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Late harvest as factor affecting esca and Botryosphaeria dieback prevalence of vineyards in the Alsace region of France

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Summary

The decline of grapevines due to esca and Botryosphaeria dieback (Bot. dieback) is a serious problem in the Alsace region of France. A survey of 82 vineyards over 8 years showed that among a set of agronomical and cultural variables, esca and Bot. dieback prevalence correlated to the harvest dates, especially late harvest dates for the production of sweet wines. The interpretation of this finding that points to the carbon balance of the vine and its reserves status as possible causation is discussed. Under this hypothesis the data also point to climatic variables as factors in the disease epidemiology, with a lag phase of about one year.

K e y w o r d s : cultural practices, carbohydrate reserves, climatic factors, source to sink relationship.

Introduction

The wood diseases of the grapevine, esca, Eutypa dieback and Botryosphaeria dieback (Bot. dieback) are serious cultural problems in many worldwide grape growing countries (Moller *et al.* 1974, Dubos *et al.* 1980, Kassemeyer 1987, Van Niekerk *et al.* 2006, Surico *et al.* 2006, Savocchia *et al.* 2007, Larignon *et al.* 2009, Urbez-Torres 2011). Cultural practices and environmental factors, including edaphic and climatic factors, are known to be important for these diseases as shown below.

To reduce Eutypa dieback, timing of winter pruning and pruning wound protection were found to be especially important tools (John *et al.* 2005, Weber *et al.* 2007, Sosnowski *et al.* 2008, Urbez-Torres and Gubler 2011, Rolshausen *et al.* 2010). For esca and Bot. dieback, pruning date and pruning wound protection had no effect on disease incidence and cordon spur pruning showed a slightly higher symptom expression rate compared to Guyot cane pruning (Dumot 2010, Dumot *et al.* 2012). In another study on the contrary Guyot cane pruning resulted in a higher esca symptom expression rate than cordon spur pruning (Cahurel 2009). Regardless of the pruning modes, there was more esca/Bot.dieback expression at lower planting density (Cahurel 2009, Dumot 2010, Dumot *et al.* 2012).

Edaphic factors are also important. In Bordeaux region, it was shown that soils with higher water reserves and no limited water supply have higher expression rate of diseases (Destrac *et al.* 2007). In the south of France, there was a positive correlation between Bot. dieback disease prevalence and rainfall either in May or one month before symptoms appeared, and a negative correlation between symptom expression and potential evapotranspiration (PET) during the period of symptom expression (Larignon 2009).

The expression and development of grapevine trunk diseases broadly depends on environmental factors and on cultural practices. The purpose of this study is to provide a more detailed characterization of the role of these factors as they relate to these diseases.

Material and Methods

T h e v i n e y a r d s : This study is based on the network of the National grapevine wood diseases survey programme that started in 2003. Thirty farms in Alsace participated in the survey. Observations were carried out on one plot of each of three cultivars: 'Riesling', 'Auxerrois' and 'Gewürztraminer' on each farm, 90 plots in total (Fussler *et al*. 2008). The number of plots concerned by the present study is 82, because of the withdrawal of 8 plots that had been uprooted. One plot of each of these cultivars was randomly selected from each farm. Because the two diseases are often found together in declining vines (Kuntzmann *et al*. 2010), Esca and Bot. dieback prevalence were considered together under the acronym EBDA (Esca /Black Dead Arm). Eutypa dieback notation ceased after 2008. The age of the plots in 2003 ranged from two to more than fifty years old, with a mean age of 21 years. Within the whole network only thirteen plots had been treated with arsenite. Moreover the treatments were carried out many years before the beginning of the survey and thus unlikely have had a significant impact on the results of the study. Data on cultural practices and agronomical variables were gathered by field observations and by interviews with the growers. As cultural practices were considered for example soil management, application of sodium arsenite, farming mode, claimed AOC, frequency of late harvest and as agronomical variables soil classification, variety, kind of selection for planting material, rootstock, planting density, vigour and vegetative expression.

T h e A l s a c e w i n e r e g i o n : Located between latitudes 47.5° and 48.4° North, the Alsace wine region is less than 10 km wide and has a mostly Easterly orientation,

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stretching in a north-south direction for 100 km from Strasbourg in the north to Mulhouse in the south. The climate is oceanic under continental influence, with hot summers and cold winters. The average rainfall of 600-750 mm falls mainly as summer thunderstorms. The winters are relatively dry. The diversity and variability of soil types found in Alsatian vineyards are a product of the complex geology of the region. Soils change over very short distances and include granitic, chalky and alluvial soil types (Party, 1990). In Alsace 12 grape varieties are grown and three AOC designations are produced. AOC Alsace and AOC Grand Cru are for still wines; AOC Crémant d'Alsace is for sparkling wines. Two additional classifications for sweet wines made from grapes affected by noble rot are Vendanges Tardives (VT) and Sélections de Grains Nobles (SGN). From those 12 varieties, only a few can be used to elaborate wines covering the whole range of AOC designations and additional classifications.

D a t a a n a l y s i s : Data were analyzed with SAS statistical software package (SAS 9.2). Data were divided into dependent variables and predictor variables. Because of the large number of variables and their different natures, an exploratory approach by multivariate analysis, principal component analysis (PCA) and multiple correspondence analysis (MCA), were undertaken first. This led to the suppression of some variables. Additional variables were suppressed following Partial Least Squares Regression (PLS Regression), an analytical method designed for cases where variables are numerous and correlated to each other. Finally the PLS Regression model was built with the variables listed in Tab. 1. To clearly separate the variety effect from the harvest date effect on trunk disease expression, and because both are linked by the designated AOC, a complementary analysis was done with R software (R 2.12.1.) in 'Gewürztraminer' for the harvest variables and AOC. EBDA prevalence data were transformed into classes with the same limits and in order to aggregate a similar number of plots for each year.

Results

G e n e r a l t r e n d o f g r a p e v i n e t r u n k d i s e a s e e x p r e s s i o n i n t h e n e t w o r k : Most of the network's vine growers observed an increase in the prevalence of wood disease symptoms in the beginning of the two-thousands, some others in the 1990s, followed in a few cases, by a decrease in expression. On average, Eutypa dieback prevalence varied between 0.4 and 0.9 % from 2003 to 2008, with a peak of 2.5 % in 2005 for the most affected variety Auxerrois (data not shown). Between 2003 and 2011 average prevalence of EBDA varied between 1.5 and 8 % (Fig. 1). The worst affected variety was 'Gewürztraminer' (2-10 % symptomatic vines) and the least affected variety was Auxerrois (1.5-4.5 % symptomatic vines). In 'Riesling', expression rates matched the global mean (1.1-8.5 % symptomatic vines). However, a very wide variation between plots within varieties was observed. During this period the rate of disease expression in some badly affected 'Gewürztraminer' and 'Riesling' plots reached 25-30 % (data not shown). The trend of disease expression in relation to age of vine does not show any difference on average (data not shown).

P L S R e g r e s s i o n : The PLS Regression model retained 9 components which explain 77.92 % of the predictor variables and 65.72 % of the dependent variables.

The first component on the value axis (Fig. 2) is principally constructed with the quantitative variables altitude, farm area and age (plot age). It also sets the modalities *late harvest frequently*, 'Gewürztraminer', and AOC Grand Cru against *late harvest never*, 'Auxerrois', AOC Crémant and AOC Alsace respectively. The second component on the category axis sets the modalities *arsenite yes* and 'Auxerrois' against *arsenite no* and 'Gewürztraminer'. The representation of the dependent variables (Fig. 3) shows a similar behavior between the different years despite slight differences for 2003 and 2009. By comparing Figs 2 and 3, it can be established that the older plots, at higher altitudes,

Table 1

Description of variables

Variables	Nature	Unit/Modalities
Rate of EBDA (from 2003 to 2010)	Quantitative	Percentage
Late harvest	Qualitative	always, frequently, seldom, never
vigour 2010	Qualitative	high, medium, low
AOC	Qualitative	Alsace, Crémant, Grand Cru
Harvest at opening	Qualitative	always, frequently, seldom, never
Qualitative level	Qualitative	high, medium, low
Sodium Arsenite	Qualitative	yes, no
Planting density	Quantitative	vines/hectare
Yield 2010	Qualitative	high, medium, low
Observers	Qualitative	IFV, Adar, CA68, SRPV/FREDECA
Altitude	Quantitative	meters
Slope	Qualitative	plain, hillside
Vine variety	Qualitative	Gewürztraminer, Riesling, Auxerrois
Age	Quantitative	Years (age of the plot in 2010)
Farm area	Quantitative	hectares

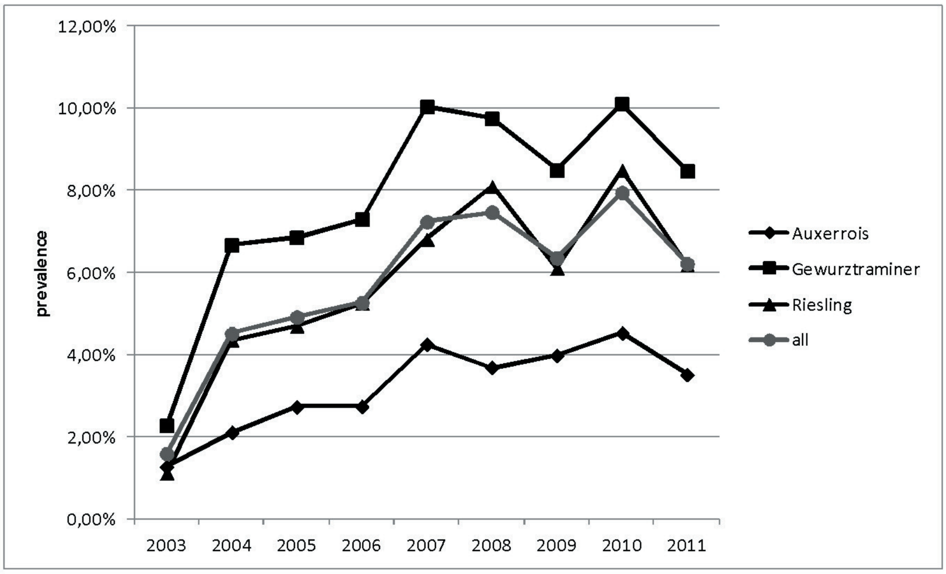

Fig.1: prevalence of EBDA from 2003 to 2011.

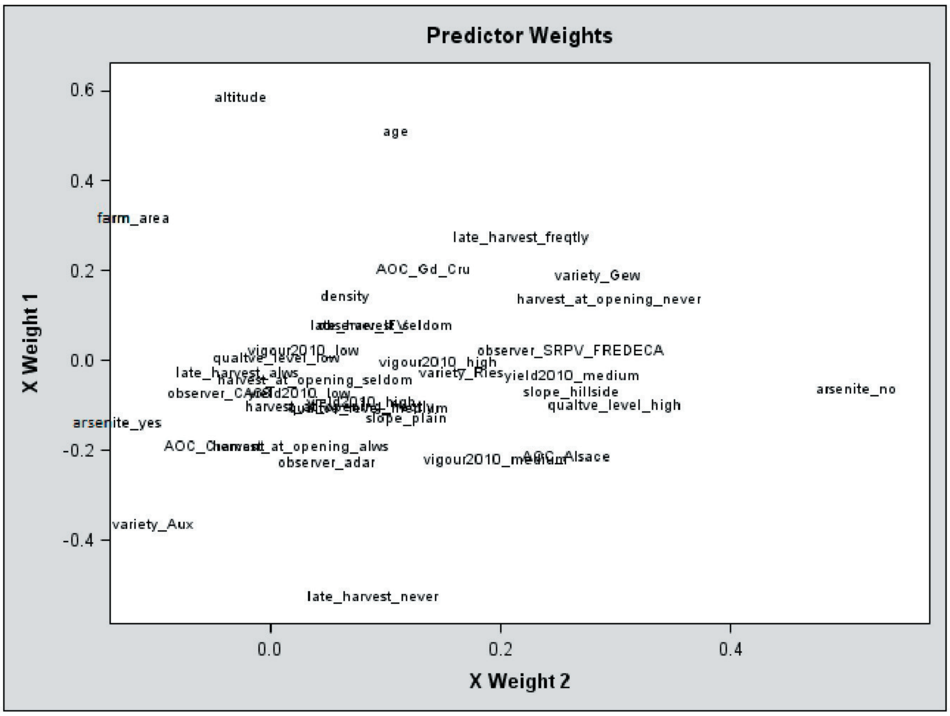

Fig.2: Representation of the projection of predictor variables on the plane of the two first components.

belonging to a farm growing a larger vineyard area were more affected by disease. In addition, the plots of 'Gewürztraminer' AOC Grand Cru that were frequently harvested late have a higher rate of EBDA than the plots of 'Auxerrois' variety, AOC Crémant or Alsace that have never been late harvested. The plots of 'Gewürztraminer' never treated with arsenite have higher rates of EBDA than the plots of 'Auxerrois' treated once with arsenite.

In 2010 plots with higher yields and vigour were more affected by the disease in the years 2004 to 2008 than the plots with lower yields and vigour (Figs not shown).

The relationship between altitude and farm area and the rate of EBDA, is similar to the relationship between the variables AOC and late harvest and the rate of EBDA (Figs not shown).

Multiple Component Analysis (MCA) on Gewürztraminer plots: Two components were kept, explaining 40 % of the total inertia, which is correct for an MCA. High rates of EBDA (EBDA_3) are associated with the modality late harvest frequently, contrary to low rates of EBDA (EBDA_1) which are associated with the modality *late harvest never* (Fig. 4). The variable *harvest at opening* has no important weight here. This can be explained by the fact that almost all plots of 'Gewürztraminer' in the network are never harvested at the opening date of the corresponding AOC Alsace or Grand Cru.

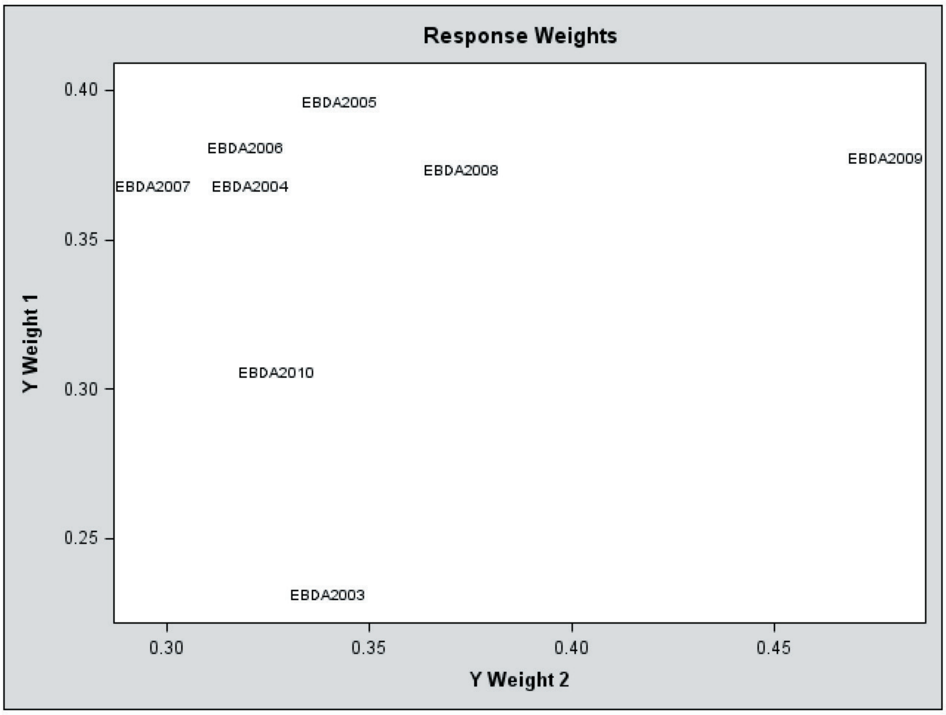

Fig.3: Representation of the projection of dependent variables on the plane of the two first components.

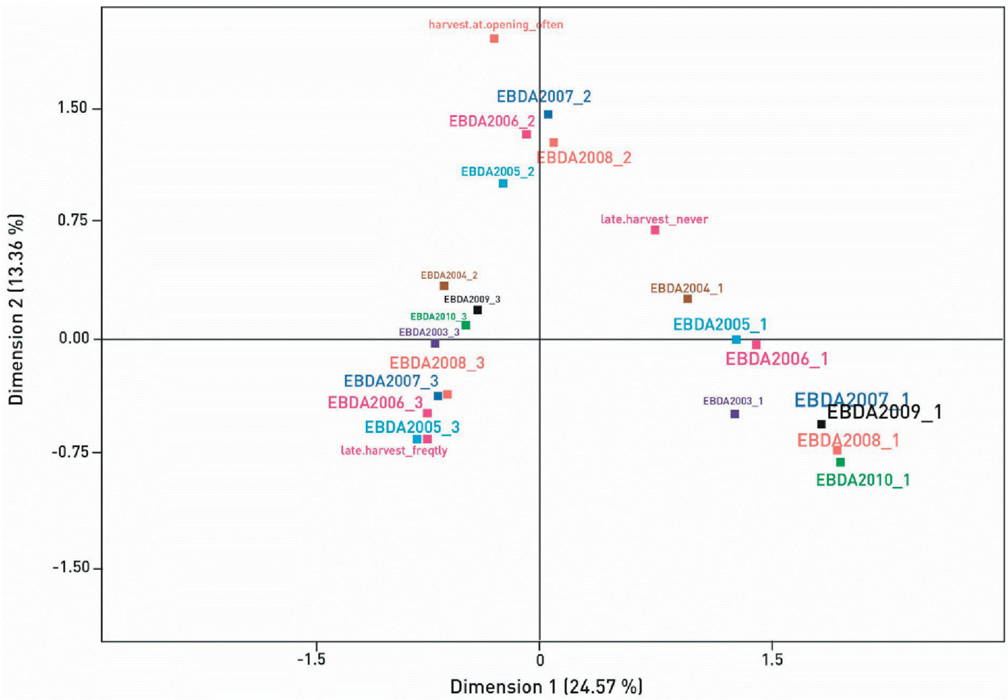

Fig.4: Projection of the modalities on the two first components plane of the MCA.

Discussion

Relationship between Esca and Bot. dieback and harvest date: The influence of harvest date is a key factor in EBDA expression. The impact of this cultural practice lies in the partitioning of carbohydrates and nitrogen between vegetative growth, formation and ripening of the fruits and the constitution of the reserves, where grape berries are more attractive sinks than carbohydrate or nitrogen storage in the perennial parts (Hale and Weaver 1962, Koblet 1969, Stoev and Ivantchev 1977). Hofäcker (1977) showed that the concentration of total nonstructural carbohydrates (TNC) in young potted plants bearing grapes was always lower than in non-bearing plants. However, the real effect of harvest date on TNC concentrations in the perennial parts has been poorly studied with no relationships found for one year old wood (Wample and Bary 1992, Hamman et al.

1996). In fact, the only way to approach the effect of harvest date on carbohydrate storage levels, is to consider it in the way of a source-sink relationship. Several authors have studied the impact of manipulating source to sink relations on the reserve status of the grapevine and showed either a decrease in TNC concentrations, or a decrease in the ratio between starch and soluble sugars for the perennial parts, when reducing the leaf to fruit ratio or when doing selective leaf removal (Candolfi-Vasconcelos and Koblet 1990, Duchene *et al*. 2003, Bennet *et al*. 2005, Smith and Holzapfel 2009, Jermini *et al*. 2010, Zufferey *et al*. 2012). The depletive effect of harvest date in commercial vineyards is probably weaker than the treatments implemented in these trials, but strong enough to be significant, possibly in association with other factors.

Esca, Bot. dieback, and other variables: The analysis indicates that the older the vineyard, the higher the prevalence of esca and Bot. dieback. This observation is in contrast to that of Fussler *et al*. (2008) who found that the highest level of esca was associated with vines in their 15 to 25 years old class, although differences in sample plots and years concerned by the survey make it difficult to have direct comparisons with the study reported here. For the effect of arsenite treatments shown by the model, the structure of the corresponding data is in favour of a relation with the variety and its use and the arsenite effect is just a coincidence.

An association is shown between high yield and high vigour as estimated in 2010 and the prevalence of esca/Bot. dieback. Although the yield effect could be related to the replenishment of the reserves, such an association was not established. In addition some data (personal data, unpublished) indicate a decrease from around 10-12 % in 2001-2002 to around 3-5 % in 2009-2011 in the prevalence of esca/Bot. dieback in a vineyard where nothing else including yield changed, but vigour was increased from 38 to 55 grams per cane.

Climatic conditions and esca/Bot. dieback prevalence: In case of an implication of the reserves in the epidemiology of esca/Bot. dieback it would be logical to observe an effect of climatic conditions too, through the effect of climate on the whole plant physiology, particularly photosynthesis. Climatic effects on the disease prevalence can be considered at two different scales, both differing in the length of the lag phase between climatic conditions and disease expression.

First, one might focus on an intra-annual scale. In Italy and for esca, no specific weather conditions could be put in relation with the disease evolution over several years (Surico *et al*. 2000). Yet it seemed that a fresh and rainy summer was more favorable to the chronic form of esca, while a hot and dry one was more conducive to the acute form (apoplexy). In Bordeaux esca leaf symptoms are inhibited by water stress (Destrac *et al*. 2007). In Alsace for esca/Bot. dieback there was no climatic factor responsible for the timing of leaf symptom apparition along the season (Kuntzmann *et al*. 2010). The same analysis as Larignon (2009) was done on data from 2004 to 2010 for 12 vineyards from the network but the regression analysis did not show any particular relationship. In this real-time based first approach, it could be hypothesized that in Alsace, esca and Bot. dieback symptoms variation is currently seldom limited by climatic conditions unlike in southern France.

Secondly, one can consider an inter-annual scale, especially for perennials, with a greater lag-phase of about one year existing in this case. As a climatic variable the insolation duration can be considered first. With light stress photosynthesis is reduced (Keller and Koblet 1994). Defoliation stress during the ripening period leads to a retranslocation of carbon reserves from the woody parts into the fruits and thereafter a depletion of stored carbohydrates (Candolfi-Vasconcelos *et al*. 1994). It could be supposed that a photosynthesis reduction by light stress has a similar effect on the carbohydrate storage level. When looking at data in Fig. 1 and Tab. 2, the prevalence increase between 2006 and 2007 could have been triggered by the lack of insolation during the ripening period in August 2006. However in discrepancy between this theory and the facts, 2011 shows a decrease in the disease as it should have been the opposite in regard of 2010 August's insolation. The explanation for that could lie in the potential yield, far lower in 2010 than in 2006 because of a severe winterfrost, which

Table 2

Selected climatic data Colmar/France (source: MétéoFrance)

Year	Rainfall May (mm)	Rainfall June (mm)	Rainfall one month before symptom appearance (mm)	Daily mean potential evapotranspiration from beginning of symptom expression to 80 % of symptom expression (mm)	Monthly insolation duration (h)	
					August	September
2002					196	159
2003					308	247
2004	36.8	54.4	69.4	4.5	180	193
2005	43.4	44.4	39.4	4.5	219	186
2006	80.6	46	53.8	5.1	149	172
2007	68.8	82	68.8	3.2	223	176
2008	61.5	49	81.5	4.1	212	161
2009	54.5	42.5	60.5	4.1	260	192
2010	56	37.5	37.5	4.0	165	205

resulted in an improvement of the leaf to fruit ratio. So far in the discussion, it could be possible to underline here an interaction of climate and cultural practices.

Over the last one or two decades, vigour and vegetative expression of the vines in Alsace has globally strongly decreased as a consequence of cover-cropping development and fertilization reduction. Vineyards with cane weight lower than 30 g and as low as 10 g are frequent (personal data), in contrast of what is observed in other viticultural areas (BENNET *et al*. 2005, VASCONCELOS and CASTAGNOLI 2000) or what should be the objective in cool climates (VASCONCELOS and CASTAGNOLI 2000). Cultural practices have changed without consideration of natural variations of climate. As shown in Fig. 5, average annual temperature has increased in two steps from the end of the eighties to the beginning of the two-thousands and seems to have been decreasing since. But annual rainfall shows cyclic variations between 500 and 650 mm, with a local minimum in the beginning of the nineties and a local maximum 10 years later, and a decrease since (Fig. 6). These rainfall changes coupled with those in cultural practices might be responsible for a decrease in vigour and vegetative expression with consequences on the photo-assimilation capacity of the vine. Indeed it was shown a depressive effect of inter-row covercropping on vine vegetative expression, with a decrease in leaf area and pruning weights (CHANTELOT *et al*. 2004). The effect on the yield was less pronounced, thus cover cropping worsened the leaf to fruit ratio. Interaction between climate and cultural practices like soil cultivation or fertilization may account for the general increasing trend in disease and especially for early harvested varieties where harvest date does not play such a role. The first upsurge of disease indicated by grapegrowers corresponds to the local rainfall minimum in the beginning of the nineties, the second to its decrease since early two-thousands.

Rainfall acting through drought stress is another climatic variable which can be implicated. Alsace is a rather dry region where water stress situations may occur some years depending on the soil. For example in 1992 leaf pre-dawn water potential lower than -0.6 MPa could be observed all

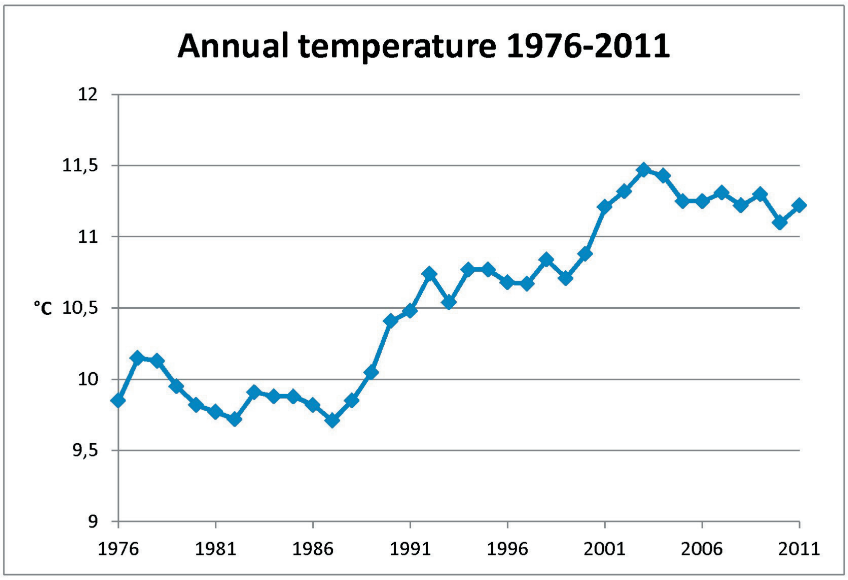

Fig.5: Annual temperature in Colmar/France 1976-2011, 5 year simple running mean (data: MétéoFrance)..

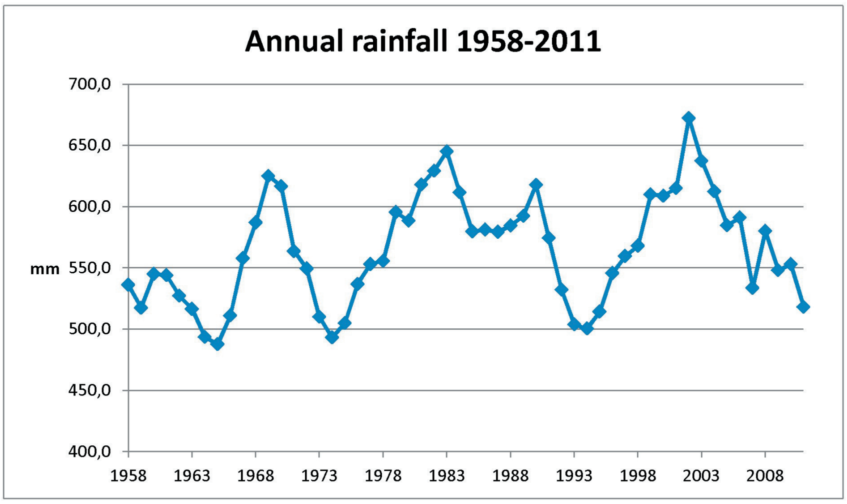

Fig.6: Annual rainfall in Colmar/France 1958-2011, 5 year simple running mean (data: MétéoFrance).

along the ripening period (LEBON *et al*. 2003). Such low values are not recommended during ripening (DELOIRE *et al*. 2003). In Germany where climate and training systems are quite similar to Alsace, the threshold for irrigation is by -0.25 to -0.3 MPa (SCHULTZ 2003). Water stress from fruit set until veraison showed a potential reduction of stored carbohydrates in the trunk at leaf fall (COOLEY *et al*. 2006). The effect of post-veraison water stress was not investigated but is likely to occur. In 2009 it was very dry during the whole ripening period and drought symptoms were frequent in vines established on thin, sandy or gravelly soils. No measurements were done in Alsace, but for Riesling and similar environmental and cultural conditions, the leaf pre-dawn water potential decreased from -0.6 Mpa to -1.2 Mpa on the same period, for the control in an irrigation trial (PRIOR 2011). Undoubtedly such a water stress could be responsible for the prevalence increase between 2009 and 2010 as shown in Fig. 1.

Vine carbon balance and decline: In a review of decline in forest trees, both hydraulic dysfunction and a carbon balance deficit were mechanisms forwarded as explanations (BRÉDA *et al*. 2006). These authors showed a one-year delay in the impact of drought on the crown vitality via the amount of stored carbohydrate. Similarly the delay of one year for the effects of climatic factors observed in this study of grapevine wood diseases could be in favour of an implication of the vine carbon balance in the epidemiology of the diseases.

The carbon balance hypothesis is placed much upstream in the chain of events which lead to the decline. It could explain a preliminary step, that is why the wood would be invaded by fungi like *Botryosphaeriaceae*, whose toxins (MARTOS *et al*. 2008, DJOUKENG *et al*. 2009) could be involved in the symptoms expression on the vegetative aerial parts (REGO *et al*. 2009).

This hypothesis is in line with numerous references indicating the need for "stress or non-optimal growth conditions" for disease expression on trees or vines for *Botryosphaeriaceae* (SLIPPERS and WINGFIELD 2007, VAN NIEKERK *et al*. 2011) or for *Phaeomoniella chlamydospora* (FISCHER and KASSEMEYER 2012). A mix of changing cultural practices under changing climatic conditions acting on the carbon vine balance seems a candidate among these stress factors as shown by this study. However as pathogenicity of *Botryosphaeriaceae* has firmly been proven in controlled conditions (VAN NIEKERK *et al*. 2006, SAVOCCHIA *et al*. 2007, URBEZ-TORRES and GUBLER 2009, LARIGNON 2010, WUNDERLICH *et al*. 2011), the precise role of environmental stress factors requires a better characterization.

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