FUNGI INFECTIONS IN VINE

Climatology plays a fundamental role in the last stages of vine development. In particular, high humidity and temperature above 25°C as the season progresses, increase the risk of fungal infections, especially powdery mildew, downy mildew and botrytis (but also infections by Aspergillius, or Penicillinum), which, in turn, weaken the plant and become an entry vector for other fungi. These infections can cause significant losses in production if they are not properly controlled and treated, both due to the reduction of the harvest, the affectation of the vine or the loss of quality of the must produced. In addition, the infection produces a reduction in the available nitrogen (which may compromise the fermentation process), a loss of total acidity and an increase in volatile acidity that subsequently complicates the entire winemaking process.

Powdery mildew (*Uncinula necator*) attacks any green part of the vine. In the leaves it is recognized because an ash colored powder appears on both sides; on shoots and branches shoot fuzzy dark green to black spots; and the clusters are covered with a powder that stops the growth of the skin of the grain, producing oxidative changes and putrefactions in the pulp.

The temperature between 25 and 28°C, humidity and lighting are the factors that condition the development of this fungus that, carried by the wind, spread the disease to any green part of the plant.

Downy mildew (*Plasmopara viticola*) is one of the most well-known and serious diseases. It attacks all the green parts of the grape in the phenological period of awakening, the vegetative period, in spring. Then it remains dormant in the autumn in the form of an oospore in the plant remains until the next cycle. Once germinated and spread by the wind, they penetrate the tissues of the plant through the stomata, giving rise to an intercellular mycelium and to what is known as primary contamination. Then the secondary infestation will begin and the symptoms will manifest on the vine in the form of yellowish spots on the leaves, limited by the nerves, and a white and cottony formation of spores on the underside, from which it will reach the cluster, which will turn grey -moved first and dehydrated later.

Esca is associated with infection by fungi *Stereum hirsutum Per.* and *Phellinus igniarius Fr.* that penetrate the wood through significant wounds produced by pruning. The symptoms usually begin in full bloom or already in the middle of summer and consist of the appearance of internervial discolorations, and on the edges of the leaves, yellowish in the white and reddish varieties in the inks that come together and dry up in the center. The leaves end up falling, one or more arms of the strain can die (even all of it). Berries are not fattening properly and may not reach maturity; and in the most extreme cases, especially with high temperatures, the death of the vine occurs.

The anthracnose of the vine is a wood disease caused by the fungus *Glocosporium ampelophagum* that attacks all the green organs of the vine, producing lesions characterized by the presence of a whitish central area surrounded by a black halo. but it is mainly in the herbaceous shoots where attacks mainly occur. The leaves dry and fall off, leaving irregularly shaped holes surrounded by a purplish black border. The branches appear burned, are short, winding, crooked and have numerous secondary and tertiary ramifications that give the strain a scrubby appearance. In the inflorescences when the attack is very intense, they dry completely and the loss of the harvest is total. On the fruit, if it has survived, black spots appear that fade through its center, turning grayish-white and peeling off the affected skin.

Botrytis (*Botrytis cinerea*) is caused by a fungus that can attack all the green organs of the strain, but the greater severity is due to the attack on the clusters during veraison, when the accumulation of water, sugars, and polyphenols begins in the grape. The infection produces a dense cottony film that covers the grain, which begins a process of putrefaction, oxidation and acidification, to end up withered and parched. However, when the fungus infects the ripe berry, once veraison has been overcome and at the maximum level of sugars and polyphenols, the drying effect of the fungus (noble rot) produces a concentration of sugars and acids that results in an extremely appreciated must for its organoleptic characteristics.

In all cases, the infection causes a significant change in the metabolism of the berry, altering its chemical and organoleptic characteristics. A recent study (1) found that powdery mildew infection resulted in decreased vanilla-like aromas due to a variety of very subtle changes, including decreased levels of vanillin, octanoic acid, and ethyl isobutanate ester , 2-methylbutanoate acetate, and 3-methylbutyl acetate, all of them compounds that contribute positively to the aroma of the wine, resulting in 'flat' wines, with little appeal. In contrast, botrytis infection produced

an increase in lactones (fruit odors), isobutanol, isoamyl alcohol, furaneol, and homofuraneol (roasted and vanilla), among others, which resulted in an increase in positive aromas.

Although in many cases it is possible to detect infected bunches with the naked eye, visual detection requires the implementation of specific selection procedures, either manual or automated, which cannot be applied to large quantities of grapes in a quick time, nor is it effective. When the infection exists, it does not manifest itself visibly in the form of damage to the berry. The alternative is detection using adequate analytical methods that indicate the presence of specific markers. Some of these infection markers look for compounds generated by the metabolism of the fungus. Among them, glycerol, gluconic acid and lacassa stand out. Of all of them, gluconic acid is the most used due to its easy implementation in the laboratory and its high correlation with the level of infection and its use is preferable to that of the measurement of lacassa (non-existent in the case of Botrytis infection) or glycerol (due to the variability associated with very low levels). The enzymatic gluconic measurement method is an officially adopted method by the OIV (2) for both must and wine.

Gluconic acid is a metabolic product of the oxidative aerobic fermentation of glucose produced by numerous species of fungi, and has been widely studied in the case of Botrytis infection. The glucose oxidase from the fungus oxidizes the C1 aldehyde from glucose to carboxyl, producing glucolactone which, in turn, is spontaneously hydrolyzed to gluconic. By itself it is not toxic nor does it contribute unpleasant aromas to the wine, but its presence is indicative of the metabolism of the fungus. In this sense, the determination of gluconic has been routinely implemented in many Protected Designations of Origin as a quality parameter both in the reception of the grape and in the acquisition of must or wine and is one of the elements considered in the evaluation of the same. The enzymatic method validated by the OIV as the official type II method (resolution OIV-OENO 622-2019) is based on the transformation of gluconic into ribulose with production of NADPH and the reaction is monitored by means of the variation of absorbance at 340 nm. This reaction is very fast and allows accurate results of the gluconic concentration to be obtained in approximately 2-3 minutes, and the process can be automated on chemical analyzers.

Sinatech has dedicated reagents for the determination of gluconic acid for the Y15 / Y25 and Dionysos systems (and easily adaptable to other automatic or manual systems) according to the official OIV method, with a measurement range of 0.06 to 2.00 g / L and analysis time of 3 minutes.

Code	Format	Test / Kit	Measure range
SY2405	2x40 mL + 1x12 mL	314	0.06 – 2,00 g/L
SD6005	2x30 mL + 1x15 mL	233	

References

(1) Lopez-Pinar et al. Effects of Bunch Rot (Botrytis cinerea) and Powdery Mildew (Erysiphe necator) Fungal Diseases on Wine Aroma. Front. Chem., 28 March 2017 | https://doi.org/10.3389/fchem.2017.00020
(2) Resolución OIV-OENO 622-2019: http://www.oiv.int/public/medias/6830/oiv-oeno-622-2019-en.pdf

For more than 10 years, Sinatech's commitment to the winemaker has been working side by side to provide the most appropriate analytical solutions to the control and monitoring of the winemaking process. Automated methods easily adaptable to any work routine, with a personalized advisory team to help you quickly and smoothly implement.

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