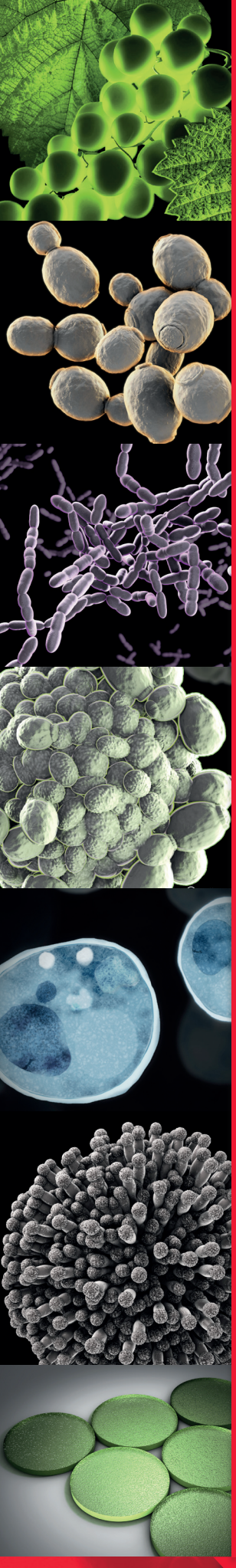

Lalvigne Botryless™ for controlling botrytis and acid/sour rot

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Botrytis and acid/sour rot can impact the quality of grapes and wine, as well as significantly decreasing yield.

The control of these two types of vine diseases is limited both by the pre-harvest interval (PHI) of products available on the market and by the small number of solutions permitted in organic farming.

CRUCIAL ACTION POINTS

Research published in 2020 by González–Domínguez *et al*. analysed the results of 116 studies on the effectiveness of different defence strategies for botrytis control. In particular, the authors studied the best time to act, namely when the treatments would be most effective. The study clearly demonstrated the importance of acting at the end of flowering for successful control of this pathogen. At this stage, the fungus can develop in the floral organs, which are then a source of inoculum for subsequent infections. The second crucial moment to act is the pre-closure of the cluster, which is important for limiting the fungal spores inside before it closes.

Time of action for a preventive procedure is of strategic importance when using natural methods with low environmental impact.

WHEN ACID/SOUR ROT ALSO OCCURS

Acid/sour rot, linked to the development of acetic bacteria (*Acetobacter spp*. and *Gluconobacter spp.*, among others) and apiculate yeasts (*Kloeckera apiculata, Saccharomycopsis vini, Hanseniaspora uvarum, Candida spp*.), normally occurs because of grape microlesions with effects increasing as ripening progresses or when the sugar content is higher. In addition to rainfall, contributing factors also include cluster firmness and thin skin, as well as damage from insects such as moth (*Lobesia botrana*) and *Drosophila suzukii* fruit fly.

The presence of powdery mildew damage is also closely related to the onset of acid/sour rot and numerous publications find there is a directly proportional connection also with the presence of botrytis, both as a triggering agent and as a consequence of the fact that both infections have the same causes.

CHITOSAN FROM 100% *ASPERGILLUS NIGER*: CHARACTERISTICS AND EFFICACY

For both acid/sour rot and botrytis, protection often extends up to the pre-harvest period, so there is extensive research into natural solutions that can work without residue and that do not interfere with alcoholic fermentation.

In 2022, chitosan from 100% *Aspergillus niger* (CAS no. 9012-76-4) was approuved as a basic substance and in 2023, allowed in organic farming for the control of fungal diseases and bacteria, without PHI, according to EC Reg. 1107/2009. Lallemand Oenology, the specialist in microbial solutions, has developed a specific chitosan from 100% *Aspergillus niger* with a low molecular weight that favours its efficacy and speed of action: LalVigne BOTRYLESS™. From an operational point of view, this formulation does not cause phytotoxicity problems and is compatible with plant protection products. Even in advanced phenological phases, its use does not interfere with alcoholic fermentation.

The control of botrytis and acid/sour rot by Botryless™ is based on the synergy of three fundamental characteristics:

1. antimicrobial action;
2. elicitor effect;
3. creation of a protective bio-film.

These characteristics and production stability, guaranteed by controlled microbiological production mean that LalVigne BOTRYLESS™ (LB) has better performance and characteristics compared to chitosan hydrochloride extracted from the processing of derivates from various crustaceans. For this reason, the European legislator has also identified chitosan hydrochloride of animal (crustacean) origin with a different CAS, namely 70694-72-3. Furthermore, chitosan from crustacean origin can be an issue in terms of allergens, whereas LalVigne BOTRYLESS™ is not.

In a trial conducted on the development of different botrytis isolates in petri dishes, the inhibition rates found for LB were over 70%, while the crustacean chitosan hydrochloride used at the same dosage never went beyond 30% inhibition (Figure 1). The direct fungicidal effect is just one of the mechanisms by which LB acts to counteract the proliferation of botrytis.

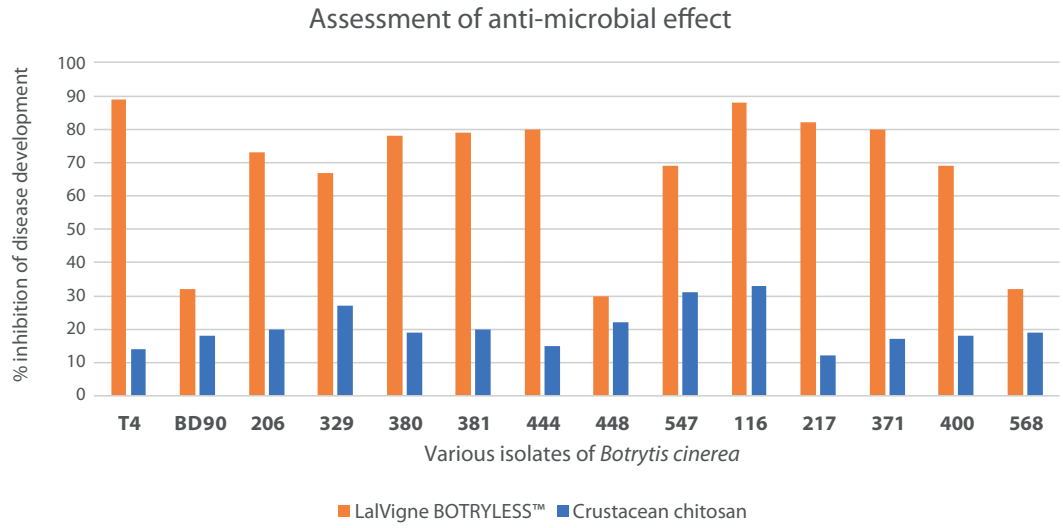

Figure 1. Results of a test carried out by the University of Salamanca (Spain) on the effect of LalVigne BOTRYLESS™ compared with a crustacean chitosan for inhibiting growth of different botrytis isolates. Tests performed in petri dishes with MEA culture medium. Data expressed as percentage of inhibition of fungus proliferation compared to untreated petri dish.

Based on its intrinsic characteristics and the numerous experiments carried out, the most appropriate strategy for the use of LB as a preventive measure has been defined as able to ensure good levels of efficacy for the control of botrytis. This strategy involves three applications, each at a dose of 100g/hL, at the end of flowering, pre-cluster closure, and early veraison stages.

EFFICACY TESTS IN VINEYARDS

Efficacy tests conducted over the years, on different grape varieties and in areas with high disease pressure have confirmed what has been observed in the laboratory.

In 2023, a year of high pressure, the effectiveness of the preventive strategy was evaluated in a trial conducted in Friuli on Sauvignon Blanc, applying treatments performed with the timing and dosages indicated above. The use of LB has reduced the incidence and severity of botrytis infection (Figure 2).

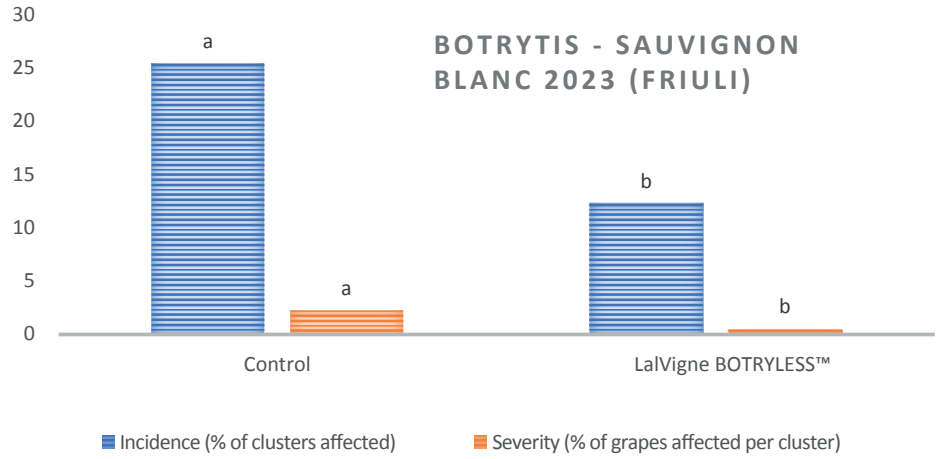

Figure 2 - Evaluation of the effectiveness of the use of LalVigne BOTRYLESS™ in the control of botrytis, surveys carried out at harvest date. In the sample, three treatments were performed: at the end of flowering, at pre-closure of the clusters, and at the beginning of veraison. Different letters indicate significant differences between samples for P<0.05 (t-test).

In the same vineyard, the test was also conducted in 2022, but this was a year in which no botrytis infection was found. However, an acid/sour rot infection began to be observed, with a very rapid and virulent development. In this situation, it was decided to perform the third treatment at a higher dosage (600 g/ha) both in the control (curative strategy) and in the sample already treated with two preventive applications (Figure 3). In this case, the preventive treatment was able to reduce the incidence of infection significantly. The curative approach was able to contain the proliferation of the infection with a single application, making it possible to arrive at harvest with a lower number of affected berries than the control.

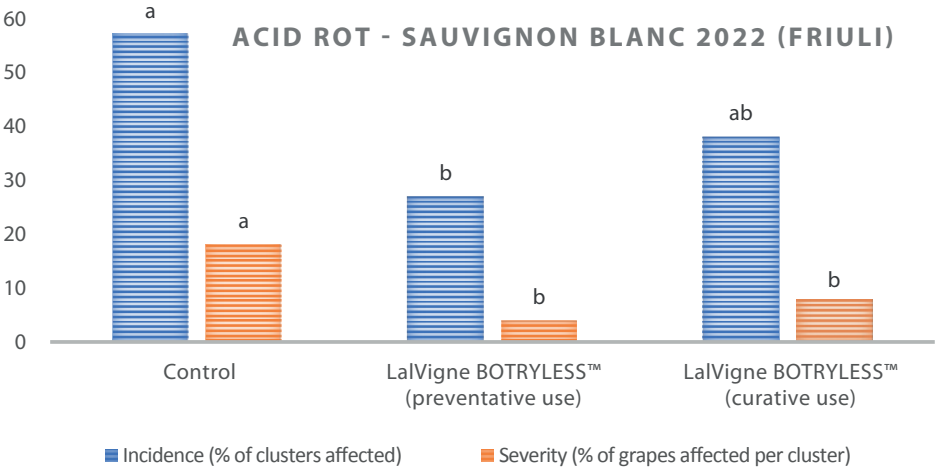

Figure 3 - Evaluation of the efficacy of the use of LalVigne BOTRYLESS™ in the control of acid rot, measurements carried out at harvest date. In the preventive strategy, three treatments were put in place (pre-closure of the cluster and start of veraison at 300 g/ha, and treatment at the end of veraison at 600 g/ha). In the curative strategy, a single treatment was performed at the beginning of veraison at 600 g/ha. Different letters indicate significant differences between samples for P < 0.05 (t-test).

In addition to the parameters of incidence and severity, the gluconic acid content was analysed in a trial conducted in Spain on Bobal in 2019. Two samples were compared with the untreated control, one with LB at 300 g/ha and one with a conventional anti-botrytis reference in the market at the regular dosage. In both cases, a single application was performed at the beginning of veraison, at the onset of the first symptoms. The incidence of botrytis at harvest was 43.5% in the control compared to 18.9% in the anti-botrytis treated sample, and 11% in LB. At the same time, a significant reduction in the content of gluconic acid in the must was observed (Figure 4), with substantial positive repercussions on winemaking and wine quality.

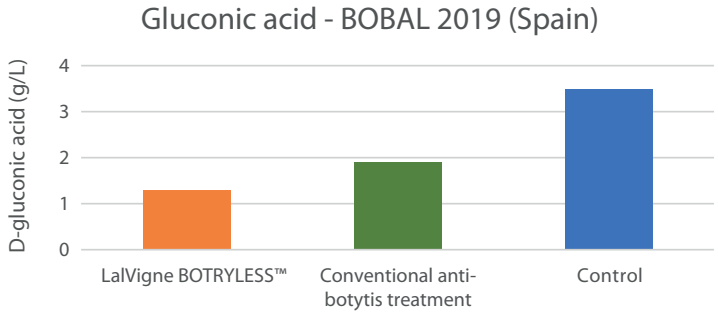

Figure 4 - Effect of different botrytis control strategies on gluconic acid concentration. In the samples considered, a single veraison treatment was performed: LalVigne BOTRYLESS™ at 300 g/ha and a conventional anti-botrytis reference for the market at label dosage. Analysis performed by Excell Iberica at the harvest.

IMPACT ON CLUSTER MICROBIAL BIODIVERSITY

In a trial conducted by CREA-VE in 2023, the effect of a single LB treatment was evaluated both on bacteria and fungi associated to botrytis and acid rot, and on the entire microbial population present on the cluster. To perform this monitoring, the innovative metagenomics technique was used.

Metagenomics

Metagenomics represents a recent approach to the study of entire microbial communities in their natural environment, that is to say, in the case of vineyards, soil, grapes, bark, leaf surface, roots. Indeed, only about 1% of the microbial population can grow in laboratory conditions. Conversely, metagenomics enable DNA extraction and sequencing (also called NGS, Next Generation Sequencing) of all the species present in a sample and not just those that can be cultivated. There are several metagenomic analysis techniques, including the one used in this study (16S and ITS-NGS, also called microbial metabarcoding), which consists of sequencing a specific region of a particular gene that codes for the ribosomal RNA of bacteria and fungi respectively. This approach allows a quick identification of the species present in microbial communities of a given environment, making it possible to study their composition and structure. The analysis of the complex data obtained through the new generation sequencing of the analysed samples is carried out with specific bioinformatic and statistical analyses.

The trial was conducted in the Piave area (Veneto, Italy) on Pinot Grigio, where a single LB treatment was carried out five days before harvest at a concentration of 100 g/hL. Monitoring was performed before treatment (T0) and four days after application of specific chitosan (T4).

Analysis of fungal population data (Figure 5) showed that botrytis increased significantly (from 21% at T0 to 85% at T4) in untreated plants (Control) four days after the start of the trial (T0). This abundance influences the development of other microorganisms negatively and limits the fungal biodiversity of the cluster. The use of LB significantly reduced the incidence of botrytis (34%), confirming a specific fungistatic effect. At the same time, some genera known as potential fungal disease biocontrol agents, like *Aureobasidium* and *Sporobolomyces*, show an increase, taking advantage of the reduced prevalence of botrytis in the treated grapes.

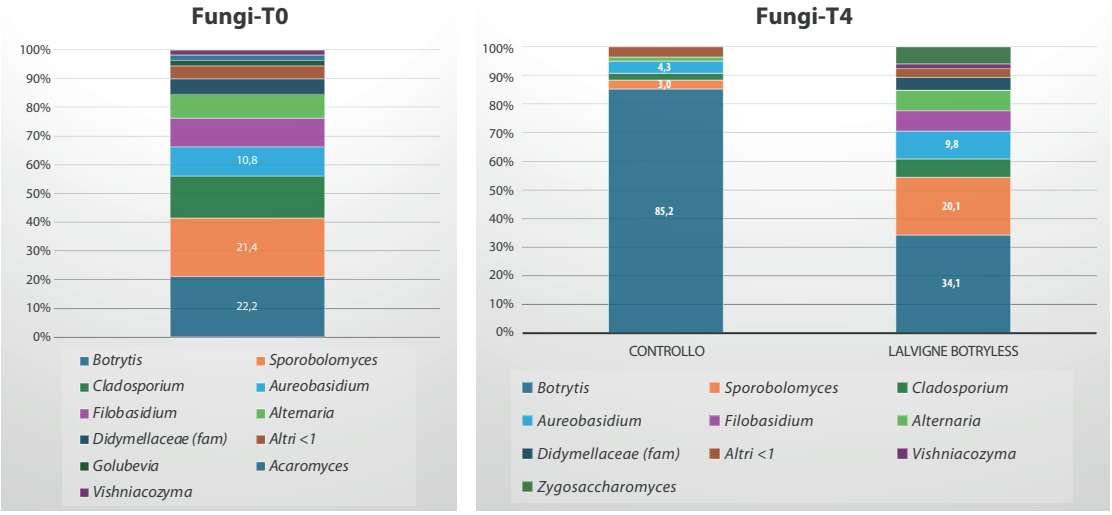

Figure 5 - Relative abundance of the fungal population quantified by metagenomic analysis before treatment (T0) and four days (T4) after treatment with LalVigne BOTRYLESS™ compared to control.

As far as the bacterial population is concerned, the treatment was successful in controlling *Acetobacter, Gluconobacter* and *Komagataeibacter*, genera related to acid/sour rot, which, after four days of treatment, were 35.5% of the population in the control compared to 1.7% in the LB treated sample (Figure 6). In this specific case, the bacterial biodiversity index increased in the control due to the large assortment of bacteria involved in acid rot.

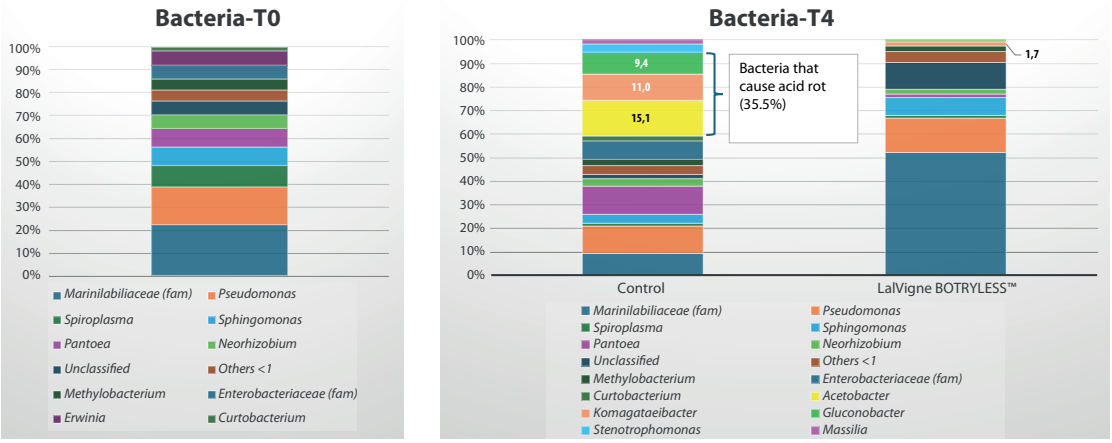

Figure 6 - Relative abundance of the bacterial population quantified by metagenomic analysis before treatment (T0) and four days (T4) after treatment with LalVigne BOTRYLESS™ compared to control.

The effect of the treatment on overall biodiversity, which can be estimated with the Shannon Biodiversity Index (H') shown in Figure 7, is different for the two communities, fungal and bacterial. This is probably due to the different initial situation: in the case of yeasts and fungi, the strong preponderance of botrytis in T0, especially in the control, limited the development of other genera, consequently chitosan allowed an increase in biodiversity. In the case of bacteria, the initial microbial community (T0) after four days (control) is quite varied, as can be seen from the high H' index. Consequently, in this case, the effect of the treatment, which has an impact on several genera (including the three belonging to the Acetobacteraceae described above), reduces the overall biodiversity.

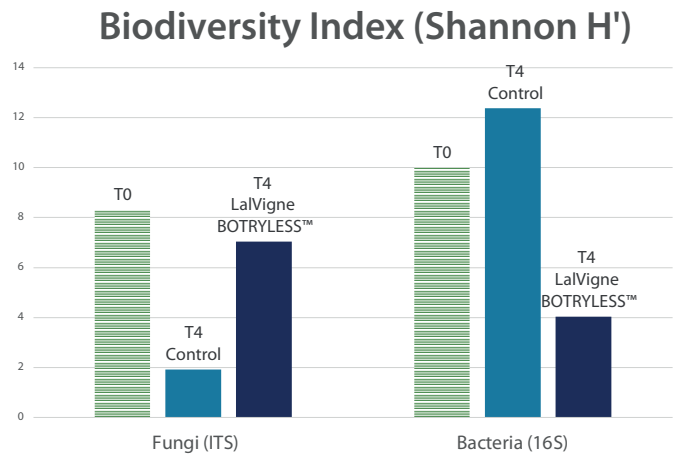

Figure 7 - Shannon Biodiversity Index (H'). Indicates the microbial biodiversity detected in the different samples at different sampling times.

THE RESPONSE TO VINEGROWERS NEEDS

The search for natural solutions allowed in organic viticulture for the control of rot and botrytis, without PHI and interference on alcoholic fermentation, has always been an important demand in the production world. A new chitosan of fungal origin, which has all the abovementioned characteristics, has been tested to evaluate its efficacy against these cryptogams. This specific chitosan has been shown to be highly effective against the bacteria responsible for acid/sour rot and botrytis, as inferred from the positive results of field trials carried out on problematic varieties and regions.

The bibliography is available upon request to the editors.