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CURRENT STATUS OF GRAPEVINE FLAVESCENCE DORÉE AND BOIS NOIR IN BULGARIA (2022-2024)

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Abstract

The present article examines the monitoring of phytoplasma diseases in grapevines during the period 2022-2024, with a special focus on the distribution, symptoms, diagnosis, and control methods of these diseases. Phytoplasma diseases, such as Flavescence dorée and Bois noir, pose a serious risk to viticulture due to the significant economic losses they cause. Molecular methods were used in the study to detect phytoplasmas. A total number of samples was more than 660 were analyzed, of which around 6 indicated a positive result for the phytoplasma disease Bois noir. And for the time being, the phytoplasma disease Flavescence dorée is not present in Bulgaria. Regardless of the status of the two phytoplasmas, from quarantine to non-quarantine controlled, according to phytosanitary legislation, the risk of their spread poses a significant threat to viticulture in Bulgaria. The obtained results highlight the need for integrated control strategies, including early diagnosis, vector control, and the use of resistant grapevine varieties, to prevent the introduction of this phytoplasma disease.

***Key words**: Phytoplasma diseases, Flavescence dorée, Bois noir, Bulgaria.*

INTRODUCTION

Viticulture is a key subsector in Bulgarian agriculture, ranking second in monetary terms after tobacco production (Avramov & Mihaylov, 2019). Following this period, it is positioned directly after fruit growing and vegetable production (Agrostat, 2021). Despite its economic importance, viticulture faces numerous challenges, including grapevine yellows - a widespread global issue representing serious diseases transmitted through infected planting material. The diversity of symptom manifestations leads to the naming of various phytoplasma diseases in grapevines (Avramov, 2019). Phytoplasmas are submicroscopic bacteria lacking a cell wall, characterized by high pleomorphism and the absence of flagella or spores. They infect the plant's vascular system, primarily affecting the phloem. This results in severe disruptions to cellular structures, including the destruction of chloroplasts and reduced photosynthetic activity. In natural conditions, the transmission of phytoplasmas occurs primarily through cicadas and leafhoppers. These pathogens multiply in the body cavity and hemolymph of their vectors. The vectors can transmit the pathogen after feeding or within a few hours to days. In Bulgaria, data on phytoplasma diseases in grapevine plantations are scarce and do not meet modern scientific research standards (Dobrev, 1909; Trifonova et al., 1952; Abrascheva, 1977; Kovachevski et al., 1999; Avramov et al., 2007; Avramov et al., 2018). Grapevine yellows (GY), caused by various phytoplasmas worldwide, cannot be diagnosed based solely on symptoms. The most severe form of this disease, Flavescence dorée (FD), causes significant damage in major viticultural regions in France, Italy, Spain, Slovenia, Croatia, and Serbia (Belli, 2010). In Bulgaria, the FD phytoplasma is classified as a quarantine pest. Another important type of GY in Europe is Bois noir (BN), associated with the stolbur phytoplasma (STOL) or 16SrXII, which is primarily transmitted by the cicada *Hyalestes obsoletus* (Avramov et al., 2007; Avramov et al., 2011). The hosts of the Grapevine flavescence dorée phytoplasma include *Vitis vinifera* and other species and hybrids of the genus *Vitis* (Eveillard et al., 2016). Its spread has been reported in eight major EU member states involved in viticulture, such as Austria, Croatia, France, Hungary, Italy, Portugal, Slovenia, and Spain, as well as in Switzerland and Serbia

(Jeger et al., 2016). The most effective vector for transmitting this phytoplasma is *Scaphoideus titanus* (Mori et al., 2002; Schvester et al., 1963). The vector of Grapevine flavescence dorée, *Scaphoideus titanus*, originating from North America (Papura et al., 2012), is distributed in 18 European countries (Jeger et al., 2016). The phytoplasma is transmitted to a new host plant through the insect's saliva (Wang et al., 2024). Symptoms typically become more pronounced during the summer months. In some cases, individual shoots of the vine may be affected, while in others, the entire vine shows signs of infection (Belli et al., 1973; 2010; Caudwell, 1964). In cases of late infection, the process of wood formation in the vine stops. The leaves undergo changes in color, and their edges curl downward. If the infection occurs early in the season, reduced fruit formation is observed, with inflorescences drying up and falling off. In later infections, the grape clusters turn brown and shrivel, and their stems dry out. The spread of Flavescence dorée in grapevines occurs primarily through two means: the transfer of infected plant material and the dispersal of infected vectors that migrate from neighboring areas, carried by planting material or through transportation. In 1961, a disease of the "yellows" type was first recorded in the viticultural regions of northeastern France, known as "Bois noir" (BN). A few years later, similar symptoms were observed in Germany, in the wine-growing regions of the Mosel and Rhine valleys, where the disease was named "Vergilbungskrankheit". Subsequent research confirmed that it was the same disease (Pierro et al., 2024). In the following years, BN was reported in numerous countries across the Euro-Mediterranean region, as well as in some parts of other continents, causing significant agricultural losses (Mori et al., 2024). In 2005, the first cases of BN disease in Bulgaria were recorded in the regions of Sliven, Targovishte, and Veliko Tarnovo, affecting the grapevine varieties Chardonnay and Merlot. According to observations in subsequent years, the number of affected areas gradually increased (Avramov & Mihaylov, 2019). In the Euro-Mediterranean region, '*Candidatus* Phytoplasma solani' is primarily transmitted between plants by the polyphagous vector *Hyalesthes obsoletus*, which is widely distributed across Europe and present in many European countries (Weintraub & Beanland, 2006; Johannesen et al., 2008; Sémétey et al., 2018; Avramov, 2022). The cicada is responsible for the widespread dissemination of stolbur during different periods in countries such as Israel, Spain, Italy, Greece, and others (CABI, 2021). Infected grapevines are not a significant source of inoculum, as the cicada prefers other food sources and only turns to grapevines as a last resort. This explains the relatively limited spread of BN and the possibility of infection even with a high population density of the vector in the vineyard area. However, the situation changes rapidly and dramatically when new vectors of phytoplasmas appear in the vineyards (Kosavac et al., 2019). Bois Noir in grapevines causes yellowing of the leaves in white varieties and reddening in red varieties. Characteristic symptoms also include downward curling of the leaves, stunted growth, as well as shriveling and drying of grape clusters and berries. Young plants may die after infection, while older ones usually recover (Pierro et al., 2024). The severity of the symptoms depends on the susceptibility of the specific variety (EFSA, 2016). The signs of the disease do not differ from those caused by grapevine flavescence dorée (EPPO, 2018). The main symptom associated with BN is the loss of yield due to berry deformation, leading to significant economic consequences, especially in sensitive varieties (EFSA, 2014; Mori et al., 2024). The article aims to raise awareness about the serious threat posed by phytoplasma diseases, such as FD and BN, to viticulture due to the significant economic losses they cause. It emphasizes the need for integrated control strategies, including early diagnosis, vector control, and the use of resistant grapevine varieties. Ultimately, the article seeks to draw the attention of specialists, producers, and regulatory authorities to the importance of prevention and control of phytoplasma diseases in order to safeguard Bulgarian viticulture from potential economic losses.

MATERIALS AND METHODS

The materials were received at the laboratory from various locations across Bulgaria. During the period 2022-2024 a total number of samples 667 were obtained, plant samples collected from vineyards (466 samples), mother vineyards (171), rooting beds, rootstocks, scions, and cuttings (30) were analyzed. The number of samples for the year 2022 was 244, of which 161 samples were from vineyards and 83 samples were from mother vineyards. In 2023, a total of 230 samples were analyzed, including 180 samples from vineyards, 31 samples from mother vineyards, 12 samples from rooting beds, 6 samples from rootstocks, and 1 sample from scions. In 2024, the total number of samples analyzed was 192, of which 125 samples were from vineyards, 57 samples from mother vineyards, and 11 samples from cuttings. The diagnosis of phytoplasmas is routinely performed using PCR and can be divided into three phases: general extraction of DNA from symptomatic and asymptomatic tissue; PCR amplification of phytoplasma-specific DNA; and characterization of the amplified DNA through RFLP analysis or nested PCR with group-specific primers (Avramov, 2023).Total DNA was extracted from plant tissue (Doyle and Doyle, 1990) or a pre-determined individual in CTAB buffer following the protocol by Marzachi et al. (1998). Laboratory analysis was conducted using Nested PCR with two pairs of universal primers, P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1996) and U3/U5 (Lorenz et al., 1995), as well as specific primers f01/r01 (Lorenz et al., 1995), followed by RT-RFLP analysis using Rsal/AluI restriction enzymes for final identification and species classification (Lee et al., 1998).

RESULTS AND DISCUSSIONS

For the period 2022-2024, a total of 1,346 laboratory analyses were conducted on 667 plant samples, and phytoplasma infections were detected in 6 samples. No FD infection was found in any of the samples during this period. The results were presented in Table 1.

Table 1. Laboratory Analyses and Infections, 2022-2024

Year	Total samples	Total Analyses	BN infection	FD infection
2022	244	492	+	-
2023	230	462	+	-
2024	193	392	+	-
Total	667	1346	+	-

+ = positive result; - = negative result.

In 2022, one BN infection was detected in a vineyard in the Montana region and another BN infection in a vineyard in the Burgas region. In 2023, only one BN infection was recorded in a vineyard in the Ruse region. In 2024, two BN infections were identified in the Ruse region from mother vineyards (Table 2).

Table 2. Number of samples and infections of BN by year

Year	Mother vine yards	Infec tion	Vine yard	Infec tion	Root stocks	Infec tion	BN+
2022	83	-	161	2	-	-	2
2023	31	-	180	1	19	-	1
2024	57	2	125	1	11	-	3
Total	171	2	465	4	30	-	6

In 2024, there is an increase in cases in the Ruse region, which may indicate a localized risk of spread (Table 3).
The infected plant materials originate from three varieties - Chardonnay (4 positive results); Cabernet Sauvignon and Misket Hamburg and separated us following in a regions - Ruse 3 positive samples with confirmed detection of BN on Chardonnay and Misket Hamburg; Montana region with detection of BN on Cabernet Sauvignon and Chardonnay; Burgas region on Chardonnay (Table 3).

Table 3. FD and BN Infections by year and region

Region	Number of samples	Infection	
		FD	BN
Blagoevgrad	27	-	-
Burgas	65	-	1
Varna	74	-	-
Vidin	37	-	-
Vraca	44	-	-
Veliko Tarnovo	28	-	-
Dobrich	30	-	-
Lovech	12	-	-
Montana	51	-	2
Paazardjik	19	-	-
Pernik	1	-	-
Pleven	4	-	-
Plovdiv	43	-	-
Ruse	40	-	3
Sliven	49	-	-
Stara Zagora	21	-	-
Haskovo	16	-	-
Shumen	43	-	-
Yambol	49	-	-
Total for the country	677	6	

The recommended measures for the detected infections include adherence to quarantine measures, and uprooting of the vineyards is not recommended. Out of 1,332 laboratory analyses of 667 samples, only 6 positive cases were detected. No cases of Flavescence dorée (FD) were found, which is a positive indicator of the phytosanitary status of Bulgarian vineyards. Bois noir was first recorded in Bulgaria in 2005 in the regions of Sliven, Targovishte, and Veliko Tarnovo, affecting the wine grape varieties Chardonnay and Merlot. The result of the work confirms the date of previously detection, that the infection spread to additional viticultural areas, and had already been detected in all major wine-producing regions of the country (Avramov, 2023). Data from 2022 to 2024 indicate a stable level of BN spread, with small down level of changes, as 6 infections were found among 667 analyzed samples. The distribution of infections by grape variety shows that wine varieties are the most affected (1.08%), followed by local and table grape varieties (0.58%) (Table 4).

Table 4. Infected varieties by BN for 2022-2024

Region	Infected varieties by BN	
Ruse	Chardonnay (2)	Misket Hamburg
Montana	Chardonnay	Cabernet Sauvignon
Burgas	Chardonnay	-

This confirms the observed higher susceptibility of wine grape varieties to phytoplasma infections. The Chardonnay variety appears to be particularly vulnerable, with BN detected in 4 samples from various regions of the country. Although BN was identified in 1 analyzed grapevine varieties, the overall infection rate remains relatively low, possibly due to the absence of favorable factors for its spread, such as the origin of planting material, varietal specificity, vineyard age, agricultural practices, and pest management systems. This suggests that some varieties are more susceptible to BN infection, while others are more resistant (Avramov, 2023). During the most recent analyzed period 2022-2024, a significant decrease in BN cases has been observed compared to previous years. Although the overall prevalence of BN remains low, an increase in cases was noted in the Ruse region in 2024. This may indicate a localized risk of infection spread in this area. The decreased frequency of BN infections in recent years may be attributed to improved phytosanitary control, the effective application of integrated pest management strategies, and strict monitoring of planting material. However, the localized increase in cases in Ruse requires attention and additional preventive measures. Key risk factors for BN spread are: presence of vectors (*Hyalesthes obsoletus*) - although grapevines are not the primary host of this vector, an increase in its population in a given area can raise the risk of infection transmission; use of infected planting material - despite strict regulations, the possibility of BN spreading through infected cuttings remains. Recommended measures to limit BN spread are**:** Enhanced monitoring and surveillance - in regions with confirmed cases (Ruse), more active diagnostics are necessary to prevent further spread; Vector control - integrated pest managment strategies to control *Hyalesthes obsoletus*, including biological control and agricultural practices, can reduce the risk of transmission; Strict control of planting material - ensuring healthy planting material through in-depth laboratory testing before replanting and Vineyard management-improving agricultural practices, including sanitary pruning and optimizing plant protection systems.

CONCLUSIONS

In conclusion the data from the 2022-2024 period indicate a significant decrease in BN cases in Bulgaria compared to previous years. The registered increase in cases in the Ruse region in 2024 highlights the need for intensified monitoring and the application of effective preventive measures. The absence of Flavescence dorée cases is a positive indicator of the health of Bulgarian vineyards, but continued systematic control and adherence to phytosanitary measures remain crucial for limiting the spread of phytoplasma infections in the country. The present study provides up-to-date information on the status of phytoplasma diseases in grapevines in Bulgaria for the period 2022-2024, with a focus on FD and BN. The data highlight the need for continued strict monitoring and the implementation of integrated control strategies, including early diagnosis, vector management (primarily *Hyalesthes obsoletus*), and the use of resistant grapevine varieties.

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