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Powdery mildew on grapevine: the date of primary contamination affects disease development on leaves and damage on grape

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Abstract: The temporal evolution of the disease on leaves and damage on grape was monitored at different scales in order to study the effect of early primary contamination on epidemic development and its relationship with damage on grape. At a vine scale, on vines artificially inoculated with 12 days delay, the disease evolution on leaves was delayed. At flowering, early contaminated vines were 60% more diseased than late contaminated vines. On bunches, the progression and final disease were significantly different with, at veraison, an average severity of 99% for early contaminated vines versus 62% for late contaminated ones and 29% for uncontaminated ones. A significant difference for the Incidence-Severity relationship at the leaf scale was quantified indicating the difference in symptoms according to the date of contamination. At the plot scale (330 vines), we compared the maps of frequency of diseased leaves per vine at different scoring dates with the maps of frequency of bunches with a given level of damage. Epidemics initiated earlier were characterised by higher severity for a given level of frequency of diseased leaves (at the vine scale) and higher final diseased leaves frequency (at the plot scale). Early difference in the number of diseased leaves led to significant differences in the number of highly damaged clusters (> 25%) and in the average clusters severity. The analysis of disease maps demonstrated the spatial relationship between the frequency of diseased leaves at flowering and the frequency of severe damaged bunches.

Key words: primary infection, incidence – severity relationships, permutation, powdery mildew.

Introduction

Powdery mildew (*Uncinula necator*) is the most widespread disease on grapevine worldwide and it is the one of main target of fungicides used on *Vitis vinifera*. Because of the absence of effective forecast system for the epidemic initiation due to ascospores and of the difficulty to early detect the disease in the vineyard without expert sampling, the disease may be difficult to control. In France, the control of powdery mildew typically relies on an average of seven routine applications of fungicide within the growing season costing around 60 millions euros per year (U.I.P.P. 2003). Initial experimentation suggests that effective control of powdery mildew is possible using fewer applications provided these are applied at certain strategic periods during epidemic development (Cartolaro et al. 2005; Delière et al. 2005). The efficacy of such strategies is however variable depending on the type of epidemic. Factors of variation may be as different as number and distribution of primary foci, earliness of ascospore release, isolate aggressiveness, or host susceptibility. The major effect of ontogenic resistance of the organs on powdery mildew development (Doster & Schnathorst 1985; Ficke, Gadoury & Seem 2002; Ficke et al. 2003; Gadoury et al. 2003) may also enhance the importance of the date of primary contamination for the risk of invasion of the disease on leaves and for the severity of damage on clusters.

In this study, we describe and demonstrate the effect of early primary contamination on epidemic development and its relationship with damage on grape. This was studied at two experimental scale: at the vine scale by comparing the temporal evolution of the disease on leaves and bunches for early, late and uncontaminated vines and at a plot scale by showing the spatial relashionship between maps of frequency of diseased leaves with maps of the frequency of damage on bunches.

Material and methods

Vine scale – Temporal Analysis

Experimental design and treatments

Experiments were conducted in 2001 on three-vine micro-plots (cultivar Cabernet-Sauvignon), with five repetitions per treatment fully randomized. Each micro-plot was separated by three vines within the row. Spacing was 1 m between vines and 1.5 m between rows. Treatments were: D1, early contamination on one shoot on the central vine (In1) or on one shoot on each vine (In2), D2, late contamination (In1 or In2), Un, uncontaminated (lately contaminated by natural inoculum dispersed from other plots). Inoculation was performed by dispersing spores from two sporulating leaf disks placed at the top of a Cellophane funnel attached around a shoot. Funnels were stapled allowing air circulation and left in place for 24 hours. Inoculations were performed on the 4th of May at BBCH 13-53 for D1, and 16th of May at BBCH 55 for D2.

Sampling and Variables

Disease incidence and disease severity were assessed on central vine: 10 leaves per shoot, two shoots per vine (30 May, 8 and 18 June and 2 July) and 20 clusters (22, 29 June, 9 and 26 July). Severity was expressed as mean percentage of sporulating area.

Statistical analyses

Only the disease evolution on the central vine is analysed here. Temporal incidence data on leaves (proportion of diseased leaves per vine) are fitted to logistic models $y = \frac{A}{1 + e^{K} e^{(-R.t)}}$ by using non-linear regression. Parameters *A* and *K* are linked either to the maximum (*A*) or to the initial (*K*) level of disease, and parameter *R* indicates the rate of progression of disease with time (t). Models (treatments) are compared pairwise by Akaike's procedure: for each hypothesis (one model for both data sets or two different models) an Akaike corrected criterium *(AICc)* is calculated AICc=N.ln(SS/N)+2K+2K(K+1)/N-K-1 with N=number of data points, SS=sum-of-squares, K=number of parameter+1, and based on this, the likelihood of the model (probability that the more complex model is correct) is calculated P =exp(-0.5 ΔAICc)/(1+exp(-0.5ΔAICc)) with ΔAICc the difference in AICc for the two models compared.

Temporal severity data on bunches (average severity per vine) were fitted to linear models by using lienar regression, then treatments were compared by F test.

The relationship between leaf incidence (I) and leaf severity (S) was analysed after fitting data to the equation $I=1-\exp(-aS^{b})$ by non-linear regression (McRoberts, Hughes, and Madden 2003). Treatments were compared by Akaike's procedure.

Plot scale – Spatial Analysis

Experimental design, sampling and variables

Experiments were conducted in 1999 on a 330 vine isolated plot of cultivar Cabernet-Sauvignon. Spacing was 1 m between vines and 2 m between rows. Epidemics were initiated naturally.

Disease incidence was assessed on each vine: 5 to 10 leaves per shoot (following vine growth), two shoots per vine (12, 26 May, 2, 15 June, 1, 20 July) and all clusters (5 July). Bunches were scored on a 0 to 4 visual scale reflecting the proportions of diseased berries: C0, no visible powdery mildew; C_1, < 25% diseased berries; C_2, 26 – 50% diseased berries; C_3, 51 - 80% diseased berries; C_4, > 80% diseased berries.

Statistical analyses

Data were the frequency of diseased leaves and frequency of bunches with a level of severity. The data were represented on a grid of I vines and J rows, with the values at the nodes. The principle was 1) to examine the spatial structure of data for leaf disease and grape disease, and 2) test for independence between the two spatial distribution (images) of the frequency of diseased leaves per vine early in the season and the frequency of diseased clusters with different levels of damage at bunch closure. The second step was carried out in order to establish if there was spatio-temporal relationship between the disease on leaves and those on bunches.

Studies of the spatial structure. Independency between values was tested as follows. Either *total independency* (tested hypothesis: the probability to get a value at a given point is the same at any point, all values are independent and there is no spatial structure for disease data) or *independency between rows* (tested hypothesis: values between rows are independent, which fit with the hypothesis of progression of the disease along the row). To do this, the procedure used was to:

– *calculate a statistic on the observed data*: the variogram (average of the square of the difference of values for vines at a distance *d*):

$$\gamma(d) = \frac{1}{I(J-d)} \sum_{1<i<I} \sum_{1<j<(J-d)} \left(X_{(i,j)} - X_{(i,j+d)}\right)^2$$

the variogram here is used as an indicator of the structure of the data.

– *perform permutations of the data (400 times)*, either total randomisation of values to test for total independency, or row randomisation by performing rotations on the row (identical neighbours on the row, but different neighbours between rows, to test if the progression on the row is favoured).

– *calculate the variograms*, on each permuted grid.

– *build on a confidence band* for each distance d, with a quantiles at α=0.05, to measure the adequacy of the data with the hypothesis of independence.

– *Test of the independency between images.* The principle is to test for *independency between the spatial structure* of disease on leaves and those of damage on bunches. The procedure used was to:

– *calculate a statistic on the observed data*: the covariogram (average of the product of the difference of values on leaves with the difference of values on grape for vines at a distance *d*):

$$C\gamma(d) = \frac{1}{I(J-d)} \sum_{1<i<I} \sum_{1<j<(J-d)} \left(X_{(i,j)} - X_{(i,j+d)}\right)\left(Y_{(i,j)} - Y_{(i,j+d)}\right)$$

with X the values of disease on leaves and Y the values of disease on clusters. The covariogram here is used as an indicator of the spatial correlation between disease data on leaves and on bunches.

– *make a translation from one image compared to the other*. The spatial structure of both images is conserved but the distance between structures is increased.

– *calculate the covariograms*, on each translated grid.

– *build on a confidence band* for each distance *d*, with a quantiles at α=0.05, to measure the adequacy of the data with the hypothesis of independence between maps.

Results and discussion

Vine scale – Temporal analysis

On leaves, disease dynamics overtime was of the logistic type. Disease dynamic on the central vine for plots inoculated at the earlier date with a high level of inoculum (D1In2), may present a higher rate of progression with time than for plots inoculated with a lower level of inoculum (D1In1) (Fig 1 A). However only one global model (1 curve) can be accepted to describe the average progression on these central vines inoculated early (Probability it is correct 0.756, ΔAICc=-2.27). For plots inoculated later, disease dynamic on the central vine is very similar for both level of inoculum (D2In2 and D2In1) (probability to accept a global model 0.94, ΔAICc=-5.86). Disease evolution on plots inoculated at the two different dates is significantly different with, with for D2, a delay of 8 days to reach the same level of disease. At the end of the season, disease maximum are however not significantly different for D1 and D2 (probability to accept a global model based on difference for K= 99.9, ΔAICc=21.58) (Fig 1 B). On the uninoculated vines, disease progress of the is significantly different than for the lately inoculated vine (probability to accept a global model = 0.0006, ΔAICc=14.82) with different final level of disease (Fig. 1B). At flowering (D160), early contaminated (D1) vine suffered 60% more disease than late contaminated (D2), and uncontaminated vine (Un) have 60% less disease than late contaminated. Severity is on average higher for vines inoculated earlier due to a higher frequency of symptoms with a severity up to 15% (Fig. 2). The relationship between leaf incidence and leaf severity was also significantly different for early contaminated vines (D1) and lately contaminated vines (D2 and Un) (probability to accept a global model = 0.0087, ΔAICc=9.47) (Fig. 3), supporting a higher agregation (higher severity for identical incidence) for vines inoculated earlier, which may lead to a higher level of sporulation.

On bunches, the average severity evolved linearly over time. The rates of progression were significantly different for D1 (0.22), D2 (0.17) and Un (0.08) ($F=17.15$, $df=90$, $P<0.0001$) (Fig 1 B). The final level of disease were also significantly different at veraison (D210), with average damage of 99% (D1), 62% (D2), 29% (Un).

Plot scale – Spatio-temporal analysis

Description of the disease on leaves and grape. The epidemic spread from the four primary foci detected at first scoring (12 of May) and reach 14.2% of diseased leaves at the end of flowering (15 June) and 83% at the last scoring (20 July) (Fig. 4). At bunch closure, if 95% of bunches were diseased, those with high damage (severity > 25%) appeared to be mostly located around primary foci.

Spatial structure on leaves and grape. The variogram on leaves for the 2nd of June (beginning of flowering) was under the independence assumption (under the confidence band, indicating aggregated values) for vines as close as three meters (average focus size) (Fig. 5A). At the 15 of June, the variogram was under the independence assumption for distances to 7 meters. On the inter-row variogram, values were not different from random on the 2nd of June (Fig.5B), whereas rows close to each other started to have similar levels of disease on the 15 June. On bunches, at 5th July, data were highly structured with clumped values for vines at distance of 19 meters for diseased bunches, 15 meters for bunches with more than 25% of severity (Fig.5C), and 2 meters for 50 and 75% of severity respectively (Fig. 5D).

Comparison of spatial structure for leaves and grape disease. Observed co-variogram calculated from the data of the frequency of diseased leaves from the beginning of flowering (2nd June) to the end of flowering (15 June) and the frequency of bunches with at least 25% of damage, do not match the confidence band (mean, min, max calculated from the translated

maps). This result indicates the dependence between disease data on leaves and on bunches. (Fig. 5E). This is further increased when restricted to higher levels of damage (Fig. 5F). These links between frequency maps of diseased leaves early in the season, and maps of damage on grapes at bunch closure indicate the significant effect of the date of primary infections on leaves.

Therefore, the amount of disease on leaves very early in the season governs the extent of damage on grapes. Furthermore, at bunch closure, very high a level of damage on grape bunches is restricted to the location of primary foci on leaves. This means that early detection and quantification of disease early in the season are crucial in order to forecast damage on grapes and could be used as a risk indicator in IPM strategies.

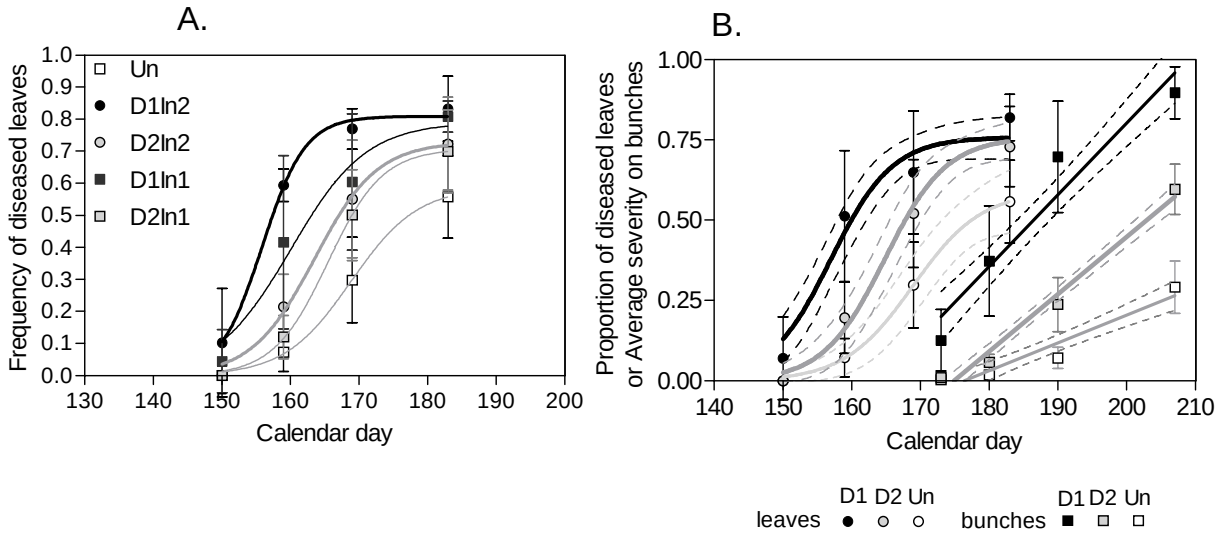

Fig. 1. Disease progress curves on the central vine of plots inoculated either the 4th of May (D1) or the 16th of May (D2) with low (In1) or high (In2) level of inoculum, or for uncontaminated plots. Each point is an average of 5 replicates, vertical bars indicate the standard deviation of the data and broken lines indicate the confidence interval for the fitted model.

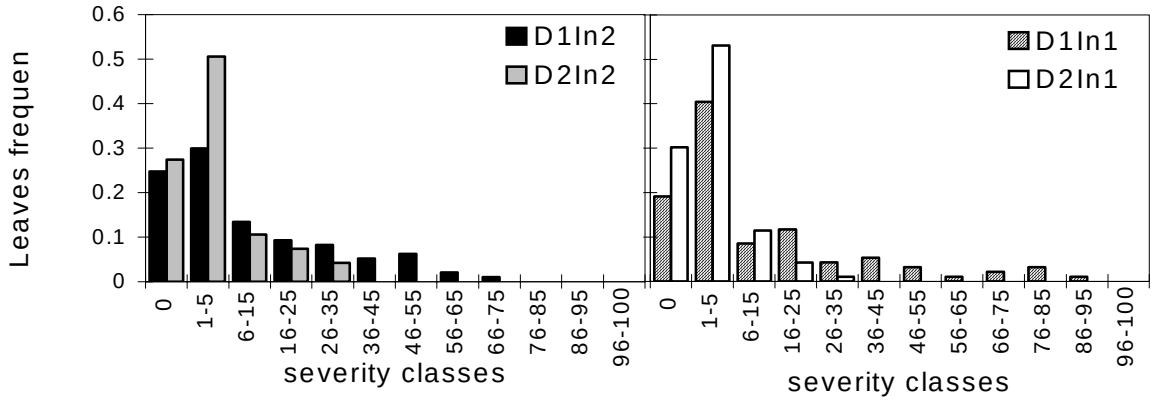

Fig. 2. Distribution of severity classes for leaves of central vines according to the different treatments.

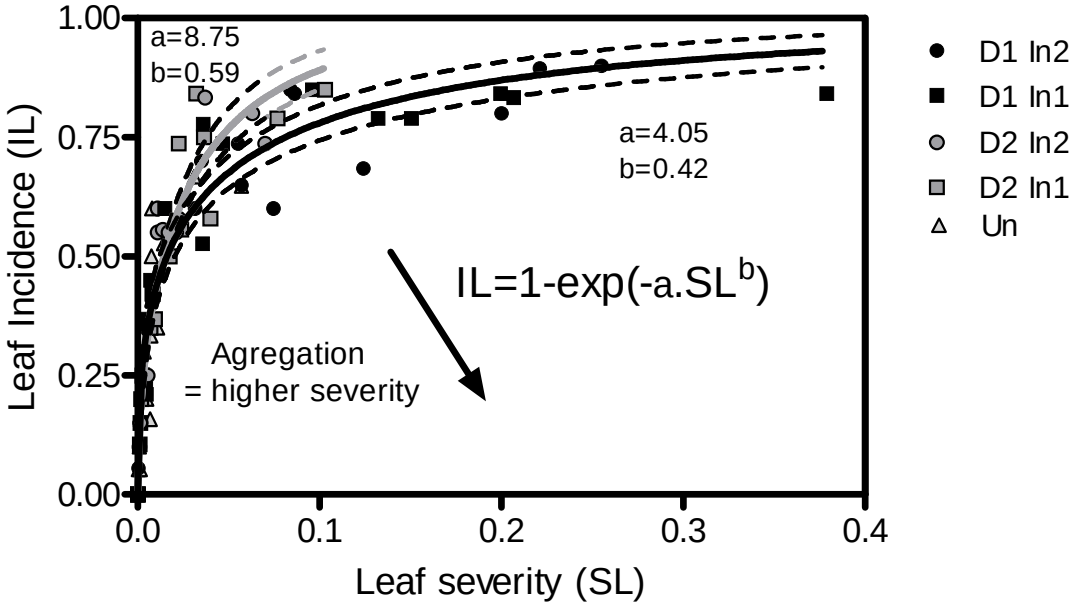

Fig. 3. Relationship between Incidence and Severity at the leaf scale for central vines according to the different treatments.

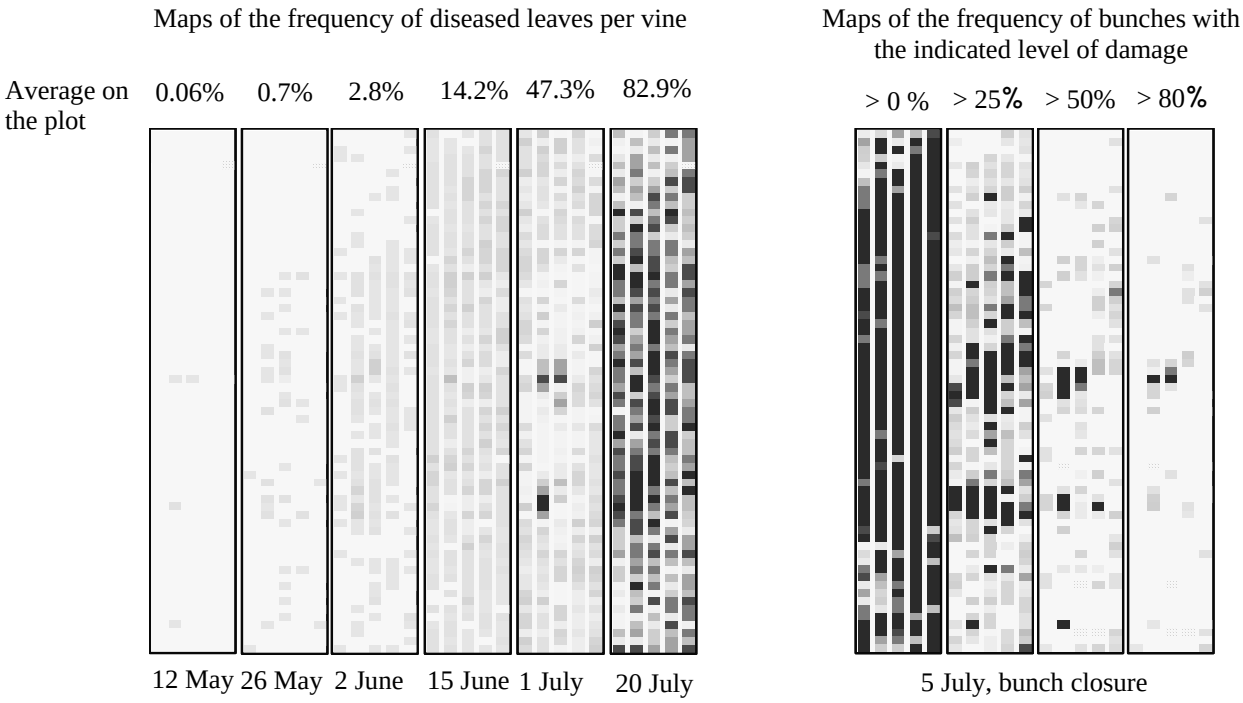

Fig. 4. Maps (5 rows x 66 vines) of the frequency of diseased leaves per vine for different scoring dates (6 maps on the left) and the frequency of diseased clusters with the indicated level of severity (4 maps on the right). Frequencies vary from 0 (white) to 1 (black).

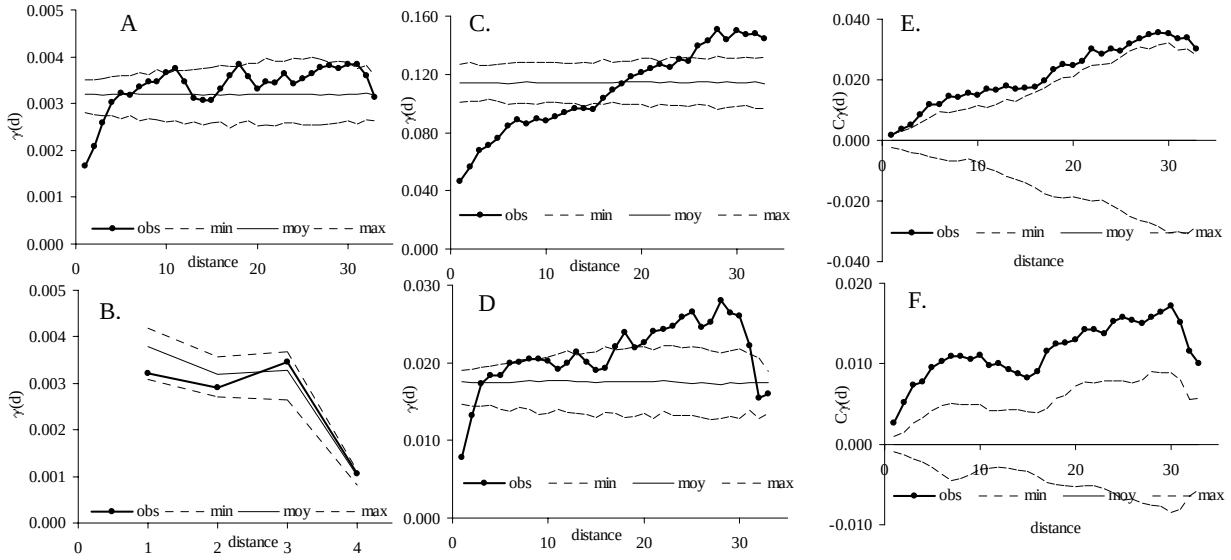

Fig. 5. Variograms on the frequency of diseased leaves (2 June) along the row (A) or between rows (B), on the frequency of diseased clusters (5 July) with at least 25% of damage (C) or 75% (D), and covariograms between the frequency of diseased leaves (2 June) and the frequency of diseased clusters (5 July) with at least 25% of damages (E) or 80% (F).

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