturally infected cultivated and wild grapes (*Vitis labrusca, V. vinifera, Vitis* interspecific hybrids, *Vitis riparia,* and *Vitis aestivalis*) well as from Virginia creeper (*Parthenocissus quinquefolia*) (Figure 1). Small pieces of sporulating plant tissue (leaves, clusters, stems, or tendrils) were stored in 95% ethanol and sent to INRA (the French version of the USDA, Bordeaux, France) for genetic analysis. DNA was isolated and purified according to established protocols. Specific polymerase chain reaction (PCR) primers were used to amplify four different nuclear genes (28S, ITS, tubulin, actin). These genes are commonly used to study diversity of fungi at the species level. PCR products were sequenced and the obtained DNA sequences were analyzed for similarity using a computer program. A phylogenetic tree was constructed showing groupings of isolates that are similar to each other.

2) Assess morphological diversity among P. viticola isolates from different grape hosts

Plant tissue samples supporting sporulation of downy mildew were collected as described in objective 1 and were stored in a fixative solution until analysis. Ten sporangia per isolate were measured under the microscope using a stage micrometer and photos were taken of sporangia and sporangiophores. Sporangial size was analyzed by genetic grouping using the statistical program StatGraphics.

Summary of the expenditures during the period.

Budget category	Original Budget ($)	Expenditures ($)
Salary	3,840	3,541
Fringe	0	420
Travel	700	0
Materials and supplies	1,000	1,641
Other direct costs	350	288
Total	5,890	5,890

Results and conclusions of the project

1) Determine genetic diversity among P. viticola isolates from different grape hosts

DNA was successfully isolated from 109 downy mildew samples from Michigan and compared to DNA of 14 European and two Canadian isolates. DNA sequences were generated by PCR for four different nuclear genes (28S, ITS, tubulin, actin). Phylogenetic analysis yielded four different clades (A-D) corresponding to different host plant groups. The information obtained from each of the four genes was congruent. Figure 2 shows the different groupings of the isolates based on analysis of the 28S gene. Only selected isolates are shown in the tree for space reasons. Clade A contains downy mildew isolates from *Vitis riparia* (riverbank grape) and *Vitis* hybrids, such as Chancellor and Vignoles. Clade B contains downy mildew isolates from *Vitis labrusca* and *Vitis eastivalis* (summer grape). The European isolates appeared identical and fell into this clade. Clade C contains isolates from *Vitis vinifera* and table grapes (Vitis spp.), whereas Clade D was quite distinct and contained isolates from *Parthenocissus quinquefolia*.

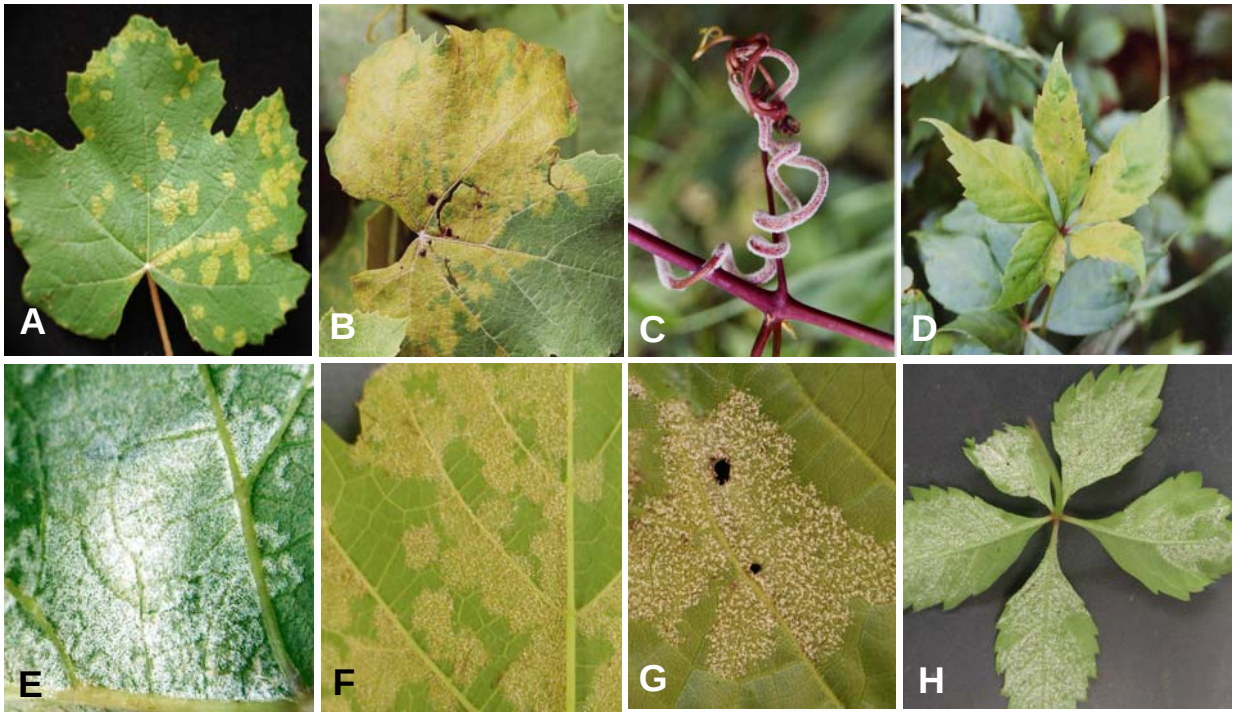


Figure 1. Downy mildew infection and sporulation on different host species used in genetic and morphology study. A) symptoms on *Vitis labrusca* 'Niagara', B) *Vitis aestivalis,* C) *V. riparia,* D) *Parthenocissus quinquefolia,* and sporulation on E) *Vitis labrusca* 'Niagara', F) *Vitis aestivalis,* G) *V. riparia,* and H) *Parthenocissus quinquefolia*.

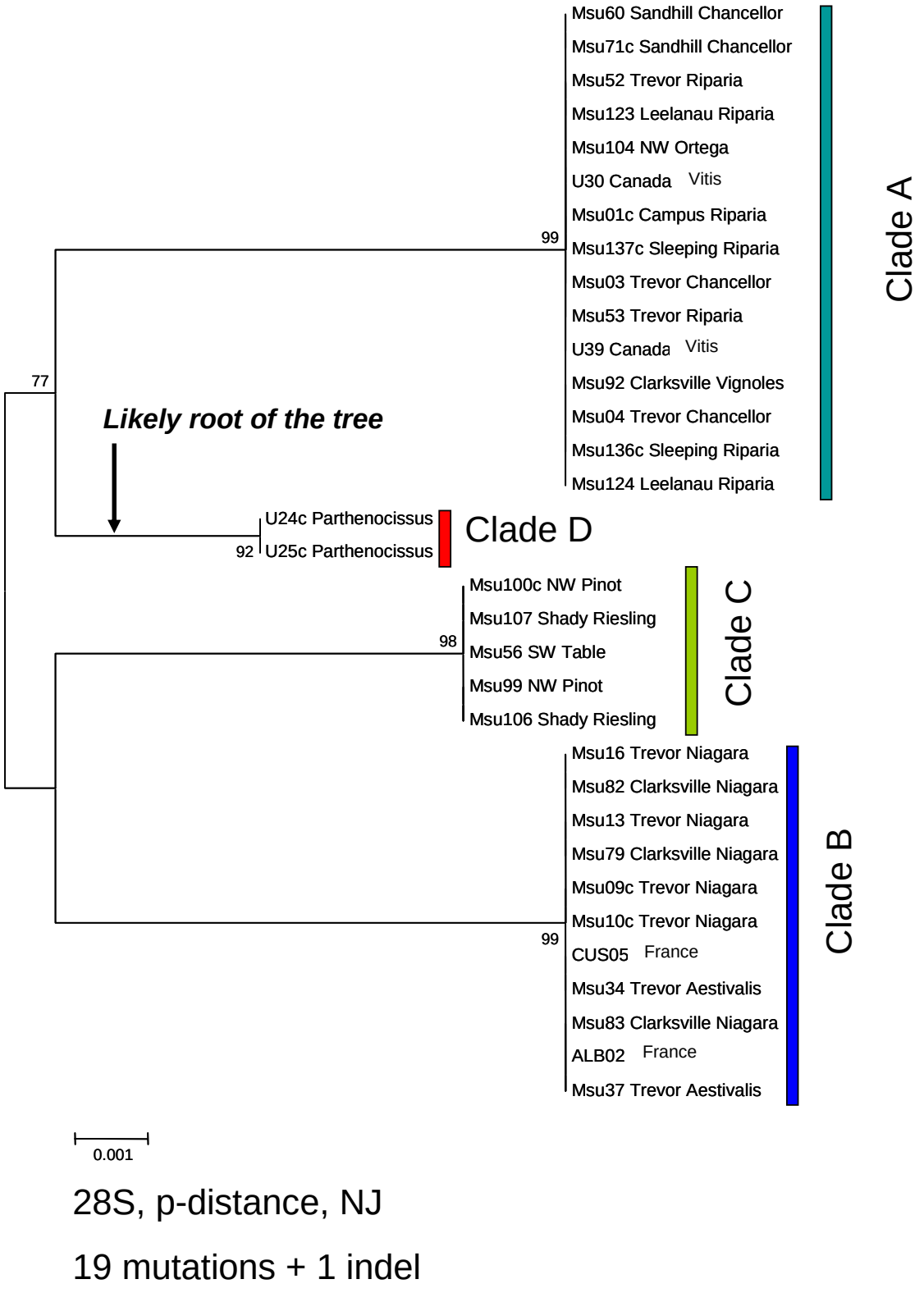
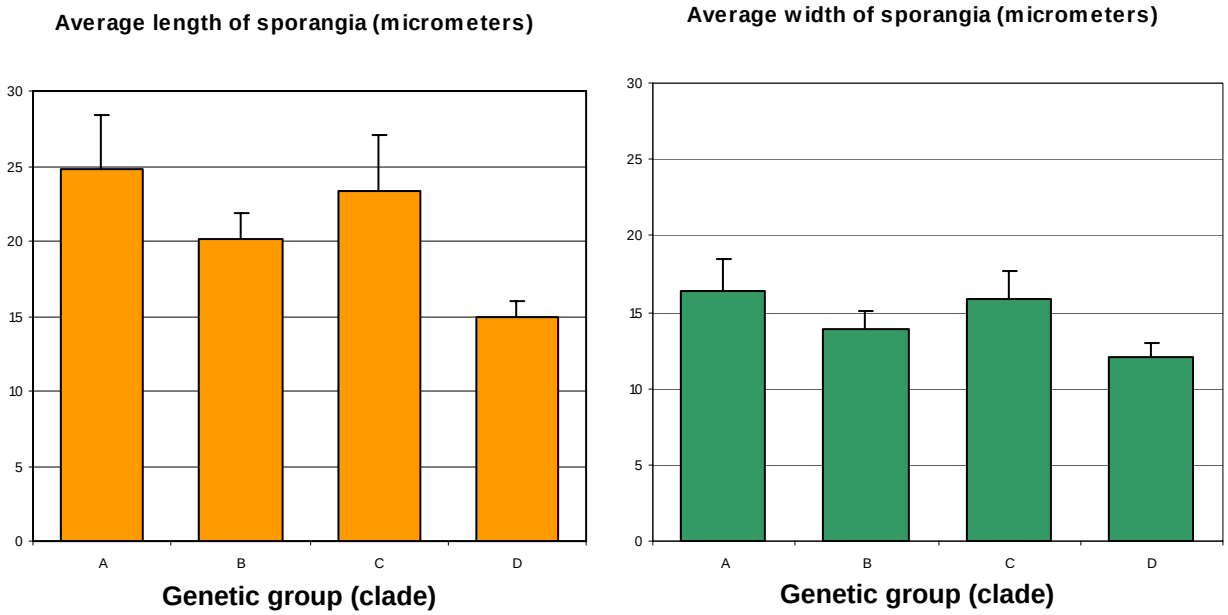


Figure 2. Phylogenetic tree (based on DNA sequence of the 28S gene) showing relatedness of *Plasmopara viticola* isolates collected from various grape and other hosts in Michigan, Canada, and France. Isolates are representative of a larger group of 109 isolates which are not shown.

2) Assess morphological diversity among P. viticola isolates from different grape hosts

While sporangial size of downy mildew isolates supposedly is variable and influenced by the substrate the pathogen is growing on, we found that sporangial size differed significantly among the genetic groups we identified. Isolates from Virginia creeper (*Parthenocissus quinquefolia*) were the smallest, while isolates from Clade B (*Vitis labrusca* and *Vitis aestivalis*) were smaller than those of Clade A (*Vitis riparia* and *Vitis* hybrid isolates) and Clade C (*Vitis vinifera* and table grape isolates). The morphological differences support genetic differences among isolates from different host groups. These data suggest that downy mildew isolates on different hosts in the Vitaceae family are either different species or subspecies. Further studies are needed by downy mildew taxonomists to decide this.



Overall conclusions

This research project has shown that downy mildew populations on cultivated and wild grapes in Michigan are not uniform and vary genetically and morphologically. Genetic groups (or clades) are formed according to the host that the downy mildew isolates originated from. While extensive sampling was not possible in each clade, e.g., due to limited occurrence of downy mildew in Michigan *Vitis vinifera* vineyards, there appear to be four distinct groups: Clade A, which contains isolates from *Vitis riparia* (riverbank grape) and *Vitis* hybrids; Clade B, which contains isolates from *Vitis labrusca* and *V. aestivalis*; Clade C, which contains isolates from *Vitis vinifera* and table grapes (Vitis sp.), and Clade D, which was quite distinct and contained isolates from *Parthenocissus quinquefolia*. Past pathogenicity tests support the hypothesis that there are biological limitations to cross infection, such that genetically separate downy mildew populations can co-exist in the same location. It appears likely that wild *V. riparia* may serve as a source of inoculum for hybrid grapes, but not for *V. labrusca* juice grapes. Downy mildew on Virginia creeper is a separate population that is restricted to Virginia creeper. The research has also yielded information regarding European isolates, which cluster with isolates from *Vitis labrusca*. This raises an interesting question on the origin of these isolates, which heretofore were thought to have been introduced into Europe with *V. rupestris* rootstocks in the mid 1800's. This merits futher investigation.

Communications Activities, Accomplishments, and Impacts:
The impact of this study is a better understanding of the biology and population structure of *Plasmopara viticola* on grapes. This may lead to better methods of managing the disease, for instance, by treating downy mildew on juice grapes and wine grapes as two separate pathogen populations that may have biological differences as well. In addition, the study showed that grapevine breeding programs need to consider using mixtures of isolates from different *Vitis* hosts to properly screen for downy mildew resistance in grapevine breeding lines. However, most of the impact will be in the longer term, as research collaborations have been established with the National Agricultural Research Institute (INRA) in France. The goals of the INRA programs are to develop wine grape varieties with durable resistance to downy mildew as well as to understand the origin of downy mildew introductions into Europe. Future plans are to screen hybrid grape lines for downy mildew resistance at MSU using Michigan *P. viticola* isolates due to the limited diversity of downy mildew populations in Europe. These lines may become available for sustainable wine grape production in the future. In addition, genetic testing for a mutation conferring fungicide resistance in downy mildew isolates from Michigan maybe conducted by the INRA labs in the future. Future goals of the MSU-INRA team include a survey of downy mildew isolates throughout the East Coast of the USA to pinpoint the potential source of the European *P. viticola* isolates. The research results will be presented at upcoming grape extension and scientific meetings. In addition, submission of a publication to a scientific journal (e.g., Phytopathology) is planned for the fall of 2008.

Funding Partnerships:
An application to the Viticulture Consortium East for additional funding was not granted in 2007 due to elimination of the Viticulture Consortium funds that year. However, MGWIC funding was supplemented by funding from INRA France, which conducted the DNA extraction and genetic analyses (valued at more than $10,000).