Portable biosensor-based device for mapping the risk of grape infection by Botrytis cinerea in the vineyard (WINBIOTOOL)

PHASE 1/2020

Objective

The main objective pursued in this stage was the validation at the laboratory level of the biosensor for Botrytis cinerea and the preliminary development of: (1) the sensor for lacasse, (2) the portable detection device and (3) the specifications for application support for the management of the risk of fungal infection in the vineyard.

Results

(1) Quality and quantitative assessment of grapes infected with *Botrytis cinerea*. Laccase activity analysis on infected grapes

The risk assessment of *Botrytis cinerea* fungus has been achieved in Grape growing Centre Valea Calugareasca, on 5 wine grapevine cultivars: three highly susceptible varieties to gray mould – Chardonnay, Sauvignon Blanc, Feteasca Alba, and two varieties with medium resistance – Feteasca Neagra and Cabernet Sauvignon. The evolution of weather conditions over the 2020 season have not created favourable conditions for Botrytis c. Pathogen, along the fruit set, berries growing and bunch closure growth stages, except of some isolated attacks on leafs and bunches determined mainly by cultivars' related susceptibility and vineyards' specific micro-climate.

Between the laccase activity of resulting juice of *Botrytis cinerea* infected bunches and the severity of infection observed in the vineyard blocks a moderate to good correlation (R = 0.64) has been highlighted over the measurements carried out over veraison (BBCH81 – 88), and a very good correlation (R = 0.88) has been highlighted for the measurements carried out over the full ripening growth stage (BBCH 89).

(2) Establishing preparatory specifications and implementing grapevine fungal infection risk management in the vineyard

A Data base over a 10 years' time period (2010 – 2019) was completed in order to establish the agricultural environment conditions (weather, soil) and environment indicators (climatic and edafic) involved in the gray mould (*Botrytis cinerea*) infection occurrence and display. The **VITISMED** data base provides two components: daily grapevine weather (CZV) and monthly grapevine weather (CLV). According to this, 7 environment indicators have been calculated for each grape growing year as follows: Bacchus, Broome, helio-thermal, edafo-climatic, grapevine, hydro-thermal, dry, and standard rainfall. The data were statistically processed using Sigma Plot 14 software application.

The monthly weather indicators that have had the greatest influence on the occurrence and display of the disease were: mean temperature, relative humidity, total rainfall, mean temperature during leaf wetness and leaf wetness (time period). Following the determining of correlations among indicators there were developed two equations for Botrytis infection forecast:

Risk Index 1 = 0.765 + (0.00582 * Sunshine period) - (0.178 * Hydrothermal indicator), (R=0.939),

Risk Index 2 = -2.552 + (0.00575 * Sunshine period) + (0.0517 * Relative wetness) - (0.146 * Hydrothermal indicator), (R=0.964).

(3) The analysis of environment and technological factors that determine the occurrence and development of gray mould in the vineyards

For establishing the influence of environment and technical conditions which determine the occurrence and development of gray mould (*Botrytis cinerea*) in the vineyards, two grapevine varieties were studied: Cabernet Sauvignon with medium resistance to Botrytis infection, and Chardonnay with high susceptibility.

The analysis of main weather elements revealed that 2020 season benefited of a very high thermal resource with multiple influences in growth stages evolution.

The favourable conditions for fungus development were recorded in the first half of June, when the rainfall has fostered the primary infection, but the high temperatures over July and August and the lower level of rainfall, have led to low level of infection incidence, the level of varieties resistance to fungus attack being good and very good.

In addition to weather conditions which have not favour the development of Botrytis infection, severe water stress influenced in a negative way the evolution of main physiological processes in the grapevine, mainly photosynthesis, because to eliminate de water losses, stomata shut down and do not allow carbon dioxide intake.

Shoots growth was extremely poor for both varieties; consequently the favourable micro environment for fungus development was not achieved.

ICDVV collaborated with International Centre of Biodynamics in carrying out the following activities:

Act. 1.1. Development, characterization and validation at the laboratory level of the electrochemical immunosensor for *Botrytis cinerea*

In order to make available to the collaboartor the spores produced by different strains of *Botrytis cinerea*, ICDVV Valea Calugareasca, isolated and purified several strains of *Botrytis cinerea* using as source infected grapes.

The initial material was represented by berries with visible sign of Botrytis infection, Sauvignon and Chardonnay varieties.

Isolation of pure culture of *Botrytis cinerea* was achieved by using Kritzman and Netzer medium (1978) which is selective for Botrytis. Around the colonies developed on this medium a brown halo is formed, due to the oxidation of tannic acid, this being a component of the medium.

After 3-4 days, non-confluent fungal colonies developed on the surface of the medium, some of them being surrounded by a brown halo, indicative of laccase production and secretion**.**

From the edge of the colonies with brown halo, small pieces of mycelium were taken and cultured in Petri dishes with Potato Dextrose Agar medium. The Petri dishes were incubated at 24° C in the dark. Subcultivation of the mycelium was repeated until pure cultures of *Botrytis cinerea* were obtained.

Selected Botrytis strains were analyzed in terms of the morphological properties of the colonies (color, texture, shape, edge), and according to characteristics of spores, mycelium and conidiophores.

Act. 1.2 Development of an electrochemical sensor in order to measure the laccase activity "on field" and characterization at the laboratory level.

Under the coordination of ICB București, an electrochemical method was developed that allows the precise and rapid determination of laccase in the must and which can be adapded for "on field" measurements. The method has been described in the paper published by Gaspar et. al, 2020 (https://doi.org/10.3390/chemosensors8040126).

(4) Working visits, scientific events and dissemination of results

PROJECT MEETINGS

Two online "Kick-off meeting" of WINBIOTOOL via Microsoft teams were organized on 23th July and 29th October in order to introduce the team members, present their institutions and discuss the project activities for 2020.

In addition, ICB Bucharest and EPI-SISTEM partners held visits work at ICDVV Valea Calugareasca related to the development of the portable laccase analysis system and the determination of the risk of infection with *Botrytis cinerea*.

DISSEMINATION OF RESULTS

Two scientific papers have been published:

Liliana Pircãlabu, Elena Brinduse Marian Ion, 2020, "Prediction of botrytis risk in vineyards based on weather indicators", Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Horticulture, Vol. 77, nr.2, pg. 58 – 63. http://dx.doi.org/10.15835/buasvmcn-hort:2020.0038

Gáspár S, Brinduse E, Vasilescu A, „Electrochemical evaluation of laccase activity in must", Chemosensors 2020, 8(4), 126. https://doi.org/10.3390/chemosensors8040126