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# Evidence for sporangial dispersal leading to a single infection event and a sudden high incidence of grapevine downy mildew

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In a German vineyard (PRO) between 10 and 13 June 2004, the incidence of grapevine downy mildew (*Plasmopara viticola*) increased abruptly from 0 to 99%. Infected vines bore on average between 45 and 60 lesions each, corresponding to about 220 000 lesions ha$^{-1}$ in a non-aggregated distribution. A second vineyard (FUT), approximately 50 m distant from PRO, had been inoculated 3 weeks before the abrupt increase in incidence of disease in PRO. Using microsatellites to ascertain the sources of inoculum and likelihood and extent of interplot spread from FUT to PRO, 555 samples were collected and 20 unique genotypes were identified, of which one caused 80% of the sampled lesions in both vineyards. Three genotypes responsible for 95% of the lesions in FUT and PRO were identified as the genotypes originally established through earlier inoculations in FUT. This is the first report of definitive and quantitative evidence of sporangial migration up to 130 m in a single infection event. The utility of molecular tools to address practical epidemiological issues in this pathosystem is illustrated. The results of this study provide an example of how *P. viticola* was able to rapidly colonize European vineyards after the pathogen was introduced from North America in 1878.

*Keywords*: epidemiology, microsatellites, *Plasmopara viticola*, spore dispersal, *Vitis vinifera*

## Introduction

Downy mildew, caused by the diploid oomycete *Plasmopara viticola*, is a destructive disease of European grapevine (*Vitis vinifera*) in temperate climates. Following introduction to France in 1878, the pathogen dispersed throughout France and Italy by 1879, to the Mosel region of Germany by 1880 (Müller & Sleumer, 1934) and by 1881 to the Messinia region of Greece (Gennadios, 1889). The pathogen overwinters as sexually produced oospores in fallen leaves and berries. The disease purportedly starts when germinating oospores cause primary infections early in the growing season. Oospore viability was believed to diminish rapidly between bud break and shortly after bloom. Abundant secondary infection is also assumed to drive increase of disease thereafter (Blaeser & Weltzien, 1979; Schruft & Kassemeyer, 1999). Spatial spread of the disease is poorly understood but is believed to rely on the dispersal of sporangia produced on foliar lesions (Zachos, 1959; Lafon & Clerjeau, 1988; Blaise *et al*., 1999).

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Microsatellite-based studies of *P. viticola* indicated that oosporic infections are abundant, randomly distributed within plots and may occur throughout the growing season. Initial incidence of downy mildew on untreated vines may be very low, approx. 1000 lesions ha$^{-1}$. The genetic structure of the *P. viticola* populations colonizing the vines at the first disease stage is highly differentiated; almost every lesion is caused by a different *P. viticola* genotype. About 30% of the genotypes in each population undergo asexual cycles throughout a survey period. The distance of sporangial dispersal per secondary cycle is usually less than 20 m (Rumbou & Gessler, 2004; Gobbin *et al*., 2005, 2006), although the foregoing reports could not exclude longer-distance transport as reported for other oomycetes such as *Peronospora tabacina* (Aylor & Taylor, 1982) and *Bremia lactucae* (Wu, 2001).

At the State Institute for Viticulture in Freiburg, Germany, an abrupt and severe increase in incidence of downy mildew was observed over a 4-day period in 2004 in a 1300 m$^2$ untreated vineyard (PRO). Lesions were randomly distributed and their density was estimated at about 220 000 lesions ha$^{-1}$. An adjacent vineyard approximately 50 m distant (FUT) had been inoculated artificially 3 weeks earlier. The objective of this study was to use

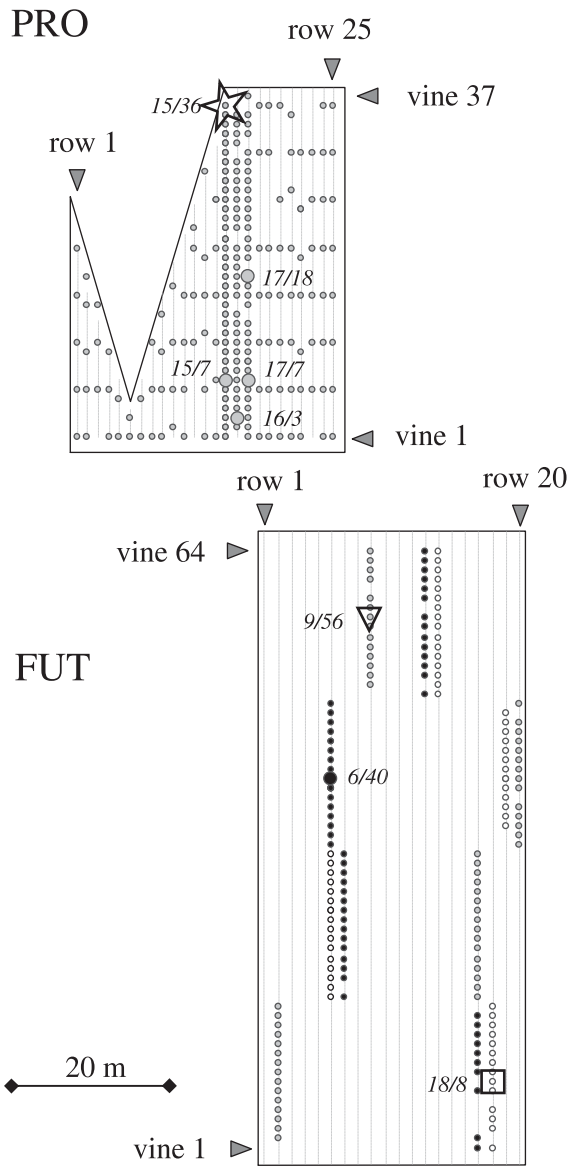


Figure 1 Shape and scaled relative position of the PRO and FUT plots. Rows are indicated by parallel discontinuous lines. Vines are regularly ordered along the rows and shown only if sampled. Independent vine co-ordinates are given for the two plots. Sampled vines are indicated by circles; black and white circles indicate that the vines were treated with the product Tr01 and Tr02, respectively; grey circles indicate untreated vines. In PRO, large grey dots show the four intensively sampled vines; in FUT, the large black dot indicates the sampling position of the four lesions derived from artificial inoculations. The lower and upper limits of maximal sporangia migration distance are comprised between triangle and star, and square and star, respectively.

previously developed microsatellite-based methods (Gobbin *et al*., 2003) to analyse the genetic structure of the pathogen populations in FUT and PRO to ascertain the sources of inoculum (oosporic or secondary) and likelihood and extent of interplot spread from FUT to PRO.

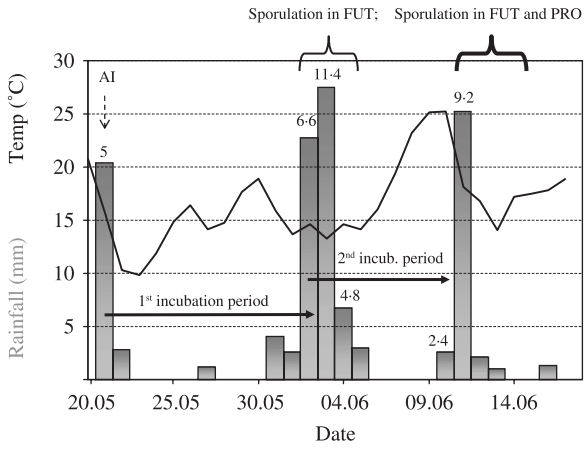


Figure 2 Rain amount (bars, mm), maximal rain intensity (numbers above bars if > 2·0 mm $h^{-1}$), average temperature (line, °C) recorded in Freiburg during the period 20 May–17 June 2004. The vertical dotted arrow indicates the time of artificial inoculations in the FUT plot (AI); horizontal arrows indicate the days required to fulfill the process of incubation following the forecasting model VitiMeteo Plasmopara; light and bold braces indicate oil spot appearance in the FUT plot and in both plots, respectively.

## Materials and methods

At the State Institute for Viticulture in Freiburg (Germany) two vineyards were selected; FUT and PRO. The shape and layout of both vineyards is shown in Fig. 1. The FUT vineyard (fungicide test), planted with the susceptible Pinot Noir (PN) variety had been used to evaluate various fungicides against *P. viticola*. Products were tested in four replicates of 16 vines per row and their effect was compared with four similar untreated replicates. On 21 May 2004 (BBCH stage: 16, Lorenz *et al*., 1994) in FUT, four leaves per replicate (320 leaves in total) were artificially inoculated with a sporangial suspension ($2 \times 10^4$ $mL^{-1}$). The sporangial suspension was derived from a mixed population collected in 2003 in Freiburg that was frozen over the 2003–2004 winter and multiplied in spring 2004 in a glasshouse on potted vines for 2–3 inoculation/harvesting cycles (Rumbolz *et al*., 2002; Unger *et al*., 2007).

The PRO vineyard was a mother plot for the propagation of clones of PN and various hybrid cultivars and was not treated with fungicides until the first occurrence of oil spots. Both FUT and PRO were checked weekly for the occurrence of foliar symptoms. Disease incidence and severity were assessed according to EPPO guidelines (Anonymous, 1996). Meteorological data were recorded by means of a weather station (Opus 200, Lufft, Germany) placed in one corner of the plot. Incubation periods were estimated using the forecast system VitiMeteo Plasmopara (Viret *et al*., 2005).

On 21 May 2004, artificial inoculations were performed in FUT prior to rainfall (Fig. 2). According to the forecast system VitiMeto Plasmopara, on 2 June the incubation

Table 1 Investigation of grapevine downy mildew caused by *Plasmopara viticola* in Freiburg vineyards. Plot characteristics and sampling details. Samples were taken from heavily sporulating oilspot lesions. Fungicides were applied to 76 replicates of 80 in FUT and no application was done in PRO

| Plot | Grape variety[a] | No. rows[b] or no. vines/row[c] | Total no. vines[d] or area ($m^2$)[e] | Source of sample identified by row number (R) or vine within row number (V) | Sampling frequency (no. of lesions/no. of vines or % of lesions) | No. of samples |
|---|---|---|---|---|---|---|
| PRO | R × S (rows 1–14) | 25[b]; 3–37[c] | 620[d] | R1–14; 18–25 | 1/5 | 54; 60 |
| | PN (rows 15–25) | | 1300[e] | R16–17 | 1/1 | 33; 34 |
| | | | | R15 | 2/1 | 73 |
| | | | | V15/7; V17/7; V17/18 | 30% | 16; 21; 22 |
| | | | | V16/3 | 100% | 60 |
| | | | | | | 373 (total) |
| FUT | PN | 20[b]; 64[c] | 1280[d] | R1–20 | 1/1 | 182 |
| | | | 3120[e] | | | |

[a]Riesling × Sylvaner, R × S; Pinot Noir, PN.
[b,c]Number of rows and number of vines per row, respectively.
[d,e]Total no. vines and plot area ($m^2$) respectively.

period had been completed. On this date the first symptoms were observed on the inoculated leaves (BBCH-stage 59–60) in FUT but not on uninoculated leaves in FUT, or on leaves in PRO. Disease incidence on leaves in FUT on 2 June was < 1% and was 0% in PRO. After the second rainy period lasting from 31 May to 4 June, sporulation of the oil spots was visible on 4 June. In the same time-span the wind blew in all directions at variable wind speeds. On 13 June, at the conclusion of a second rain event lasting for 4 days, disease incidence increased to 99% on vines and to 45% on leaves in PRO. In the FUT vineyard in the four replicates with fungicide treatments Tr01 and Tr02, and in the four untreated replicates, disease incidence increased to 28% on leaves.

Once the sudden increase of disease in PRO had been observed, heavily sporulating lesions were collected from untreated vines in PRO and from vines in FUT treated with Tr01 and Tr02. At least one lesion was collected per five vines in both PRO and FUT. Within PRO, the number of samples was increased within the rows 15, 16 and 17 and within four randomly chosen vines (Table 1) to improve quantification of the genetic diversity of the *P. viticola* population. On vine 6/40 (40th vine in row 6) located in FUT, four samples were collected from the lesions resulting from the artificial inoculations performed on 21 May 2004. Those lesions were larger (> 5 $cm^2$) than the recently appeared oil spots (< 3 $cm^2$), partially necrotic and with a non-circular shape. Samples were processed and genotyped using microsatellite analysis according to the procedure described by Gobbin *et al.* (2003).

## Results and discussion

From within PRO and FUT, 373 and 182 samples were collected, respectively. Twenty unique genotypes were identified among the 555 samples (Table 2). Fourteen of the 20 genotypes were found in single copy (G04-17), while six genotypes were found in multiple copies (clonal genotypes; G01-03, G18-20). G04-17 may have been the result of natural oosporic infection that took place during the second rainy period (31 May–4 June), while G18-20 were probably derived from a primary cycle triggered by the first rainy period and followed by a secondary cycle which occurred on the second rainy period. These 17 genotypes (27 lesions) were randomly distributed within the plots. Two of the three remaining genotypes, G01, G02 and G03, played a key role in the epidemic: G01 comprised 14% of the total samples collected (79 lesions), G02 comprised 80% of the samples collected (444 lesions) and G03 was identified five times; all three genotypes were identified among the four samples collected from the artificially inoculated vine 6/40 in FUT.

The contribution of G02 and G01 to the total disease incidence was similar in any sub-area of FUT and PRO. Collecting one lesion every five vines or up to all lesions in a single vine, 73–85% and 11–22% of all the samples were G02- and G01-derived lesions respectively (Table 2). The ratio of G01:G02 in rows with different sampling intensity (0·2, 1 and 2 samples per vine), within single vines, within grapevine varieties or single treatments never significantly deviated from the average (expected) value of 0·18 ($P > 0{\cdot}1$ after chi-squared tests).

It is not possible to determine exact dispersal distances of spoarangia, as their only source with known population structure was on vine 6/40 (2, 1 and 1 sample of G02, G01 and G03, respectively; Table 2). Nevertheless, assuming the same population structure on each artificially inoculated leaf in FUT, the maximal sporangia dispersal distance ranged from 65 m to 130 m. At least 65 m spread may have occurred starting from the artificially inoculated vine 9/56 in FUT to the vine 15/36 in PRO, where G02 was identified (Fig. 1), but G02 may also have infected the remotest PRO vine after being released from vine 18/8 in FUT (130 m). It is recognized that the maximum possible dispersal during such an event has not been defined in this study. Previously, Zillig speculated that sporangia could disperse 100 m (Zillig, 1942). More recently it has been claimed that sporangia of *Bremia lactucae* can disperse

Table 2 Identification of genotypes of *Plasmopora viticola* contributing to the epidemic of grapevine powdery mildew in Freiburg vineyards. Intensive sampling was carried out in two vineyards (FUT and PRO). For details of row number and vine samples see Table 1

| | FUT plot | | | | | PRO plot | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | SS | V6/40[b] | Untreated[c] | Tr01[d] | Tr02[d] | SS | R1–14, R18–25 | R16 | R17 | R15 | V15/7[e] | V17/7[e] | V17/18[e] | V16/3[e] | R1–14[e] | R18–25[f] | Overall SS |
| G01 | 25 | 1 | 8 | 10 | 7 | 54 | 19 | 5 | 5 | 9 | 2 | 3 | 5 | 6 | 6 | 13 | 79 |
| G02 | 143 | 2 | 46 | 47 | 50 | 301 | 90 | 28 | 26 | 63 | 13 | 18 | 16 | 47 | 46 | 44 | 444 |
| G03 | 4 | 1 | 1 | 1 | 2 | 1 | | | | | 1 | | | | | | 5 |
| Others[a] | 10 | | 4 | 3 | 3 | 17 | 5 | | 3 | 1 | | | 1 | 7 | 2 | 3 | 27 |
| %G01 | 14% | 25% | 14% | 16% | 11% | 14% | 17% | 15% | 15% | 12% | 13% | 14% | 23% | 10% | 11% | 22% | 14% |
| %G02 | 79% | 50% | 78% | 77% | 81% | 81% | 79% | 85% | 76% | 86% | 85% | 86% | 73% | 79% | 85% | 73% | 80% |
| G01:G02 | 0·18 | 0·5 | 0·17 | 0·21 | 0·14 | 0·18 | 0·21 | 0·18 | 0·19 | 0·14 | 0·14 | 0·17 | 0·31 | 0·13 | 0·13 | 0·30 | 0·18 |
| Total | 182 | 4 | 59 | 61 | 62 | 373 | 114 | 33 | 34 | 73 | 16 | 21 | 22 | 60 | 54 | 60 | 555 |

SS = sample size; T = treatment; V = vine; R = row.

[a]Data from 17 other genotypes are summarized for convenience.

[b]The four lesions collected resulted from artificial inoculations on 21 May 2004. Those lesions were larger (> 5 $cm^2$) than the oil spots which appeared on 4 June 2004 (< 3 $cm^2$), partially necrotic and with a non-circular shape.

[c]Four replicates were untreated.

[d]Fungicides were applied to 76 replicates of 80 in FUT.

[e]Riesling × Sylvaner.

[f]Pinot Noir.

over a distance from 80 to 3000 m (Wu *et al*., 2001). Further studies employing microsatellite-based methods may enable more precise quantification of the risks posed by long-distance transport of sporangia of *Plasmopara viticola*.

In Freiburg, disease increase was clearly due a single asexual cycle and the clonal genotypes found in PRO could be definitively attributed to dispersal from vines artificially inoculated with the same genotypes at a previous date in FUT. Previous surveys of heavy downy mildew epidemics did not allow such a precise estimation of sporangial dispersal due to overlapping asexual cycles prior to or during samplings, thereby disturbing the epidemic pattern. The quantification of the magnitude of secondary cycles was mainly impeded by the very low sampling capacity with respect to the huge number of lesions present, often exceeding a thousand oilspots per vine in the period July–September. (Rumbou & Gessler, 2004, 2006; Gobbin *et al*., 2005, 2006). The main difference between previous studies and the one reported here may be inoculum fitness. During inoculation/harvesting cycles in the glasshouse, G01 and G02 may have been selected as the fittest within the population of the collected genotypes. Once reintroduced into the source vineyards they caused an unpredictably high amount of damage.

Although admittedly this study was of limited scope, it has demonstrated how microsatellite analysis might be used to address practical epidemiological issues in the *V. vinifera*/*P. viticola* pathosystem. Definitive and quantitative evidence is provided of dispersal of up to 130 m and of substantial increase in disease incidence in an area of 1300 $m^2$ after a single secondary cycle. The results also provided a quantitative and definitive illustration of speed with which *P. viticola* could spread across Europe in multiple but isolated events between the time of its introduction in 1878 and the widespread deployment of Bordeaux mixture beginning in 1882 (Millardet, 1885).

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