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Powdery mildew development is positively influenced by grapevine vegetative growth induced by different soil management strategies

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ABSTRACT

In various crop species, high levels of powdery mildew infection and severity have been associated with high vegetative vigour. In grapevine this relationship has also been observed by vine growers, though it has not been quantified. This study was undertaken to investigate the relationship between the development of powdery mildew on leaves and berries and canopy growth, and thus to quantify the relationship between the pathogen and its host. Over a two-year period (2005 and 2006), an experiment was carried out in a vineyard (cv. Aranel) near Montpellier, southern France. Several levels of canopy growth were generated by implementing four soil management strategies: i) perennial cover crop in the inter-row, ii) annual cover crop in the inter-row, iii) chemical weed control over the entire soil surface, iv) chemical weed control all over the soil surface and drip irrigation and fertilization in the row. Powdery mildew was artificially inoculated on experimental sub-plots with *Erysiphe necator* [Schw.] Burr. conidia. The most vigorous vines developed a larger number of diseased leaves and a higher percentage of mildewed berries compared to low-vigour vines. The major explanatory variable highlighted in these experiments was the shoot leaf number, mainly early in the season. A higher leaf population generated a larger number of powdery mildew colonies close to grapes and consequently a higher probability of berry infection. Our experimental results provide evidence of a positive relationship between powdery mildew development and grapevine vegetative development. These results provide an opportunity to develop new IPM strategies in vineyards.

1. Introduction

Obligate parasites are recognized as particularly sensitive to ontogenic resistance i.e. to change the susceptibility of their organs during plant development (Develey-Rivière and Galiana, 2007). The former was documented for the main diseases of grapevine: black-rot (Hoffman et al., 2002), botrytis (Salzman et al., 1998; Kretschmer et al., 2007), powdery mildew (Ficke et al., 2003; Gadoury et al., 2003) and downy mildew (Kennelly et al., 2005). In various crop species, high levels of powdery mildew infection and severity have been associated with plant development under conditions of high vegetative vigour or tissue turgescence (Jarvis et al., 2002). In wheat, powdery mildew increases when nitrogen fertilization rate increases (Broscious et al., 1985). Models were developed to account for the effect of host development on disease progress (Hau, 1990). For wheat, models were developed to account for the susceptibility of host tissue and for the effects of leaf position or plant architecture on powdery mildew (Rossi and Giosue, 2003) or *Septoria tritici* Roberge (Robert et al., 2008). For apples, Lalancette and Hickey (1986) designed a model where leaf number is a key variable to simulate powdery mildew development, explaining the importance of plant growth in disease attacks.

A positive relationship between grapevine growth and susceptibility to fungal pathogens has also been observed by vine growers, pathologists and extension services (de la Rocque, 2002; Goulet et al., 2006). In several studies concerning the effects of crop practices on grapevine yield and quality, interactions between diseases and vine growth were observed (Reynolds and Wardle, 1994; Intrieri et al., 2001; Zahavi et al., 2001; Pellegrino et al., 2004; Evans et al., 2006; Morlat and Bodin, 2006). For example, grey mould incidence was positively correlated to canopy development, and variables such as leaf number, leaf dry weight and area were identified as key variables associated with the disease

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development (Valdés-Gómez et al., 2008). The development of powdery mildew was found to be higher in pruning systems favouring a high vegetative expression on the cultivar Concord (Gadoury et al., 2001). Powdery mildew incidence and severity on grapes were shown to be twice as high and five times higher respectively in a vigorous grapevine plot compared to a non-vigorous plot on cv. Chardonnay (Evans et al., 2006). Recently, a characterization of the spread of epidemics in the vineyard showed a more rapid spread of the disease on plots with higher vegetative vigour (Calonnec et al., 2009). A set of deterministic epidemiological models was developed to take into account the dynamics of the grapevine's susceptibility, its growth and/or architecture, and their interaction with powdery mildew (Sall, 1980; Blaise and Gessler, 1992; Gessler and Blaise, 1992; Calonnec et al., 2008). However, few studies have been conducted to investigate and quantify the relationship between the grapevine's vegetative development and the development of powdery mildew.

In all the examples above, the interactions between the dynamics of secondary infection of powdery mildew and plant growth certainly explain the differences in disease damage levels. Several factors could explain the positive relationship between powdery mildew development and grapevine vegetative vigour: i) a higher plant leaf number, as deduced from one experiment conducted by Gadoury et al. (2001) on Concord grapes; ii) a longer period of susceptibility of the affected organs; berries are very sensitive to infection between their setting stage and bunch closure; young leaves are very susceptible and they turn more resistant with ageing, so that any factor that slows down the maturation process of the organs may increase the plant's susceptibility to the disease (Doster and Schnathorst, 1985); iii) favourable changes in tissue properties, for example structural changes (formation of suberized epidermis, cellular necrosis etc.) or physiological and chemical changes (synthesis of proteins or other compounds, changes in cellular osmotic potential, etc.) (Goheen and Schnathorst, 1963; Adrian et al., 2000; Deloire et al., 2000; Jeandet et al., 2002); iv) more favourable microclimatic conditions; in vigorous vineyards, dense and poorly ventilated canopy and poorly illuminated bunches (Pellegrino et al., 2004), which favours the development of powdery mildew (Halleen and Holz, 2001; Zahavi et al., 2001).

Therefore, all cultural practices that favour vegetative vigour may predispose the host to an increased development of powdery mildew. High grapevine vigour could modify ontogenic resistance of leaves (particularly delaying grapevine phenological stages such as veraison or harvest (Matthews et al., 1987; Keller et al., 2001), or stretching the duration of the flowering, fruit set or bunch closure periods (Gadoury et al., 2006). This study was undertaken to investigate the relationship between the development of powdery mildew on leaves and berries for various levels of canopy growth, and thus to quantify the relationship between the pathogen and its host. To this end, several policies of soil management – irrigation, nitrogen fertilization and cover cropping – were used in order to generate various levels of nutrient supply and hence of grapevine growth.

2. Materials and methods

2.1. Experimental field and cropping practices

Field experiments using *Vitis vinifera* L. cv Aranel (a white cultivar) grafted onto Fercal rootstock were conducted in 2005 and 2006. The vines were planted in 1998 at a density of 3333 vines ha^{-1} (2.5 m × 1.2 m) in a vineyard of 1.5 ha located near Montpellier, France (43°31′N–3°51′E, 10 m a.s.l.). The area is characterised by a typical Mediterranean climate with an average annual rainfall of 749 mm with 520 mm (70%) of the rain falling in autumn and winter (from September to March). The average annual water deficit (PET—rainfall) was about 174 mm (1975–2005). The soil was deep (more than 3 m) and homogeneous, classified as calcaric Fluvisol (FAO classification), containing 31% sand, 35% silt and 34% clay. The vines were trained to a vertical shoot positioned system with a canopy height of 1.9 m, with rows aligned W–NW. In the entire experimental vineyard, shoots were topped and trimmed once per year.

Four types of cropping systems were used in order to generate various levels of canopy development:

i) Perennial cover crop in the inter-rows sown in 2002 with a mixture of tall fescue (*Festuca arundinacea* Shreb) and Perennial ryegrass (*Lolium perenne* L.) (PI);
ii) Annual cover crop of barley (*Hordeum vulgare* L.) sown every autumn in the inter-rows (1.5 m wide) and destroyed by surface tillage just after grapevine flowering (mid-June) (NPI);
iii) Chemical weed control with glyphosate (Roundup Bio Forces®, 2%) over the whole soil surface (WC); and
iv) Chemical weed control as above and drip irrigation in the row with a water application of 100% of the Penman–Monteith reference evapotranspiration (3400 m^3 ha^{-1} in 2005, 7400 m^3 ha^{-1} in 2006) from grapevine bud break to harvest (Allen et al., 1998), and fertilised with N (80 kg N ha^{-1} in 2005, 120 kg N ha^{-1} in 2006) (WCI).

In each cropping system, sub-plots of three adjacent vines were selected for vine growth and powdery mildew assessments. Each year, the sub-plots were distributed in the field using as selection criteria the pruning weight map of the previous season (2004 or 2005) in order to get the largest range of plant growth (Fig. 1). In 2005, three sub-plots per cropping systems were selected and four in 2006, except in the WC area. These sub-plots were artificially inoculated with *Erysiphe necator* conidia to get a uniform intensity of primary infection as presented in Table 1. Inoculations were performed on 29 April 2005 and 26 April 2006 (4–6 leaves unfolded) on the central shoot of the central vine, as described by Calonnec et al. (2009).

Inoculated sub-plots were protected from any fungicide spray by wrapping the three vines in plastic film at the time of spraying. The remaining vines were protected against powdery mildew infection using two fungicide treatments every year: tebuconazole (Corail®,EW 0.15 kg a.i. ha^{-1}) at flowering and tryfloxistrobin (Natchez®, WG, 0.06 kg a.i. ha^{-1}) 14 days after flowering. To control downy mildew, one treatment was applied at flowering with the fungicides cimoxanil (0.12 kg a.i. ha^{-1}) + mancozeb (1.4 kg a.i. ha^{-1}) formulated as Sitolan® WG. To control insects, three treatments were applied every year by using chlorpyrifos-ethyl (Dursban 2®, 0.37 a.i. kg ha^{-1}) and cypermetrin + diazinon (Socavers®, 1.2 l. ha^{-1}) in the whole experimental vineyard. With these treatments no disease developed in the vineyard except in the inoculated sub-plots.

2.2. Assessment of vine growth

Leaf number was measured every 10 days from 8 leaves unfolded stage to bunch closure. To identify these grapevine phenology stages, the Eichhorn and Lorenz phenological scale modified by Coombe was used (Coombe, 1995). This phenological scale is a system of growth stage identification that contains a succession of developmental events that always follow each other, having 35 stages that are easily described, and clearly identified from "winter bud" to "end of leaf fall". Leaf number was measured on twelve shoots for each sub-plot throughout the experimental period - six on the central vine and three on each lateral vine. A distance of about 30 cm separated the shoots. The

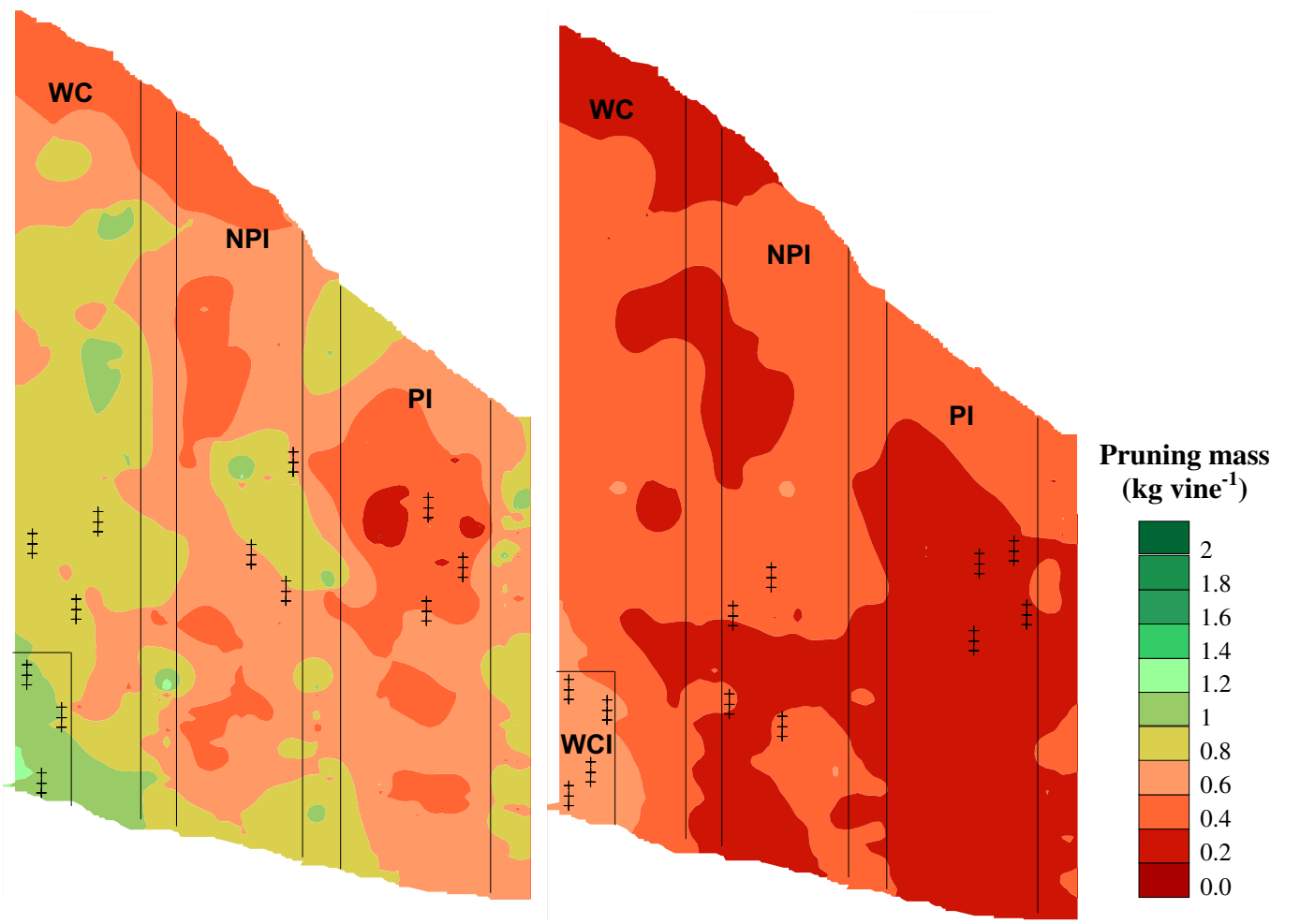

Fig. 1. Pruning mass map (kg vine^{-1}) on the experimental field for 2004 (left) and 2005 (right) seasons. Four treatments were used to create different canopy growth levels: one with a permanent intercrop (PI), another with a non-permanent intercrop (NPI), a third with chemical weed control (WC) and a fourth with chemical weed control, irrigated and fertilised (WCI). The experimental sub-plots (Ξ) were located along the field experiment. Vertical lines separate the soil management treatment.

following variables were measured or calculated: i) primary shoot leaf number (PSL) (total leaf number directly born on the primary shoot), ii) lateral shoot leaf number (LSL) (total leaf number directly born on secondary and tertiary shoots), iii) total shoot leaf number (TSL = PSL + LSL), and iv) the average leaf appearance rate (LAR) (total leaf number born on the primary, secondary and tertiary shoots per day) between May, 10th and grapevine fruit set.

Table 1
Evaluation of the uniformity of leaf number and disease development on inoculated shoots, 14 days after artificial inoculation. Mean values and standard deviation.

Growth levels issues of soil management strategies[a]	Total leaf number per shoot		Total diseased leaf number per shoot		Disease incidence on shoot		Disease severity (%)	
	mean	SE	mean	SE	mean	SE	mean	SE
2005								
PI sub-plots	10.7	1.7	4.0	0.6	0.37	0.03	11.5	1.9
NPI sub-plots	11.0	0.0	5.0	0.0	0.45	0.00	11.8	2.3
WC sub-plots	16.3	2.0	5.7	0.3	0.35	0.03	7.2	1.8
WCI sub-plots	14.0	3.5	4.7	0.3	0.37	0.07	10.2	4.1
2006								
PI sub-plots	12.3	2.0	6.3	0.6	0.54	0.08	13.3	3.2
NPI sub-plots	15.8	1.8	6.5	0.5	0.43	0.05	6.7	1.0
WCI sub-plots	16.3	1.2	7.3	0.3	0.45	0.03	9.0	2.5
Significance[b]	n.s.		n.s.		n.s.		n.s.	

[a] PI = permanent intercrop, NPI = non-permanent intercrop, WC = weed control, WCI = chemical weed control, irrigated and fertilised.
[b] ns indicates no significant differences between sub-plots of different growth levels (issue of different cropping systems) calculated using a Kruskal–Wallis non-parametric test ($P \leq 0.05$).

In December 2004 and 2005, vines were hand pruned and the collected canes were weighed immediately to assess pruning mass (PM). On the whole experimental field, about 200 vines were distributed in a regular grid in groups of three vines. Data mapping was performed using Surfer software (v. 8.0, Golden Software, Inc., Golden, Colorado, USA) and the interpolation method used in this study was based on a deterministic function (inverse distance weighting).

2.3. Assessment of powdery mildew

Disease incidence and severity were assessed on the same dates and on the same shoots as leaf number. The number of diseased leaves on primary shoots (DLP) and on lateral shoots (DLL) was recorded. A diseased leaf appearance rate was calculated (DLAR). Foliar disease severity was recorded on each infected leaf as the percentage of the leaf surface colonized by *E. necator*; it was averaged for each shoot, primary (DSLP) and lateral (DSLL).

The grape disease incidence (DIG) (proportion of clusters affected by the fungus) and severity (DSG) (percentage of the visual bunch surface colonized by the fungus) were also assessed visually on all bunches of each sub-plot. Variables were computed for the inoculated vine and for lateral vines. Incidence and severity on bunches were recorded every 7 days in 2005 and 10 days in 2006 from fruit set to harvest. The grapes were harvested on August, 30th in 2005 and on August, 29th in 2006.

For every variable, the corresponding abbreviation and sampling procedure are listed in Table 2.

2.4. Data analysis

The uniformity of artificial inoculation was verified by comparing among all grapevine growth conditions the foliar disease incidence and severity on inoculated shoots two weeks after inoculation. For this the Kruskal–Wallis non-parametric test ($P \leq 0.05$) according to the analysis proposed by Acevedo-Opazo et al. (2008), was performed. The relationship between powdery mildew development (incidence and severity) on leaves shortly before bunch closure (June, 22th), on berries at veraison (August, 1st) and with grapevine growth variables at fruit set (June, 12th) were explored by using principal component analysis (PCA). From the matrix of coordinates of the individuals on the PCA axes, the Euclidean distance between individuals was calculated and a hierarchical clustering analysis (HCA) was performed on this distance matrix to group similar individuals into homogenous clusters. Distances between clusters were recomputed by the Lance-Williams dissimilarity formula. The clustering method was that of minimum variance (Ward, 1963). Correlations between the variables and similarities between individuals were discussed. Data for disease development on bunches were fitted to grapevine growth variables by linear regression. Data analysis was conducted with StatBox software (Version 6.23, Grimmer Software, Paris) and with R (Version 2.1.1, copyright 2005).

3. Results

The initial shoot development and level of disease was similar in all sub-plots, as indicated by the comparison of shoot growth and disease development observed on inoculated shoots 14 days after inoculation in 2005 and in 2006 (Student test, $p = 0.05$) (Table 1). Disease incidence was slightly higher in 2006 than in 2005, but this was not the case for disease severity. The similar disease development in all grapevine growth conditions, at the beginning of the experiment, indicated a similar susceptibility of the shoots and then no influence of the soil management strategies on leaf susceptibility at this stage.

3.1. Dynamics of leaf appearance and disease development

In 2005, from the middle of the growing season (after fruit setting), the leaf number per shoot was significantly different between PI and NPI sub-plots (Fig. 2a) compared to the WC and WCI sub-plots on inoculated vines (Student test, $p = 0.05$). In 2006, only WCI sub-plots had a leaf number per shoot higher than PI and NPI sub-plots before grapevine flowering. Near the time of bunch closure (July, 1st in 2005 and June, 29th in 2006), WCI sub-plots showed 17% more leaves per shoot in 2006 than in 2005.

In 2005 strong heterogeneities were observed between the sub-plots, particularly within the WC ones. In 2006, the selection of sub-plots based on the pruning mass map and the experience of the previous year provided more homogeneous plant growth for all crop management conditions.

For both years, according to the rate of diseased leaf appearance and final level of disease, more disease developed on the most vigorous vines mostly under conditions of weed control and higher water and nitrogen supply. As an example, in 2005, at the time of bunch closure, the number of diseased leaves per shoot (DL) was 25–40% higher in WCI and WC sub-plots than in the other

Table 2
Variables of canopy development and powdery mildew development measured and calculated over the season with their sampling protocols.

Variables	Abbrev.	Units	Sampling per sub-plot
Vine growth			
Primary shoot leaf number	PSL	Leaf number shoot^{-1}	12 shoots (6 on the central vine and 3 on each lateral vine),
Lateral shoot leaf number	LSL	Leaf number shoot^{-1}	every 10 days, from May 10th to July 5th in 2005, and from
Total shoot leaf number	TSL	Leaf number shoot^{-1}	May10th to June 22nd in 2006
Leaves appearance rate	LAR	Leaf number shoot^{-1} d^{-1}	
Disease evolution on leaves			
Number of diseased leaves on primary shoot	DLP	Leaf number shoot^{-1}	12 shoots (6 on the central vine and 3 on each lateral vine),
Number of diseased leaves on lateral shoot	DLL	Leaf number shoot^{-1}	every 10 days, from May 10th to July 5th in 2005, and from
Total number of diseased leaves per shoot	DL	Leaf number shoot^{-1}	May 10th to June 22nd in 2006
Diseased leaves appearance rate	DLAR	Leaf number shoot^{-1} d^{-1}	
Average disease severity on leaves of primary shoot	DSLP	%	
Average disease severity on leaves of lateral shoot	DSLL	%	
Average disease severity on leaves per shoot	DSL	%	
Disease evolution on bunches			
Average disease incidence on grape	DIG	proportion of grapes diseased	All grapes in sub-plots, from
Average disease severity on grape	DSG	%	June 13th to harvest every 7 days in 2005 and every 10 days in 2006

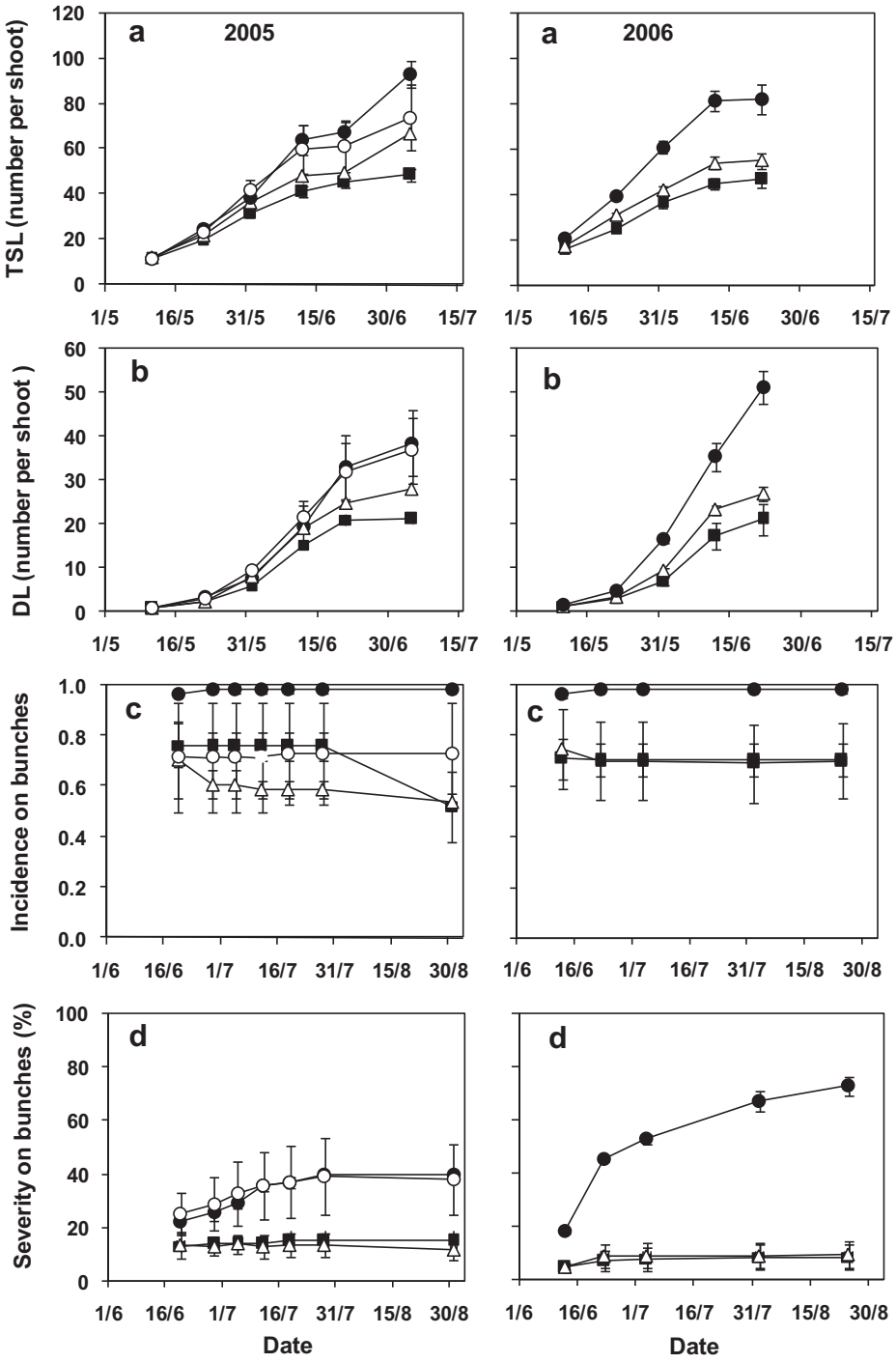

Fig. 2. Seasonal time-course of total number of leaves per shoot (TLS, a), total number of diseased leaves per shoot (DL, b) and disease incidence on bunches (c) and severity on bunches (d) on inoculated vines for years 2005 and 2006. Points are the average values for the following treatments: permanent intercrop (PI, ■), non-permanent intercrop (NPI, △), chemical weed control (WC, ○) and chemical weed control, irrigated and fertilised (WCI, ●). Error bars represented the standard error of the measurement.

treatments (Fig. 2b). In 2006, WCI sub-plots had 60% more diseased leaves than PI sub-plots. The disease severity on leaves was low during the two seasons for all grapevine growing conditions: less than 7 and 2% for inoculated vines and their lateral vines, respectively, at bunch closure (data not shown).

On bunches, disease incidence varied significantly among the different growing conditions: all bunches of the sub-plots with high vegetative growth (WCI sub-plots) were diseased against less than 80% for the sub-plots with less vegetative growth for both years. For both seasons, the disease severity was higher in sub-plots with vigorous plants (WC and WCI) than in the others sub-plots (PI and NPI) (Fig. 2d). The disease severity on the WCI sub-plots was higher in 2006 (70%) than in 2005 (40%).

In 2005, the great variability of disease development on leaves was linked to similar variability of the disease development on bunches, particularly between WC and WCI *vs*. PI sub-plots. On the lateral grapevines, the shoot growth followed the same trend as observed on the inoculated ones, in both 2005 and 2006 (Fig. 3a).

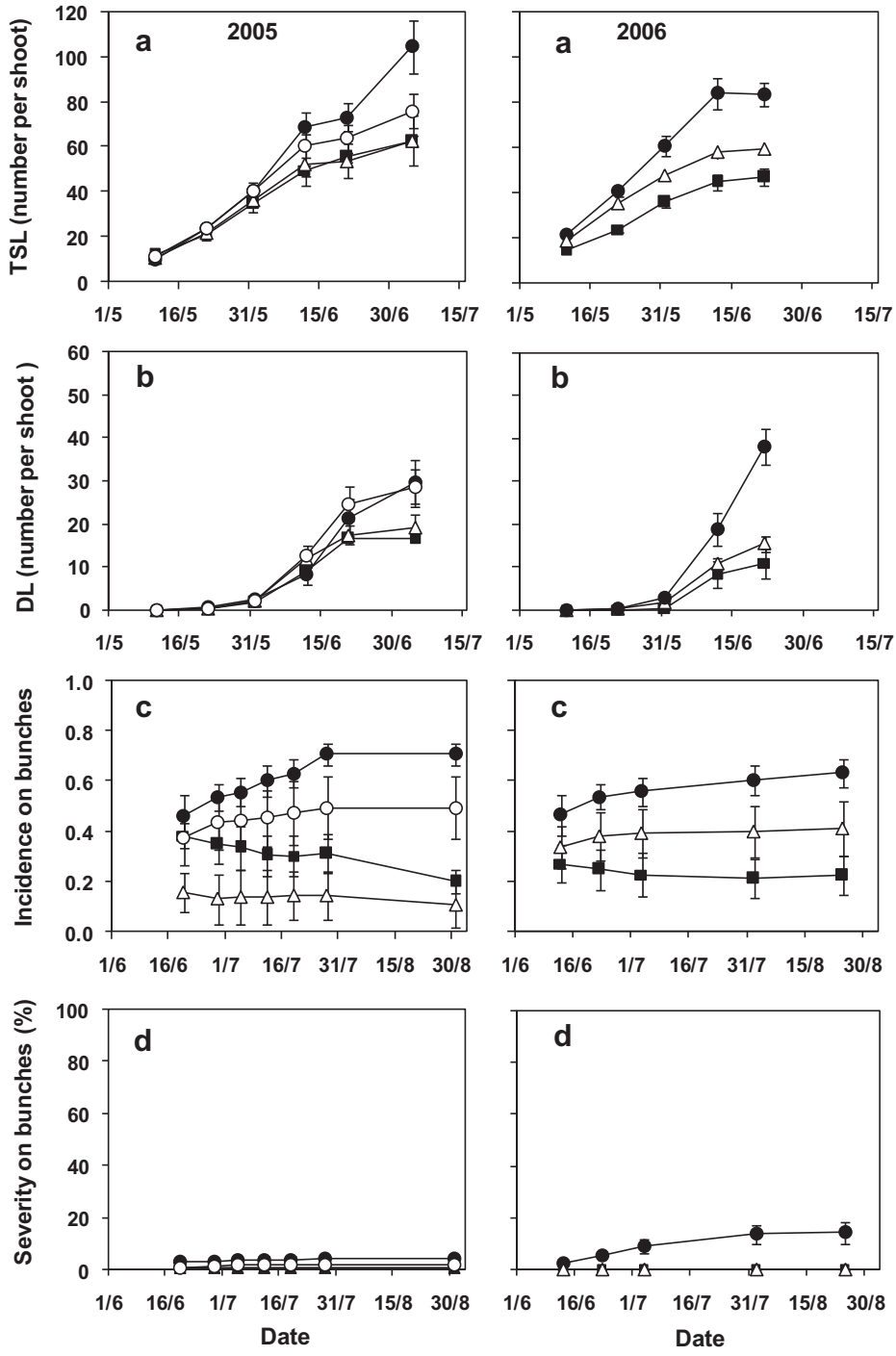

Fig. 3. Seasonal time-course of total number of leaves per shoot (TLS, a), total number of diseased leaves per shoot (DL, b) and disease incidence on bunches (c) and severity on bunches (d) on lateral vines for years 2005 and 2006. Points are the average values for the following treatments: permanent intercrop (PI, ■), non-permanent intercrop (NPI, △), chemical weed control (WC, ○) and chemical weed control, irrigated and fertilised (WCI, ●). Error bars represented the standard error of the measurement.

The disease incidence on bunches from vigorous grapevines (sub-plots of WC and WCI cropping systems) increased slowly from fruit setting to harvest, with final values lower than on inoculated grapevines (Fig. 3c). In 2005, the number of diseased bunches in the intercropped cropping systems decreased from veraison to harvest, which was explained by berry abscission due to a strong water restriction described by Celette et al. (2008), as well as by difficulty of observation of small symptoms on ripe grapes. The disease severity followed the same trend as that on inoculated grapevines, but with lower average values (less than 20%, Fig. 3d).

3.2. Relationship between shoot growth and disease development

The descriptive analysis given previously showed a significant influence of the grapevine growth on disease development but with a high variability in growth between sub-plots of the different cropping systems. Multidimensional analyses allowed selected variables from plant growth and disease to be considered together with each sub-plot as an individual, whatever its cropping systems.

The multivariate analyses (PCA and HCA) of the inoculated grapevines made it possible to distinguish the effects of grapevine

growth on the disease development (Fig. 4). Thus, when the selected variables, TSL at fruit set, LAR from May, 10th to fruit set, DLAR from May, 10th to bunch closure, DL and DSL at bunch closure and DSG at veraison, were compared in correlation analyses for both years, strong positive correlations were observed between variables of grapevine growth early in the season and variables of disease development on leaves (TSL or LAR *vs*. DL or DLAR, $R > 0.86$) and on bunches (TSL or LAR *vs*. DSG, $R > 0.92$). Also the disease development on leaves (total of diseased leaves per shoot or diseased leaf appearance rate) was strongly and positively correlated with disease development on bunches (DL or DLAR *vs*. DSG, $R > 0.86$).

In the PCA analysis, axes 1 and 2 accounted for 87.6 and 7.5% of the total variance of the data, respectively. The first factorial axis was constructed from all variables: vine growth variables at setting (TSL and LAR) which contributed positively at the rate of 35%; variables linked to diseases on leaves at bunch closure (DL, DLAR, DSL) which contributed positively at the rate of 49% and finally the variable characterising disease on bunches (DSG) (16%). This axis separated individuals with high vegetative growth at fruit setting and with heavily infected leaves and bunches from those with low vine growth at setting and little disease development. The second main axis was representative of the disease severity on leaves, with DSL accounting for 78.2%. The hierarchical clustering analysis significantly differentiated individuals into two groups: cluster 1, with individuals with the highest vegetative growth and the highest disease development and cluster 2, with the remaining individuals. Cluster 1 included all WCI sub-plots from 2006 and three out of six WC and WCI sub-plots from 2005. This analysis clearly separated WC sub-plots in 2005, which were heterogeneous in terms of both vegetative growth and disease development.

The multidimensional analyses showed that grapevine growth early in the season positively influenced foliar disease development. Thus when the rate of diseased leaf appearance (DLAR) between inoculation date and bunch closure on inoculated and lateral plants from both years was plotted against the rate of leaf appearance (LAR), a linear relationship was observed for both the inoculated and lateral grapevines (Fig. 5) whatever the growing system. The slopes of the two relationships differ, which has to be related to the date of arrival of the inoculum. Thus, for the inoculated grapevines, the fungus was artificially inoculated early in the season whereas for the lateral grapevines it arrived once the first sporulation cycles took place. These results show that the infection risk is related to the quantity of inoculum in the adjacent leaves. On bunches it depends on the earliness of the attack.

3.3. Influence of shoot growth on disease development on bunches

Among the grapevine growth and disease development variables, the average leaf appearance rate (LAR) and diseased leaf appearance rate (DLAR) were those best correlated ($p < 0.01$, R-values > 0.86 and 0.43, respectively) with the disease severity on bunches (Fig. 6). The distance to the source of inoculum played an important role on disease development as shown by i) the low values of disease severity on lateral vines, and ii) the higher variability observed on lateral grapevines than on inoculated ones.

A simple regression analysis between disease severity on grapes and leaf appearance rate at fruit setting on inoculated grapevines showed that the disease development depended significantly on the grapevine growth ($DSG = -36.7 + 49.06 \times LAR$, $R^2 = 0.77$). Also a multiple linear regression between disease severity on grapes and all grapevine growth and disease variables, carried out on inoculated grapevines, showed that disease severity on grapes is explained largely by the rate of leaf appearance (LAR) between bud break and fruit setting and the number of diseased leaves on lateral shoots

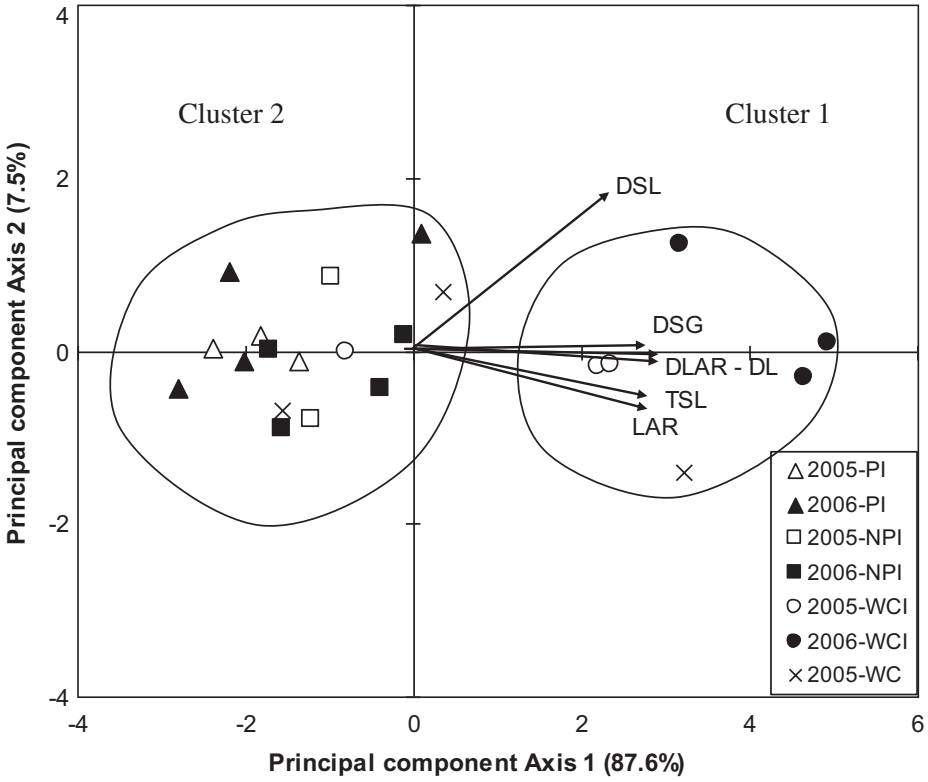

Fig. 4. Relative location on the first two axes of the principal component analysis of the PI (permanent intercrop), NPI (non-permanent intercrop), WC (chemical weed control) and WCI (chemical weed control, irrigated and fertilised) individuals and of variables characterising vine growth (TSL at fruit setting, LAR between May 10th to fruit set), powdery mildew on leaves (DLAR between May 10th to about bunch closure, DL and DSL at about bunch closure) and powdery mildew on bunches (DSG at veraison). Lines group similar individuals according to the HCA analysis. Variables as abbreviated are described in Table 2.

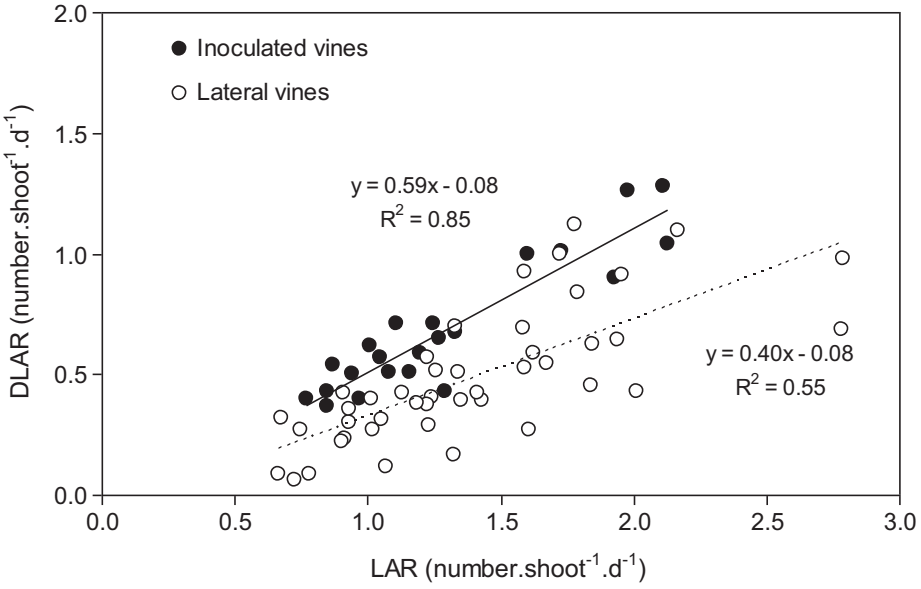

Fig. 5. Rate of diseased leaf appearance (DLAR) from May, 10th to about bunch closure on inoculated and lateral grapevines for 2005 and 2006 seasons (pooled data) function of the rate of leaf appearance (LAR) from May, 10th to fruit set.

(DLL) at bunch closure (DSG = −25.33 + 24.8 × LAR + 1.03 × DLL, $R^2 = 0.77$).

4. Discussion

The main objective of the present study was to test and to quantify the positive relationship between the grapevine's vegetative growth and the development of powdery mildew on leaves and its spread on bunches. The water and nutrient cropping systems that were implemented affected the availability of soil resources to grapevines and resulted in various patterns of vegetative growth. The differences in the total number of leaves per shoot at flowering and at bunch closure reached 50% and 55% for the two extreme treatments (PI and WCI), respectively. This strong variation in vegetative growth resulted in different levels of disease incidence and severity on leaves and consequently on bunches. Thus vigorous grapevines, which benefited from a high water and nitrogen supply, developed a larger number of diseased leaves and a higher percentage of diseased berries than low-vigour vines. The results available for other crop species like corn, barley and apples, as well as the scarce results published for grapevine, show a similar trend (Broscious et al., 1985; Lalancette and Hickey, 1986; Gadoury et al., 2001; Jarvis et al., 2002; Evans et al., 2006).

4.1. Direct effects of the dynamic of host growth on the disease

The main explanatory variable highlighted in our experiments was the shoot leaf number, as soon as early in the season (at fruit setting). Thus, a greater leaf population generated a larger number of powdery mildew colonies close to bunches and consequently a higher probability of berry infection. It is important to note that the relationship between leaf appearance and rate of disease leaves is identical for the different cropping system which is in favour of the same main mechanism for disease development. Such a mechanism could explain the results from experiments of pruning systems (balanced pruning and minimal pruning) carried out on grapevine cv. Concord (Gadoury et al., 2001). In these experiments with balanced and minimal pruning systems but with similar numbers of infected leaves per shoot, the higher shoot number in minimally pruned grapevines produced a higher disease development. The earlier the vegetative growth, the higher the damage of the disease. As an example, in our study, the irrigated and fertilised cropping system (WCI) has a vigour level on June, 1st 2006 equivalent to the one observed on June, 12th

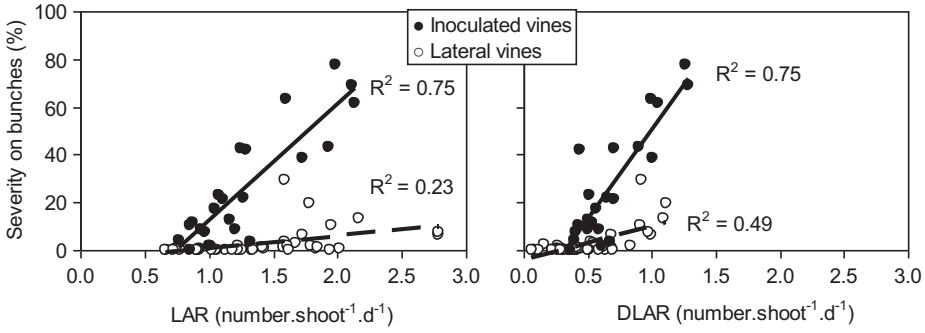

Fig. 6. Disease severity on inoculated and lateral vines for 2005 and 2006 seasons (pooled data) function of the rate of leaf appearance (LAR) and of the rate of diseased leaf appearance (DLAR).

2005 with considerable consequences for disease severity on bunches (70% in 2006 compared with 40% in 2005).

4.2. Potential difference of host growth susceptibility

Other explanatory factors of the positive relationship between disease development and grapevine growth are more difficult to analyse from the present results, but they can be suggested. As an example, in 2006, the disease incidence on grapes of lateral grapevines still increased after veraison in the most vigorous grapevines, while it remained stable in the other ones. Also, for vigorous inoculated grapevines, the disease severity on grapes increased even after veraison, which is unusual (Ficke et al., 2002, 2003; Gadoury et al., 2003). This late phenomenon could be related to a delay in maturity in vigorous plots or a longer period for bunch susceptibility. Indeed, harvest measurements of fruit composition made on vines adjacent to the experimental plots showed a sugar/titratable acidity ratio lower in the vigorous plot compared to other plots (Valdés-Gómez et al., 2008). The delay in maturity at harvest was certainly already present at veraison, since grape maturity is greatly delayed when nutrient availability does not limit growth, as suggested in several studies (Matthews et al., 1987; Spayd et al., 1994; Keller et al., 2001). Such a delay of maturity could modify the berry's ontogenetic resistance or increase the infectious period, so that infections which took place before veraison could continue to spread after this phenological stage (Kast and Stark-Urnau, 2000; Halleen and Holz, 2001). Quoted veraison date was corresponding to an overall average on the field, and then a possible delay in veraison stage in vigorous vines could also explain the increase in disease severity on grapes even after veraison in non-vigorous vines.

Changes in the leaf tissue properties could also explain some of the differences in disease development. Goheen and Schnathorst (1963) showed that conidia germination over grapevine leaves is weaker when the cellular osmotic potential (ψ_o) decreases. Thus, in their study, for ψ_o of −3 MPa and −1.1 MPa the germination rate was 4% and 35%, respectively. Water restriction modifies the ψ_o, as observed in a grapevine experiment in Greece (−1.4 MPa under no water restriction which was equivalent to a $\psi_{pd} = -0.15$ MPa and −1.8 MPa under water restriction equivalent to a $\psi_{pd} = -1.2$ MPa) (Patakas and Noitsakis, 2001). In our experiments, measurements of pre-dawn leaf water potential (ψ_{pd}) in grapevines adjacent to diseased PI sub-plots was −0.6 MPa at bunch closure and decreased to −1.4 MPa at harvest (Valdés-Gómez et al., 2009). In WCI sub-plots, ψ_{pd} was maintained close to −0.15 MPa throughout the whole season. These significant differences in ψ_{pd} could partly explain the larger number of diseased leaves observed on the vigorous vines and also disease development on berries whose ψ_o is related to the overall plant water potential.

4.3. Indirect effects through micro-climate

Lastly, a very dense, poorly ventilated and poorly illuminated canopy may create a micro-climate close to bunches favourable to powdery mildew development (Halleen and Holz, 2001; Zahavi et al., 2001). These conditions were observed for vigorous sub-plots of cropping systems WC and WCI. Canopy density measurements carried out late in the season, at harvest, on vines adjacent to diseased sub-plots by using the point quadrat method (Smart, 1988) showed that WCI sub-plots had a leaf layer number two to four times higher than the NPI and PI sub-plots (3.7, 1.9 and 1.3 for WCI, NPI and PI in 2005 and5.2, 2.5, 1.7 for WCI, NPI and PI in 2006) (Valdés-Gómez et al., 2008). Even if the evolution of canopy density is not linear with time, we can hypothesise that at the time of their maximum susceptibility bunches are more exposed to UV in less vigorous sub-plots than in higher vigorous sub-plots.

4.4. Implications for IPM

Under our experimental conditions the early artificial inoculation of the *E. necator* allowed significant disease development and a significant positive relationship with vine vegetative growth. In natural conditions and on a larger plot area any delay in primary inoculations between vigorous and non-vigorous grapevines should increase the differences. These findings may have implications for the development of IPM strategies in vineyards. We have highlighted as a possible major explanatory variable, high shoot leaf number, mainly early in the season. Thus crop practices which decrease grapevine vegetative growth could be used to keep disease incidence at a low level. Hence intercropping, pruning, leaf removal, or careful choice of plant density should be worth testing. In many viticultural regions as in Central Europe, northern and Atlantic France vineyards, where rainfalls are abundant and regularly distributed within the year, intercrops are quite used due to the potential positive impacts on grapevines and their environment. In Mediterranean regions, however, to the present, few grape growers have introduced a cover crop into their vineyard (Mezière et al., 2009) due to the risk of severe drought during spring and/or summer and their consequences on competition for soil resources between two crops (Celette et al., 2009). In our study, the non-perennial intercrop resulted in an interesting technique to decrease vine growth without significant yield decrease for both dry and humid years (Celette, 2007; Valdés-Gómez, 2007). To answer to the difficulty of the winegrower to decide to use perennial or non-perennial intercrop, Ripoche et al. (2010) tested different intercrop management plans in vineyards to classify them in relation to their ability to fulfil a particular set of objectives and to deal with climatic variability. Results showed that the most satisfactory intercrop management plans differed according to the priority given to managing production or reducing environmental impacts and depended on the soil depth. When priority was given to the environmental criterion, the cropping systems with a long intercrop period were better, regardless of the soil type.

As was stated above, vine growth expressed as shoot leaf number at an early stage was considered the main factor affecting disease development in our conditions and can show the effects of water and nitrogen constraints early in the growth cycle (even before flowering). In our conditions, this variable related well with canopy density at a later stage. Today canopy density can easily be measured in precision viticulture with embedded sensors. We still have, however, to better explore the relationship between the early leaf number, the late canopy density and the disease initiation to be able to define a level of plot vigour fitting with the lowest risk of disease.

The extrapolation of our results may be unwise in view of the cultivar (Aranel) used in the present study, which is moderately susceptible to powdery mildew. Other research will have to determine if the relationship between vine vegetative growth and powdery mildew development is of the same magnitude for other grapevine cultivars. The factors and variables responsible for the relationship should be further explored. Indeed, the time lapse between significant phenological stages such as flowering and setting, the high sugar levels in leaves (Doster and Schnathorst, 1985) as well as cellular osmotic potential, can be very different among cultivars, and thus modify the relationship between grapevine vigour and disease development.

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