PYRAZINES: the secret is to manage them in the vineyard

New techniques to reduce the content of pyrazines with the application of a specific inactive yeast during veraison

Fabrizio Battista
R & D Lallemand

The vegetal character of wines, apart from a few rare varietal exceptions and in limited areas, has always been considered a defect. The main compounds responsible for the herbaceous flavours in wines are pyrazines, identified for the first time in 1975 on Cabernet Sauvignon grape varieties (Bayonove et al. 1975), but present in many varieties.
Four main aromatic pyrazines have been identified in grapes and wines:
- iso-propyl-methoxy-pyrazine (IPMP)
- ethyl-methoxy-pyrazine (EMP),
- sec-butyl-methoxy-pyrazine (SBMP),
- iso-butyl-methoxy-pyrazine (IBMP).

Of these, only the IBMP generally reaches values above the odour detection threshold, making it the only molecule responsible for the typical defects linked to the sensations of vegetables. Due to the very low olfactory threshold of the IBMP (1-2 ng / L in the most slender wines, 7-8 ng / L in the most structured) and its stability in the wine, when present, even at low concentrations, it masks other aromatic compounds, showing herbal notes which often alter the organoleptic profile of wine (Ferreira et al., 2000). In all cases, concentrations above 15 ng / L always have a negative impact on the organoleptic profile (Moio, 2016). It is not easy to limit and reduce these aromas in winemaking without also compromising other volatile compounds which contribute to the overall quality of the wine. The most effective strategy to reduce its content is to avoid its accumulation in the vineyard and to promote its maximum degradation after veraison. Before we see how new applications in the vineyard can help reduce pyrazines, let us recap a few basic points.

Accumulation, degradation, and distribution of pyrazines

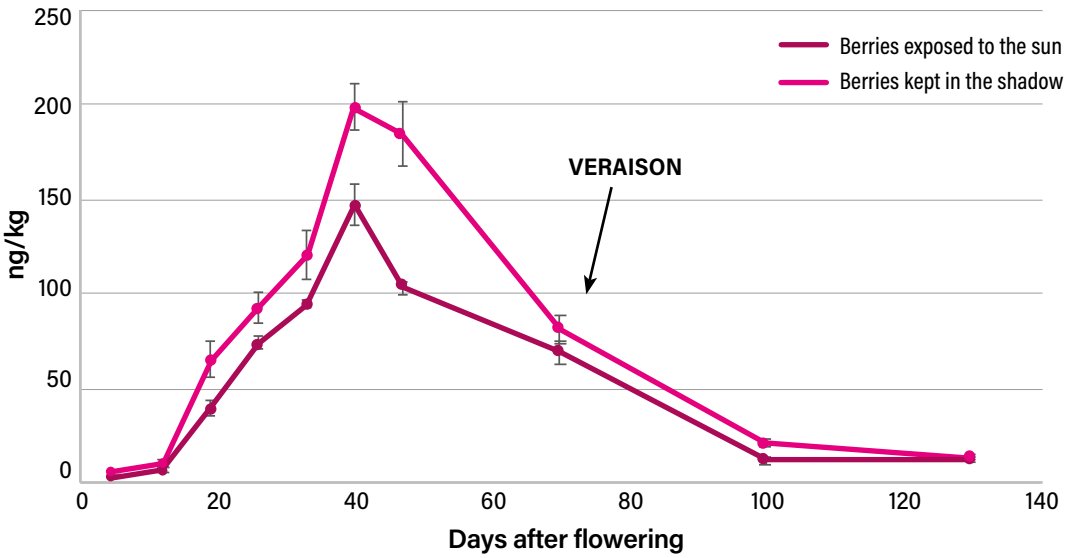
The leaves are the main production organs for pyrazines and they harbour very high contents; these pyrazines are transported via the phloem to the clusters (Ryona et al., 2008) and it has recently been demonstrated that the berries are also capable of synthesizing them directly (A Koch et al., 2010).
The pyrazine content of the grape increases from the first stages of the formation of berries until the

start of the translucent phase, three weeks before veraison (Figure 1), when the maximum content is reached. From this moment, their rapid degradation in the cluster begins, which then slows down when approaching maturation; in the leaves, their content continues to increase.

It is important to remember that the objective of a fruit, like the grape of the vine, is not to be eaten, but to protect the seed until maturity, which occurs in the vine at the veraison. When the grape seed is ripe, the plant tries to make its fruit edible to attract insects, birds and other animals that feed on it and release the seed ensuring the survival of the species. This increase in the palatability of the fruit includes the accumulation of colouring substances, aromatic compounds, sugars, the degradation of acids (malic acid) and the reduction of pyrazines.

In the bunch, the pyrazines are mainly found in the stem and in the skin whereas they are truly little present in the grape seeds and even less in the pulp. Regardless of the stem, at harvest the skins contain more than 95% of the pyrazines contained in the grape (Roujou De Boubée et al., 2002), therefore, especially in red wine making, their extraction is inevitable during a normal maceration. These compounds are chemically very stable in wine and their content in red wines is almost identical to that found in grapes at harvest. In white winemaking, clarification operations help to reduce their concentration by around 30 to 40%. Their content at harvest depends on many environmental and cultural factors, mainly the availability of water and nitrogen which determine the vigour of the plant, the light and, marginally and not quite clearly, the temperature. To understand the aspects which determine the final content of these compounds, it is important to remember that the conditions which influence their synthesis (pre-veraison) are different from those which influence their degradation (post-veraison).

Figure 1 – Evolution in the IBMP content (ng / kg) in grapes exposed to light and shade from flowering to harvest, by Ryona et al., 2008. The maximum peak in their content is produced three weeks before veraison. The exposure of the berries to the sun determines a lower peak. At the harvest, the differences were significant.



Vigour and availability of water

Many scientific articles agree that the main factor influencing the synthesis of pyrazine (pre-veraison) is the vigour of the plant. The more vigorous plants manage to accumulate greater quantities of pyrazines in the grape at veraison and their accumulation in the berry can also continue in the following weeks due to the abundance of vegetation (Allen and Lacey, 1993). This aspect is strongly linked to the availability of water in pre-veraison, when the water availability increases in pre-veraison, the pyrazine content increases at harvest. In this regard, Tandonnet (1996) evaluated the IBPM content in wines by comparing three different pre-veraison water regimes: normal, irrigated and dry. The results confirmed that pre-veraison irrigation led to an increase in the concentration of IBMP in three different years (Figure 3). Even late rainfalls in post-veraison that have caused late vegetative growth, had the effect of increasing the pyrazines content at harvest, (Belancic and Agosin, 2007). Likewise, it has become clear that severe water stress, by slowing the maturation kinetics, also has the

Figure 2 - IBMP content (average of three vintages) in wines made from grapes with normal water availability (rainfall during the year), irrigation before veraison and low water availability before veraison (land covered with cloth). The irrigation resulted in a significant increase in the IBMP compared to the control and the treatment without irrigation, modified by Tandonnet (1996).

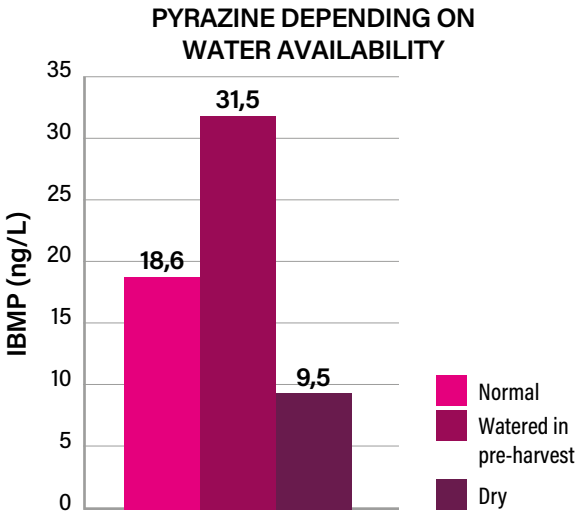


Figure 3 - Sensory impact of the treatment on Glera (with LalVigne Aroma) and Sangiovese (with Lalvigne Mature), by Tomasi et al. 2016 and Battista 2019

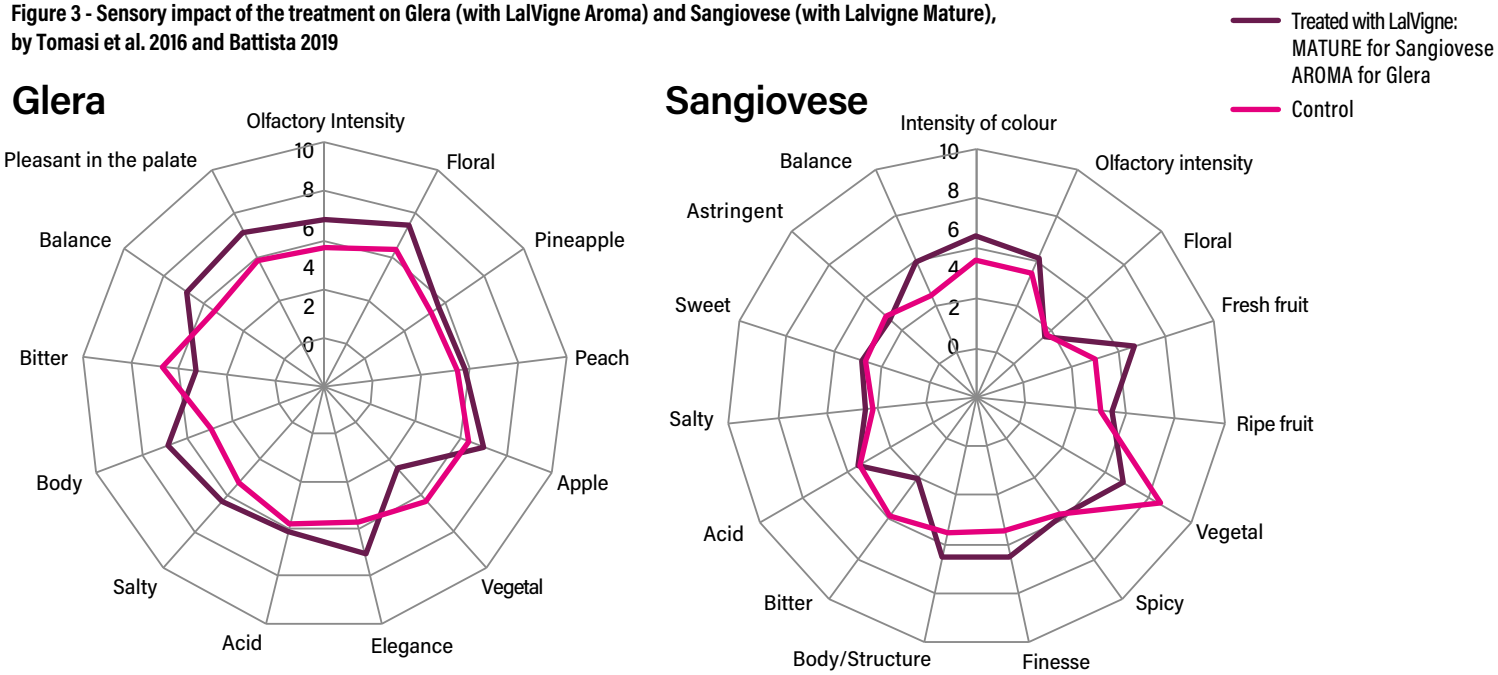
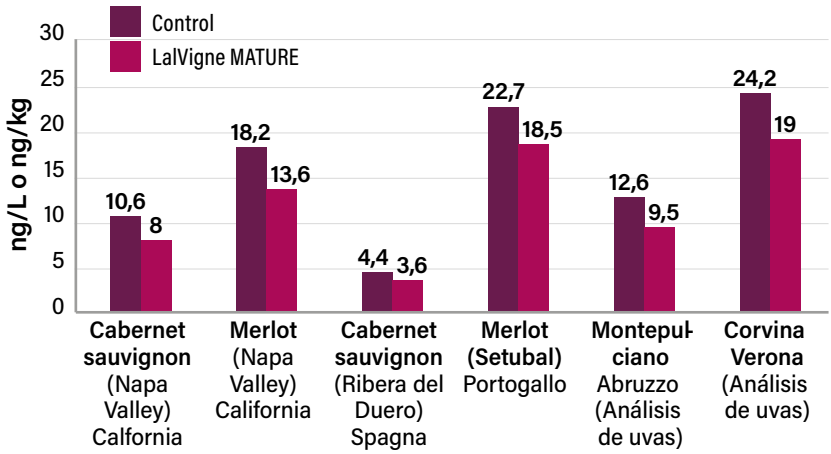


Figure 4 - Total content of methoxy-pyrazine in different regions, vintages, and varieties. Analysis performed by GC / MS on wine (except for Montepulciano and Corvina where the analysis was carried out on grapes). In all regions with different climates and yields, the content in pyrazines was considerably lower after treatment with LalVigne Mature



effect of slowing the degradation of pyrazines, leading to higher contents in the harvest (Pons et al., 2017).

Radiation and temperature

The light radiation has an inhibitory effect on the synthesis of pyrazine, in fact, greater exposure to light in the early stages of development of the grape berry determines a lower content at veraison (Figure 1), however none effect was found by exposing the clusters later (Plank et al., 2019). Even leaf stripping, which results in greater exposure and elimination of the main synthetic organs of these compounds, has not shown consistent effects; except when done early, ten days after flowering; leaf stripping at veraison or near harvest is not useful for the reduction of pyrazines (Scheiner et al., 2010). The role of temperatures on the content of these compounds is not entirely clear: in general, it has been observed that hot vintages or warmer areas have lower concentrations of pyrazines at harvest, although this could be linked to the reduced availability of water and / or to the greater water requirements of the plant due to the increase in evapotranspiration.

Climate change

One of the main effects on the vine of climate change is the gap between technological maturation, (the accumulation of sugars and the degradation of acids), and phenolic and aromatic maturation (Mira de Orduña, 2010, Sadras and Moran 2012). Even the content of pyrazines is influenced by this phenomenon, contrary to what one might expect, these do not decrease with increasing temperatures. There is more and more often an early accumulation of sugar which does not allow a good and complete degradation of the pyrazines, resulting in a high content at harvest

As part of a research project carried out for five years (2013-2017), the CREA-VE of Conegliano realized a follow-up on a Merlot vineyard by comparing the grapes and wines obtained with LalVigne Mature with their control (Tomasi et al, 2018). Chemical

analysis of the grapes from the five vintages showed a positive impact of the treatment on phenolic maturation; the subsequent organoleptic analysis in addition to highlighting an increase in volume on the palate and fruity notes for the treated wines, has always shown an important difference in the vegetal notes. In 2017, in addition to the classic parameters of phenolic maturation, the analysis of the methoxy-pyrazine content present in the grapes at harvest time was carried out (Table 1): at the same level of yield and technological maturity, the content of pyrazines was higher in the control grapes compared to those treated (Figure 5), this for the three identified pyrazines (EMP, IPMP, IBMP). The difference of only 2 ng / kg found in the grapes was sufficient to have a significant impact on the organoleptic profile of the wine assessed by the juror CREA-VE (Figure 6). This confirms the extremely low olfactory detection threshold for these compounds and how small analytical differences have a remarkably high impact on wine.

TABLE 1 - ANALYTICAL DATA OF GRAPES AT HARVEST, CHEMICAL PARAMETERS AND YIELDS, ANALYSIS OF PHENOLIC COMPOUNDS AND PYRAZINES BY GC / MS

MERLOT 2017	LM	CONTROL	Sig
Production (kg / log)	3,8	3,8	ns
Sugars (° Brix)	21,4	21,3	ns
pH	3,42	3,41	ns
Titratable acidity (g / L)	6,5	6,6	ns
Tartaric acid (g / L)	4,6	4,5	ns
Malic acid (g / L)	1,7	1,6	ns
Flavonoids (mg / kg grapes)	1834a	1651b	**
Anthocyanins tot. (mg / kg grapes)	601a	480b	*
Extractable anthocyanins (mg / kg grapes)	324a	216b	*
EMP, ethyl-methoxy-pyrazine (ng / kg of grapes)	4,7b	5,5a	**
IPMP, iso-propyl-methoxy-pyrazine (ng / kg of grapes)	3,4b	4a	*
IBMP, iso-butyl-methoxy-pyrazine (ng / kg grape)	6,8b	7,6a	**

Figure 5 - Total content of pyrazines (sum of EMP, IPMP, IBMP) in Merlot grapes treated and not treated with LalVigne Mature. The treatment resulted in a significant decrease in these compounds

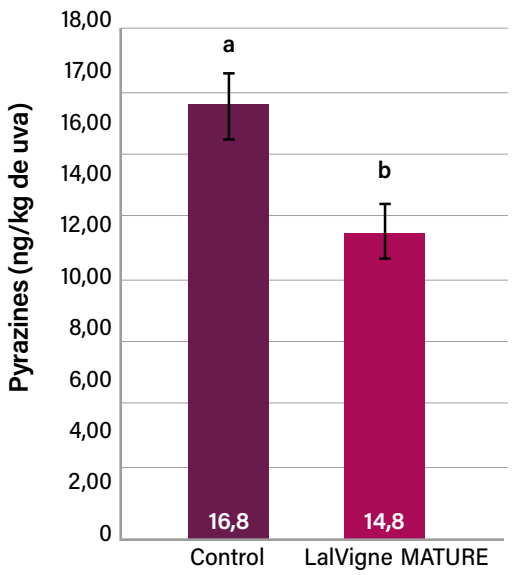
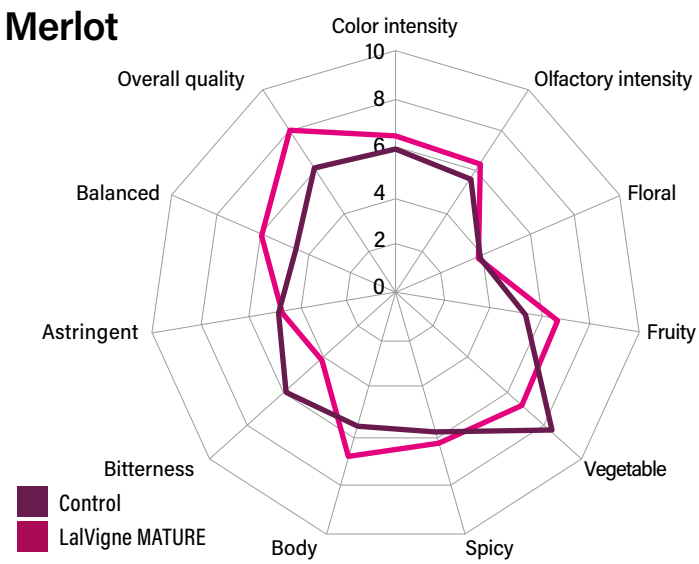


Figure 6 - Comparison of the organoleptic analysis of the two Merlot wines obtained from grapes with and without treatment with LalVigne Mature. The jury assessed the treated wine as less vegetal



THE CASE OF SAUVIGNON BLANC

Sauvignon Blanc wines are characterized by a universally recognized aromatic profile linked to the presence of various thiol compounds which determine the typical profile of this wine. There is a mistaken belief which consists in associating the boxwood notes of Sauvignon blanc with its content of pyrazines while several studies have confirmed that the "cat pee" notes are associated with 4MMP and A3MH (Darriet et al., 1993; Roland and al., 2012). Professor Vivier's team from the University of Stellenbosch in South Africa evaluated the effect of the LalVigne Aroma treatment on the aromatic profile of Sauvignon blanc (Šuklje et al., 2016). Analysis of the wines clearly showed that the treatment with LalVigne Aroma resulted in an increase in A3MH and 3MH and a decrease in pyrazines (Figure 7); similarly, no effect of the treatment was recorded on the content of 4MMP (the control and the treatment had the same content). These data confirm that with the treatment the profile of Sauvignon blanc is more complex while preserving its typicity, and expanding the notes of grapefruit, passion fruit and boxwood in a more delicate way, without the negative impact of pyrazines.

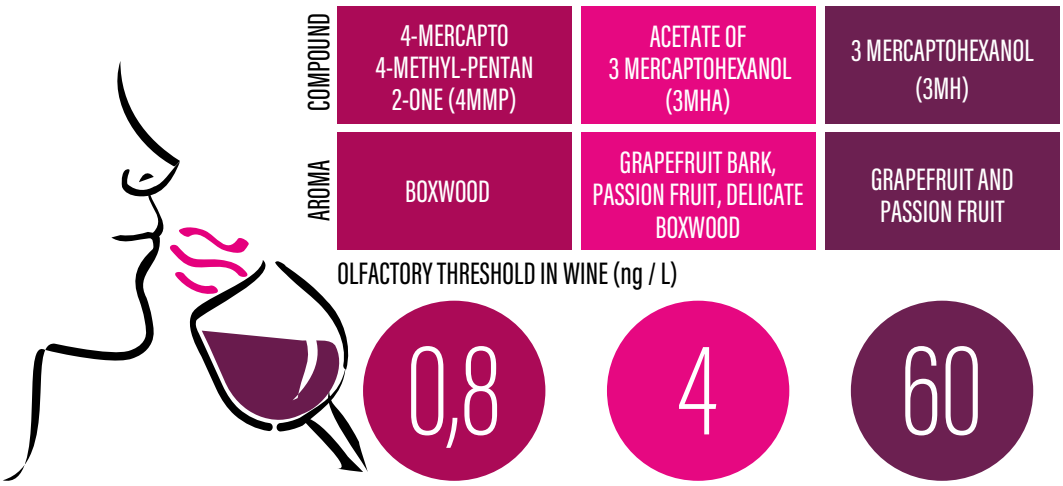
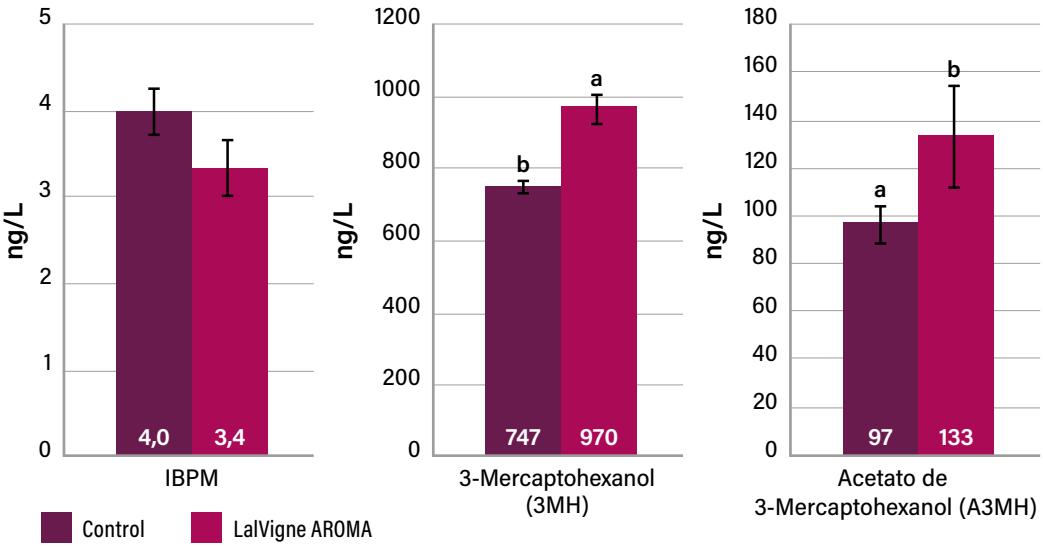


Figure 7 - Comparison of the pyrazine and volatile thiols content in a South African sauvignon blanc wine treated with Lalvigne Aroma and untreated (control)



Inactivated yeasts

Since 2015, Lallemand has developed yeast derivatives capable of stimulating the secondary metabolism of the plant without affecting the kinetics of sugar accumulation. LalVigne Aroma stimulates a greater production of varietal aromatic precursors and Lalvigne Mature promotes better phenolic maturation. Their application in veraison allows the plant to initiate more quickly the metabolic changes which cause the accumulation of the main phenolic and aromatic compounds. In addition, this treatment, which stimulates the secondary metabolism of the plant, facilitates a greater degradation of the pyrazines (Figures 4-5-7) (Šuklje et al., 2016; Tomasi et al. 2016). After numerous tests carried out in different regions, it appeared that the treatment with these two products is capable of causing a reduction in the herbal notes and vegetal off flavours in the treated wines compared to the controls (Figure 3 -6). The experience of research institutes, universities and vineyards for many years has confirmed that the treatment with LalVigne allows to reduce the vegetable notes. The effect is not only evident on varieties that contain large amounts of pyrazines such as Cabernet sauvignon, Cabernet franc, Merlot, Carménère and Sauvignon blanc, but also on native varieties such as Sangiovese, Corvina , Montepulciano, Negroamaro, Refosco, Teroldego and Glera. Analytical data, collected by universities and research institutes in different countries, on the content of pyrazines have always confirmed a decrease in these compounds in response to treatment with LalVigne.