Analysis of Nonlinear Relationships in Dual Epidemics, and Its Application to the Management of Grapevine Downy and Powdery Mildews

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ABSTRACT

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Dual epidemics are defined as epidemics developing on two or several plant organs in the course of a cropping season. Agricultural pathosystems where such epidemics develop are often very important, because the harvestable part is one of the organs affected. These epidemics also are often difficult to manage, because the linkage between epidemiological components occurring on different organs is poorly understood, and because prediction of the risk toward the harvestable organs is difficult. In the case of downy mildew (DM) and powdery mildew (PM)

of grapevine, nonlinear modeling and logistic regression indicated nonlinearity in the foliage-cluster relationships. Nonlinear modeling enabled the parameterization of a transmission coefficient that numerically links the two components, leaves and clusters, in DM and PM epidemics. Logistic regression analysis yielded a series of probabilistic models that enabled predicting preset levels of cluster infection risks based on DM and PM severities on the foliage at successive crop stages. The usefulness of this framework for tactical decision-making for disease control is discussed.

Additional keywords: *Erysiphe necator*, injury threshold, odds ratio, *Plasmopara viticola*.

Powdery (*Erysiphe necator*) and downy (*Plasmopara viticola*) mildews remain key diseases of grape, both because of their prevalence and because of the quantitative and qualitative damage they cause (4,7,11,12,15,21,23,31). In spite of continuous research, little progress has so far been achieved in terms of deployment of durable management methods for these diseases. In France, for instance, the third largest pesticide user in the world and the first pesticide market in Europe, 3% of the agricultural area is devoted to grapevine, which consumes 20% of the national pesticide inputs, 80% of which consists of fungicides (3). This is because, very often, fungicides are applied on a crop stage or calendar basis, regardless of the actual risk that diseases may pose to crop performances.

In many instances, the main objective of grapevine protection against downy mildew (DM) and powdery mildew (PM) is to protect grape clusters and ensure harvest quality. Here, we develop approaches to link the injury caused by diseases on clusters with disease progress on the foliage. The former represents a main target of protection whereas the latter may be used as a risk indicator.

The purpose of this article is twofold: first, to characterize and parameterize statistical relationships, if existing, between disease severity on the foliage and clusters for both diseases and, second, to test the hypothesis that tactical management of disease severity on clusters may be grounded on measurements of severity on the foliage. Achieving progress on the second objective would repre-

sent a step toward a sound epidemiological or statistical framework for management of these diseases in grapevine, possibly with considerable benefits for the profession, the growers, and the environment.

MATERIALS AND METHODS

Vineyard sites. The study was conducted in the 2006 and 2007 growing seasons at two experimental vineyards, Couhins and Latresne, which are located near Bordeaux and are ≈ 7 km apart. At both sites, the experiments were established on a *Vitis vinifera* Cabernet-franc cultivar and had a double Guyot training system. Spacing of vines between and within rows was 1.80 and 1.10 m, respectively, at both sites. Vines in Couhins were planted in 1982, whereas those in Latresne were planted in 2003. Both sites were equipped with standard weather stations located <300 m from the experimental areas, enabling the daily collection of minimum and maximum temperature and rainfall data.

Experimental design and disease management levels. At each site, the experimental design consisted of three disease management levels: unprotected, limited disease management, and continuous disease protection. These treatments were arranged according to a randomized complete block design with six replications. At both sites, the experimental area was surrounded by cultivated vine with conventional management. Each of the 18 individual plots at each site was 100 m² in area and consisted of five rows of 10 plants per row on which one of the three management levels was evenly applied.

Crop stages were weekly monitored according to the scale devised by Lancashire et al. (24). Whereas no fungicide treatment was applied in the unprotected plots, fungicides against DM and PM were applied according to cluster development in the limited

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disease management plots. Fungicides against DM (copper hydroxide, and fosetyl-aluminum with folpel) were applied three times, from crop stage 66 (60% of flower hoods shed) to crop stage 81 (beginning of ripening). Fungicides against PM (tebuconazole and trifloxistrobin) were also applied three times from crop stage 55 (flowers closely pressed together) to crop stage 73 (berries at 30% final size). An insecticide (*Bacillus thuringiensis*) against grape berry moth was applied once at crop stage 75 (pea-sized berries, or clusters starting to tilt downward). In the continuous disease protection treatment, pesticides were applied according to a calendar-based schedule during the growing season. This type of protection corresponds to conventional practices in the Bordeaux area (2). Fungicides against DM (copper hydroxide, cymoxanil with mancozeb, and fosetyl-aluminum with folpel) and PM (quinoxifen, spiroxamin, tebuconazole, and wettable sulfur) were applied nine times each, from crop stage 16 (sixth leaf unfolded) to crop stage 83 (majority of berries touching). An insecticide (lambda-cyhalothrin) against grape berry moth was applied once at crop stage 75 (berries pea sized), and an application against Botrytis mold (pyrimethanil) was applied once at crop stage 85 (berry maturation).

Field observations, sampling design, and disease assessment. All observations were taken on six vines located at the center of each plot; that is, at least two rows (across rows) and two vines (along rows) away from the edge of the plot. Observations, including nondestructive visual disease assessment, were thus taken on vines that were at least four rows (across rows) or four vines (along rows) away from the nearest observed vines belonging to a different experimental plot. Plants were assessed at seven crop stages, from crop stage 53 (inflorescences clearly visible) to crop stage 89 (ripe berries). These crop stages are later referred to as crop stages 1 to 7 and are given in Table 1. On each of the six observed vines, one primary shoot (growing from buds located on the canes) and one secondary shoot (shoots growing from buds located on the vine trunk, at the basis of primary shoots, or on primary shoots below the pruning zone) were chosen at random on each plant at the first assessment, and were assessed at the six following observation dates during each growing season. Three leaves were assessed on each shoot and at each assessment date: the third, fifth, and last leaf, starting from the apex of the shoot. The leaves that were observed varied from one observation to the next, thus generating a dynamic measurement of diseases as foliation, defoliation, and training occurred. Five clusters were also randomly chosen and assessed on each observed plant.

DM and PM severities on the foliage and on clusters were assessed as the percentage of tissue area covered by lesions, starting with a 1% grade and at 5% steps onward: 0, 1, 5, 10%.... Crop growth data (number of shoots, number of leaves per shoot,

leaf area, number of clusters per plant, cluster size, and number of berries per cluster) were measured on one representative plant per individual plot among the six vines where disease assessments were made.

Steps for data analysis. The dynamics of severities of the two diseases on both the foliage and clusters were first characterized graphically at the two sites in relation to weather variation. Mixed-model analyses of variance (ANOVAs) were then used to partition the sources of variation of overall epidemics, as well as of severities at successive observation dates (crop stages). Relationships between disease severities on the foliage and on clusters were then explored graphically for each disease. This led to nonlinear model analysis in order to assess and parameterize relationships between variation in disease severities on the foliage and on clusters for both DM and PM. In the last stage, probabilistic relationships between disease severities on grape and foliage were further analyzed using logistic regression at different thresholds of disease severity on clusters and at different crop stages.

ANOVAs with repeated measures. The effects of disease management levels, crop stage, and their interaction on leaf and cluster disease severity were assessed for both diseases, for each year by site combination, with a linear mixed-model analysis for repeated measures, using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). In this analysis, the level of disease management was considered to be a fixed effect, while the crop stage when assessments were made was considered to be a fixed, repeated effect and block was considered to have a random effect. Severity data were arc-sine (arc sine [severity]^{1/2}) transformed prior to analysis. In each analysis, several structures of the variance-covariance matrix of residuals were considered (26,29,33): compound symmetry (CS), with a constant variance and a constant covariance; heterogeneous CS (CSH) (i.e., with different variances on the diagonal of the matrix); first-order autoregressive (AR[1]) (i.e., appropriate when correlation between residuals declines as the time lag increases); heterogeneous AR(1) (ARH[1]); spatial power (SP[POW]) (days), which can be more appropriate than AR(1) when the periods between successive assessments are not equal (Madden and Esker, *personal communication*); Toeplitz (TOEP), which can be viewed as corresponding to an autoregressive structure of order 7 (number of assessments); heterogeneous TOEP (TOEPH); and unstructured (UN) (i.e., all variances and all covariances differ from each other). For each analysis, the model selected corresponded to the covariance-variance structure providing the lowest akaike information criterion (29).

ANOVAs on the overall levels of diseases. The effects of site, year, disease management, and their interactions on the areas under progress curves (AUPCs) (5) of disease severity on leaves

TABLE 1. List of variables used

Acronym ^a	Meaning
AUPCDV	Area under progress curve of downy mildew severity on leaves
AUPCPV	Area under progress curve of powdery mildew severity on leaves
DVi	Downy mildew severity on leaves at crop stage i
DC	Downy mildew severity on clusters
PVi	Powdery mildew severity on leaves at crop stage i
PC	Powdery mildew severity on clusters
Crop stage 1	Crop stage at assessment 1: 52–53: inflorescences clearly visible
Crop stage 2	Crop stage at assessment 2: 55–57: flowers separating
Crop stage 3	Crop stage at assessment 3: 73–75: berries pea-sized
Crop stage 4	Crop stage at assessment 4: 76–77: berries beginning to touch
Crop stage 5	Crop stage at assessment 5: 77–78: majority of berries touching
Crop stage 6	Crop stage at assessment 6: 83–85: softening of berries
Crop stage 7	Crop stage at assessment 7: 88–89: ripe berries
MAXDC	Maximum downy mildew severity on clusters over the season
MAXPC	Maximum powdery mildew severity on clusters over the season

^a Crop stages according to Kennelly et al. (21).

and clusters were analyzed for DM and PM. This was done using a mixed model, where blocks were considered as random effects and the other effects as fixed effects. The analysis corresponded to a split-split-plot design, with site as main unit, disease management level as a subunit, and year as a sub-subunit.

Nonlinear models for disease severities on leaves and clusters. This part of the analysis of field data had two objectives, the first one being to describe quantitatively epidemics on the foliage and on the clusters for both diseases and the second, more important, one being to test the relationship between dual epidemics (foliage-clusters) for both DM and PM.

The simplest possible way to model the dynamics of both diseases on leaves and clusters was sought, using as few parameters as possible. Lagged-regression or SEIR-type models were not considered, because of their comparative complexity, and also because the time-step between observations on the foliage and on clusters was much larger than the duration of latency periods in PM (8) and DM (13).

The modeling analysis was performed in two stages: first, epidemics on leaves were modeled using a logistic equation; second, epidemics on clusters were modeled using a differential equation, where disease increase on clusters intensifies with disease on the foliage and is limited by the amount of disease already present on clusters. Epidemics rarely occurred in the continuously protected treatment and, thus, were not included in this analysis. Therefore, nonlinear modeling analysis was applied to a total of 16 cases, corresponding to two diseases (DM and PM) at two sites over 2 years and under two protection levels.

Nonlinear modeling of disease severity progress on leaves. The logistic model tested was (5,25,36):

$$x_L(t) = 1 / \{1 + [(1 - x_0)/x_0] \times \exp(-r \times t)\} \quad (1)$$

where t is time in number of days from the first assessment and x_L is disease severity on leaves. The parameters to estimate are x_0 and r , where x_0 is disease severity (DM or PM) at $t = 0$ and r is the apparent rate of infection (36). Equation 1 assumes strict increase in x_L over time. Observed data indicated that this is a simplification, as in many epidemics (32). Disease intensities often showed a trend to taper off at the end of the season, and even sometimes declined when the last assessment was taken. This decline in DM and PM of grapevine may be attributed to disappearance of lesions as leaves are shed (DM) or a reduction of symptom visibility as lesions become older (PM). The last assessment was not included in the logistic model parameterization for two reasons. The first is related to the increasing ontogenic resistance of clusters to both diseases; therefore, disease (DM and PM) severity on clusters cannot be attributable to disease on the foliage toward the end of the season (6,8–10,21) (see also below). A second reason is that accounting for the last foliage observation could overestimate the possible foliage-cluster relationship. This is because including the last observation, with sometimes a decline, would tend to underestimate the overall rate of disease increase on the foliage throughout the season. For each analysis, mean DM and PM severities on leaves for each individual plot, and for the first six assessments only (i.e., six data per observation date for six dates), were used to fit the model and, therefore, a total of 36 data points was used for model fitting.

It was also assumed that protection over a cropping season had no effect on x_0 . Therefore, the value for x_0 estimated in each unprotected management level at a given site in a given year was retained in the corresponding limited management level, and only r was estimated. The logistic model was tested using the NLIN procedure of SAS (SAS Institute Inc.). The model fit was assessed using a pseudo- R^2 value computed as $1 - (\text{SSR}/\text{SST})$, where SSR is the sum of squares for residuals and SST is the total sum of squares (5,26,33).

Nonlinear modeling of disease severity progress on clusters. The model tested was

$$dx_C/dt = tc \times x_L \times (1 - x_C) \quad (2)$$

where x_C is disease severity (DM or PM) on clusters; t is time in number of days from the first assessment; x_L is the current disease severity on leaves (computed from the logistic model fitted on leaf severity progress curves); and the parameter to be estimated, tc , is the transfer coefficient between leaves and clusters. The parameter tc may be seen as the increase of disease severity on clusters per unit disease severity on leaves per day. Thus, this parameter represents a numerical link between disease severity on leaves and the increase in disease severity on clusters. Disease increase on clusters is further assumed to occur at specific crop stages only: disease increase takes place only when clusters have been formed, and disease increase does not occur at late crop stages, due to decreasing susceptibility of maturing clusters in both DM (21) and PM (6,8–10). This was translated in the model by setting tc to zero before crop stage 2 and after crop stage 5; tc being a constant parameter, to be optimized, between crop stages 2 and 5.

In each of the 16 analyses, x_L values estimated from the logistic model fitted in the previous step (severity on leaves) were used as input to the second equation. Because these x_L values corresponded to the average behavior of epidemics over six replicates, the model for severity on clusters (equation 2) was fitted using the disease severity on clusters over time averaged over the six individual plots representing replicates of a management level in a given year–site combination. Thus, seven data points were used to fit the model for each combination.

As for leaf severities, a decrease in DM and PM severities on clusters often occurred after crop stage 5, due to shedding of infected berries (DM) or to less apparent symptoms (PM). Therefore, the maximum observed severity was considered to reflect the terminal severity. The model was fitted using the MODEL procedure of SAS (SAS Institute Inc.). The model fit was assessed using the adjusted R^2 , computed by the program, and the parameter to estimate, tc , was tested for its significance with a t test.

Logistic regressions of maximum cluster severities on leaf severities at successive crop stages. The binary logistic regression models tested have the shape (1,16):

$$\text{Logit}[P(Y = 1)] = a + b \times SFi \quad (3)$$

where Y is the binary response variable, which equals 1 if the maximum severity on clusters over the growing season is larger than a given threshold and zero otherwise; a is the intercept parameter; b is the slope parameter; and SFi is the severity on foliage at crop stage i . Logistic regressions were performed for both PM and DM for five maximum cluster severity thresholds (0, 0.1, 1, 5, and 10%) and with seven predictors, representing DM or PM severities on the foliage at the successive assessment dates. Thus, 70 logistic regression analyses in total were performed, using individual plot data (i.e., the 72 combinations of years, sites, management levels, and replications).

The likelihood ratio and its associated probability provided an overall criterion of models' suitability, and the intercepts and slopes of each model were tested for their significance based on a t test (1,16). The odds ratios together with their 95% upper and lower bounds (34) provided a measure of the (foliage severity-based) risk of disease exceeding one of the preset threshold levels on clusters. The predictive ability of the models was assessed with Somer's D rank correlation, which is computed from the number of concordant, discordant, and tied pairs of observed and predicted values (16).

RESULTS

Weather variation among sites and years. Year 2006 (average minimum and maximum temperature from day 100 to 300 = 12.4 and 25.4°C and 12.9 and 25.0°C at Couhins and Latresne, respectively) was, on average, warmer than 2007, (average minimum and maximum temperature = 12.1 and 23.6°C and 11.4 and 23.2°C at Couhins and Latresne, respectively). There were few differences between the two sites in a given year with respect to maximum or minimum temperatures (Fig. 1). By contrast, there were differences in rainfall patterns between the two sites and years. Total rainfall in Couhins and Latresne in 2006 was 970 and 716 mm, respectively, and 485 and 950 mm in 2007. Rainfall events were more frequent in 2007 than in 2006. When comparing sites, differences were relatively small in 2006, with fewer rainfall events in Couhins than in Latresne before Julian day = 150, with more frequent, often less intense, rain in Latresne than in Couhins later on. Differences in rainfall patterns were quite strong in 2007, with much more frequent and stronger rainfall events in Latresne than in Couhins. Thus the combination of sites and years resulted in a range of climatic conditions, from comparatively dry and cool (especially early in the season; Couhins 2006) to humid and warm (Latresne 2007).

Variation in progress of diseases over sites and years. There were large differences in the variation in severity of both diseases between sites and years, as indicated by Figure 2. Considering disease severities on the foliage in the unprotected plots (Fig. 2A1 and C1), one may distinguish overall contexts of strong DM (severity ranges: 35 to 48, 36 to 69, and 36 to 61%) combined with weak to moderate PM (severity ranges: 0.5 to 15, 0.9 to 28, and 2 to 14%) epidemics (in Couhins 2006, Couhins 2007, and Latresne 2007, respectively), and contexts of moderate DM (severity range: 27 to 36%) and comparatively strong PM (severity range: 4 to 34%) epidemics (Latresne 2006). However, there were large variations in epidemics onset, rates of disease increase (and possible decrease), and maximum severities within these broad groups.

In 2006, epidemics of DM on the foliage (Fig. 2A1) developed relatively late in the growing season but increased rapidly in the unprotected plots, especially at Couhins. In 2007, DM epidemics

on the foliage were initiated earlier at both sites, with rates of increase that did not exceed those observed in 2006. Compared with unprotected plots, DM epidemics on the foliage were strongly reduced in plots with limited disease management (Fig. 2A2) and almost completely suppressed under continuous disease protection (Fig. 2A3).

The corresponding variation in DM severity on clusters suggested complex, nonlinear relationships with foliar severities. Although both Couhins and Latresne experienced limited severities on clusters in 2006 (Fig. 2, B1, upper two graphs), disease severities at both sites approached 100% in 2007 where no fungicide protection was ensured (Fig. 2, B1, lower two graphs). In spite of variation among plots at the same site, DM severity on clusters appeared to develop earlier and faster and reach higher terminal severities in Latresne than in Couhins in 2007. In 2006, both the limited and systematic levels of fungicide protection yielded a near-complete control of DM on clusters (Fig. 2, B2 and B3, upper graphs). It was not so in 2007, where limited fungicide protection resulted in often very large and variable DM severities on clusters in both Couhins (30 to 70%) and Latresne (20 to 90%). In that year, systematic fungicide coverage resulted in a near-complete disease exclusion on clusters in Couhins but did not prevent DM severity on clusters to exceed 10% in some plots of Latresne (Fig. 2, B3).

PM epidemics developed on the foliage at both sites and both years in the unprotected plots (Fig. 2, C1). PM epidemics were initiated earlier in 2006 than in 2007, and reached particularly high levels (and variances) in Latresne. PM epidemics on the foliage were slower in 2007 than in 2006, reaching higher (but more variable) terminal levels in Couhins than in Latresne. Overall, both the continuous and limited levels of fungicide protection yielded similar results (Fig. 2, C2 and C3), with a strong but never complete suppression of PM on the foliage.

A complex, nonlinear response of PM severity on clusters to PM severity on foliage is suggested too, with strong epidemics occurring on clusters at both sites in 2006, whereas these are negligible in 2007 in the unprotected plots (Fig. 2, D1). Contrary to epidemics on the foliage, PM epidemics on clusters were almost completely suppressed by both levels of fungicide protection at both sites and in both years (Fig. 2, D2 and D3).

Fig. 1. Weather variation at two sites and in 2 years where downy and powdery mildew epidemics were monitored. Solid vertical bars: daily rainfall; continuous lines: daily minimum temperature; dashed lines: daily maximum temperature.

Repeated-measure ANOVAs of the effects of disease management level and development stage on severities of DM and PM in each year–site combination. The covariance–variance variability matrices that were mostly selected were SP(POW) and AR(1), indicating that DM and PM severities

were autocorrelated over time (crop stages) in most cases (Table 2).

Disease management levels had a significant effect on DM severity on the foliage in all cases ($P < 0.0001$). This was the case, too, for DM severity on clusters, except in Latresne 2006,

Fig. 2. Disease severity progress of downy and powdery mildew of grapevine on leaves and clusters at two different sites under three levels of disease management in 2006 and 2007. Each group of four graphs represents disease progress of grape downy or powdery mildew, on either foliage or clusters. **A**, Downy mildew severity on foliage; **B**, downy mildew severity on clusters; **C**, powdery mildew severity on foliage; **D**, powdery mildew severity on clusters. Numbers associated with letters indicate levels of disease management, from no chemical protection (1), to limited protection (2) and continuous chemical protection (3); for instance, A1 = downy mildew progress on foliage under no chemical protection, A2 = downy mildew progress on foliage under limited protection, and A3 = downy mildew progress on foliage under continuous chemical protection. Years (2006 or 2007, rows) and sites (Couhins or Latresne, columns) are indicated in each group of four graphs. Each graph shows average disease severities (means over six grapevine plants) in each plot (i.e., six data points per observation date). Abscissa: Julian days; ordinates, disease severity (%). See Table 1 for symbols. Note the change of scale range between downy mildew severities (0 to 100%, **A and B**) and powdery mildew severities (0 to 40%, **C and D**). Lines among data points are drawn using a distance-weighted least square smoothing (37).

where the probability level was slightly >0.05 ($P = 0.061$), corresponding to low severities across all protection levels (Fig. 2, B1 to B3). In general, the F values computed for the effects of management levels on PM severity on the leaves were smaller than those computed for DM leaf severity. This yielded significant effects of disease management levels on PM severity on foliage in 2006 at Couhins ($F = 4.03$, $P = 0.032$) and Latresne ($F = 46.48$, $P < 0.0001$), and to nonsignificant disease management effects in 2007 at both sites ($F = 1.20$ and 1.51 at Couhins and Latresne, respectively). This difference between years in disease management effects on PM leaf severity reflects slower, later PM epidemics on the foliage in 2007 compared with 2006 (Fig. 2, C1 to C3), combined with large variances in PM leaf severities. In spite of small numerical differences in 2007 (Fig. 2, D1 to D3), PM severities on clusters were significantly ($P < 0.02$) influenced by disease management levels in both sites and both years.

The effect of assessment date (crop stage) on both DM and PM severities on leaves or clusters was significant in all analyses ($P < 0.0003$) (Table 2), indicating that variation of the two diseases on both tissues always occurred over time. The interaction between disease management level and assessment date (M–C) on both DM and PM severities on leaves or clusters always was significant, too ($P < 0.03$), indicating that the intensity of the effect of

management level on both diseases on each tissue varied with assessment date. This was reflected by the frequently observed differences in rates of increase in DM or PM severities on leaves or clusters among management levels (Fig. 2).

Overall effects of site, year, and management level on disease epidemics (AUPCs) over the four year–site combinations. Disease management levels had a significant ($P < 0.0001$) effect on AUPCs of DM or PM severities on both types of tissues (leaves and clusters) (Table 3). These results concur with repeated-measure ANOVAs for all year–site combinations (Table 2). Year (2006 or 2007) had a significant ($P < 0.003$) effect on areas under severity progress curves in all cases: as indicated by Figure 2, DM epidemics were stronger in 2007 while PM epidemics usually were stronger in 2006. No significant site effect was detected, except for PM on leaves ($P = 0.0059$). A significant ($P < 0.0004$) management level–year interaction (M–Y) was detected in all cases, indicating that the effect of management level on severities varied with years. For example, the effect of disease management on PM severity was much stronger in 2006 than in 2007 (Fig. 2, C1 to C3 and D1 to D3). The other interactions tested (M–S, Y–S, and M–Y–S) (Table 3) had, in general, a significant effect on AUPC of severity on leaves, whereas the Fisher values for AUPCs of disease severities on clusters were smaller, with larger associated probabilities.

TABLE 2. Analyses of variance (ANOVAs) of the effect of disease management levels and crop stages at two sites and in 2 years on successive severities of downy and powdery mildew of grapevine on leaves and clusters

		Results of ANOVA						
Disease, site, year	Tissue	SCVM ^b	Management level		Crop stage		M × CS ^a	
			<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Downy mildew								
Couhins								
2006	Leaves	AR(1)	165.20	<0.0001	156.64	<0.0001	26.31	<0.0001
	Clusters	SP	26.05	<0.0001	14.23	<0.0001	16.57	<0.0001
2007	Leaves	CS	150.77	<0.0001	192.73	<0.0001	37.09	<0.0001
	Clusters	TOEP	351.70	<0.0001	555.23	<0.0001	159.96	<0.0001
Latresne								
2006	Leaves	AR(1)	97.57	<0.0001	74.85	<0.0001	34.39	<0.0001
	Clusters	CS	3.76	0.061	4.73	0.0003	4.73	<0.0001
2007	Leaves	AR(1)	158.43	<0.0001	130.57	<0.0001	22.80	<0.0001
	Clusters	AR(1)	103.59	<0.0001	88.14	<0.0001	19.29	<0.0001
Powdery mildew								
Couhins								
2006	Leaves	SP	4.03	0.032	15.68	<0.0001	8.59	<0.0001
	Clusters	TOEP	4.95	0.018	10.28	<0.0001	4.70	<0.0001
2007	Leaves	SP	1.20	0.337	43.89	<0.0001	4.92	<0.0001
	Clusters	AR(1)	8.27	0.002	9.49	<0.0001	12.51	<0.0001
Latresne								
2006	Leaves	SP	46.48	<0.0001	18.51	<0.0001	6.79	<0.0001
	Clusters	AR(1)	23.55	<0.0001	22.99	<0.0001	14.09	<0.0001
2007	Leaves	SP	1.51	0.262	29.77	<0.0001	2.16	0.022
	Clusters	AR(1)	16.12	<0.0001	9.76	<0.0001	9.02	<0.0001

^a M × CS: management level–crop stage interaction.

^b SCMV: structure of the covariance-variance matrix used in ANOVA: CS: compound symmetry; SP: spatial power; AR(1): first-order autoregressive; TOEP: Toeplitz (see text for details).

TABLE 3. Analyses of variance of the effects of year, site, and disease management level on the area under disease progress curves of grapevine downy and powdery mildew severities on leaves and clusters

Effect	Downy mildew				Powdery mildew			
	Leaves		Clusters		Leaves		Clusters	
	F	P	F	P	F	P	F	P
Management level (M)	299.47	<10 ^{−4}	206.67	<10 ^{−4}	44.74	<10 ^{−4}	16.57	<10 ^{−4}
Year (Y)	158.41	<10 ^{−4}	770.07	<10 ^{−4}	11.71	0.0018	13.6	0.0023
Site (S)	3.36	0.0769	3.07	0.092	9.06	0.0059	2.04	0.1731
M × Y	36.35	<10 ^{−4}	210.11	<10 ^{−4}	12.51	<10 ^{−4}	11.69	0.0003
M × S	2.97	0.0668	2.52	0.101	9.8	0.0007	2.08	0.1463
Y × S	26.61	<10 ^{−4}	4.74	0.0375	23.96	<10 ^{−4}	2.55	0.1321
M × Y × S	9.16	0.0008	3.59	0.0402	15.35	<10 ^{−4}	2.73	0.086

Fig. 3. Scatterplot diagrams of downy and powdery mildew severities on clusters against foliage severities at successive crop stages and against areas under foliage severity progress curves. **A to F**, Downy mildew and **G to L**, powdery mildew; **A to E** and **G to K**, severities on foliage at successive crop stages (abscissas) versus maximum severity on clusters (ordinates); **F** and **L**, areas under leaf severity progress curve (abscissas) versus maximum severity on clusters (ordinates). See Table 1 for symbols. Stages 1 and 2 have been omitted because most values were null. Filled circles: unprotected plots; shaded triangles: plots with limited fungicide protection; open squares: plots with continuous disease protection. Pearson correlation coefficients and associated probabilities are indicated in each scatterplot. Arrows indicate different patterns of foliage-cluster severity relationships (see text for details).

Relationships between disease severity on the foliage and clusters. Scatterplots (Fig. 3) linking the mean maximum severities per individual plot during an entire growth season and the severities on leaves at the successive observation dates (crop stages) were drawn for DM (Fig. 3A to E) and PM (Fig. 3G to K). Only plots from observations 3 to 7 are given, because most leaf severities at observations 1 and 2 were null for both diseases. These scatterplots indicate a shift in the foliage-cluster severities relationships as epidemics of both diseases develop.

In the case of DM, a strong association between current leaf severity and maximum cluster severity is apparent at crop stages 3 and 4 (Fig. 3A and B), with a small variation on leaves (0 to 30%) corresponding to a wide variation on clusters (0 to 100%). From crop stages 5 to 7 (Fig. 3C to E), the relationship becomes more complex as leaf severity increases, and progressively with two patterns emerging: one parabolic-shaped structure with a maximum cluster severity approaching 100%, suggesting a less-than-additive increase of severity on clusters with increasing leaf severity; and a regularly increasing, approximately linear leaf-cluster relationship, in which the levels of maximum cluster severity remain <15%, even though leaf severities may reach 50%. The former pattern was mostly associated with unprotected plots or plots with limited disease protection while the latter pattern corresponded to plots where late epidemics developed on the foliage. This evolution of patterns corresponds to that of the correlation coefficients between leaf and maximum cluster severities, initially strong (crop stages 3 and 4) when the relationship appears univocal, then weaker (crop stages 5 and 6) when late foliage epidemics were initiated, and finally higher (crop stage 7) as leaf severity increases. These patterns (parabolic-shaped increase in the unprotected and partly protected plots; regular and limited cluster increase in plots where late foliage epidemics developed) are summarized in Figure 3F, where the maximum DM severity on clusters is plotted against the area under DM severity progress curve on the foliage.

In the case of PM (Fig. 3G to L), the relationship between leaf and maximum cluster severity at successive dates generally assumed shapes that are similar to those observed for DM. A main difference was that the foliage-cluster severity relationships appeared to be fuzzier in the case of PM, with large variances of both foliage and cluster severities. However, the range of severity on the foliage was also much smaller (0 to 35%), and so was the range of severity on clusters (0 to 32%).

Nonlinear models for leaf and cluster severities. The logistic model was successfully fitted to leaf severity data in all disease-site-year-treatment combinations (Table 4) except one (PM, Latresne 2006, limited protection), where the optimization algorithm did not converge. The model usually described a large fraction of the variation in observed leaf severity over time, and yielded large F values (14 to 353), generally large R^2 (0.267 to 0.932), and small corresponding probabilities (≤ 0.0001). Inspection of R^2 values indicates that the model fit was better when describing (i) DM than PM epidemics on the foliage and (ii) for epidemics occurring in unprotected plots. Estimates for x_0 were larger in 2007 than in 2006 for DM, whereas the opposite result was obtained for PM. Estimates for the apparent infection rate (r) were 0.01 to 0.11 day⁻¹ and were in the same range for both diseases across sites and years. Estimates for r were always smaller in the limited-protection treatment compared with the unprotected treatment for both diseases.

The linked nonlinear model for severity progress on clusters (Table 5) was successfully fitted in 14 of 16 cases. The model could not be fitted in the case of PM, Latresne 2006, limited protection (because severity estimates on foliage had not been derived) and in the case of DM, Latresne 2006, limited protection (where the iterative algorithm did not converge). The model fit was very good (R^2 of 0.75 to 0.99) in the case of DM but less so

(R^2 of 0.36 to 0.95) for PM. It was also better for PM in the unprotected plots (R^2 of 0.69 to 0.95) compared with plots with limited protection (R^2 of 0.36 to 0.42). Estimates for the leaf-cluster transfer coefficient (tc) always were significant ($P < 0.0001$ to 0.05). The transfer coefficient appeared smaller under limited protection compared with epidemics in unprotected plots: when comparing unprotected to partly protected treatments, the estimated tc values were larger in the former than in the latter in five of six cases. The one case where tc was larger in the partly

protected treatment was DM, Couhins 2007, where values were numerically close (0.227 and 0.280) and not significantly different ($P < 0.05$). Table 5 further indicates that tc values were in general larger for PM (0.039 to 1.269) than DM (0.007 to 0.374).

Logistic regressions of cluster severity on leaf severity. Results of a series of logistic regression analyses, where a series of increasing threshold values for DM severity on clusters were considered at successive observation dates (crop stages), are

TABLE 4. Nonlinear model analyses of disease severity progress curves of downy and powdery mildew of grape on leaves at two sites and 2 years^a

		Statistics for model fit and parameters ^b				
Disease, site, year	Management level	<i>R</i> ²	<i>F</i>	<i>P</i>	<i>x0</i>	<i>r</i>
Downy mildew						
Couhins						
2006	Unprotected	0.932	352.89	<0.0001	2.1 × 10 ⁻³ (5.1 × 10 ⁻³)	0.052 (0.004)
	Limited	0.716	112.72	<0.0001	SV	0.039 (0.001)
2007	Unprotected	0.814	165.68	<0.0001	2.6 × 10 ⁻² (8.3 × 10 ⁻²)	0.029 (0.003)
	Limited	0.390	59.14	<0.0001	SV	0.010 (0.001)
Latresne						
2006	Unprotected	0.778	81.45	<0.0001	3.3 × 10 ⁻⁴ (2.7 × 10 ⁻⁴)	0.062 (0.011)
	Limited	0.343	23.45	<0.0001	SV	0.031 (0.002)
2007	Unprotected	0.802	168.37	<0.0001	2.6 × 10 ⁻² (8.3 × 10 ⁻²)	0.028 (0.003)
	Limited	0.546	101.22	<0.0001	SV	0.018 (0.001)
Powdery mildew						
Couhins						
2006	Unprotected	0.594	32.62	<0.0001	3.8 × 10 ⁻⁵ (1.4 × 10 ⁻⁵)	0.068 (0.025)
	Limited	0.444	34.47	<0.0001	SV	0.049 (0.001)
2007	Unprotected	0.753	63.19	<0.0001	1.9 × 10 ⁻⁷ (2.4 × 10 ⁻⁸)	0.109 (0.071)
	Limited	0.410	29.89	<0.0001	SV	0.076 (0.002)
Latresne						
2006	Unprotected	0.522	41.72	<0.0001	1.1 × 10 ⁻² (2.0 × 10 ⁻²)	0.025 (0.006)
	Limited	NC	...	...	...	...
2007	Unprotected	0.414	14.74	<0.0001	7.3 × 10 ⁻⁶ (1.7 × 10 ⁻⁶)	0.070 (0.041)
	Limited	0.267	18.58	0.0001	SV	0.041 (0.002)

^a The model tested was: $x_L(t) = 1 / \{1 + [(1 - x_0)/x_0] \times \exp(-r \times t)\}$, where t is time in number of days from the first assessment and x_L is disease severity on leaves. The estimated parameters are x_0 and r , where x_0 is disease severity at $t = 0$ and r is the apparent rate of infection.

^b Parameter estimates are followed by their standard error in parentheses. NC = the iterative algorithm did not converge and SV = set value: the parameter estimated in the unprotected management level in the same site-year was retained to estimate r in the limited management level.

TABLE 5. Nonlinear model analyses of disease severity progress curves of downy and powdery mildew of grape on clusters at two sites and 2 years^a

Disease, site, year	Management level	Model R^2	Statistics for tc parameter ^b		
			Estimate ^c	t test	$P > t $
Downy mildew					
Couhins					
2006	Unprotected	0.938	0.029 (0.0021)	13.88	<0.0001
	Limited	0.755	0.007 (0.0008)	8.87	0.0001
2007	Unprotected	0.972	0.227 (0.0175)	12.95	<0.0001
	Limited	0.979	0.280 (0.0133)	21.11	<0.0001
Latresne					
2006	Unprotected	0.897	0.008 (0.0008)	10.01	<0.0001
	Limited	NC	...	...	...
2007	Unprotected	0.996	0.374 (0.0143)	26.14	<0.0001
	Limited	0.989	0.189 (0.0061)	30.81	<0.0001
Powdery mildew					
Couhins					
2006	Unprotected	0.953	0.538 (0.0345)	16.63	<0.0001
	Limited	0.420	0.039 (0.0132)	2.93	0.0261
2007	Unprotected	0.730	1.269 (0.2366)	5.37	0.0017
	Limited	0.367	0.518 (0.2065)	2.51	0.0459
Latresne					
2006	Unprotected	0.913	0.050 (0.0045)	11.14	<0.0001
	Limited	...	...	...	...
2007	Unprotected	0.689	0.361 (0.0716)	5.04	0.0023
	Limited	0.356	0.191 (0.0757)	2.53	0.0449

^a The model tested was: $dx_C/dt = tc \times x_L \times (1 - x_C)$, where t is time in number of days from the first assessment; x_L is disease severity on leaves (computed from the nonlinear model fitted on leaf severity progress curves, Table 4); and x_C is disease severity on clusters; the parameter estimated is tc , which is the transfer coefficient between crop stages 2 and 5 (tc was set to zero for all other crop stages). NC = the iterative algorithm did not converge and – = the model was not tested because no logistic model for x_L could be determined from nonlinear regression (Table 4).

^b Statistics pertaining to the parameter tc .

^c Estimates for the tc values are followed by their standard error in parentheses.

shown in Table 6. Useable results were obtained, depending on the threshold and the crop stage.

When considering a threshold DM severity on clusters of 0%, a logistic relationship with a significant likelihood ratio (28.5, $P < 0.001$), a high Sommer's D value (0.76), and slope (2.52, standard error [SE] = 1.20) with a significant t value (2.09, $P = 0.036$) was found at crop stage 4. At earlier crop stages for this threshold of 0%, no useable relationship were found, because of absence of disease on the foliage (crop stage 1), nonsignificant ($P = 0.264$) likelihood ratio (crop stage 2), or slope with a large standard error, small t value (1.62), and large associated probability ($P = 0.104$; crop stage 3). With crop stage increasing from 4 to 7 (and the corresponding increase in DM severity range on the foliage), significant logistic equations were identified (likelihood ratios with $P < 0.001$ and large Sommer's D values), with significant ($P \leq 0.03$) slope values (i.e., foliage severity parameters). A decline in slope values was also observed, together with a narrowing of SE of estimates.

The odds ratio represents the multiplicative factor by which the odds change when the independent variable increases by one unit (34). In the fourth equation (crop stage 4) for threshold 0%, each

percentage point of DM leaf severity increases the odds of being above the threshold (in this case, of DM appearing on clusters) by a multiplicative factor of 12.421, with upper and lower 95% confidence bounds of 131.237 and 1.176, respectively. Because the lower bound is >1 , DM on the foliage appears to be a genuine risk factor of disease developing on clusters at a severity above the considered threshold. Equations for a DM threshold of 0% on clusters indicate a decrease in odds ratio with increasing crop stage (and of range of DM severity on the foliage), and a narrowing of the lower and upper bounds of the odds ratio.

These observations can be expanded to the rest of Table 6 for the successive thresholds considered. One main, overall result from Table 6 is that satisfactory regressions were derived at earlier crop stage when higher thresholds were considered: whereas useable regressions were derived at crop stage 4 for threshold values of 0, 0.1, and 1%, satisfactory regressions were obtained at crop stage 3 for thresholds of 5 and 10%.

An overview of the variation of the odds ratios with varying thresholds for cluster severity and with leaf severity at successive crop stages is provided in Figure 4. For DM, the odds ratios decreased sharply as crop stage increased (i.e., as epidemics, if

TABLE 6. Downy mildew: logistic regression analyses of the relations between leaf and cluster severities at successive crop stages and thresholds

Val, crop ^a	Range ^b	Likelihood		Som D ^f	Intercept ^c				Slope ^c				Odds (95%) ^d		
		Ratio ^e	<i>P</i> (χ ²)		Est	SE	<i>t</i>	<i>P</i>	Est	SE	<i>t</i>	<i>P</i>	Ratio	Max	Min
0%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0–0.4	1.246 ^g	0.264	0.038	0.987	0.269	3.674	<0.001	54.258	1.717	31.607	<0.001	3.7 × 10 ²³	1.1 × 10 ²²	1.3 × 10 ²²
3	0–27.7	25.598	<0.001	0.646	0.039	0.334	0.116	0.908	41.988	25.845	1.625	0.104	1.7 × 10 ¹⁸	n.a.	0
4	0–23.5	28.528	<0.001	0.762	–0.198	0.363	–0.546	0.585	2.519	1.203	2.094	0.036	12.421	131.237	1.176
5	0–34.6	32.612	<0.001	0.810	–0.428	0.391	–1.095	0.274	1.322	0.585	2.258	0.024	3.751	11.812	1.191
6	0–48.7	26.228	<0.001	0.755	–0.460	0.413	–1.113	0.266	0.176	0.059	2.988	0.003	1.192	1.338	1.062
7	0.2–69.2	29.943	<0.001	0.737	–0.644	0.435	–1.481	0.139	0.173	0.060	2.905	0.004	1.189	1.337	1.058
0.1%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0–0.4	1.650 ^g	<0.001	0.042	0.651	0.252	2.584	0.010	55.525	1.681	33.030	<0.001	1.3 × 10 ²⁴	3.5 × 10 ²⁵	4.8 × 10 ²²
3	0–27.7	29.420	<0.001	0.635	–0.239	0.324	–0.739	0.460	13.641	12.789	1.067	0.286	8.3 × 10 ⁵	6.5 × 10 ¹⁶	0
4	0–23.5	36.860	<0.001	0.803	–0.691	0.372	–1.856	0.063	2.342	0.956	2.448	0.014	10.398	67.779	1.595
5	0–34.6	37.594	<0.001	0.839	–0.791	0.386	–2.048	0.041	0.902	0.332	2.716	0.007	2.463	4.721	1.285
6	0–48.7	28.089	<0.001	0.695	–0.766	0.399	–1.922	0.055	0.141	0.041	3.457	0.001	1.152	1.247	1.063
7	0.2–69.2	33.832	<0.001	0.771	–1.002	0.423	–2.370	0.018	0.142	0.040	3.524	<0.001	1.153	1.248	1.065
1%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0–0.4	2.501	0.114	0.051	0.114	0.239	0.478	0.633	57.538	4.7 × 10 ³	0.012	0.990	9.7 × 10 ²⁴	2.5 × 10 ²⁶	3.8 × 10 ²³
3	0–27.7	49.118	<0.001	0.795	–1.278	0.392	–3.261	0.001	27.073	16.118	1.680	0.093	5.7 × 10 ¹¹	3.0 × 10 ²⁵	0.011
4	0–23.5	62.286	<0.001	0.916	–2.110	0.534	–3.951	<0.001	3.374	1.085	3.109	0.002	29.200	245.35	3.480
5	0–34.6	36.542	<0.001	0.818	–1.161	0.374	–3.109	0.002	0.379	0.116	3.269	0.001	1.461	1.833	1.164
6	0–48.7	27.765	<0.001	0.667	–1.275	0.403	–3.160	0.002	0.106	0.026	4.098	<0.001	1.112	1.170	1.057
7	0.2–69.2	34.454	<0.001	0.733	–1.549	0.441	–3.515	<0.001	0.105	0.025	4.267	<0.001	1.111	1.166	1.059
5%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0–0.4	3.72 ^g	0.054	0.069	–0.465	0.246	–1.895	0.058	59.704	1.672	35.705	<0.001	8.5 × 10 ²⁵	2.3 × 10 ²⁷	3.2 × 10 ²⁴
3	0–27.7	69.785	<0.001	0.942	–2.575	0.566	–4.551	<0.001	5.789	2.341	2.473	0.013	326.752	3.2 × 10 ⁵	3.323
4	0–23.5	45.603	<0.001	0.913	–1.941	0.435	–4.459	<0.001	0.600	0.177	3.390	0.001	1.821	2.576	1.288
5	0–34.6	23.653	<0.001	0.741	–1.406	0.364	–3.859	<0.001	0.164	0.045	3.607	<0.001	1.178	1.287	1.078
6	0–48.7	11.144	0.001	0.513	–1.314	0.397	–3.312	0.001	0.055	0.018	3.145	0.002	1.057	1.094	1.021
7	0.2–69.2	26.929	<0.001	0.694	–2.007	0.478	–4.196	<0.001	0.078	0.018	4.221	<0.001	1.081	1.121	1.043
10%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0–0.4	0.333	0.564	0.016	–0.535	0.248	–2.160	0.031	2.655	4.640	0.572	0.567	14.221	1.3 × 10 ⁵	0.002
3	0–27.7	69.825	<0.001	0.939	–2.878	0.639	–4.505	<0.001	4.024	1.271	3.166	0.002	55.919	675.178	4.631
4	0–23.5	43.748	<0.001	0.898	–2.031	0.440	–4.617	<0.001	0.499	0.140	3.578	<0.001	1.648	2.166	1.253
5	0–34.6	21.633	<0.001	0.731	–1.485	0.368	–4.036	<0.001	0.147	0.041	3.591	<0.001	1.159	1.256	1.069
6	0–48.7	9.935	0.002	0.505	–1.397	0.405	–3.452	0.001	0.052	0.017	2.999	0.003	1.054	1.090	1.018
7	0.2–69.2	25.134	<0.001	0.695	–2.100	0.489	–4.299	<0.001	0.074	0.018	4.149	<0.001	1.077	1.115	1.040

^a Threshold value and crop stage.

^b Leaf severity range.

^c Est = estimate and SE = standard error.

^d Maximum (Max) and minimum (Min); n.a. = value too large.

^e NC = not computed.

^f Sommer's D.

^g Covariance matrix quasi-maximum likelihood adjusted.

any, develop). As threshold levels become smaller, the odds ratios become simultaneously larger (Fig. 4) and are attached to larger uncertainty (i.e., to broader 95% confidence bounds) (Table 6).

In the case of PM (Table 7), no logistic model was found for a 0% threshold, which had (i) a significant likelihood ratio, (ii) a minimum odds ratio >1, and (iii) a significant *t* value attached to the model's slope estimate. However, for thresholds of $\geq 0.1\%$, satisfactory models were found. These three criteria were met at crop stage 6 for a 0.1% threshold and at crop stage 3 for thresholds set at 1, 5, and 10%. Similarly to DM, logistic models for PM showed a decrease in slope value, a decrease in odds ratios, and a narrowing between the 95% upper and lower bound values about the odds ratios as crop stage (observation date) increased. The relationships between the odds ratio with both crop stage and threshold level (Fig. 4) resembled those obtained for DM. However, the odds ratios for PM tended to have a much greater value than for DM.

DISCUSSION

Characterization of grape DM and PM epidemics generated within the experimental design. A wide range of grape DM and PM epidemics were generated by the three management levels used at two sites and in 2 years. A review over a 10-year period (1995 to 2005) of the bulletins of the French National Plant Protection warning system in the Aquitaine province (S. Savary, *unpublished*) indicates that the levels that may occur in unprotected, reference farmers' plots are well within the range of (minimum and maximum) DM and PM severities observed in this experiment. Mixed-model ANOVAs indicated that the observed variation in foliage and cluster severities (Fig. 2) indeed reflected significant effects of management levels, years, and sites at successive dates (Table 2) and throughout seasons (Table 3). In addition to disease levels, variation occurred with respect to (i) the shape of disease progress, from exponential (e.g., PM 2007 on leaves) (Fig. 2C1, lower graphs), sigmoid (e.g., DM on clusters 2007 in the unprotected plots) (Fig. 2B1, lower graphs), and sigmoid with decline (e.g., PM on foliage 2006 in the unprotected plots) (Fig. 2C1, upper graphs); (ii) the rates of disease increase (e.g., DM on foliage in 2007) (Fig. 2A1 to A3, lower graphs); as well as (iii) the onset time of disease on the foliage (early DM epidemics in 2007 versus late epidemics in 2006 [Fig. 2A1] and early PM epidemics in 2006 versus late epidemics in 2007 [Fig. 2C1]).

For both DM and PM, large variations within replicates in severities on the foliage and clusters were observed (Fig. 2). The origin of such variation is beyond the scope of the present study but suggests dynamics that were inherent to individual plots and small interplot interferences (20). This variability was exploited when performing logistic regression analyses, where individual plots were considered.

Management level, year, and their interactions (Tables 2 and 3) were the most important sources of variation of severities of the two diseases. The effect of year can be mainly associated with differences between weather patterns (Fig. 1), particularly rainfall, observed between 2006 and 2007 (8,15,23). The year 2006 was drier than 2007 and, thus, more favorable to PM and less favorable to DM than 2007; conversely, 2007 had recurrent rainfalls, favoring DM and not PM. The significant effect of management level on disease severity in most instances is self-explanatory, because increased protection levels corresponded to a decrease in DM and PM severities (Fig. 2). However, disease management had no significant effect on severity in a few cases, when epidemics appeared late in the season and escaped the effects of fungicides (PM on foliage at both sites in 2007) (Fig. 2C1 to C3, lower graphs) or when no epidemic developed in the unprotected plots (DM on clusters at Latresne in 2006) (Fig. 2B1 to B3, upper right graphs).

Graphical relationship between leaf and cluster severity.

One important feature of the analyzed data set is the nonlinearity of foliage-cluster severity relationships, with a range of shapes. This is well illustrated in the unprotected plots for DM on the leaves (Fig. 2A1) and on clusters (Fig. 2B1) with (i) the fairly strong spontaneous epidemic on the foliage at Couhins 2006, which was associated to a very weak epidemic on clusters (Fig. 2B1) and (ii) the strong epidemics on the foliage at Couhins 2007 and Latresne 2007, which did coincide with very strong epidemics on clusters. In the case of PM, nonlinearity is exemplified by (i) the very strong epidemic on leaves in Latresne 2006 (Fig. 2C1), corresponding to a very strong epidemic on clusters (Fig. 2D1); (ii) the moderate epidemic on leaves in Couhins 2006, conversely corresponding to a moderate response on clusters, but (iii) the fairly strong, if delayed, epidemics of Couhins 2007 and Latresne 2007, corresponding to negligible epidemics on clusters. A progressive disconnection between leaf and cluster epidemics is apparent over successive observations for DM (Fig. 3A to E), which is suggested only with PM (Fig. 3G to K), with some plots experiencing variable, and sometimes strong, epidemics on the foliage with similarly strong disease on clusters whereas, in other plots, fairly strong epidemics on the foliage may develop while disease on clusters remains limited. The overall pattern is partly summarized using areas under severity progress curves (Fig. 3F and D for DM and PM, respectively), except for the shifts in cluster response over time.

Fig. 4. Odds of a disease severity on clusters being above a threshold as a function of foliage severity at successive crop stages, and of preset thresholds for injuries on clusters. **A**, Downy mildew and **B**, powdery mildew. Only odds ratios pertaining to significant ($P < 0.01$) logistic models (Tables 6 and 7) were retained. Note that odds ratios vary with both the likelihood ratios and leaf disease severity; see text for details.

The parabolic shape of relationships between cluster and foliage disease severities suggested in Figure 3 resembles relationships between disease intensities at different levels of a host hierarchy (19,25,27,28,38). The tissues (leaves and clusters) considered here do not truly belong to different hierarchy levels. However, they do correspond to vastly different population sizes: whereas the leaf area index (data not shown) often reached 2 m² m⁻² during these experiments, the grape berry area index seldom exceeded 0.04 m² m⁻², a difference in range which approaches the difference in the numbers of sites one would involve in comparing, e.g., severity and incidence data (25,38).

The hypothesis is forwarded that two phases exist in both diseases in leaf-cluster severity relationships. The first phase is materialized by a saturation, parabolic curve that corresponds to epidemics that develop early in the season, and affects both the leaves and the clusters. The second phase is associated to a linear, small-slope curve that corresponds to late epidemics intensifying only on leaves. The discontinuity of leaf-cluster severity relationships is, in addition, ascribed to two main factors: a steep and sudden change in cluster susceptibility over time (6,8–10,21) and the large discrepancy in the number of host sites on the foliage compared with clusters. DM data appear to provide better sup-

porting ground for such interpretations than PM data do (Fig. 3). The relevance of discontinuity in the leaf-cluster relationships is further addressed through nonlinear model analysis.

Nonlinear models for leaf and cluster severity. Nonlinear modeling enabled satisfactory descriptions of epidemics on leaves (Table 4) and on clusters (Table 5) with only three parameters. The fit of models generally was better for DM than for PM. Each parameter has a biological meaning, even if each actually captures an array of processes. Therefore, the approach used here may have a general value and be useful to address epidemics developing in crops where different plant organs are affected by a disease.

More importantly, a significant ($P < 0.05$) value for the transmission parameter, tc , was found in all cases. This value varied greatly according to the disease (DM or PM), sites, and years (Table 5). We hypothesize that this variation reflects the fact that tc summarizes several processes, which depend on environmental and host factors (LAI and berry susceptibility) and which, in turn, varied among years and sites. For DM, tc values, for instance, were almost 10 times larger in 2007 (0.189 to 0.374) than in 2006 (0.007 to 0.029), and this may be related to the fact that the 2006 (leaf) epidemics were delayed compared with 2007. As a result, a

TABLE 7. Powdery mildew: logistic regression analyses of the relations between leaf and cluster severities at successive crop stages and thresholds

Val, crop ^a	Range ^b	Likelihood		Som D ^f	Intercept ^c				Slope ^c				Odds (95%) ^d		
		Ratio ^e	$P(\chi^2)$		Est	SE	t	P	Est	SE	t	P	Ratio	Max	Min
0%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
3	0–2.4	9.516	0.002	0.339	1.099	0.320	3.430	0.001	466.375	31.402	14.852	<0.001	n.a.	n.a.	0
4	0–14.0	6.866	0.009	0.309	1.142	0.326	3.504	<0.001	20.511	19.110	1.073	0.283	8.1×10^8	1.5×10^{25}	0
5	0–22.6	8.053	0.005	0.375	1.018	0.343	2.973	0.003	8.893	6.890	1.291	0.197	7.3×10^3	5.3×10^9	0.010
6	0–25.6	12.201	<0.001	0.596	0.700	0.370	1.894	0.058	1.526	0.812	1.879	0.060	4.597	22.574	0.936
7	0–33.8	2.482	0.115	0.147	1.085	0.405	2.680	0.007	0.123	0.094	1.306	0.192	1.131	1.360	0.940
0.1%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
3	0–2.4	13.402	<0.001	0.345	–0.265	0.266	–0.993	0.320	13.846	9.394	1.474	0.140	1.0×10^6	1.0×10^{14}	0.010
4	0–14.0	8.639	0.003	0.228	–0.136	0.265	–0.514	0.608	2.123	2.723	0.780	0.436	8.356	1.7×10^3	0.040
5	0–22.6	15.879	<0.001	0.395	–0.400	0.285	–1.403	0.161	3.433	1.865	1.841	0.066	30.580	1.2×10^3	0.801
6	0–25.6	20.699	<0.001	0.593	–0.730	0.327	–2.234	0.025	0.741	0.260	2.850	0.044	2.098	3.493	1.261
7	0–33.8	3.850	0.050	0.104	–0.285	0.321	–0.889	0.374	0.095	0.056	1.703	0.089	1.100	1.226	0.986
1%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
3	0–2.4	22.198	<0.001	0.535	–1.669	0.347	–4.811	<0.001	9.264	3.866	2.396	0.017	1.1×10^4	2.5×10^7	5.403
4	0–14.0	19.969	<0.001	0.430	–1.628	0.357	–4.565	<0.001	4.884	3.111	1.570	0.116	132.143	5.9×10^4	0.297
5	0–22.6	31.572	<0.001	0.715	–2.078	0.423	–4.912	<0.001	3.993	1.597	2.500	0.012	54.209	1.2×10^3	2.368
6	0–25.6	18.022	<0.001	0.589	–1.830	0.391	–4.685	<0.001	0.364	0.134	2.706	0.007	1.439	1.872	1.105
7	0–33.8	6.228	0.013	0.190	–1.597	0.385	–4.149	<0.001	0.115	0.053	2.174	0.030	1.122	1.245	1.011
5%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
3	0–2.4	23.136	<0.001	0.830	–3.254	0.642	–5.067	<0.001	5.946	2.305	2.580	0.010	382.066	3.5×10^4	4.172
4	0–14.0	23.136	<0.001	0.828	–3.130	0.606	–5.167	<0.001	1.069	0.389	2.744	0.006	2.911	6.244	1.357
5	0–22.6	22.984	<0.001	0.969	–3.196	0.621	–5.149	<0.001	0.481	0.154	3.112	0.002	1.617	2.189	1.195
6	0–25.6	9.684	0.002	0.793	–2.775	0.501	–5.428	<0.001	0.179	0.064	2.814	0.005	1.196	1.355	1.056
7	0–33.8	2.088	0.148	0.117	–2.487	0.496	–5.014	<0.001	0.073	0.048	1.521	0.128	1.076	1.182	0.979
10%															
1	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
2	0	NC	...	...	...	...	...	...	...	...	...	...	1	1	1
3	0–2.4	15.063	<0.001	0.955	–3.329	0.648	–5.138	<0.001	3.212	1.119	2.869	0.004	24.828	222.748	2.767
4	0–14.0	17.376	<0.001	0.952	–3.410	0.686	–4.973	<0.001	0.700	0.261	2.683	0.007	2.014	3.358	1.208
5	0–22.6	20.111	<0.001	0.965	–3.707	0.717	–5.168	<0.001	0.417	0.108	3.845	<0.001	1.517	1.877	1.227
6	0–25.6	5.397	0.020	0.765	–2.957	0.558	–5.304	<0.001	0.138	0.057	2.403	0.016	1.148	1.285	1.026
7	0–33.8	3.446	0.063	0.260	–3.005	0.591	–5.086	<0.001	0.098	0.051	1.934	0.053	1.103	1.219	0.999

^a Threshold value and crop stage.

^b Leaf severity range.

^c Est = estimate and SE = standard error.

^d Maximum (Max) and minimum (Min); n.a. = value too large.

^e NC = not computed.

^f Sommer's D.

small amount of disease was present on the foliage in 2006 when berries were susceptible, whereas this amount was large in 2007.

In equation 2, the parameter tc is defined as the increase of disease fraction on clusters per unit time, per unit disease fraction on leaves, and per unit fraction healthy tissue still available on clusters. Therefore, the underlying hypothesis is that disease increase on clusters is due to infections from an inoculum originating from the leaves. Because tc is significantly ($P < 0.05$) different from 0, and because the linked models for foliage and clusters adequately described cluster severity data, our analyses do not reject this hypothesis. Plant pathology has frequently illustrated that the fit of a model is not a proof of validity of its underlying hypotheses (17,25,30,35). For instance, we chose not to incorporate the last foliage assessment in the model parameterization. This is based on simplifying assumptions, one of them being that ontogenic resistances on clusters to both diseases (6,8–10,21) develop abruptly. It could have been possible to develop a weighed model, where contribution of foliage diseases (DM and PM) to cluster infections (DM and PM) progressively decline. However, that would have been at the expense of the simplicity we sought and would have rendered the results strictly specific to the analyzed data set. Quantitative, detailed experimental work is needed on both diseases to test the hypothesis that disease increase on clusters mainly originates from lesions present on the foliage.

Disease increase on clusters may be ascribed to two other sources: (i) autoinoculum; that is, lesions present on clusters that multiply, and (ii) inoculum produced by sexual reproduction (release of oospores in DM and of ascospores in PM). In the first case, one should expect a logistic progress of disease. Given the short period during which berries are susceptible to infection (6,8–10,21), it seems likely that the contribution of this source of inoculum usually is small. The second possibility is supported by recent epidemiological (22) and genetic (14) work in the case of DM. However, to our knowledge, a quantification of the respective importance of the different possible sources of inoculum of epidemics on clusters has not been reported.

The optimization algorithm led to highly correlated ($r > 0.95$) estimates of parameters $x0$ and r ; therefore, spontaneous epidemics on leaves (Table 4, unprotected) cannot be compared on the basis of individual parameters only. However, estimated r values may be compared among limited and unprotected management levels and, in all 15 available cases, the r values in the former were lower than r values in the latter (Table 4), showing the general r -reducing effect of fungicide protection (39). In general, the estimated tc values were also lower in the limited protection than in the unprotected management levels (Table 5). This may indicate that fungicides lowered the increase of both diseases on clusters relative to the current levels of leaf severity. Fungicides may, for example, reduce the lesion sporulation on leaves or reduce the infection efficiency of spores that are produced on leaves and are deposited on clusters.

Logistic regressions. For both DM and PM, logistic regression analyses (Tables 6 and 7) enabled the determination of probabilistic relationships between severity on clusters and severity on the foliage at a range of preset thresholds for cluster severities and at different stages of the crop. However, the results do indicate that determination of such relationships is comparatively easier in the case of DM, due to large variances in PM severity on clusters and foliage (Fig. 2). For instance, no reliable relationship of the odds of clusters being infected with PM (cluster severity $> 0\%$) with severity on the foliage at any crop stage could be determined in this study. This difference between the two diseases can, in turn, be linked with the nature of their symptoms, with conspicuous and often well-delimited symptoms for DM as opposed to mostly discreet and transient PM symptoms, which necessarily translate in measurement errors. Comparison of the odds ratios (Fig. 4) of clusters being infected at preset severity levels

indicates both higher odd ratios and steeper decline with crop stage in the case of PM. This may be associated with a higher transmission of disease from the foliage to clusters, which is also suggested by nonlinear modeling results (Table 5), or a lower precision in disease measurements. The larger uncertainties attached to odds ratios for PM (Table 7) compared with DM (Table 6) favor the latter interpretation.

Nevertheless, the main conclusion from these analyses was the same for both DM and PM: logistic regression analyses yielded a series of significant probabilistic relationships between the risk of cluster infection and foliage severity, considering simultaneously a range of preset levels of disease on clusters, and different crop stages.

Analysis of dual epidemics. Many plant pathogens can infect different types of organs during a cropping season. Important examples of fungal diseases affecting leaves and reproductive organs (fruit and grain), for instance, include apple scab (18), *Septoria nodorum* blotch on wheat, and rice blast. The behavior of dual epidemics may depend on the timing of organ development as a crop grows, and on inoculum exchange between the two types of organs, as this analysis suggests in the case of PM and DM of grape. In dual epidemics, decision-making may be, at least in part, grounded on one component of the system as a predictor, whereas the other component is the target for protection. In this work on grapevine diseases, the foliage was the component used as a predictor, while clusters were the targets.

Conclusions. Our results enabled statistical and probabilistic linkages between components of dual epidemics in two grapevine diseases. The statistical linkage is not evidence of processes that link the two components but strongly suggests its possibility. More research is needed to document this key element of disease development and of the occurrence of qualitative and quantitative damage. Disease on leaves causes physiological injuries that hampers crop growth and reduces yield (31), whereas disease on clusters reduces yield but also quality (31) and, thus, causes direct economic loss. The probabilistic linkage should offer a basis for tactical decisions for disease management and, therefore, help reduce pesticide use. More research is necessary to devise injury thresholds that incorporate quality losses and, considering perennial crops, incorporate the effects of diseases over successive years on the host physiology.

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