

# ВИВІВАННЯ

# Viticulture

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## SANITARY CERTIFICATION IN THE PRODUCTION OF GRAPEVINE PLANTING MATERIAL BIOLOGICAL CATEGORIES: EUROPEAN EXPERIENCE AND UKRAINIAN REALITIES

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**Key words:** clonal selection, sanitary control, grapevine viruses, crown gall disease, grapevine phytoplasma and fungal diseases, ELISA, PCR.

### Abstract

The history and the perspective of Ukrainian grapevine planting material certification system are presented. The peculiarities of sanitary control system, developed in NSC "Tairov Research Institute of Viticulture and Wine-Making" based on viral, bacterial, phytoplasma and fungal diseases monitoring and laboratory testing by ELISA and PCR is demonstrated.

Grapevine planting material certification is the main method for the good and long-term vineyards functioning [1]. The main objects of such systems in viticultural countries of the world are viral, bacterial, phytoplasma and grapevine trunk diseases [2, 3, 4, 5, 6].

Grapevine planting material certification system creation was started at Tairov Research Institute of Viticulture and Wine-Making in the 80s of last century.

The aim of research was to create a methodological and technological foundations of grapevine planting material certification system for Ukraine on the basis of genetic and sanitary components.

Clonal selection was carried out from 1968 till 2014 in Odessa, Mykolaiv, Kherson, Zakarpatja regions and Crimea on the total vineyards area nearly on 2000 hectares of vineyards ( wine, table grapes and rootstocks.) After clones trials in two vegetative generations 56 clones of 25 wine varieties, 43 clones of 21 table varieties and 12 rootstocks clones were included into certification scheme of grapevine and recommended for commercialization [7].

In addition, 12 wine varieties, 27 table varieties and 3 rootstocks newly selected varieties were included into certification scheme of grapevine on the base of sanitary control.

European requirements for the production of biological categories of vine planting stock require strict sanitary control of viral, bacterial, phytoplasma pathogens, as well as grape trunk fungal diseases. The complex program for the creation of the Ukrainian system of certified vine planting material production started more than 30 years ago, and all stages of clonal breeding were accompanied by constant sanitary control.

Each stage of clonal selection and reproduction was accompanied by phytopathological studies and laboratory sanitary control of the causative agents of viral, bacterial, phytoplasmic and fungal diseases of Ukrainian viticulture.

The purpose of this study was to generalize the results of field and laboratory studies of phytopathogens in vineyards from 2005 to 2017 for the scientific substantiation of the sanitary control system proposed for viticulture in Ukraine. To do this, we had to perform the following tasks:

- to estimate the distribution of viral, bacterial, phytoplasma and grape trunk diseases in Ukraine's vineyards in the dynamics over the last decade compared with the period 1985 - 2000;
- establish levels of visual and latent infection to these pathogens;

- assess the risks of natural spread of these diseases with planting material and natural vectors, as well as temporal and spatial distribution within one plot (vineyard)

### Material and methods

Field studies were carried out in the course of the research: (a route survey of vineyards, a study of the spatial-temporal distribution of sick vines within sites) and laboratory methods (selective media method, ELISA for the identification of viral pathogens (GFLV, GLRaV 1, GLRaV 3, GFkV, GVA, GVB, ) PCR for identification of viral diseases, crown gall disease, phytoplasma diseases, grape trunk diseases agents.

### Results and discussion

#### *Field distribution, visual and latent levels of infection*

In the period 1985 - 1995 years, the control was mainly aimed at detecting of grapevine leafroll associated viruses (GLRaV)-1,3, rugose wood complex viruses (A, B), grapevine fanleaf virus (GFLV) and grapevine fleck virus (GFkV) by ELISA. Since 2002, the RT-PCR method has been used to identify these viruses. With the help of these methods, a large array of data was obtained regarding the spread of viruses in Ukraine's vineyards, their ecology and epidemiology.

Among the most spreaded diseases included for mandatory testing for planting material certification are viral diseases, such as grapevine leafroll disease (GLRaV-1,3), grapevine fanleaf virus (GFLV) and grapevine fleck virus (GFkV).

The Chardonnay variety and Muscat Hamburg were to a small extent infected with the grapevine leafroll associated virus 3 serotype and Italy variety contained grapevine leafroll associated virus 1 serotype .

Comparison of two periods of observation showed a visual increase in the leafroll infection (possibly due to the increase in the duration of dry periods during vegetation), while the latent infection remained relatively insignificant and did not exceed 3 to 5%, depending on the origin of the planting material.

Simultaneously with the detection of viral infection, all selected grapevine clones underwent testing for the causative agent of crown gall disease, because in the climatic conditions of Ukraine due to frost damages this disease manifests itself in a high degree. Diagnosis is carried out by first isolating the pathogen on a semi-selective medium of Roy and Sasser, followed by PCR identification.

When checking the varieties of grapes Cabernet Sauvignon, Chardonnay, Riesling Rhine, Muscat Hamburg, Italy, it was found that the most infected by crown gall agent was the Cabernet Sauvignon variety (nearly 11.04%), to a lesser extent Italy (5%) and Muscat Hamburg (4%).

When comparing two periods, it is shown that visual and latent infection with crown gall disease tend to be somewhat lower at last decade, probably due to a decrease in the frequency and strength of frost damages.

In 2002 - 2005, for the first time in Ukraine a phytoplasma infection was detected on vineyards. The phytoplasma infection in Ukraine was first determined visually, later identified by PCR with electrophoretic detection, as well as real-time detection as a Bois noire (BN). The main known carrier of BN, leafhopper *Hyalesthes obsoletus* was determined on Ukrainian vineyards, too. The dynamics of phytoplasma infection increasing on Chardonnay variety demonstrated on Figure 1.

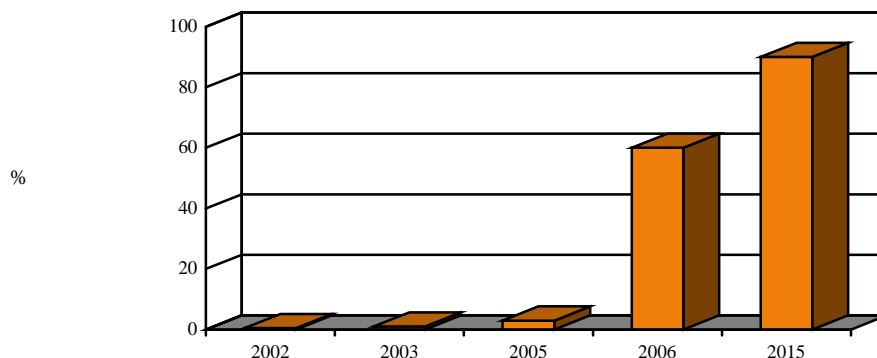


Figure 1. Dynamics of BN infection on Chardonnay variety (Ovidiopol district, Odessa region)

Approximately at the same time, the symptoms of grapevine esca complex, belonged to the group of grapevine trunk fungal diseases were first detected on Ukrainian vineyards. The leaf symptoms were detected on many wine and table grapevine varieties, wood endophyte symptoms were detected on rootstocks. The character of endophytic lesions on new rootstock Dobrynya demonstrated on Figure 2.

As a result of vineyards sanitary status monitoring, it was established that the most progressive diseases in Ukraine are phytoplasma and grape trunk ones, the second place is taken by crown gall disease



Figure 2. Circular affected zone of endophytic lesion by esca (Dobrynya rootstock)

### *Natural spread and temporal-spatial distribution of diseases*

From the first part of the study, it can be seen that visually and latently, the most common of 4 groups of diseases are leafroll (among viral ones), bacterial crown gall disease, phytoplasma diseases (Bois noire), and esca (among the grape trunk fungal disease).

For each of these diseases, the pathways and rates of natural spread as well as spatial and temporal distribution within the plot (vineyard) were assessed, which also serves as a basis for estimating the rate of natural transfer (Table 1).

Table 1

Natural spread and temporal-spatial distribution of the main common viral, bacterial, phytoplasma and grape trunk diseases (2002 – 2017)

Disease	Number of plots and years of study	Pathways of distribution (on the base of diseased plants spread)	Speed of diseased plants increasing	Risk of natural spread on vineyards
Grapevine leafroll disease (GLRaV 1, 3)	2 (2002-2006)	Planting material mainly	Up to 0,24 % per year	Low
Crown gall disease (Agrobacterium vitis)	4 (2002-2006)	Planting material and soil	2,6 % for year in average (after frost damage)	High
Phytoplasma diseases	1 (2002-2010)	Insects and planting material	Up to 7 % per year in average	High
Grapevine esca	2 (2014-2017)	Planting material and natural spread of causal agents	Up to 1,3 % per year	High

### **Conclusions**

1. The most common diseases in Ukraine among diseases included into sanitary control system for the grapevine planting material of biological categories production are viral disease (grapevine leafroll disease), bacterial diseases (crown gall disease) phytoplasma diseases (Bois noire), fungal trunk diseases (esca).
2. Progress in spreading is observed for phytoplasma disease (up to 7 % per year) crown gall disease (up to 2,6 % per year after frost damage) and grapevine esca (up to 1,3 % per year). So these diseases have a greatest risks of natural spread on Ukrainian vineyards.

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