Contributions of oospore inoculum to epidemics of grapevine downy mildew (*Plasmopara viticola*)

CORNELL

MEGAN M. KENNELLY (1) C. Eugster (2) D.M. Gadoury (1) C.D. Smart (1) R.C. Seem (1) D. Gobbin (2) C. Gessler (2)
(1) Dept. Plant Pathology, Cornell University NYSAES, Geneva NY 14456 USA;
(2) Dept. Plant Pathology, Swiss Fed. Inst. Technol., CH-8092 ETH Zurich, Switzerland.

INTRODUCTION

Plasmopara viticola, causal agent of grapevine downy mildew, is a heterothallic oomycete that overwinters as oospores in the leaf litter and soil. In the spring, oospores germinate to produce macrosporangia, which under wet conditions release zoospores. Zoospores are splashed by rain into the canopy, where they swim to and infect through stomata. After 7-10 days, yellow lesions appear on foliage. During favorable weather, the lesions sporulate and new secondary infections occur.

Although the period during which oospores germinate may last several weeks (Figure 1, and refs. 5-7, 9, 10) forecasting models for the disease generally assume that epidemics are driven by secondary cycles following the initial oosporic infection event (1,2,6). However, recent DNA microsatellite analyses have suggested that oosporic infections play a much more substantial part in epidemic progress, and that secondary (clonal) spread may sometimes be a minor component of the epidemic. Environmental conditions required for primary (oosporic) and secondary (sporangial) infection differ substantially (6). Accurate forecasting will require a precise description of both the period of oospore germination, and the relative contribution of oospores to disease progress. Our objective was to examine the relative contribution of oosporic and sporangial inoculum to an epidemic of downy mildew using both a microsatellite analysis and a bioassay.

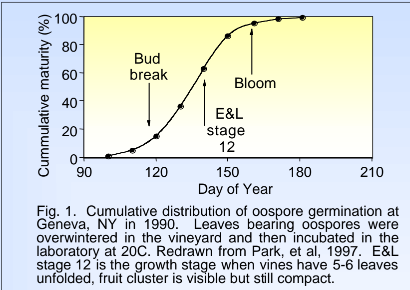

Fig. 1. Cumulative distribution of oospore germination at Geneva, NY in 1990. Leaves bearing oospores were overwintered in the vineyard and then incubated in the laboratory at 20C. Redrawn from Park, et al, 1997. E&L stage 12 is the growth stage when vines have 5-6 leaves unfolded, fruit cluster is visible but still compact.

MATERIALS AND METHODS

Microsatellite assay:

Gobbin et al. (4) identified four microsatellite markers to distinguish among clones. Isolates which shared identical allele lengths at all loci were considered to be derived from the same parent. These markers were used to characterize epidemics in Germany and Greece, and in both locations, new genotypes, presumably from oospore infection, appeared throughout the season. Although there was substantial secondary spread, in general, it was restricted to the increase of a few dominant clones (3, 8).

Our microsatellite experiment was carried out in an unsprayed vineyard of the *Vitis* interspecific hybrid cultivar 'Delaware', whose foliage is highly susceptible to downy mildew. Beginning with the first discovered infections, lesions were tagged, and their spatial coordinates were carefully mapped. One-half of each lesion was then collected, leaving the remainder to continue sporulating, and thus contribute to the population development (Figure 2).

For the first four sampling dates (12 to 20 June) we collected samples from all lesions. For the next four sampling dates (23 June to 8 July) we collected all lesions from vines bearing 1-3 lesions, 4 lesions from vines bearing 4-100 lesions, and 6 lesions from vines bearing more than 100 lesions. On the final sampling date, 22 July, disease severity was very high, and we collected 3 lesions from each vine in the plot.

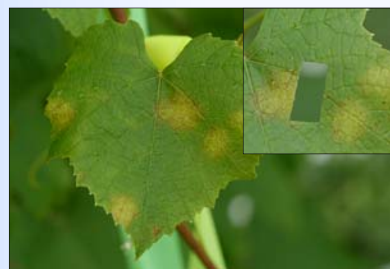

Fig. 2. Oilspots were tagged, and a portion of oilspot removed for genetic analysis

Fig. 3: DNA extractions, PCR, and fragment analysis were performed as described in Gobbin et al., (4).

Lesions were freeze-dried, the DNA was extracted, and PCR reactions and fragment analysis were conducted as described by Gobbin et al., (4). Different fragment lengths were considered to be different alleles. If lesions shared identical fragment lengths for all 4 loci they were considered to be progeny of the same primary lesion. Conversely, any differences were interpreted to mean that isolates had descended from different primary lesions.

Bioassay:
Grape seedlings were used to assess the seasonal distribution of oospore germination. In 2004, we removed oospore-infested soil from the Delaware plot used in the 2003 microsatellite studies and moved it to a location approximately 0.5 km distant from any vineyards. The vineyard soil was spread on three 2X2 m plots of killed turf to a depth of 2 cm. Twice per week we placed 10 potted seedlings in each soil plot, as well as beneath the trellis of the 2003 experimental vineyard (Figure 4). After 3-4 days exposure, the seedlings were placed in a screened house, and after 7 days we checked for symptoms of downy mildew, and for production of sporangia (Fig 5).

Fig. 4: Seedlings were placed on oospore-infested soil removed from vineyard. New seedlings were placed on the soil every 2-5 days.

Fig. 5: Downy mildew sporulating on grape seedling. After being removed from the soil, seedlings were allowed to incubate 1 week then were checked for disease symptoms.

RESULTS

During the period of 12 June to 28 July 2003 we collected 960 lesions in 10 samplings, and 869 lesions yielded sufficient PCR product with all 4 sets of primers. Locus GOB was the most polymorphic (Table 1). Combining the information from all 4 loci, we identified 386 putatively distinct genotypes. Four genotypes were identified more than 30 times each, and 287 were collected only once each.

Locus	# Alleles	Size Range (b.p.)
ISA	5	120.5 - 131.0
BER	12	156.3 - 183.5
CES	25	123.4 - 180.7
GOB	33	111.7 - 658.0

Table 1: Number of alleles detected and size ranges for each marker.

Unique genotypes represented the majority of the lesions in the early stages. By 12 days into the epidemic, the proportion of lesions with unique genotypes appeared to stabilize at about 50 percent, and remained at that level through day 40 (Figure 6). This suggested that oospores continued to germinate and cause a significant proportion of the overall disease. Perhaps incongruously, this stabilization of 50% "unique" genotypes in each sample occurred at a time when disease incidence was increasing exponentially. There was evidence of extensive secondary spread within single genotypes (Fig. 7). In the case of the 5 most frequently recovered genotypes, there was a strong correlation between frequency and date of first recovery: the genotypes which appeared first had more time to spread.

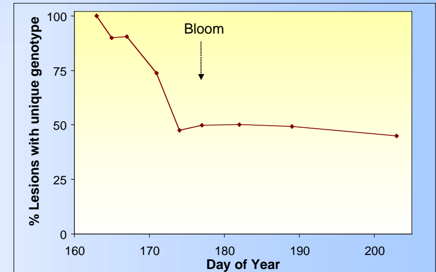

Fig. 6. Percentage of sampled oilspots that had unique genotypes. The first day downy mildew was detected was 12 June 2003. Data points represent the days when we sampled.

The seedling experiment also indicated a protracted period of oospore germination, (Fig. 8). The first infections occurred when mature vines had reached Eichorn-Lorenz growth stage 12 (5-6 leaves unfolded) which was expected. We continued to observe oospore infections for at least several weeks past bloom and are continuing to monitor for infection throughout the remainder of the season.

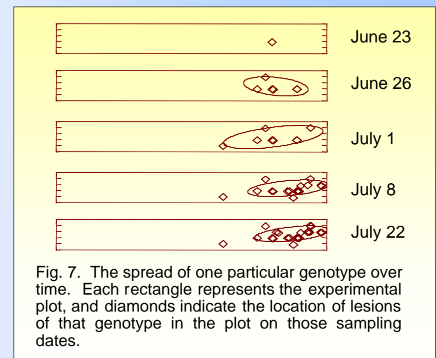

Fig. 7. The spread of one particular genotype over time. Each rectangle represents the experimental plot, and diamonds indicate the location of lesions of that genotype in the plot on those sampling dates.

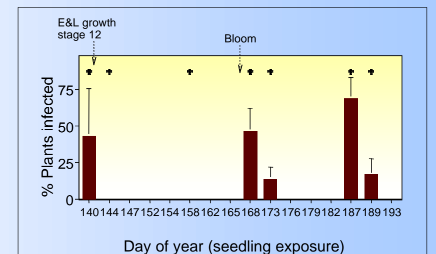

Fig. 8. Percentage of seedlings infected when exposed to oospore-infested soil. Seedlings were placed on oospore-infested soil for 2-5 days and then checked for disease development. The x-axis represents the day of year when a group of seedlings was first exposed, and the following date indicates when they were removed and the next group of seedlings exposed. A '+' indicates days with weather favorable to primary infection. Error bars represent one standard error of the mean.

DISCUSSION

Both the microsatellite assay and the seedling experiment indicated a protracted period of oospore germination, and thus argue for the revision of forecast models such as DMCast (6). The microsatellite study suggested that oosporic infections in 2003 were responsible for at least 50% of the total amount of disease as late as 40 days into the epidemic cycle. While this may seem difficult to reconcile with the extensive secondary spread which was observed, and the correlation of isolate frequency with date of first recovery, it should be noted that the absolute density of the oosporic inoculum may have been extremely high at this research vineyard. The vineyard has had very high disease levels in recent years, potentially leading to a large number of oospores. Given the unknown (but potentially large) size of the oosporic population, the high frequency of recovery of oosporic isolates in the microsatellite assay may have principally reflected a dense primary inoculum source. The results obtained at the site may therefore not be representative of epidemic development in many commercial vineyards with presumably lower levels of primary inoculum, where secondary increase of clones would be the principal component of disease increase Furthermore, though the seedling experiment also indicated a protracted period of primary infection, the disease incidence and severity did not increase over time, as would be expected if oospore infection increased over time.

CONCLUSIONS

- **The microsatellite assay and seedling assay both indicate that oospore infection occurs at least 7-8 weeks into the epidemic.**
- **The microsatellite assay suggests an increase of absolute numbers of oospore infection over time.**
- **The seedling assay indicates a more constant level over the season.**
- **Since weather conditions conducive to primary infection differ from those for secondary infection, we may need to include potential late-season primary infections in disease forecasting.**

This research was partially funded by the Viticulture Consortium-East through a grant to Cornell University-NYSAES, under Agreement #34360-7382 and the New York State Wine and Grape Foundation.

REFERENCES

1. Bleyer, G. 1997. A strategy for the controlled management of *Plasmopara viticola*. Vitic. and Enol. Sci. 52:168.

2. Cortesi, P., and Hill, G. K. 1991. Simulation of grapevine downy mildew epidemics and control with the P.R.O. model. First International Workshop on Grapevine Downy Mildew Modeling, Geneva, NY.

3. Gobbin, D., Loskill, B., and Gessler, C. 2002. Genetic diversity and disease dynamics of *Plasmopara viticola* in two non treated German vineyards. 4th International Workshop on Powdery and Downy Mildew in Grapevine, Napa, California.

4. Gobbin, D., Pertot, I., and Gessler, C. 2003. Identification of microsatellite markers for *Plasmopara viticola* and establishment of high throughput method for SSR analysis. European J. of Plant Pathology 109:153-164.

5. Gregory, C. T. 1915. Studies on *Plasmopara viticola* (downey mildew of grapes). International Congress of Viticulture, July 12-13, 1915, San Francisco.

6. Park, E. W., Seem, R. C., Gadoury, D. M., and Pearson, R. C. 1997. DMCast: a prediction model for grape downy mildew development. Vitic. Enol. Sci. 52:182-189.

7. Ronzon-Tran Manh Sung, C., and Clerjeau, M. 1988. Techniques for formation, maturation, and germination of *Plasmopara viticola* oospores under controlled conditions. Plant Dis. 72:938-941.

8. Rumbou, A., Gobbin, D., and Gessler, C. 2002. Epidemics and genetic variability of *Plasmopara viticola* in Greek populations. 4th International Workshop on Powdery and Downy Mildew in Grapevine, Napa, California.

9. Tran Manh Sung, C., Strizyk, S., and Clerjeau, M. 1990. Simulation of the date of maturity of *Plasmopara viticola* oospores to predict the severity of primary infections in grapevine. Plant Dis. 74:120-124.

10. Zachos, D. G. 1959. Recherches sur la biologie et l'epidemiologie du mildiou de la vigne en Grece. Ann. Inst. Phytopathol. Benaki 2:193-335.