MARGAUX, MAY 9, 2007

GLOBAL WARMING: NEW OENOLOGICAL CHALLENGES

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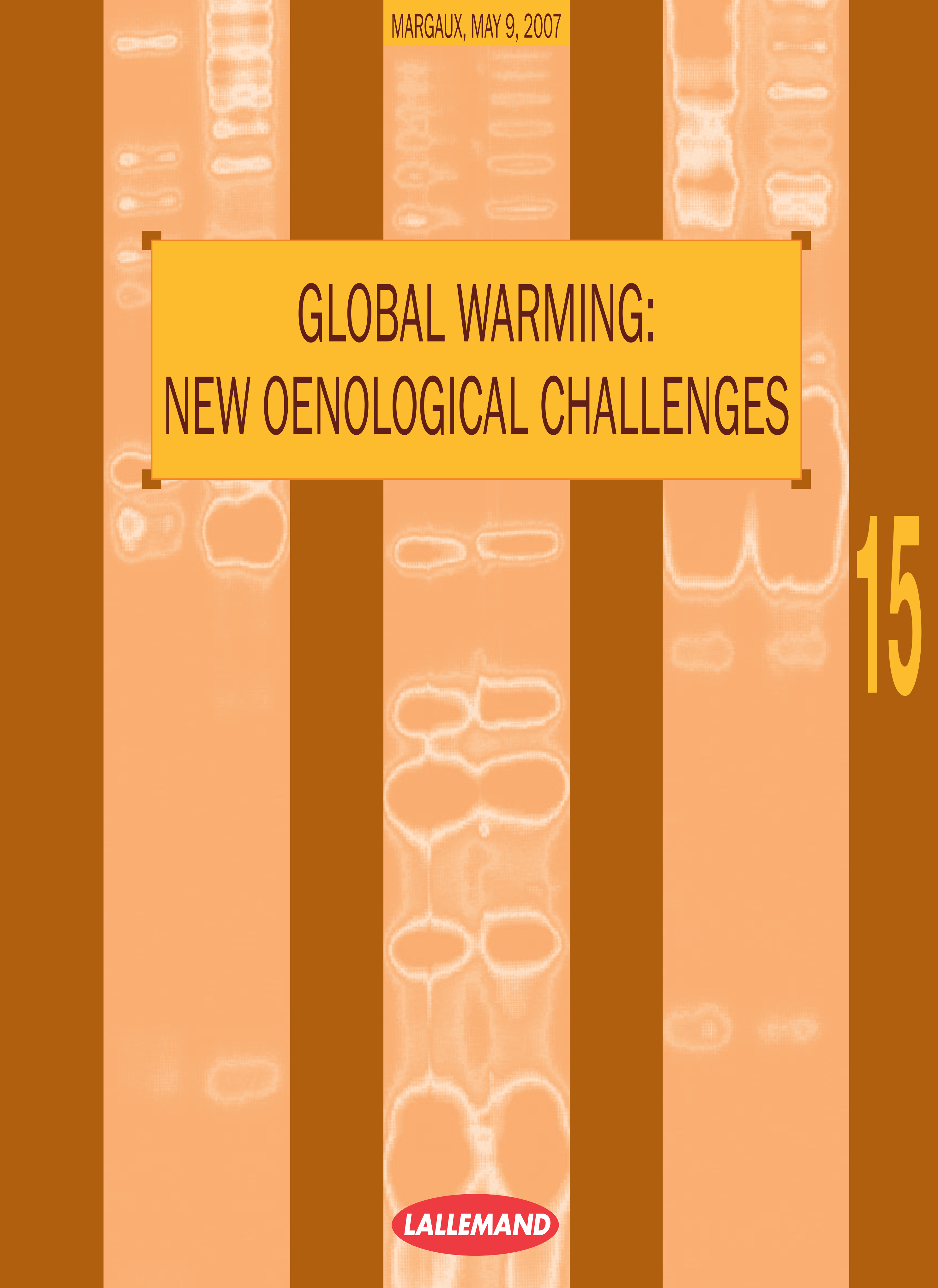

LALLEMAND

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PROCEEDINGS
OF

LES XIX^{es} ENTRETIENS SCIENTIFIQUES LALLEMAND

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FOREWORD

Scientists, oenologists and wine professionals from around the world gathered at the Relais de Margaux, near Bordeaux, France, for the *XIX*^{es} *Entretiens Scientifiques Lallemand*. This technical meeting brought together nearly 150 vine and wine specialists to discuss the impact of climate change on vineyards and winemaking and the issues this raises for the profession.

Following a report on global wine markets by Patrick Aigrain (VINIFLHOR – OIV), Jean-Pierre Gaudillère (INRA Bordeaux) outlined the consequences of different climate change scenarios on vineyards and the quality of wine. To respond to the problem of high concentrations of sugar in the grapes, one of the approaches suggested by the INRA Montpellier is to select yeast strains that produce less ethanol during alcoholic fermentation.

Ramón Mira de Orduña, from Cornell University in the United States, addressed the impact of global warming on lowered acidity in grapes, which could compromise proper fermentation.

Based on observations that the wine harvest is occurring ever earlier, Fernando Zamora, from the Universitat Rovira i Virgili in Tarragona, Spain, noted, "Global warming accelerates the accumulation of sugar in the grape. However, the grape skins and seeds may not be sufficiently mature (the phenolic maturity), resulting in unbalanced wines that are too bitter and astringent, with herbal aromas."

Climate change is now a certainty and winemaking must adapt accordingly. The new conditions will help orient the research carried out at Lallemand to better respond to the specific needs of the users of microbiological products.

CONTENTS

One of the great contributors to the *Entretiens Scientifiques Lallemand* passed away in early 2008. Professor Michel Feuillat had an outstanding career, contributing to the knowledge of wine microbiology to an extent that will be remembered for a long time. He was involved in some of the first *Entretiens Scientifiques Lallemand*, and continued to do so until the 2006 meeting in South Africa. His research has made us better aware of the impacts of yeast and yeast derivatives. Most importantly, we will remember him for his friendship and kindness.

To honour him, we would like to dedicate the *XIX^{es} Entretiens Scientifiques Lallemand* to Professor Michel Feuillat.

The Fermented Beverage Team – Lallemand

THE CONJUNCTURE OF THE WORLD WINE SECTOR AND THE EUROPEAN COMMON MARKET ORGANISATION FOR WINE REFORM

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The study of the evolution of the potential wine production in the world shows a reversal from the previous 20-year trend, ending the rapid reduction in the growth of world vineyards that began in the early 1980s. This reduction has been followed by a slight recovery, with total vineyards now nearing 8 million hectares (young vines and all types of grapes included).

Currently, Europe (including the former USSR) represents only 59% of the surface area of vineyards in the world, ahead of Asia, which is oriented mainly towards the production of table grapes and raisins. Next are North America and South America, ahead of Africa, then Australia and New Zealand combined.

The regression at the end of the 20th century occurred mainly in Europe, notably under the influence of European Union premiums for the permanent abandonment of wine-growing, a program that ceased at the end of the 1990s, and also due to decreased winegrowing in Eastern Europe subsequent to the fall of the Berlin Wall. Not until the end of the 1990s to the early 21st century did the growth of non-European vineyards – in Asia (China), Australia and New Zealand, South America (Chile), North America (when the United States started to recover from the phylloxera crisis in 1995) and South Africa – start to compensate for the regression in European vineyards, whose reduction was lessened due to the cessation of the “grubbing-up” program for the massive permanent abandonment of vineyards and the progressive stabilization of vineyards in Central and Eastern Europe.

The three major European winegrowing regions – Spain, France and Italy – are still in the lead, but Asia is rising in the ranking of the 12 main world winegrowing areas. However, not all Asia’s grapes are destined for winemaking, which is the case notably in Turkey and Iran, and also in the Chinese grape-growing region, despite the recent development in winemaking.

World grape production, whether for wine, table grapes or raisins, is also on the rise, but more noticeably and for longer than the production potential, due to a particularly marked progression of yield between the beginning and the end of the 1990s. This progression reflects both the more favourable weather conditions and the more frequent recourse to irrigation in the southern hemisphere that accompanied the partial relocation of the world’s vineyards. Although Europe, at nearly 47%, remains the premier grape-producing continent, the differences in growing practices increase the relative weight of the continents in terms of production potential, most notably in North and South America, and Australia and New Zealand. Furthermore, traditionally the production of vineyards not destined for winemaking has been greater than the production for wine, especially in Europe. Italy, which is both a major winegrowing region and a significant producer of table grapes, is the premier world producer of grapes, ahead of France, while Spain suffers a water deficit over a large part of its vineyards that limits production, except where growers resort to irrigation, a growing trend in the past 10 years. Egypt also appears in this ranking due to the high productivity of table grapes.

Globally, on average from 2001 to 2005, out of 625 million hundredweights (100 kg) of grapes produced, 27% was for eating as fresh fruit, 7% for drying into raisins, and 66% was pressed. While only 58% was turned directly into wine, a major portion of the crushed grapes not made into wine was reincorporated into the following grape harvest, notably for enriching it (chaptalization), as the production of grape juice is evaluated at a little more than 10 million hectolitres.

The evolution of world wine production reflects the reversal in the trend at the end of the 20th century previously noted, but there is great inter-year variability. Thus, a few years ahead of the projections for 2010, world production reached 300 million hL (except for juice and musts) in 2004, while the 2007 forecast of 266 million hL should lead to a level of production similar to 1998.

With more than two thirds of world production, Europe is still far ahead as the world's premier producer of wine, ahead of North and South America. Note that the relatively low proportion of Asian production is due to its orientation towards non-wine products. Inversely – compared to its weight in the potential for production – the high proportion of Australia and New Zealand reflects the high yield of its winegrowing vineyards, like other countries in the "new world" (with the exception of New Zealand, whose importance in wine production is moderate).

As regards wine consumption, it is important to note that, in terms of the profile for the consumption of alcoholic beverages, a certain convergence can be observed in the relative breakdown of commercial alcohols in developed countries. While the traditional winegrowing countries see their proportion of wine decrease, inversely the traditional brewing countries are seeing their proportion of wine increase, to the detriment of beer. But in the long term, this "model" of evolution is not applicable everywhere, particularly in developing countries where wine, which is mainly imported, remains an expensive beverage, or in China, where the consumption of wine, despite recent developments, does not yet represent 1% of the alcohol consumed in this country.

For a little more than 10 years, the world consumption of wine has progressed at the rate of approximately +1.8 million hL/year and now stands at a little more than 240 million hL.

Europe remains by far the premier market for wine (at two thirds). Although consumption in traditional producer countries is decreasing (and is only partially compensated by the progression of Northern Europe, and recently by Russia), it is ahead of North and South America (at a little more than 20%), progressing notably in the North (in the United States and Canada), while Asia is also progressing (notably in China).

As regards the evaluation of wine consumption in China, depending on whether you refer to an evaluation, as is the case for the Organisation Internationale de la Vigne et du Vin (OIV), assimilating the balance with consumption of wine equivalent (apparent consumption), or whether you try to identify as far as possible the consumption of "European-style" wines, you must divide by a little more than two the level of wine consumption in this country.

When we compare the current world levels of production and consumption, we could quickly conclude the existence of overproduction in the wine market, but that would ignore the needs for the industrial uses of wines (brandy, vinegar, etc.) that can be evaluated on the world level at nearly 35 million hL.

Measuring the balance in the world wine market is therefore complex, due notably to the fact of the strong interannual variability of production. By taking into account the fluctuation not only of wine, but the musts as well, and the quantity of wine grapes left on the vine (as is the case in Australia, before the low 2007 harvest), the average overproduction in this market from 2001 to 2005 can be estimated at about 17 million hL, or approximately 7% of world consumption, which can normally be absorbed by the variations in stock. But this average must not distract us from the major variations in the harvest, such as in 2004, which led to a surplus apparently higher than 30 million hL, which, until the low 2007 harvest, had contributed to depressing the world market until recently, even though this depression was unevenly distributed among types of wines and markets.

The world wine market (defined here as the sum of exports from all countries) is growing rapidly and now exceeds 80 million hL traded. Although European countries remain the clearly dominant exporters, we can easily perceive the proportion filled by Australia and New Zealand in this trading. The pace of development for Australia's exports is indeed very rapid, even though that of Spain, a "new world" country, given the organization of its usual channels, is also important.

Globally, the United States and countries in the southern hemisphere are taking world market share from traditional European exporters, France in particular, and to a lesser degree from Italy.

At the time of our meeting (the *XIX*^{es} *Entretiens Scientifiques Lallemand*), the European Commission had presented its reform. Since then, the political compromise that came out of the last Council of European Union agriculture ministers (in December 2007), which is not yet formalized, clearly differs from the application of certain proposals, but does not put into question the overall orientation of the project. After a wave of pulling out grapevines (reduced in terms of quantitative objectives over the initial project), the European market will be gradually liberalized. Thus, for the great majority of producers (except those whose wine production benefits from a strong international reputation and who could continue to get value from production constraints), the necessity of playing "cost-volume" strategies will become increasingly important. At which point the average productivity of the European vineyard, the main factor for the division of per unit production costs for wine, should increase to resist ever stronger competition, with its corollary, notably in the Mediterranean zone: resorting to irrigation.

This evolution of the next Common Market Organisation (CMO) for wine assembly, in a context of climate change marked notably by the increasingly delicate problem of water management already apparent in Australia and southern Spain, is an important reason to immediately begin discussing what technical strategies for adaptation should be implemented, especially if we consider the increase in the degree of alcohol in wines that such climate change could accentuate, while consumption trends in developed countries, still in the majority as regards the world importing of wines, are not particularly inclined towards a laissez-faire policy for world alcohol consumption.

CLIMATE CHANGE AND VITICULTURE

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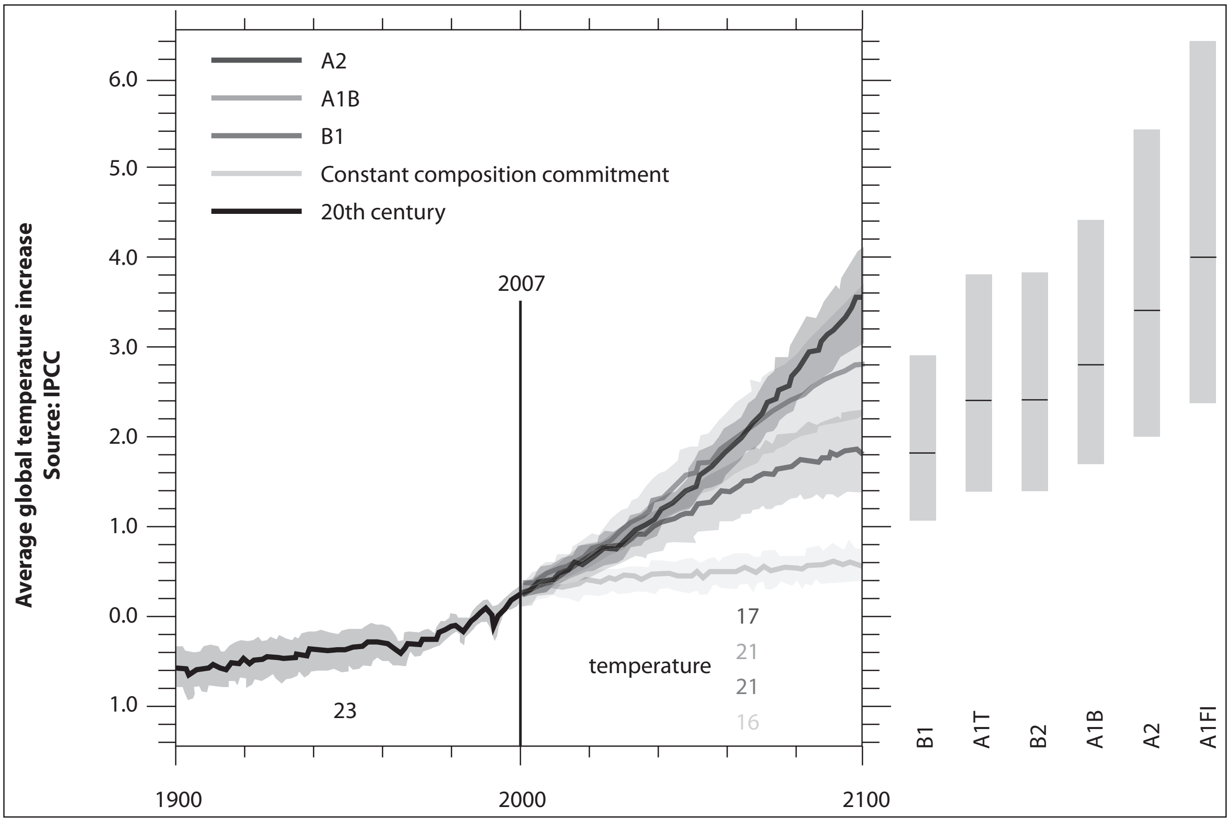
Introduction

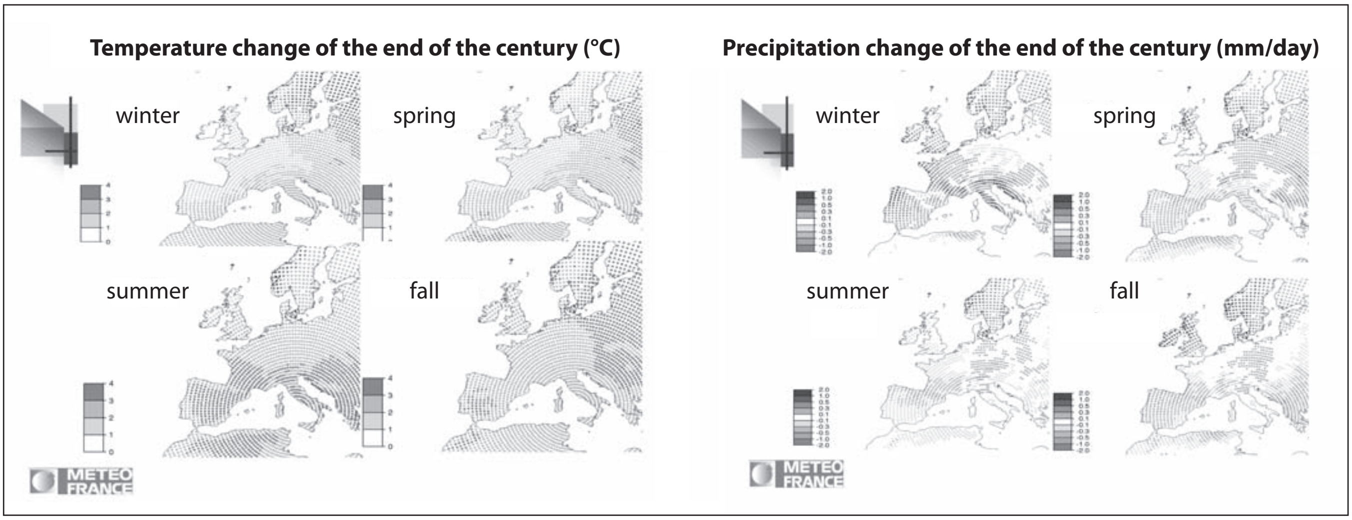
The greenhouse effect is a natural phenomenon resulting from the presence of gases in the atmosphere that absorb the infrared thermal rays emanating from the Earth's surface, and without which the average temperature of the planet would be about −18°C instead of +15°C. In the early 1970s, the observation of a noteworthy increase in the concentration of certain greenhouse gases – clearly tied to human activity – led to forecasting the possibility

FIGURE 1. Evolution of global temperature for several climate models and scenarios for the evolution of CO_2.



The French models CNRM and IPSL contributed to the recent report, and the CNRM report allows us to obtain scenarios with a spatial resolution of 50 km, sufficiently precise to evaluate regional trends.

Figure 2. Regional climate scenarios for 2070 by the CNRM (Perarnaud et al., 2005).



of climate change due to the reinforcement of this greenhouse effect. At the top of the list of greenhouse gases is carbon dioxide (CO_2), whose current level exceeds 380 ppm, compared to 260 ppm in the pre-industrial era, and which is likely to reach 450 ppm to 1,000 ppm by the end of the 21st century, depending on the evolution of energy policies.

Since this realization of the influence of humans on the global climate (which is only one of the components of what is called global change), the forecasts by climate specialists have become progressively more refined and have earned a degree of confidence. There is now a broad consensus in the community on the very strong probability of the realization of the scenarios presented by the Intergovernmental Panel on Climate Change (IPCC) 2001 experts and confirmed in the recent 2007 report. These scenarios include an increase in the planet's temperature of between 2°C and 6°C (depending on the model, and on the hypothesis for the evolution of CO_2, see Figure 1); a greater contrast in precipitation levels, tending to increase in wet regions and seasons and to decrease in dry situations; with a trend towards an increase in variability and to extreme episodes.

Impact on viticulture

Research from a few years ago had already allowed us to assess the probable consequences on agriculture in France, particularly for annual production (essentially wheat and corn) and for grasses (Delecolle et al., 1999; Soussana, 2001). However, little research had been done on grapevines until the article by Schultz (2000), well known in French winegrowing circles, who was the first to ask the question. The observations on the evolution of flowering dates for fruit trees led to the question of grapevines, and to launching research programs in the field.

Photosynthesis stimulated by the increase in CO_2 at the end of the century

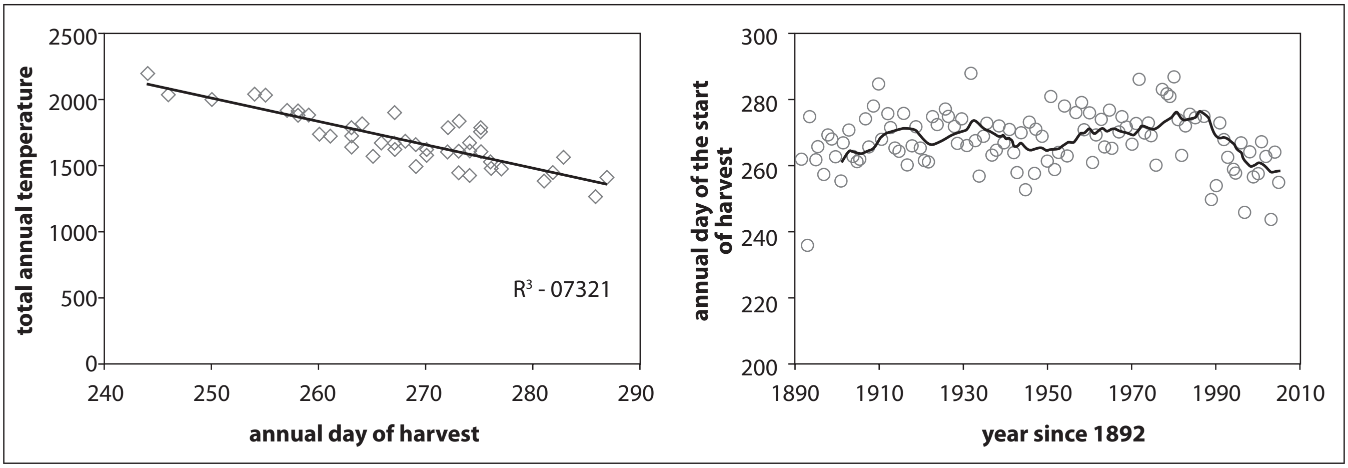
It must first be noted that, like all vegetation canopies, grapevines are directly concerned by the increase in the atmospheric concentration of CO_2, which is in large part responsible for the human reinforcement of the greenhouse effect. The stimulation of photosynthesis related to this increase would reach 20% to 30% (according to the hypothesis of the concentration doubling that of the 1990s, to about 700 ppm), depending on the what is grown and the environmental conditions, and the resulting 15% to 20% increase in biomass, given that respiration will also be reinforced. Moreover, water use efficiency would be increased (by about 10%), given the effect on stomatal resistance.

These effects could introduce a higher degree of complexity in the evaluation of the consequences on grapevines. It is evident that increased biomass production, with the simultaneous increase in water efficiency, will lead to significantly different eco-physiological functioning, and would bring us to reconsider agricultural practices as a whole, taking into account the purely climatic effects that we will discuss now.

Phenological changes are already perceptible

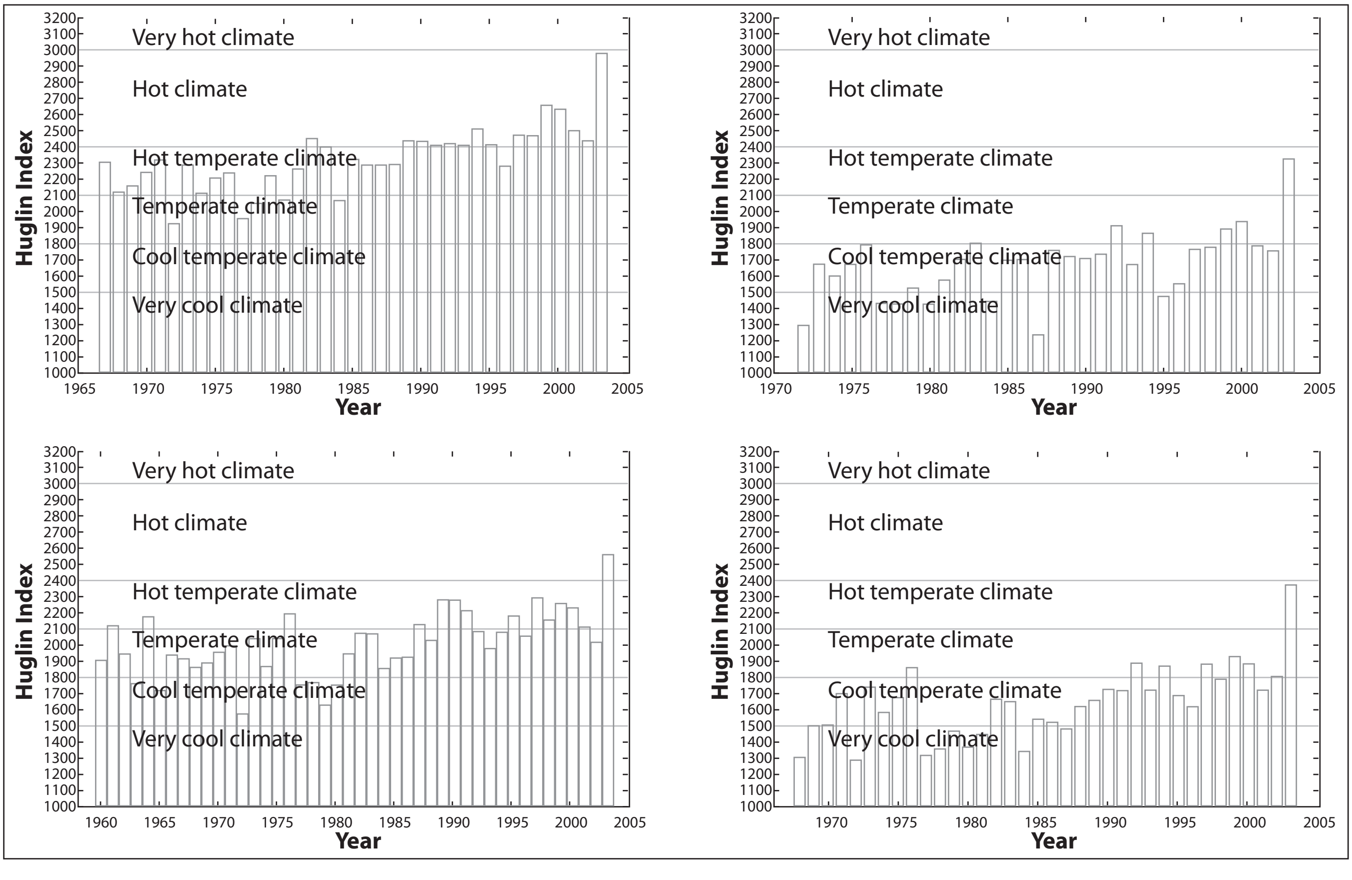
For the moment, the phenological changes concern mainly the temperature factor, which is clearly central to the issue and the most obvious in the climate scenarios (Seguin, 2003b). Indeed, this factor is responsible for the changes noted above, related to the climate warming in

Figure 3A. Relation between the annual temperatures (the sum of daily temperatures higher than 10°C).
Figure 3B. Evolution of harvest dates in Saint Emilion since 1890.



urban France over the past century of about 0.9°C (Moisselin et al., 2002), and more specifically in the past decade, of about 0.4°C to 0.6°C. This evolution affects practically all the phenological manifestations of perennial crops, particularly the flowering dates, which have advanced by two or three weeks in 30 years for both grapevines and fruit trees. And the wine harvest has advanced by nearly a month in 50 years in the Côtes-du-Rhône and 15 days in Bordelais, changes that cannot be explained, at least in part, by changes in viticultural practices. The harvest dates observed at Saint Emilion over the past 40 years show the very significant relationship between the total annual temperature and the harvest date (Figure 3A). Historical material for Saint Emilion (Figure 3B) shows variable but on average stable harvest dates throughout the 20th century (on average the 270th day of the year, i.e. September 27). Starting in 1990 we observe a trend to ever-earlier harvests (in 2007, the 258th day, i.e. September 15).

Figure 4. Evolution of the Huglin Index from 1968 to 2003 for Avignon (top left), Bordeaux (top right), Colmar (bottom left) and Dijon (bottom right).



Temperature is the dominant, perhaps exclusive, factor (Figure 3A). A significant development involved the integration of phenological observations in a Phenoclim database, common to fruit trees and grapevines. The study brought together partners in the field of research (in this case, ITV for grapevines) and their historical data, and was coordinated by INRA Avignon, to see if there was a relationship in the local climate data on the sites concerned. Over and above the analysis of the evolution of temperatures, this database resulted in improved models, instead of the classic utilization of total temperature, and serves as a support for deepening the knowledge of this thermal effect on development. These models were then utilized to follow up in real time, on the one hand, and integrated in the mechanistic models simulating the functioning of the crop on the other hand, such as the STICS-vigne model that will be discussed later.

The consequences of the recent evolution of the climate on the *terroirs*

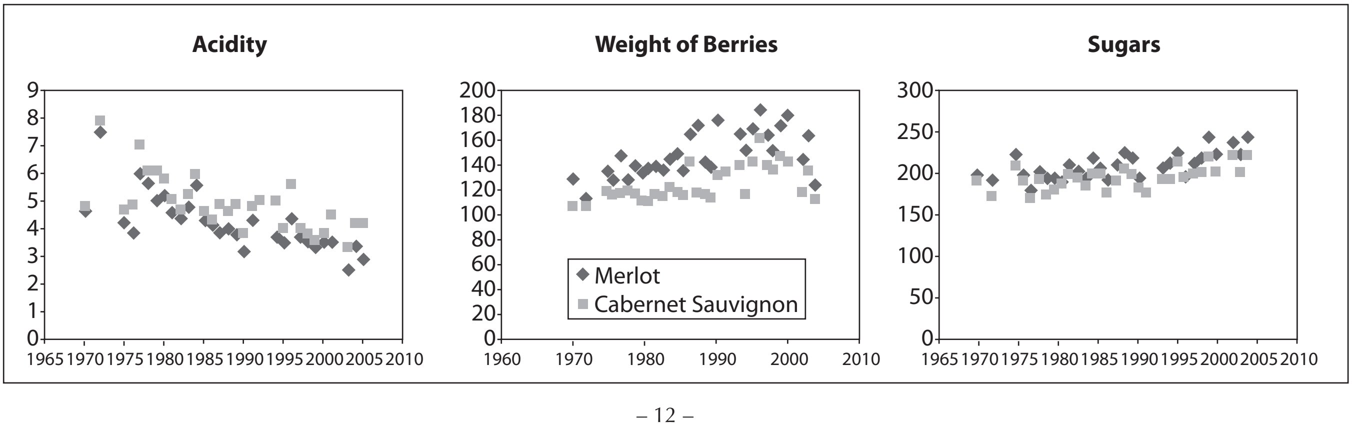
First, it was possible to assess the significance of the recent evolution by utilizing intermediate tools between the temperature data alone and the more elaborate models under development (Figure 4). These tools often take the form of indexes, such as the Huglin Index (1978), which is based on temperature and forecasts the harvest date.

The retrospective analysis of harvest dates over the past 30 years at seven INRA sites that have climate data from the network managed by Agroclim (Angers, Avignon, Bordeaux, Colmar, Dijon, Montpellier and Valence) made obvious a clear and common trend, among all the sites, to higher indexes, with more or less variability in the past 10 to 15 years. This climate, manifestly warmer and more regular, is clearly favourable to viticultural production, as the information from professional circles on the increase in sugar levels and the decrease in acidity during this period can attest (completed by the findings of Duchêne and Schneider, 2005, on the Alsace vineyard).

However, analysis of the Huglin Index and the sums of temperature led us to observe that this evolution comes with a clear trend towards extending into the future the limits of adaptation for varietals in climate zones as they were defined based on the supposedly stationary climate of the past (Figure 6). The exceptional case of the summer of 2003 (Figure 4), with summer temperatures 3° to 4°C higher than normal (which made it the hottest on record since 1370, based on the historical analysis of harvest dates in Burgundy), and a marked drought starting in June, fortunately showed the limits of such an empirical approach to the effects of climate change: even though the 2003 vintage is particular and does not lend itself to long aging of the wines, it was not the total catastrophe that the Huglin Index might have led us to suppose it would be. Of course, identical conditions in the future will lead to wines with a typicity that is different from those we have known for centuries, but various strategies for adapting can be imagined. This qualitative evolution of the harvest can already be observed in the data collected in standardized conditions for about 30 years by INRA Bordeaux (Figure 5). We can note a regular decrease in the acidity of musts, an increase in the size of the berries, and an increase in the sugar levels. The qualitative evolution of Merlot grapes is more marked than Cabernet Sauvignon grapes. This trend is not totally due to the changes in temperature. The evolution of practices to better control the fruit load (through the reduction of the number of bunches) has an effect on the increase of the size of the berries.

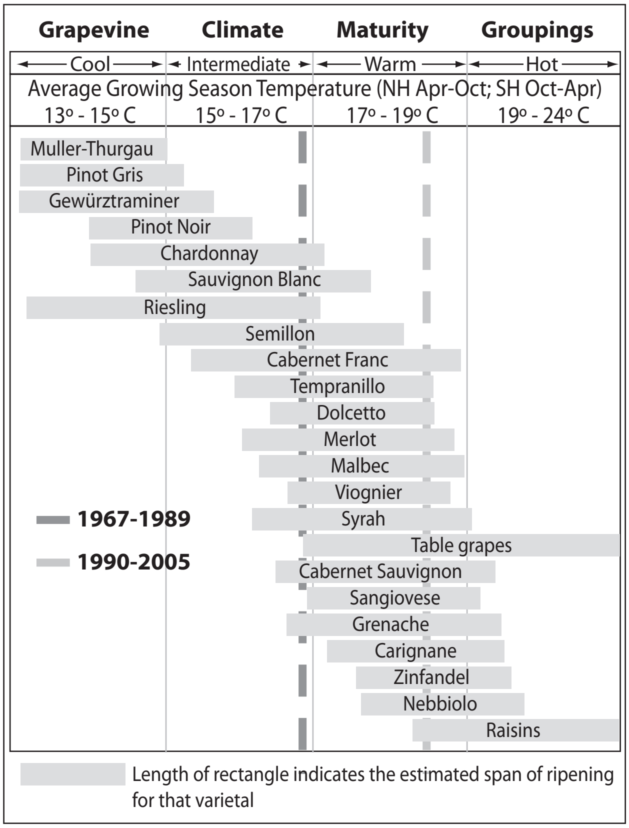
To justify these practices, more complete tools, based on the plant's eco-physiological functioning, must be utilized, such as the STICS-vigne model, whose initial components

Figure 5. Evolution of the quality of grapes at harvest over the past 35 years in the Bordeaux Pessac Leognan appellation for the Merlot and Cabernet Sauvignon varietals (INRA Bordeaux).



were designed by Brisson et al. (2002), and which was recently developed by Garcia de Cortazar (2007). The impact study of climate change on French vineyards as a whole was based on the joint utilization of the STICS model and the simulated climate data by the ARPEGE-Climat model (Météo-France). The planting structures and traditional techniques were defined for each region. The main findings show a major modification in the phenology, as well as an increase in vegetal biomass and production (except in the vineyards of Côtes-du-Rhône and Languedoc where a decrease was observed), an increase in the hydric stress level at the end of the cycle, and a major modification of the climate conditions in the veraison-harvest period. Given these results, different technical combinations have been proposed to adapt vine behaviour to the climate changes for each region. Additionally, an initial analysis of the possibility of growing a varietal (Syrah) outside its traditional zone has confirmed the empirical impression of a possible extension towards Bordelais, Cognac and the Val-de-Loire for a moderate warming (2°C), and towards Alsace or Champagne for a 3° to 4°C warming, but, in any case, the product would be different from the traditional one.

Figure 6. Climate requirements of varietals and average temperatures registered at Bordeaux between 1967 and 1989 (blue), and 1990 and 2005 (red).



Conclusion

Global warming will have a significant effect on viticultural production throughout the world, and a little more so in Europe where warming will be more pronounced than in the southern hemisphere. Also, it will surely be more controversial, given the link to the *terroir* (Seguin and Garcia de Cortazar, 2005). Current AIC regulations do not allow us to easily foresee the possibility of adapting varietals to new conditions, but this is obviously one of the options that can no longer be neglected in the future. The other is to seek, as far as possible, to maintain the traditional system, by trying to limit as far as possible temperature increases in the topoclimates (hills), microclimates (valley bottoms at night) or phytoclimates (height and spacing, foliage density), etc. The objective is to slow the maturation process of the grapes and encourage the development of secondary metabolites involved in sensory characteristics of wines. Indeed, oenology will have a role to play in safeguarding typicity, and even creating new typicity, based on the components of the harvest that will have changed considerably, with more sugar and alcohol, less acidity, and more subtle components on which the impact of climate change is still poorly understood.

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MANAGING EXCESS ALCOHOL IN WINE: A NEW CHALLENGE FOR WINE YEAST

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In the past 20 years, alcohol levels in wine have increased significantly. This trend, observed in numerous wine-producing regions, is related to a variety of factors, including global warming and the selection of varietals that produce high levels of sugar, as well as the evolution of winemaking practices that encourage the harvest of very mature grapes. The increase in the degree of alcohol in wines is a concern for several reasons. On the one hand, in strong concentrations ethanol can affect the aromatic properties of wines due to its interaction with certain aroma molecules. On the other hand, the current market is in tune with consumers' health concerns and prevention policies, and is thus more interested in easy-to-drink wines with moderate alcohol levels. Moreover, in certain countries there are economic constraints due to the taxes imposed on the degree of alcohol. Therefore, there is a strong demand for wines with lower alcohol levels. This demand has led the industry and researchers to consider various strategies that rely on interventions at different levels, such as the selection of varietals or physical intervention at different phases in the fermentation process. One attractive option consists of utilizing yeast starters that produce less alcohol for the same amount of sugar consumed. The objective of this article is to expose advances in research in this field. After presenting the situation and the scientific issues related to the development of strains that produce less alcohol, we will describe the main approaches developed recently to reach this objective, as well as strategies for the future.

Introduction

During fermentation, yeast transforms 1 mole of sugar into 2 moles of ethanol and 2 moles of CO_2 via the glycolysis (Embden-Meyerhoff) pathway, according to the $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ reaction. A significant portion of the sugars is utilized for the formation of biomass and other by-products of fermentation (e.g., glycerol, organic acids, esters and higher alcohols), thereby lowering the effectiveness of the conversion of sugars into alcohol, which reaches 92% to 93%. Ethanol production during oenological fermentation is 0.47 g of ethanol per gram of sugar, which represents the quantity of sugars necessary to produce one degree of alcohol per 16.8 g of sugar.

The possibility of selecting a yeast starter from among the oenological strains that produces less ethanol is limited by the fact that *Saccharomyces cerevisiae* yeast presents low diversity in terms of the conversion of sugars into alcohol. A study based on the comparison of the ethanol production of 60 commercial yeast strains during fermentation under standardized laboratory conditions demonstrated that the quantity of sugars necessary to form one degree of alcohol varies according to the strain, from 16.5 to 17.0 g. With such a small differential, we can expect only very slight variations in the degree of ethanol, less than 0.5°.

As the objective is to lower the ethanol level by 1° to 2°, obtaining starters that produce low levels of alcohol relies on metabolism modifications that redirect part of the sugars towards by-products other than ethanol. Bear in mind that to obtain one degree less of alcohol, a large quantity of sugars (16.8 g per degree of alcohol) must be redirected towards other metabolites. Thus, the choice of target metabolites is crucial, as their accumulation must not alter the sensory properties of the wine. Moreover, the properties of the yeast (e.g., their fermentation and growth capacity, etc.) must be preserved. Given these major constraints, the development of strains that produce lower levels of ethanol is a challenge for science and has been a field of active research for a decade.

Strategies to lower the ethanol production of yeast

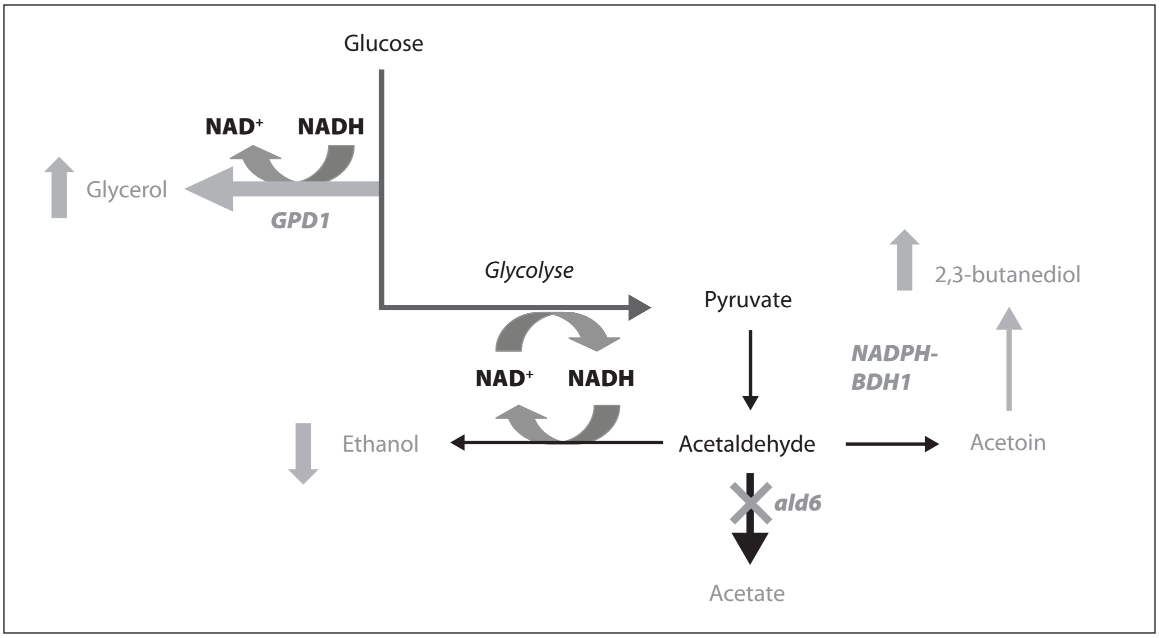
Metabolic engineering strategies are powerful approaches for remodelling metabolism. Based on the modification of existing enzymatic reactions or the introduction of new reactions, these approaches can be useful for diverting the sugars or the glycolytic intermediaries of the main pathway, glycolysis. Different strategies have been envisaged.

Malherbe et al. (2003) expressed the glucose oxidase of *Aspergillus niger* in *S. cerevisiae*. The expression of this enzyme, which is capable of oxidizing the sugars into gluconolactone and H_2O_2, results in lowered ethanol production due to the lower quantity of sugars entering the glycolytic pathway (the Embden-Meyerhoff pathway). The main drawback of this strategy is related to the conversion of gluconolactone, through the chemical pathway of the product of this reaction, into gluconic acid, a compound that has a strong tendency to combine with SO_2. Furthermore, the oxidation reaction of glucose requires oxygen, which in even small quantities can foster the production of oxidized compounds during fermentation.

Another approach developed by the INRA in Montpellier is based on the deviation of pyruvate, at the end of the glycolytic pathway, towards the formation of lactic acid to the detriment of the alcohol pathway, by the expression of a bacterial lactate dehydrogenase (Dequin et al., 1994). Lactic acid is an interesting compound due to its absence of flavour and acidifying properties. Increased production of this compound, formed in trace amounts by yeast metabolism, could rescue the lack of acidity often associated with high-alcohol wines. In the yeast cell, lactic acid plays the role of accepting the electron equivalent to that of ethanol. The deviation of the metabolism towards the production of this organic acid allows a concomitant decrease in alcohol production, without affecting the intracellular oxidoreduction balance. However, this approach is limited by the amount of lactic acid acceptable in the wine. Considering that its level must be under 10 g/L, in order to avoid excessive acidification, the expected decrease in alcohol yield remains under 0.5°. On the other hand, this strategy is very relevant for correcting acidity problems in wines (Dequin et al., 1999).

More recently, a strategy based on the expression of a bacterial NADH oxidase in yeast has been considered. This is about lowering the intracellular level of cofactor NADH, which is essential to the activity of the fermenting alcohol dehydrogenase. Indeed, this strategy could drasti-

FIGURE 1. Strategy for lowering ethanol production based on deviating sugars towards the formation of glycerol and 2,3-butanediol.



cally lower ethanol production, but results in a strong accumulation of acetaldehyde, which disrupts yeast growth. These effects can be limited by a slight modulation in the oxygen necessary for NADH oxidase activity. However, the production of oxidized compounds is strongly increased due to the limitation of the flow through ADH, and to the presence of oxygen (Heux et al., 2006a, Heux et al., 2006b).

A potentially very interesting strategy is based on the deviation of the carbon flow towards the production of glycerol. Glycerol is the most abundant by-product of alcoholic fermentation after ethanol and CO_2. With no flavour of its own, it can contribute to the wine's mouthfeel, and, over 25 g/L, to its viscosity. The increase in the level of synthesis of the glycerol-3-phosphate dehydrogenase by the overexpression of the GPD1 or GPD2 genes coding for two isoforms of this enzyme has permitted the effective decrease in ethanol production by up to 15% to 20%, which represents the most marked deviation obtained to date (Michnick et al., 1997; Remize et al., 1999). This decrease results both from the deviation of the carbons towards glycerol and the decreased availability of the NADH, utilized preferentially for the synthesis of glycerol. However, this deviation comes with major modifications in the production levels of other metabolites, including some that are undesirable in the wine, notably acetate and acetoin. Acetate production can be reduced by the deletion of the ALD6 gene coding for an acetaldehyde dehydrogenase (Remize et al., 2000; Cambon et al., 2006). Very recently, efforts have been focused on the reduction of acetoin production, a compound accumulated in strains that are high producers of glycerol (about 15 g to 20 g/L) at several grams per litre, while its olfactory detection threshold is about 150 mg/L. The acetoin produced by the yeast is naturally completely converted into 2,3-butanediol. In yeasts that hyperproduce glycerol, this reaction, catalyzed by the butanediol dehydrogenase, is limited, leading to a strong accumulation of acetoin. The increase in the level of synthesis of this enzyme, as well as the overexpression of a NADPH-dependant mutated form, can strongly lower the accumulation of this compound (Ehsani et al., Brevet FR 07/03279) by encouraging its conversion into 2,3-butanediol, a sensory-neutral compound.

This strategy, based on deviating sugars towards the formation of glycerol and 2,3-butanediol, permits a 15% to 20% reduction in ethanol output, which can be modulated according to the level of glycerol overproduction. The foreseeable impact on the level of ethanol is from 1.5° to 2°. Research currently underway aims to study the impact of these modifications on aroma compounds and on yeast metabolism overall.

Conclusion and Outlook

Numerous studies have been completed over the past 10 years to develop yeast strains that produce less alcohol. The approaches tested have been largely based on metabolic engineering, which allows the remodel of the metabolism in a directed way, based on the knowledge of the pathways and genes involved. While several of these approaches have proved relevant for lowering ethanol output, they have also revealed a certain number of difficulties. The strategies based on oxygenation during the fermentation process, however restrained, are to be avoided, because they lead to an accumulation of oxidized metabolites. A major difficulty is the management of side effects, particularly the accumulation of undesirable products, related to the redirection of the flows. Among the different strategies studied, one very promising approach is the redirection of carbons towards glycerol, as the associated side effects have been mastered. For this strain, where modifications were introduced to existing genes, the approach is self-cloning. In certain countries, this strategy benefits from special status. In Japan, for example, self-cloning is not considered to be a genetic modification approach (Akada, 2002).

To date, the utilization of genetically modified (GM) oenological yeast strains is not very well accepted, which has encouraged "classic" approaches to genetics that are less controversial. Breeding techniques based on crossing spores have led to the improvement of numerous existing traits. These approaches have been used to select hybrids that overproduce glycerol (Eustace and Thornton, 1987; Prior et al., 1999; Prior et al., 2000). However, they are limited by the level of overproduction reached. Hybrids producing 12 g to 15 g/L have been obtained in synthetic media, but their production in natural must has been lower, at about 9 g/L, which does not result in a significant reduction in the degree of alcohol.

An alternative to the metabolic engineering approaches is the selection of mutants through directed evolution. Based on the selection of variants, most often obtained by maintaining a strain through numerous generations in selective conditions to force adaptation, these approaches have had some success in recent years in improving certain traits.

Such innovations can also be expected from the choice of target. For example, reorienting a part of the carbons towards the pentose phosphate pathway is foreseeable. This pathway, which plays a vital role in yeast in order to provide NADPH and precursors for biosynthesis, is an alternative to glycolysis for the degradation of sugars. As some of the carbons that take this pathway are eliminated in the form of CO_2, an amplification of the pentose phosphate pathway could lower the quantity of carbons available for the formation of ethanol. Strategies for the deviation of the carbon flow towards the pentose phosphate pathway, based on metabolic engineering approaches and directed evolution approaches, are currently under study in our laboratory as part of the PNRA VDQA "Quality wines with reduced alcohol levels" research, coordinated by J. L. Escudier, INRA Pech Rouge.

Whatever the method, it is essential to analyze the effects of metabolic reorientations globally, at the cell level. One can wait for the utilization of post-genomic tools (transcriptomics, proteomics and fluxomics, etc.) for a better understanding of the effects of modifications as well as the identification of the phenotypes obtained through the selection or evolutionary adaptation approaches. One of the limits of the current approaches to improvement is the poor understanding of the metabolic network as a whole, and the mechanisms involved in the interconnections between pathways. Systems biology – an emerging discipline whose aim is to analyze the metabolic network as a whole, through steps associating biology with modelling – will generate this type of knowledge, so essential for designing more rational improvement strategies and putting them to work.

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CONTROLLING MALOLACTIC FERMENTATION IN A CHANGING CLIMATE

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Abstract

Several temperature models suggest that climate change will affect regional suitability for winemaking. Specifically, temperature increases that lead to grapes and musts with higher sugar concentrations and pH values are now encountered more frequently, and this evolution may require alternative viticultural practices and vinification decisions. On the winemaking side, the high pH and increased sugar concentrations, which lead to wines with higher alcohol content, may increase the risk of microbiological spoilage and cause more difficult alcoholic and malolactic fermentations. Malolactic fermentation (MLF) is a secondary fermentation carried out in most red and some white wines, especially in cool climates, and leads to acid reduction and flavour modifications. Encouraging spontaneous MLF, carried out by bacteria naturally present in musts and wines, can lead to unpredictable results and spoilage, especially in the context of evolving grape quality. The availability of commercial freeze-dried preparations of wine lactic acid bacteria with known characteristics, as well as their combination with nutrients, has led to more controllable MLF and wine quality. Yet, in spite of these advances, MLF may continue to be difficult in some wines. This article will present some of the challenges of, and possible alternatives to, traditional fermentation management.

Introduction

Current observations and various weather models clearly indicate the world climate has changed dramatically and will continue to evolve at a similar pace (http://www.ipcc.ch). Evidently, it is expected that climate modifications will also affect winegrowing regions (White et al., 2006). While overall an increase in temperature is expected, the actual extent may vary widely, as may the weather conditions in individual winemaking regions (Schär, 2004). Like other plants, vines react to environmental factors, including light, temperature and water availability. Changes in these factors, especially water availability and temperature, eventually influence grape composition and thus the must used in winemaking. Higher temperatures generally lead to higher potassium uptake and lower malic acid content, and affect grape and must pH values, and also lead to higher sugar concentrations translating into higher potential alcohol contents (Coombe, 1987). Accordingly, wines with high alcohol concentrations and higher pH values are now more frequently encountered, in cooler climates as well.

Higher pH values increase the spectrum of microorganisms capable of colonizing and spoiling food and, consequently, food acidification has been one of the traditional cornerstones of food preservation. Beer, which typically has pH values somewhat higher than wine (> pH 4), is affected by a larger number of spoilage microorganisms, which may include *Enterobacteriacae*, *Bacilli*, *Micrococci* and *Staphylococci* in addition to various lactic acid bacteria and spoilage yeasts, which are also relevant in winemaking (Priest and Campbell, 2003). Certainly, while the effect of pH on the microbiology of beer and wine cannot be denied, the overall lower ethanol content of beer also contributes to its higher spoilage susceptibility. At the same time, the high sugar concentrations that are now often found in grape musts lead to alcohol levels that can be high enough to not only inhibit potential spoilage microorganisms, but also the growth and performance of the production organisms required for vinification. Consequently, sluggish and stuck fermentations, where the fermentative activity of production microorganisms slows down, or completely stalls, remain a serious problem in the wine industry and may leave unfinished products exposed to oxidation and microbial spoilage.

During alcoholic fermentation, the production organisms – typically yeast of the species *Saccharomyces cerevisiae* – may be osmotically stressed by high sugar concentrations (Zuzuarregui and del Olmo, 2004). At the same time, their production of ethanol, a cytotoxic compound, may also lead to sluggish or stuck fermentations (Ansanay et al., 2001) leaving varying amounts of residual sugars in wines, which may become unsuitable for sale. In the production of most red wines and some whites (mainly those grown in cool climates) a secondary fermentation, called malolactic fermentation (MLF) may also be encouraged, and MLF most often occurs during the final phases of alcoholic fermentation (AF), or after its conclusion (Davis et al., 1985). MLF is carried out by lactic acid bacteria (LAB) and leads to the deacidification of the wine and to aroma modifications (Lonvaud-Funel, 1999). While both AF and MLF may result spontaneously from the activity of yeast and bacteria naturally present in musts and wines, for some years highly concentrated freeze-dried preparations of yeast and bacteria have been used to induce AF and MLF, respectively (Lonvaud-Funel, 1999). This, combined with better nutrient management, has led to faster and more predictable fermentations and wine quality. Yet, especially considering the increase in challenging alcohol concentrations, MLF remains difficult to accomplish in some wines or can be slow, mainly because of the strong combined inhibitory effect of ethanol and acidity in wines (Wibowo et al., 1988; Vaillant et al., 1995).

While MLF may have a less important role in hot climates because the increased temperature is associated with a decrease in total acidity, MLF remains an essential tool in cool climates and for most red wine fermentations, and, thus, viable alternatives to traditional processes are sought for cases when MLF is difficult. Alternatives may be found at different stages of the winemaking process, and involve various techniques. For example, musts with high sugar concentrations may be diluted, or sugar reduction treatments may be used, to decrease the potential alcohol level where allowed and desirable. Certainly, technological solutions for the reduction of alcohol in wines after alcoholic fermentation also exist. Besides technological approaches, the modification of the microbiological factors is an interesting option. For example, the utilization of specifically adapted microorganisms to address vinification problems appears interesting (McBryde and Jiranek, 2003; McBryde et al., 2006). At the same time, optimizing the yeast and bacterial inoculation protocol can also improve fermentation kinetics.

In fact, successfully inducing simultaneous AF and MLF, where the must is inoculated with both yeast and bacteria, could be beneficial in regards to the microbiological and technical aspects. This would allow more efficient malolactic conversion in difficult wines (e.g., wines with low pH) because of the low alcohol concentrations and the higher nutrient content present in fermented grape musts compared to wines. In addition, MLF would benefit from the heat released during AF, which can be relevant in cellars with little temperature control. Wines obtained after successful AF/MLF would be immediately ready for downstream treatments, such as racking, fining and the addition of sulphur dioxide, thus increasing microbiological stability and processing efficiency.

However, and in spite of the considerable interest in this technique, its application is not very common due to fears of wine quality depreciation by LAB activity in musts, and the limited scientific data available. Specifically, it has been suggested that bacterial activity in high sugar content musts may lead to acetic acid formation, and/or to yeast inhibition (Lonvaud-Funel et al., 1988). Table 1 shows results from a multiparameter study with New Zealand Chardonnay, where simultaneous AF/MLF was compared to traditional consecutive inoculation protocols. More recent results from a multi-pH study with French Chardonnay in the 3.2–3.65 pH range are also presented.

Results

MULTIPARAMETER STUDY WITH NEW ZEALAND CHARDONNAY

Results from this study were published in the January issue of *Applied and Environmental Microbiology* (Jussier et al., 2006). By definition, wines were considered to be dry once the residual sugar fell below 0.1% (1 g/L). In consecutive treatments, this was the case after 21.6 days (Table 1), and thus, the time point of inoculation with bacteria.

Table 1. Delay observed to reach dryness (combined glucose and fructose levels below 1 g/L), and L-malic acid concentrations below 500 and 100 mg/L during fermentations of Chardonnay must with *Saccharomyces cerevisiae* CY3079 combined with *Oenococcus oeni* EQ54 or Alpha strains (consecutive treatments were inoculated with bacteria after reaching dryness at 21.6 days).

Malolactic strain	*Oenococcus oeni* EQ54			*Oenococcus oeni* Alpha		
Treatment	Simult.	Consec.		Simult.	Consec.	
		time since inoculation	total time		time since inoculation	total time
Glucose + fructose < 1 g/L [days]	19.50	21.60	n.a.	20.51	21.60	n.a.
Malic acid < 500 mg/L [days]	7.5	28	49.6	7.5	8	29.6
Malic acid < 100 mg/L [days]	27	n.r.	n.r.	20	44.4	66

Simult.: simultaneous
Consec.: consecutive
n.a.: not applicable
n.r.: not reached
Source: Jussier et al., 2006.

The degradation of glucose and fructose was similar during the first three weeks of AF regardless of the time of inoculation with MLB (Figures 1A and 1B). However, after three weeks, the sugar concentrations differed greatly (inserts in Figures 1A and 1B). While wines with traditional, consecutive AF and MLF had combined glucose and fructose concentrations of approximately 700 mg/L, wines produced with simultaneous inoculation of yeast and bacteria had no detectable glucose or fructose residues (inserts in Figures 1A and 1B, and Table 2). Table 1 shows the fermentation times required to achieve malic acid concentrations of less than 500 and 100 mg/L, with the latter being generally recognized as the threshold for a complete MLF (Henick-Kling and Park, 1994). Overall, all treatments with simultaneous inoculation of yeast and bacteria led to faster (Table 1) and complete (Table 2) malic acid degradation. Even when the delays after inoculation with MLB are compared (Table 1), MLF remained faster when bacteria were inoculated together with yeast. The difference was specifically considerable for strain EQ54, which failed to reduce malic acid levels below 100 mg/L after consecutive inoculation management.

Figure 1. Time course of glucose and fructose concentrations during AF and MLF of Chardonnay with *S. cerevisiae* CY3079 and *O. oeni* Alpha. A, simultaneous inoculation with yeast and bacteria. B, inoculation with bacteria after completion of AF (arrow indicates inoculation with MLB in consecutive treatment); ☐ glucose; ○ fructose. Inserts magnify last 30 days of fermentations. Source: Jussier et al., 2006.

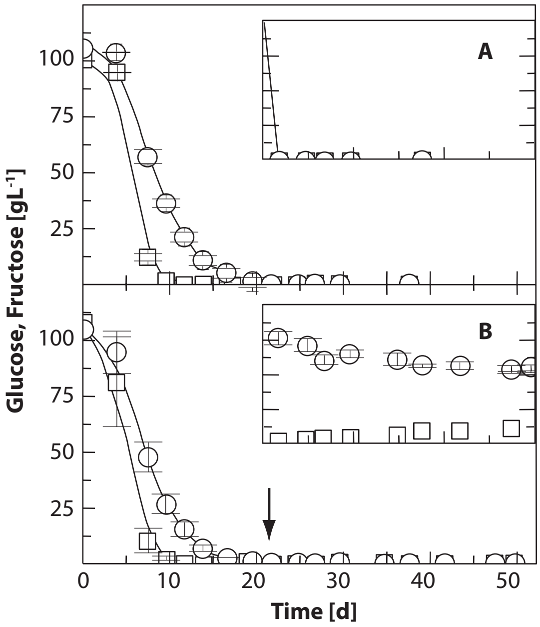

Figure 2. Time course of citric acid degradation and acetic acid formation during AF and MLF of Chardonnay with *S. cerevisiae* CY3079 and *O. oeni* Alpha. A, simultaneous inoculation with yeast and bacteria. B, inoculation with bacteria after completion of AF (arrow indicates inoculation with MLB in consecutive treatment); ☐ citric acid; , ○ acetic acid. Source: Jussier et al., 2006.

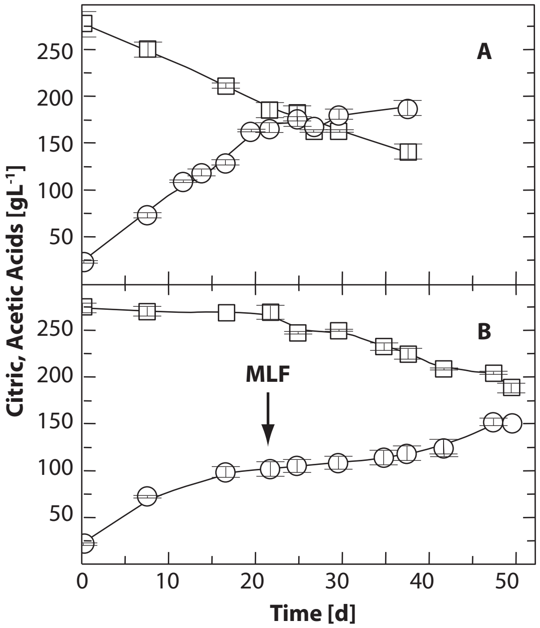

TABLE 2. Values of wine parameters after stabilization with sulphur dioxide. Wines from simultaneous and consecutive AF and MLF with two different malolactic strains (EQ54 and ALPHA) are compared. Student's t-test was used to ascertain statistically significant differences. All values are expressed in mg/L ± SE unless otherwise stated.

Malolactic strain	*Oenococcus oeni* EQ54		*Oenococcus oeni* ALPHA	
Treatment	Simult.	Consec.	Simult.	Consec.
Parameter				
pH	3.54	3.52	3.53	3.53
Total acidity [g/L]	6.38±0.08	6.75±0.06	6.46±0.08	6.55±0.06
Ethanol [% vol.]	13.0±0.3	13.9±0.3	13.6±0.2	14.1±0.3
Acetaldehyde	5.5±1.8	10.9±1.7	6.8±1.6	9.5±1.9
Bound SO_2 /total SO_2 [%]	46.1±0.06	45.6±0.17	46.7±0.07	48.2±0.37
Glycerol	5.3±0.08	5.3±0.02	5.2±0.15	5.3±0.2
Ammonia	0.0±0	0.0±0	0.0±0	0.0±0
Urea	0.0±0	0.0±0	0.0±0	0.0±0
Arginine	0.0±0	0.0±0	0.0±0	0.0±0
Citrulline	0.0±0	0.0±0	0.0±0	0.0±0
Ornithine	0.0±0	0.0±0	0.0±0	0.0±0
Malic acid	0.0±0*	356±4.6*	0.0±0*	40±3.8*
Acetic acid	196±2*	149±1.2*	188±7.5	168±7.0
Citric acid	120±20.7	201±3.6	141±8.6	165±0.3
Lactic acid [g/L]	7.970.07	7.780.05	7.830.05	7.930.02
Fumaric acid	1.410.01*	1.840.01*	1.350.13	1.690.09
Glucose	0.0±0*	140±17.6*	0.0±0*	140±5*
Fructose	0.0±0*	517±17*	0.0±0*	561±21*
Glucose + Fructose	0.0±0*	657±47*	0.0±0*	702±17*

*Statistically significant difference at 0.01 confidence interval
Simult.: simultaneous
Consec.: consecutive
Source: Jussier et al., 2006.

Citric acid was degraded more rapidly in all treatments with simultaneous yeast and bacteria inoculation, and acetic acid formation was visibly correlated to citric acid degradation in these cases, which is shown in Figure 2. During fermentations with bacterial inoculation after completion of AF, acetic acid levels increased during the first two weeks of AF without a strong correlation to citric acid degradation. Final citric acid concentrations in all wines were similar, while small differences could be measured for acetic acid concentrations, which were higher in treatments with simultaneous inoculation of yeast and bacteria (Table 2).

No statistically significant differences were found for any combination of treatments for the concentrations of lactic acid (Ø = 7.88±0.04 g/L), glycerol (Ø = 5.27±0.05 g/L), ethanol (Ø = 13.7±0.25% vol.), bound SO_2 per total SO_2 (Ø = 46.7±0.56%), the total acidity (Ø = 6.53±0.08 g/L) or the pH (Ø pH = 3.53) in the final wines (Table 2).

Sensory evaluation of all wines by a triangle discrimination test with a semi-expert panel revealed no statistically significant differences for any treatment combination regardless of the bacterial strain used and the timing of malolactic fermentation.

SUMMARY OF RESULTS WITH FRENCH CHARDONNAY (MULTI-pH STUDY)

In the consecutive fermentations at different pHs, bacteria were inoculated once the combined glucose and fructose values were below 1.5 g/L, i.e., after 13.5 days. The sugar residues were relatively homogenous (Table 3) across treatments. Except for pH 3.2, simultaneous fermentation always led to complete and faster malic acid degradation (Figure 3). In consecutive fermentations, malic acid degradation was delayed (Figure 3, upper row), and successful only at pH 3.65 and pH 3.5. The time gain from simultaneous AF/MLF was between 41 and 52 days (Table 4).

TABLE 3. Wine parameters after stabilization with sulphur dioxide. Wines from simultaneous and consecutive alcoholic and malolactic fermentations with *S. cerevisiae* CY3079 and *O. oeni* MBR31 are shown. Student's t-test was used to ascertain statistically significant differences. All values are expressed in mg/L ± SE unless otherwise stated.

Parameter	pH	Consec.	Simult.	Parameter	pH	Consec.	Simult.
Glucose	3.2	18.3±0	20.0±1	Ethanol [% vol.]	3.2	12.3±0.05	12.8±0.25
	3.35	25.2±0.1	31.6±1		.35	12.8±0.1	12.7±0.15
	3.5	36.9±1	62.8±5		3.5	12.9±0.15	12.6±0.05
	3.65	47.4±1*	92.1±2.7*		3.65	12.7±0.2	12.6±0.05
Fructose	3.2	1390.0±17*	526.0±6*	pH	3.2	3.13±0	3.14±0
	3.35	210.0±10	109.0±7		3.35	3.26±0	3.33±0
	3.5	143.0±0.1	209.0±11		3.5	3.51±0	3.51±0
	3.65	92.0±2*	397.0±7*		3.65	3.74±0	3.71±0
Glucose and Fructose	3.2	1408.0±17*	546.0±7*	Acetaldehyde	3.2	29.6±0	19.0±1
	3.35	235.0±10	141.0±7		3.35	30.4±0.5*	12.5±0.1*
	3.5	206.0±1	272.0±13		3.5	16.0±4	15.4±0.1
	3.65	137.0±1*	489.0±8*		3.65	12.6±0	7.3±0.4
Acetic Acid	3.2	280.0±10	310.0±0	Citric Acid	3.2	-	-
	3.35	265.0±5 *	355.0±5 *		3.35	-	-
	3.5	310.0±10	345.0±5		3.5	-	-
	3.65	320.0±0 *	415.0±5 *		3.65	-	-
Bound SO2*	3.2	71.5±15	59.5±7	Lactic Acid	3.2	1.12±0.02	1.14±0.09
	3.35	84.5±11	57.0±7		3.35	1.51±0*	4.24±0.1*
	3.5	64.5±4	59.0±4		3.5	3.88±0	4.12±0.1
	3.65	64.0±2	45.0±6		3.65	4.07±0.1	4.33±0.3
Glycerol [g/L]	3.2	4.69±0.4	5.46±0.04	Malic Acid	3.2	2.04±0.01	2.58±0.04
	3.35	5.55±0.2	5.05±0.1		3.35	1.52±0.02*	0.05±0.00*
	3.5	5.30±0.1	5.17±0.1		3.5	0.11±0.01	0.03±0
	3.65	5.38±0	5.40±0.1		3.65	0.02±0	0.03±0.001

*With identical free SO_2 (30 mg/L) – *Statistically significant difference at 0.01 confidence interval
Consec.: consecutive – Simult.: simultaneous

FIGURE 3. Course of malic acid (squares) and lactic acid (circles) concentrations during AF and MLF of Chardonnay with CY3079 and *O. oeni* MBR31. Top, simultaneous; bottom, consecutive yeast-bacterial inoculation (arrow shows inoculation with bacteria in consecutive treatments).

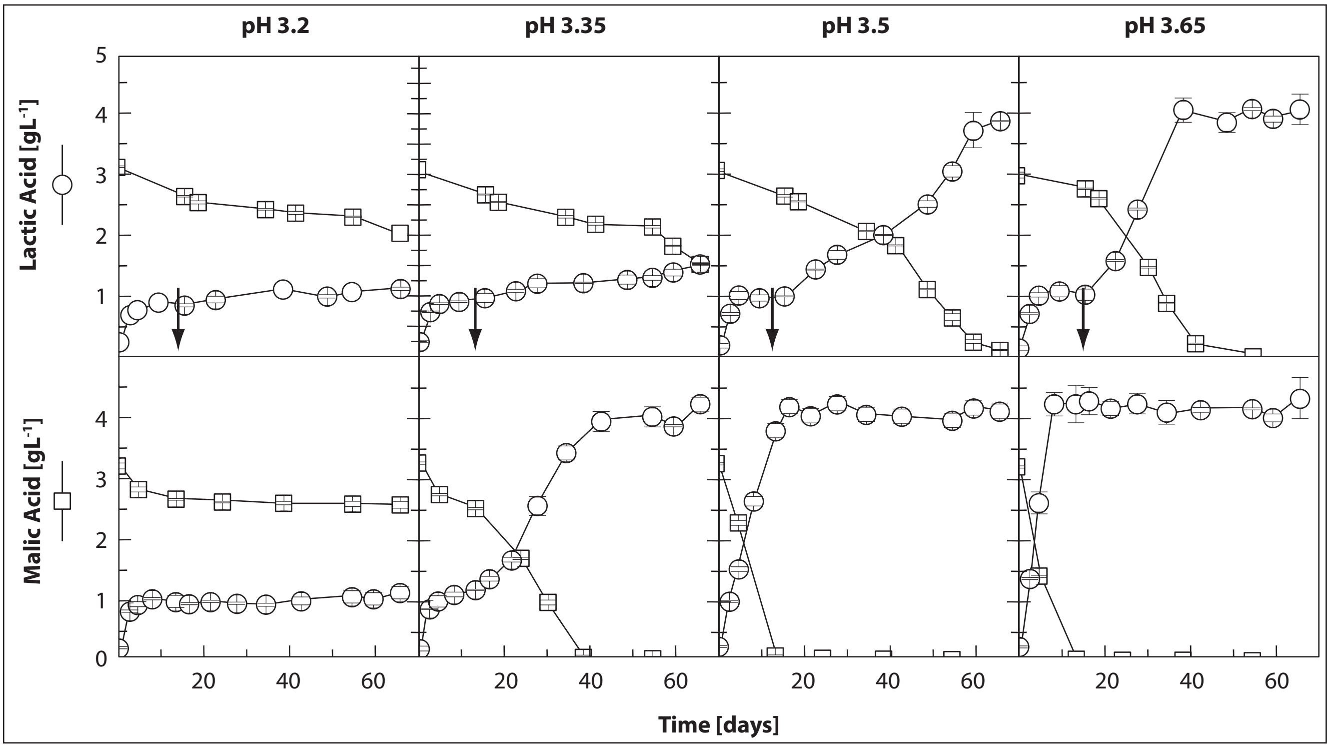

Table 4. Delay observed to reach dryness (combined glucose and fructose levels below 1 g/L and L-malic acid concentrations <100 mg/L) during fermentations of Chardonnay must with *S. cerevisiae* CY3079 combined with *O. oeni* MBR31 strain (consecutive treatments were inoculated with bacteria after reaching dryness at 13.5 days; values in parentheses show malic acid residues in finished wines, where MLF was incomplete).

pH	Simultaneous AF/MLF	Consecutive AF/MLF	
	Days after Inoculation with Yeast and Bacteria	Days after Inoculation with Bacteria	Total Duration after Yeast Inoculation
3.2	n.r. (2.58 g/L)	n.r. (2.04 g/L)	n.r. (2.04 g/L)
3.35	38.5	n.r. (1.52 g/L)	n.r. (1.52 g/L)
3.5	13.5	41.5	65
3.65	13.5	31	54.5
n.r.: not reached			

Consecutive fermentations displayed higher maxima for citric acid levels and the citric acid degradation was somewhat delayed compared with simultaneous fermentations. However, the final concentrations were identical. In contrast, the acetic acid levels increased faster in simultaneous fermentations and higher final values were reached, which were of statistical significance at pH 3.35 and 3.65.

Discussion

In this paper, the results from two experiments comparing consecutive and simultaneous alcoholic and malolactic fermentations are shown. While the alcohol levels present in the final wines were only relatively high in the New Zealand Chardonnay, in both experiments the combined inhibitory effects led to reduced malolactic fermentability when a traditional, consecutive fermentation protocol was used. In contrast, co-inoculation with yeast and bacteria almost always ensured complete malolactic conversions and the malic acid degradation was always faster. While both fermentation success rates and fermentation time reductions were superior after simultaneous AF/MLF, the method did not lead to an inhibition of the alcoholic fermentation or sensory degradation. While slightly higher acetic acid levels were found after simultaneous AF/MLF, the increases measured were neither of practical (sensory) nor legal relevance. Most wine parameters studied were not or were only slightly affected by the fermentation method. However, differences observed with regards to the course of the acetaldehyde concentrations suggest that simultaneous AF/MLF should be considered more carefully in red wine fermentations.

The examples presented clearly indicate that microbial selection, as well as the comprehensive study of microbiological growth and adaptation, and the kinetics of metabolic transformations, can contribute to establishing tailored vinification protocols that meet current and future challenges.

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PHENOLIC MATURITY AS A CRITERIA FOR CHOOSING THE BEST WINEMAKING STRATEGY

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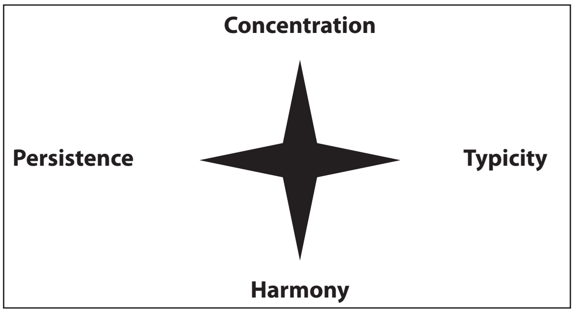
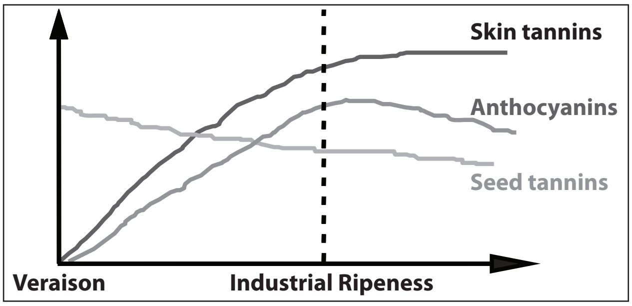
Nowadays, deeply coloured and full-bodied red wines are highly valued by the market. For that reason, winemakers apply themselves to making these wines, which are necessarily very tannic (Ribéreau-Gayon et al., 2000; Zamora, 2003). However, excessive phenolic compound extraction may occur during winemaking, making the wine more astringent and affecting its quality (Bertuccioli et al., 2002; LLaudy et al., 2004). In fact, the current trends in the wine market are oriented towards the production of red wines that present the following four attributes: concentration, harmony, persistence and typicity (Figure 1).

FIGURE 1. Current trends in high quality red wines.



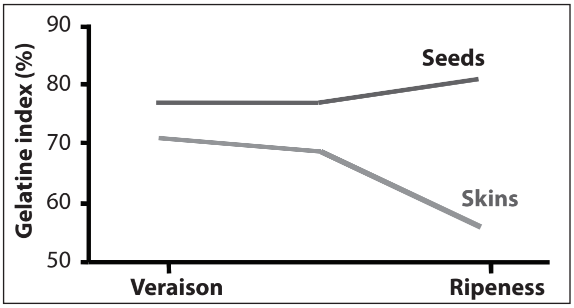
Nevertheless, wines like this are not easy to produce. While there are many indispensable aspects in their production, probably the only aspect that is truly essential is that the grapes be very ripe, especially their skins and seeds. That is the concept of phenolic maturity (Glories and Agustin, 1993; Zamora, 2002). Figure 2 shows the evolution of phenolic compounds throughout the ripening process.

FIGURE 2. Evolution of phenolic compounds throughout the ripeness process.



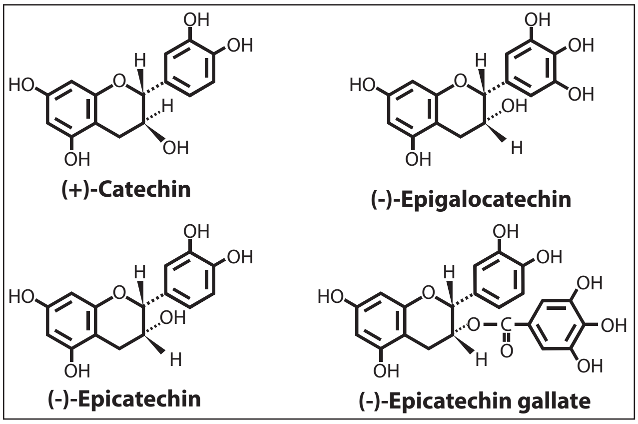
After veraison, anthocyanins and skin tannins increase progressively until they reach a maximum. Later, anthocyanins and skin tannins decrease slightly or remain stable. However, the concentration of seed tannins decreases throughout the ripening process. Figure 3 shows the evolution of the astringency of tannins during the maturation period.

FIGURE 3. Evolution of the astringency of tannins throughout the ripening process.



The behaviour of the astringency of skin tannins and seed tannins is different. Whereas the astringency of skin tannins decreases throughout ripening, the astringency of seed tannins remains relatively stable. Therefore, red wines produced from grapes that were not well ripened will present a high proportion of seed tannins, which are always very astringent, and a low proportion of skin tannins. Inversely, red wines developed with well-ripened grapes will present a high proportion of skin tannins, which are not very astringent, and a low proportion of seed tannins. The reason why seed tannins are more astringent than skin tannins is related to their composition. Figure 4 shows the structure of monomeric tannin units (Zamora, 2003).

Figure 4. Structure of monomeric tannin units.



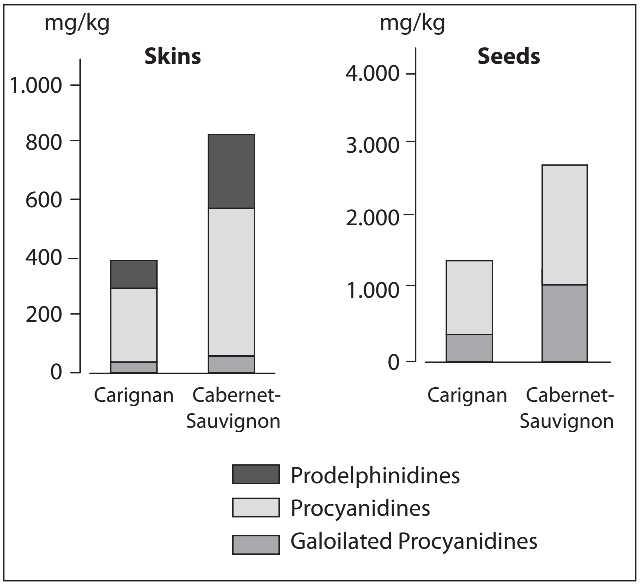
Condensed tannins, also called proanthocyanidins, are polymers of these four subunits. Their astringency is conditioned by their molecular size and by the proportion of epicatechin gallate. The greater the degree of polymerization and the proportion of epicatechin gallate, the greater the sensation of astringency (Vivas and Glories, 1996; Gonzalez-Manzano et al., 2004; Vidal et al., 2003).

The composition of skin tannins and seed tannins is not exactly equal (Figure 5).

Skin tannins present a higher molecular weight than seed tannins, and they have epigalocatechin in their composition (Gonzalez-Manzano et al., 2004; Vidal et al., 2003). On the other hand, seed tannins have a greater proportion of epicatechin gallate, which is probably the reason why they are more astringent.

Without a doubt, climate change is now one of the issues of greatest public concern. The increasing consumption of fossil fuels is largely responsible for the increase in the concentration of carbon dioxide in the atmosphere that provokes the well-known greenhouse effect. This phenomenon, which now affects the Earth's climate, also affects the grape-ripening process (Jones et al., 2005; Zamora, 2005). Figure 6 synthesizes the major effects of climate change on the grape-ripening process.

Figure 5. Tannin distribution in grape berries.



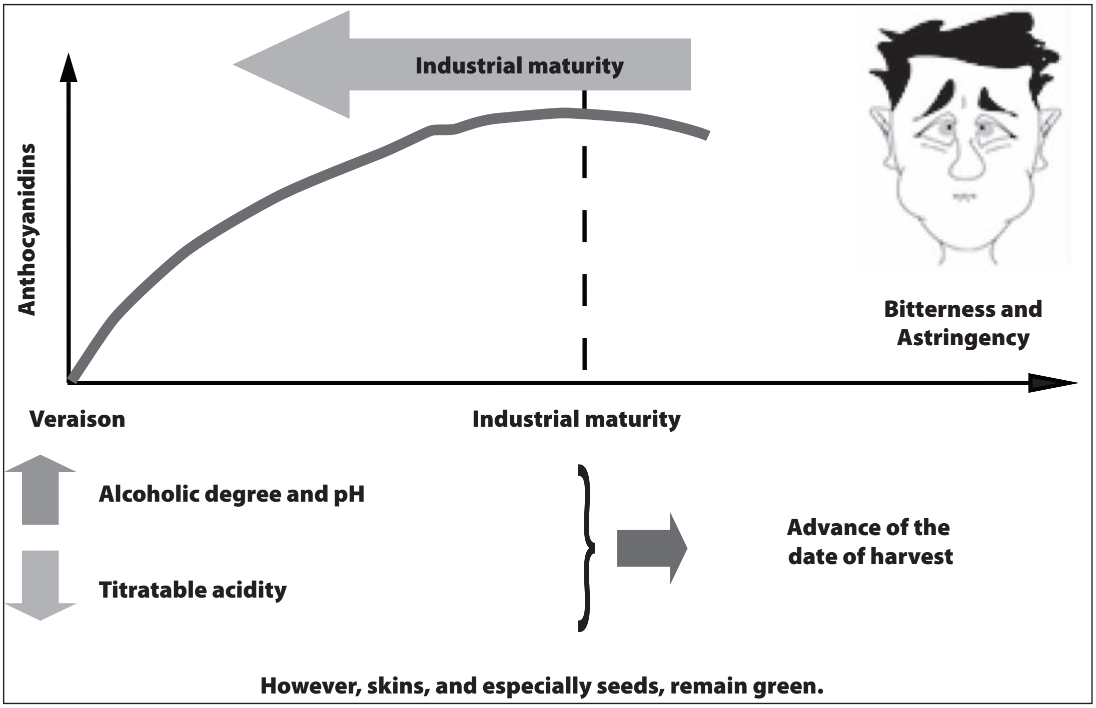
Simply put, global warming results in grapes that store sugars and degrade acids faster than in normal conditions. Therefore, grapes develop a very high potential alcoholic degree and pH sooner than usual, advancing the harvest date. However, the skins, and especially the seeds, remain green. As a consequence of climate change, there is a greater imbalance between industrial or sugar maturity and phenolic maturity.

A better understanding of the effects of this phenomenon on the quality of red wines requires analyzing the kinetics of the dissolution of phenolic compounds during winemaking (Figure 7) (Zamora, 2003).

Colour intensity and anthocyanin concentration follows a similar behaviour pattern. They increase progressively until they reach a maximum level. Later they decrease slightly. Skin tannins start to dissolve immediately after maceration begins and continue to increase throughout the maceration process. However, seed tannins do not start to dissolve at the beginning of the maceration process because they require the presence of ethanol and rising temperatures. Considering the kinetics and the evolution of phenolic maturity reveals some interesting relationships (Table 1).

With well-ripened grapes, the macerating time depends on the type of red wine desired. A short maceration time is sufficient for making wines to be consumed early, and a longer maceration is required for red wines for oak aging (Ribéreau-Gayon et al., 2000; Zamora, 2003).

Figure 6. Effects of global warming. An increasing imbalance between industrial and phenolic ripeness.



However, when grapes are not well ripened, the winemaker has a very difficult decision to make. With a short maceration, the wine would not have sufficient colour. But with a long maceration, the risk of extracting astringent, herbaceous and bitter tannins is very high.

So what are the options in such a situation? I think there are only two possibilities: harvesting when the alcoholic degree and/or pH are at the correct level then adapting winemaking to the conditions of green grapes; or waiting for complete maturity and harvesting when the grapes are really well ripened then applying techniques for lowering alcoholic degree and pH. Figure 8 shows the strategies possible for making red wine from grapes that are not well ripened (Zamora, 2002).

Figure 7. Extraction kinetics of phenolic compounds throughout the maceration/fermentation process.

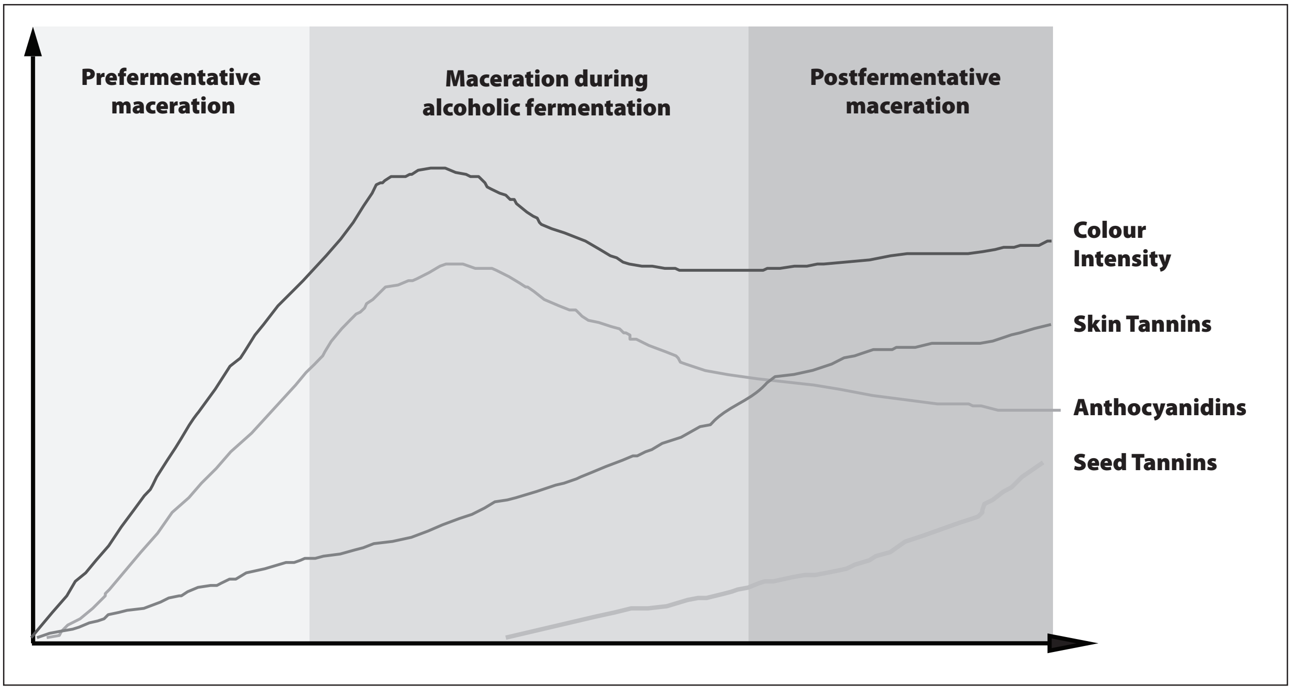
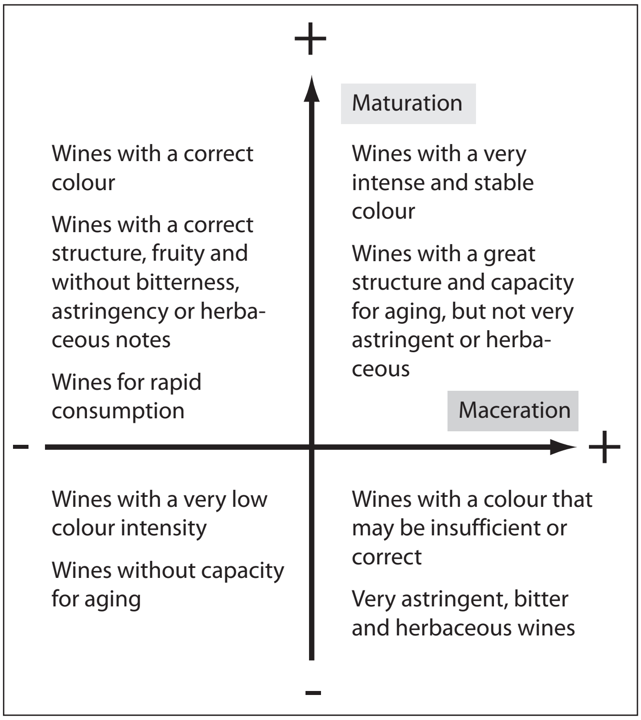


Table 1. Relationships between maturation and maceration.



Wines with a correct colour

Wines with a correct structure, fruity and without bitterness, astringency or herbaceous notes

Wines for rapid consumption

Wines with a very intense and stable colour

Wines with a great structure and capacity for aging, but not very astringent or herbaceous

Wines with a very low colour intensity

Wines without capacity for aging

Wines with a colour that may be insufficient or correct

Very astringent, bitter and herbaceous wines

Figure 8. Possible winemaking strategies when the grapes are not sufficiently ripe.

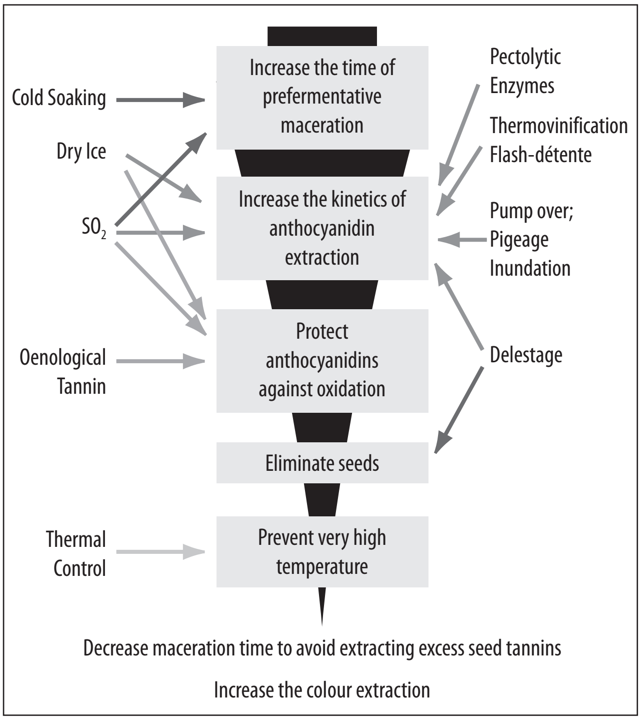
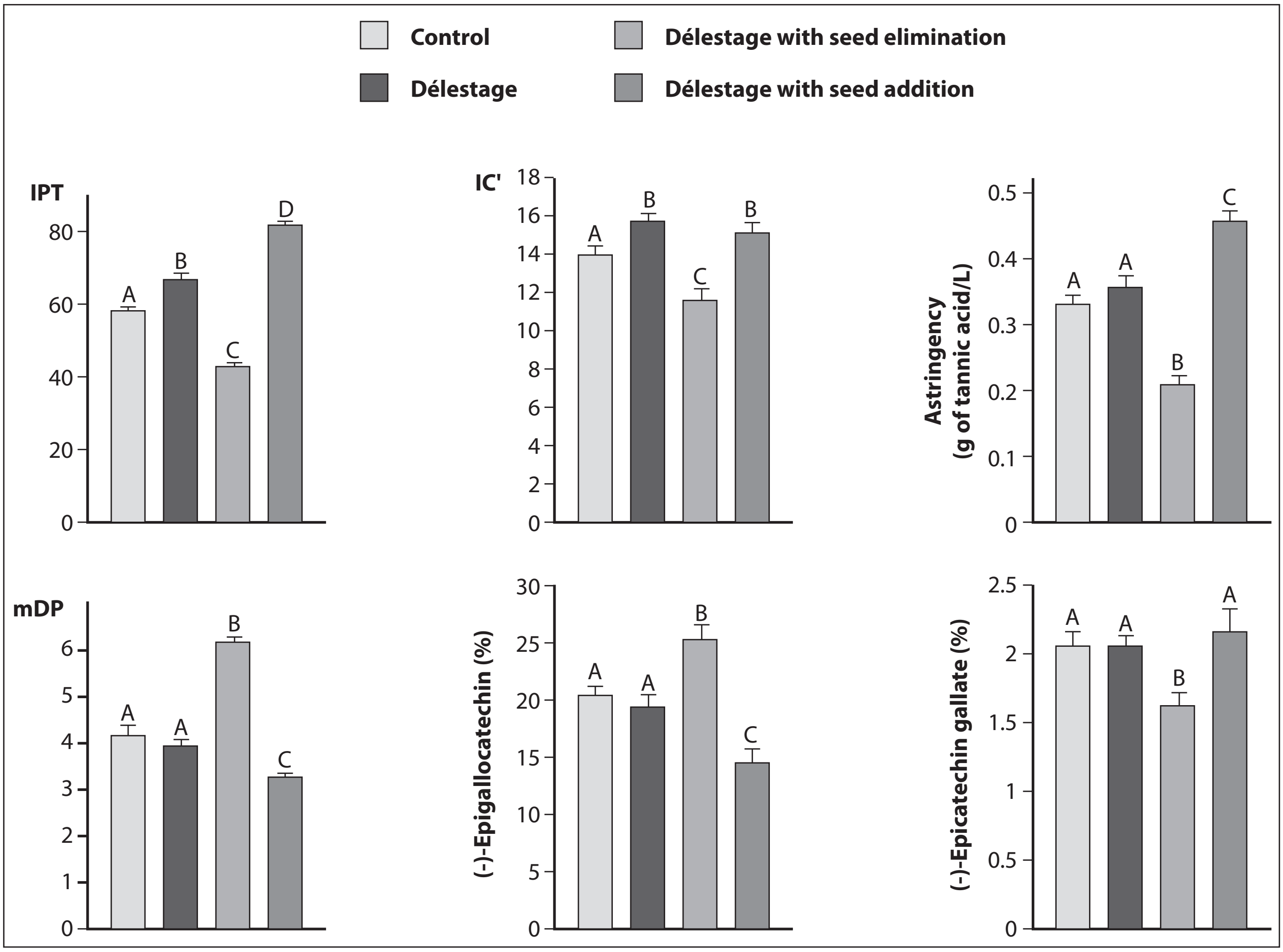


Figure 9. Influence of eliminating and adding seeds.



The winemaker must try to decrease maceration time to avoid extracting excess seed tannins, while simultaneously trying to increase colour extraction. To this end, the winemaker can try increasing the prefermentative maceration time by means of cold soaking (Zamora, 2004). We can protect anthocyanins by the correct use of carbon dioxide, sulphur dioxide or even oenological tannins, and we can increase the kinetics of anthocyanin dissolution by means of a pectolytic enzyme treatment, by applying a thermal treatment, or by means of an increased mechanical treatment of the cap (pump over, pigeage, inundation or délestage). Another interesting strategy may be the elimination of the seeds, by means of délestage (Zamora, 2005). Figure 9 illustrates the effects of eliminating and adding seeds.

Eliminating the seeds decreases the red colour and total phenolic concentration (Canals et al., in press), but drastically decreases astringency, making the wine more pleasant. This decrease in astringency is due mainly to the lowered presence of epicatechin gallate from seed tannins.

As mentioned, the other possibility is to wait for complete maturity and harvest the grapes when they are really well ripened, then apply the techniques for lowering alcoholic degree and pH. The possible strategies to correct high alcoholic degree and high pH are:

1. Selecting varietals and clones that ripen later
2. Adapting growing techniques to this new situation
3. Selecting yeasts with lower sugar/ethanol transformation
4. Decreasing the concentration of sugars in the must through inverse osmosis
5. Partially dealcoholizing the wine with the "spinning cone column"
6. Lowering pH through cation exchange or electrodialysis.

The first three points are probably very interesting, but rarely used. More scientific research is necessary to study their utilization. Points 4, 5 and 6 are better understood, although more work is necessary to improve their capacities and applications. Figure 10 shows how inverse osmosis can be used to lower the potential alcoholic degree.

This technique is now a reality. However, it does present some disadvantages: it is expensive, and the grape juice must be thoroughly filtered; to lower the potential alco-

FIGURE 10. Lowering the sugar concentration in the must.

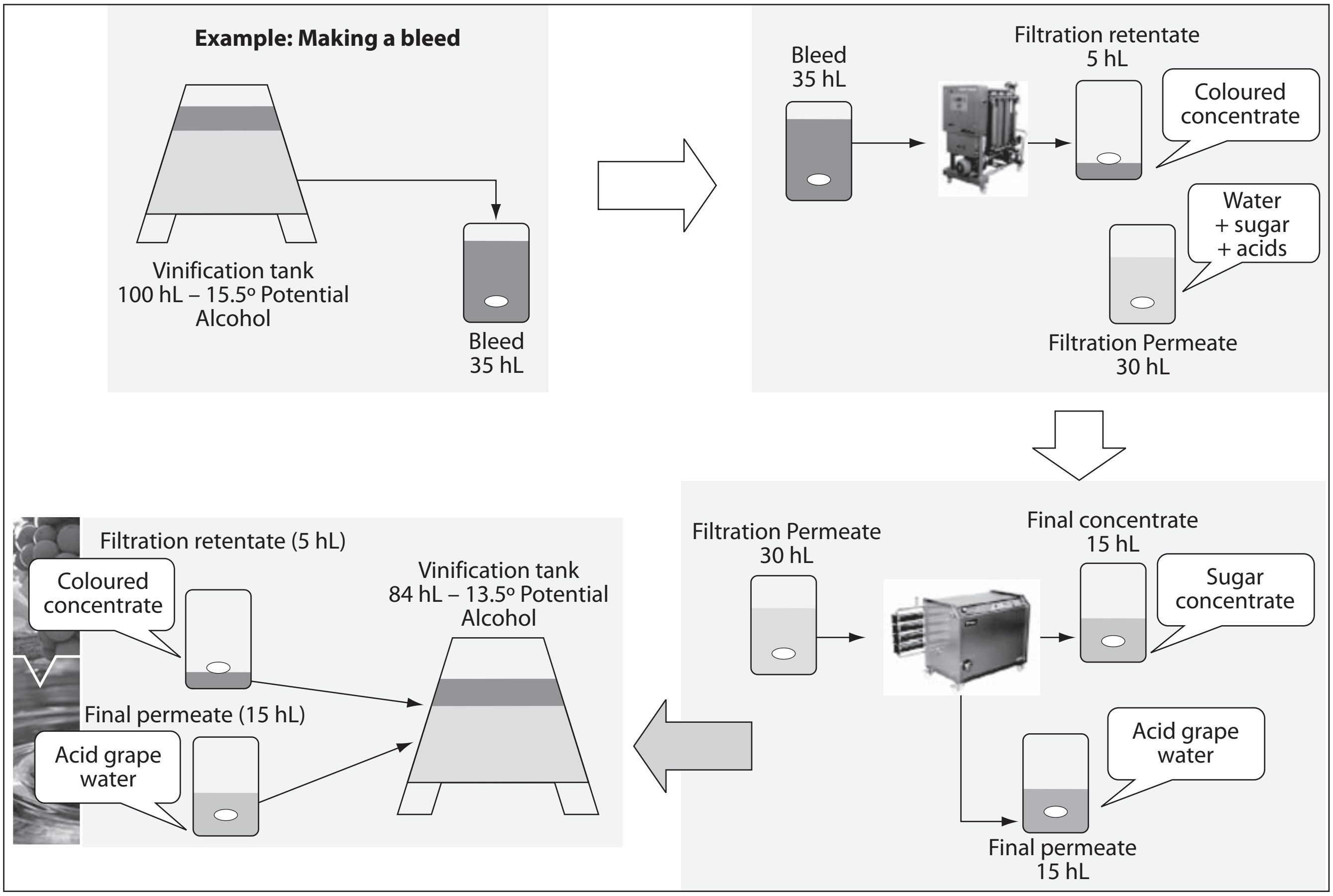
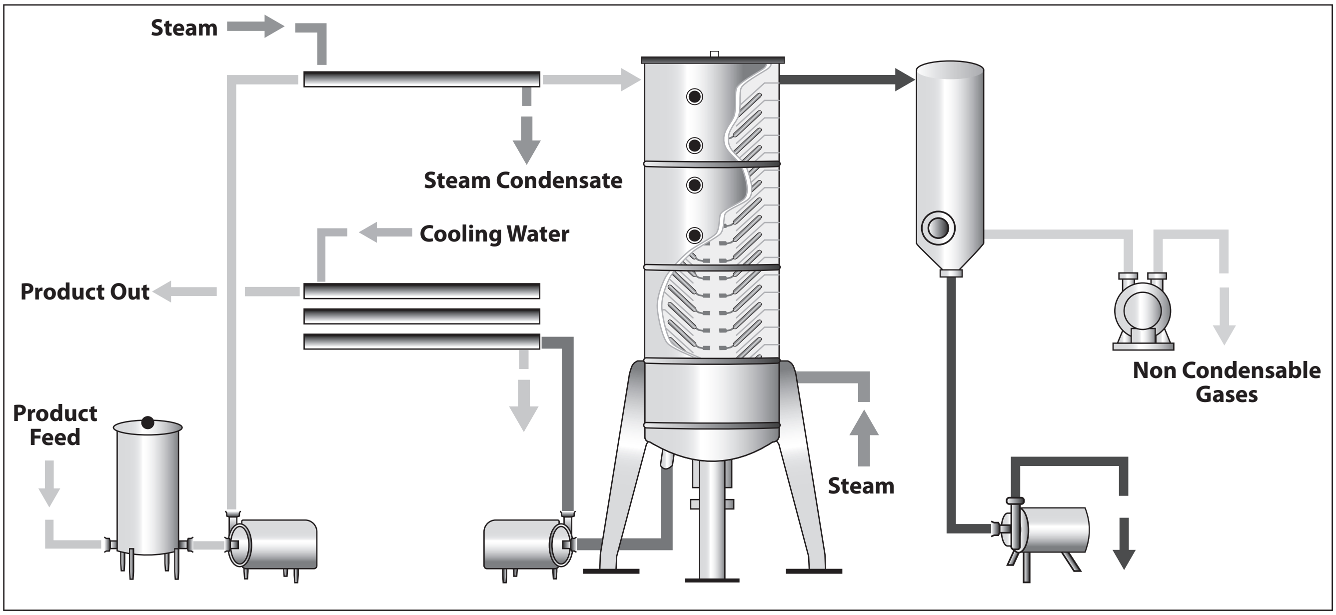


Figure 11. Partial dealcoholization of wine: The Spinning Cone Column.



hol by 1 degree, the volume of wine must decrease by approximately 7%. Another possibility is the partial dealcoholization of the wine by means of the spinning cone column (Figure 11).

The spinning cone column can partially or completely eliminate the ethanol in the wine. This technique evaporates the ethanol at a relatively low temperature. To facilitate the evaporation, the wine goes through spinning plates that increase the evaporation surface, and vacuum is applied. Figure 12 illustrates this process.

However, the spinning cone column is currently an expensive technique. Moreover, during the elimination of ethanol a great amount of aromas is also eliminated. Theoretically, these aromas can be recovered through cold condensation.

To lower pH, there are only two physical methods: cation exchange and electrodialysis. Both techniques are very useful for that objective. Table 2 shows how both methods perform (Walker et al., 2004).

Clearly, climate change is inevitable. We can only adapt and try to mitigate the effects. The techniques discussed are currently available and can be very useful for compensating the effects of global warming on wineries. However, global warming is a major problem and the real solution is found elsewhere (Figure 13).

Without trying to be alarmist, global warming is nevertheless an unfortunate and indubitable reality. We must prepare for this new situation. I hope the doctor's orders will be followed strictly. Only by applying this prescription will the great wines of the 21st century continue to be produced in the traditional regions, and not in the Norwegian fjords or Siberia!

Figure 12. Diagram of Spinning Cone Column process.

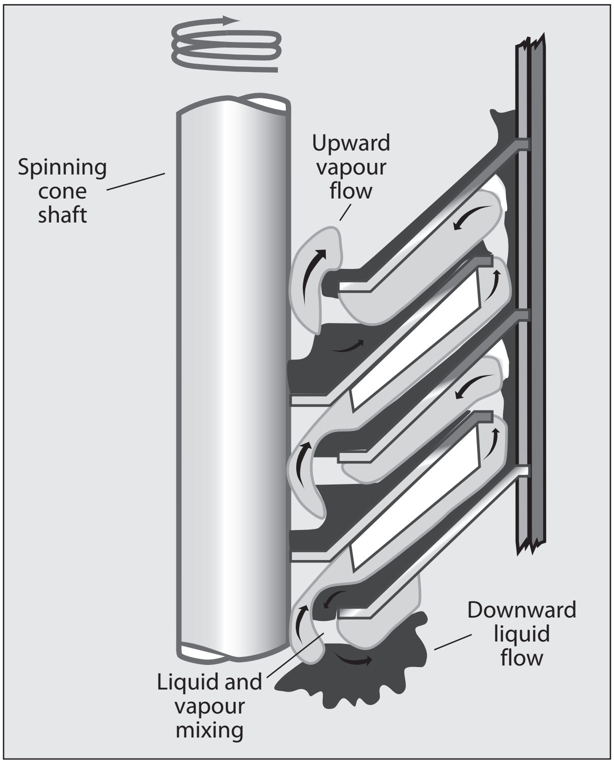
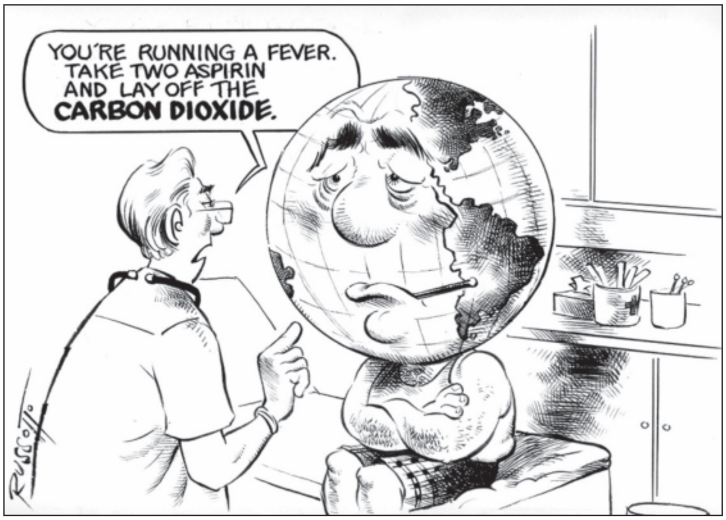


Table 2. Influence of cation exchange and electrodialysis over pH, acidity and colour.

		pH	ATT	TH2 (g/L)	K (mg/L)	A520 nn	IPT
Control		4.24	4.48	2.5	2138	4.13	48.8
Cation Exchange	Amberlite resin	3.48	5.92	2.55	1294	5.87	42.1
	Lewatit resin	3.48	5.98	2.53	1314	5.81	41.5
Electrodialysis	Ionics membrane	3.49	6.43	2.54	1184	6.16	44.3
	Nafion membrane	3.47	6.35	2.56	1200	6.13	44.0
	Ultrex membrane	3.49	6.01	2.52	1243	5.90	44.0
Adapted from Walker et al., 2004							

Figure 13. The only solution for climate change.



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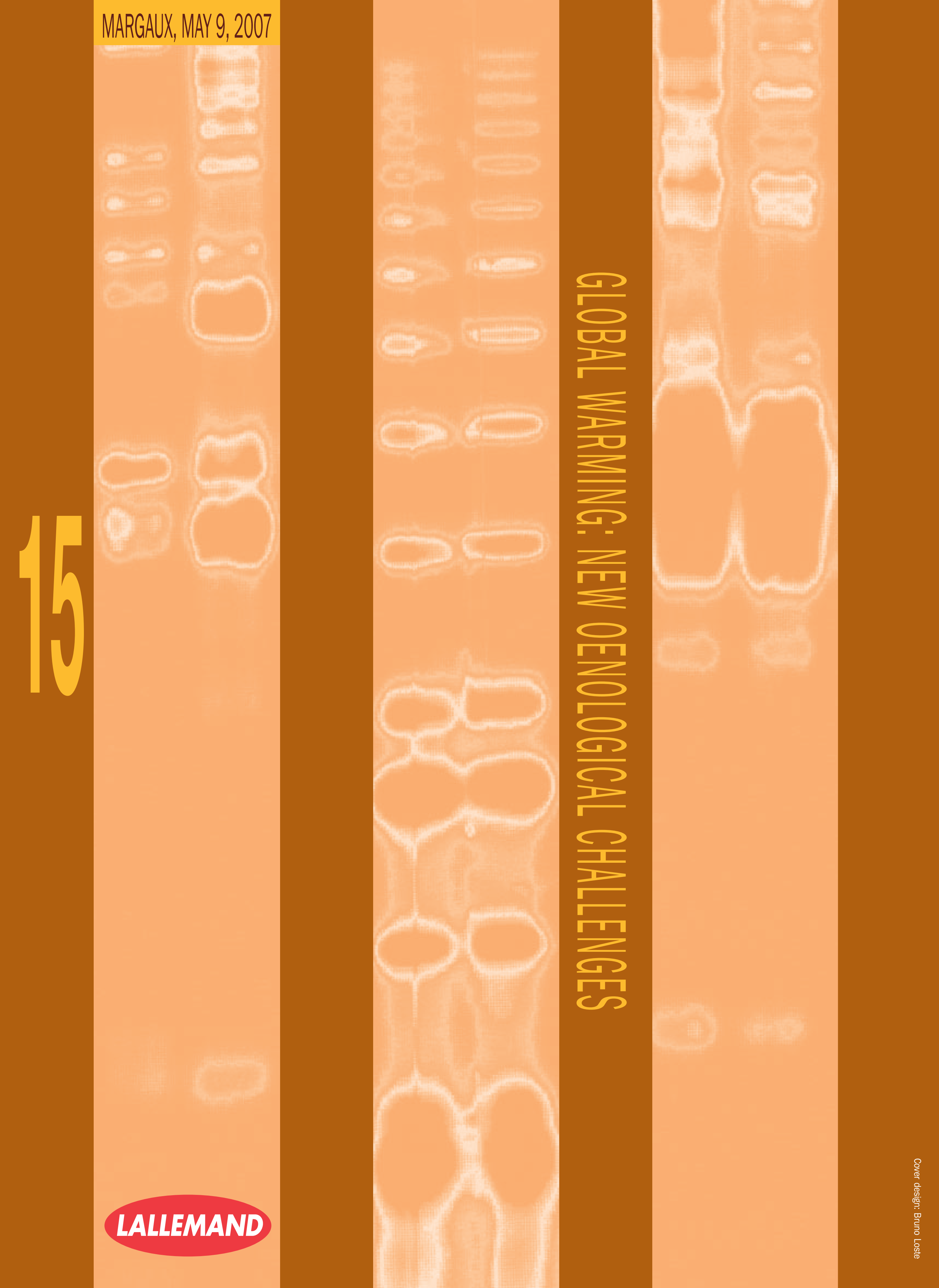

MARGAUX, MAY 9, 2007

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GLOBAL WARMING: NEW OENOLOGICAL CHALLENGES

LALLEMAND

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