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REACTION OF NEW INTRODUCED WALNUT CULTIVARS TO ANTHRACNOSE AND BACTERIOSIS IN BULGARIA

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Abstract

The Walnut bacterial blight caused by Xanthomonas arboricola pv. juglandis and walnut anthracnose caused by the fungus Gnomonia leptostyla (sexual stage) and Marssonina juglandis (asexual stage) are the most economically significant walnut diseases in Bulgaria's climate. This study compared the susceptibility of newly introduced walnut cultivars Valmit, Valeris, Sebin, and Yalova 1 to these diseases with that of the prevalent Bulgarian cultivars Izvor 10 and Silistrenski. Observations for disease symptoms on leaves were conducted twice annually in the spring and autumn in a young walnut orchard at the Fruit Growing Institute – Plovdiv. The average percentage of walnut leaf spots showed that the Romanian cultivar Valmit (31.44%) was most affected. Another Rumanian cultivar Valeris was reported with the lowest percentage of infection to fungal disease with 22.30% infected leaves. From the observed cultivars, the most affected by bacterial blight was the Bulgarian one - Izvor 10 in degree 8.6%, which determinated it as a high susceptible to this disease. In our research the Turkish cultivar Yalova1 was reported as the lowest susceptible with 4.74% infected leaves.

***Key words**: cultivars, Gnomonia leptostyla, infection, Juglans regia L., Xanthomonas arboricola pv. juglandis.*

INTRODUCTION

Walnut is a nut crop fruit belonging to the *Juglans* genus of the *Juglandaceae* family. Widely cultivated in different regions of the World, the walnut has an important role in human health due to its rich nutritional content (Carvalho et al., 2010). Walnut anthracnose, caused by the ascomycetous fungus *Gnomonia leptostyla* (Fr.) Ces. et de Not. (anamorph *Marssonina juglandis* (Lib.) Magn.), poses a significant threat to walnut production worldwide (Yang et al., 2021). This disease is recognized as one of the most serious and widespread walnut afflictions, impacting nearly all walnut-growing regions. Its severity is closely linked to environmental conditions, with wet and rainy weather, particularly during spring, creating ideal conditions for rapid disease development and spread. The pathogen attacks various parts of the walnut tree, including leaves, twigs, shoots, and husks. Infected tissues develop irregular necrotic patches, often surrounded by distinctive small chlorotic halos. These lesions not only diminish the photosynthetic capacity of the tree but also directly impact fruit yield and quality (Belisario et al., 2008). Early infections can lead to fruit deformation, premature fruit drop, and reduced marketability. Furthermore, repeated defoliation, a common consequence of severe anthracnose infection, can have long-lasting detrimental effects on tree growth and vigor, occasionally leading to tree decline and even death. The economic impact of walnut anthracnose is substantial, with reported field losses reaching up to 50% in severely affected areas (Woeste et al., 2001). This underscores the critical need for effective disease management strategies to protect walnut orchards and ensure sustainable production (Yang et al., 2021). Bacterial pathogens are causing a lot of destructive crop diseases, which globally can have significant economic losses. There are over 25 genera and 200 species of bacteria that are pathogenic on plants (Sharma et al., 2022), many causing devastating outbreaks and substantial crop losses. Among damaging species belong to the genera *Xanthomonas* (Sharma et al., 2023). Host plant resistance plays a crucial role in the effective management of numerous plant diseases (Mehlenbacher, 1995), evaluating disease and pest resistance as a critical component of cultivar development. Deploying anthracnose-resistant cultivars offers a promising approach to disease management and can be effectively integrated with other control strategies, such as targeted chemical applications, to minimize disease pressure (Berry, 1981). These two diseases target different parts of the walnut tree. *O. leptostyla* primarily infects leaves, petioles, and fruits, while *X. arboricola* pv. *juglandis* can infect a broader range of tissues, including leaves, catkins, female flowers, green branches, and nuts. Walnut blight, in particular, is known to significantly reduce both yield and the quality of harvested nuts (Arnaudov et al., 2014), impacting marketability and profitability. Research has demonstrated a strong cultivar-dependent response to *G. leptostyla* infection. Analysis of disease progression revealed significant variations in the number of leaf spots, the extent of fungal growth, and overall disease severity among different walnut cultivars (Dastjerdi, 2023). This variability in susceptibility underscores the importance of identifying and promoting the cultivation of resistant or tolerant cultivars as a key strategy for sustainable walnut production.

MATERIALS AND METHODS

In 2019-2021 period, monitoring the phytosanitary condition of walnut experimental orchards was conducted through route surveys in various phases of plant development. The surveyed plantations included two Turkish cultivars Yalova and Sebin, two cultivars from Romanian Valeris and Valmit and two Bulgarian cultivars Izvor 10 and Silistrenski which were grafted on common walnut (*Juglans regia* L.) rootstock.

The studied cultivars were monitored at Fruit Growing Institute in Plovdiv, the city has a humid subtropical climate with considerable humid continental influences. There are four distinct seasons with large temperature jumps between seasons. The development of the disease on the leaves is expressed by the attack index, which is calculated in percentages according to the formula of McKinney (1923). An infectious index was calculated for each cultivar, and the individual cultivars were classified according to their degree of susceptibility. The monitoring of walnut orchards was carried out two times per season, 100 leaves of each cultivar were randomly collected in May and September. The assessment of plant disease infections was conducted using the widely accepted six-point phyto-pathological scale (Table 1).

The data were statistically processed following Duncan's test (Steele & Torrie, 1980).

Table 1. Phyto-pathological scale

Degree of Susceptible	Degree of attack	
	Gnomonia leptostula Marssonina juglandis (%)	*Xanthomonas arboricola* pv. *juglandis* (%)
Immune	0	0
Resistant	1-5	to 1
Low susceptible	6-25	2-5
Moderately susceptibility	26-25	6- 10
Susceptible	36-50	11-20
Highly susceptibility	over 50	over 20

RESULTS AND DISCUSSIONS

The walnut cultivation area has become extensive, covering several regions in Bulgaria. The main diseases of walnut anthracnose and bacteriosis occur to varying degrees in different regions because of location, soil and cultivation conditions. Therefore, it is important to investigate the walnut level of susceptibility to anthracnose and bacterial blight in each cultivation region, especially newly introduced cultivars. On the other side, the effect of global climatic changes can lead to many adverse consequences, such as reduced yields, deteriorating fruit quality, susceptibility to diseases and pests, and reflect on the life of trees. Data analysis (Figure 1) showed the average rainfall and average temperature in the 2019-2021 period.

Moderate rainfall is observed throughout the 2019 year, with the wettest months being May and June, and the driest - August. The highest amount was recorded in June, with a significant peak of over 150 mm.

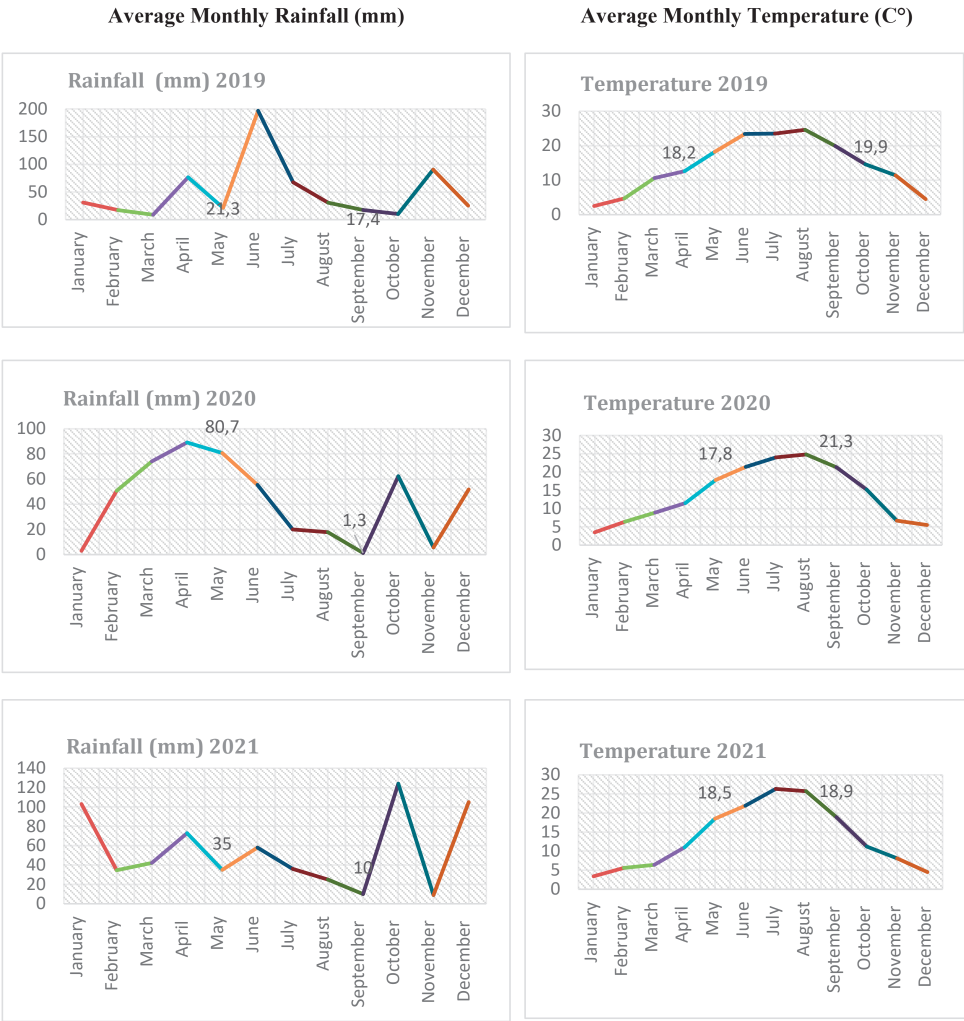

Figure 1. Climate data, registered for walnut vegetation in 2019-2021 for Plovdiv, Bulgaria

These climatic conditions create a favourable environment for the development of diseases, especially in the spring. 2020 was a year with variable rainfall, with the wettest months being May (21.3 mm), April, September (17.4 mm), and October. The driest month was January, followed by August.

The graph displays the monthly rainfall totals for 2021 in Plovdiv region. Overall, the year is marked by a relatively even distribution of rainfall. April has a significant increase in rainfall making it one of the wettest months. Rainfall in May was high but started to slightly decrease compared to April. The graph for 2021 shows the average monthly temperatures, the year follows a typical seasonal pattern of temperature change. The analysis data on climate shows that rainfall and temperature in three years were suitable for the development of the fungal disease (*Gnomonia leptostyla*) infection and secondary infections. In this period also accumulation of inoculum, which, when coupled with the frequent rainfall of autumn, leads to the development of the pathogen. From the end of May to the beginning of June, the first monitoring of symptoms of bacterial blight and walnut leaf spots on the leaves was done. Results of the study on the attack of leaves of walnut cultivars to anthracnose caused by the wintering form of fungus *Gnomonia leptostyla* in the Plovdiv region (Table 2). The three-year study (2019-2021) on the degree of anthracnose infection in different walnut cultivars was analysed, and the Bulgarian cultivars Izvor 10 and Silistrenski work as a baseline for comparison.

A B C

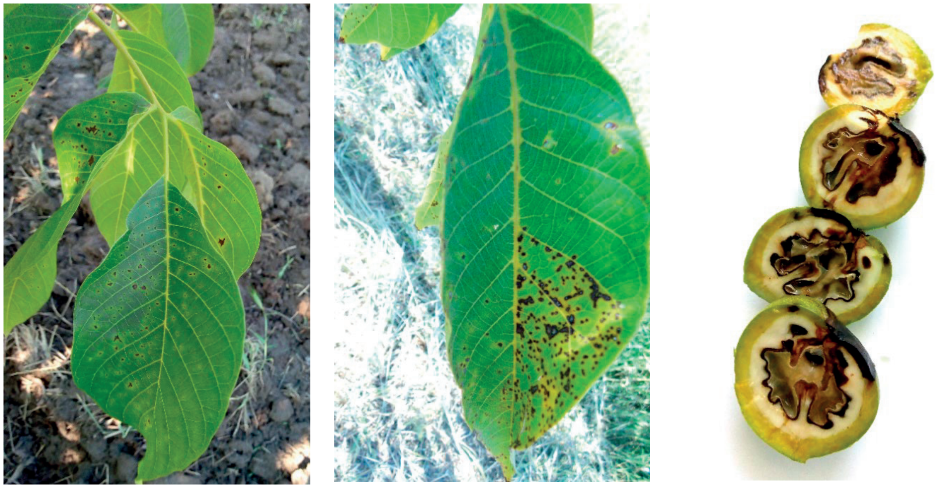

Figure 2. Symptoms of (A) bacterial blight (*Xanthomonas arboricola* pv. *juglandis*), (B) walnut leaf spot (*Gnomonia leptostyla*) and (C) apical necrosis

Table 2. Infection index on leaves of walnut cultivars

Cultivar	Leaves infection of *Gnomonia leytostyla and Marssonina juglandis* (%)								
	2019		Average	2020		Average	2021		Average
	I	II		I	II		I	II	
Yalova 1	18.33	31.43	17.02	3.40	30.37	9.29	5.80	61.14	33.47
Silistrenski	10.53	34.29	13.83	4.80	54.55	16.04	4.00	46.80	25.40
Valmit	18.66	52.63	22.49	0.60	25.63	6.71	8.20	96.00	52.10
Izvor 10	19.01	48.00	21.51	2.75	15.78	5.32	6.00	95.60	50.80
Sebin	20.00	25.14	16.28	2.20	23.45	6.96	5.20	98.00	51.60
Valeris	11.11	25.14	11.84	2.38	7.27	3.01	7.40	85.92	46.66

In comparison with Bulgarian cultivars in 2019, Izvor 10 (21.51%) showed similar levels of infection to Yalova 1 (17.02%) and Valmit (22.49%), but higher than Silistrenski (13.83%) and Valeris (11.84%). In 2020, Izvor 10 showed lower levels of infected leaves 5.32% than all other cultivars, except for Valeris, which had the lowest values with 3.01%. In 2021, Izvor 10 showed significantly higher levels of infestation 50.80% than all other cultivars, except for Sebin, which had similar values of 51.60%. The cultivar Izvor 10 showed the highest level of variable resistance to anthracnose over the years. Another Bulgarian cultivar Silistrenski in 2019 showed the lowest percentage of infected leaves. In 2020 Silistrensk showed higher levels of infected leaves than most cultivars. In 2021, Silistrenski showed lower levels of infestation than all studied cultivars. The comparison between Bulgarian cultivars Silistrenski showed better resistance to anthracnose compared to Izvor 10. Rumanian cultivar Valeris was measured with the lowest percentage of infected leaves in 2019 and 2020 the cultivar presented resistant stable to walnut leaf spots. The Turkish cultivar Yalova 1 showed lower resistance to anthracnose compared to Silistrenski and Valeris, but similar to Izvor 10. Atack of leaves of Shebin showed great variability in its resistance to anthracnose over the years. While it was with lowest infection index to the first report in May, it was highly attacked in September. The analysis of the data of the reaction of walnut cultivars can conclude that Silistrenski showed the best and most stable resistance to anthracnose among the studied cultivars, including in comparison with the other Bulgarian cultivar Izvor 10.

Table 3. Infection index on leaves of walnut cultivars

Cultivar	Leaves infection of *Xanthomonas arboricola* pv. *juglandis*								
	2019			2020			2021		
	I	II	average	I	II	average	I	II	average
Yalova 1	9.52	2.33	5.92	1.40	8.04	4.72	1.00	6.35	3.67
Silistrenski	14.29	11.18	12.73	5.16	7.57	6.37	1.40	4.90	3.15
Valmit	9.33	12.86	11.10	2.38	4.36	3.37	1.20	11.00	6.10
Izvor 10	14.29	17.60	15.94	0.90	5.05	2.97	2.20	12.00	7.10
Sebin	11.67	11.44	11.55	2.43	7.52	4.98	0.80	8.20	4.50
Valeris	7.27	20.00	13.64	2.87	5.56	4.21	1.20	7.55	4.38

Two times per year the reaction of walnut cultivars to bacterial pathogen *Xanthomonas arboricola* pv. *juglandis*.

The Turkish cultivar Yalova 1, showed good resistance to bacterial blight in each year of study. Levels of infection in Valmit were lower than those of Izvor 10 in most years but similar to or slightly higher than those of Silistrenski.

The cultivar Valmit performs better than Izvor 10 and is comparable to Silistrenski in terms of bacterial blight resistance.

Another Rumanian cultivar Valeris demonstrates the best resistance to bacterial blight among all the cultivars tested. The cultivar was significantly lower than those of all other cultivars, including the Bulgarian and Turkish ones.

The Bulgarian cultivar Izvor 10 was the most attacked cultivar to bacterial blight among those compared. Silistrenski showed better resistance than Izvor 10 but is more susceptible than Valmit and Valeris.

The average result (Table 4) presents the degree of susceptibility of six walnut cultivars to two diseases, a fungal infection caused by *Gnomonia leptostyla* or *Marssonina juglandis* and a bacterial disease caused by *Xanthomonas arboricola* pv. *juglandis*. The average percentage in tree studied years reflects the leaf area value affected by the respective pathogen. Cultivars which were low susceptibility to walnut leaf spots were Yalova 1 (23.36%) Valeris (22.30%).

Their average reaction showed that cultivars lowest are classified as "low susceptible" to both diseases. As moderately susceptible were distributed cultivars Valmit (31.45), Izvor 10 (29.47) and Sebin (27.65).

Non-significant differences between studied cultivars.

Table 4. Degree of susceptibility of walnut cultivars
to *Gnomonia leytostyla* and *Xanthomonas arboricola* pv. *juglandis*

Cultivar	Leaves infection *Gnomonia leytostyla (%)*	Degree of susceptibility	*Xanthomonas arboricola* pv. *juglandis(%)*	Degree of susceptibility
Yalova 1	23.36 a	Low susceptible	4.77 a	Low susceptible
Silistrenski	23.36 a	Low susceptible	7.42 a	Moderately susceptibility
Valmit	31.45 a	Moderately susceptibility	6.85 a	Moderately susceptibility
Izvor 10	29.47 a	Moderately susceptibility	8.67 a	Moderately susceptibility
Sebin	27.65 a	Moderately susceptibility	7.01 a	Moderately susceptibility
Valeris	22.30 a	Low susceptible	7.40 a	Moderately susceptibility

The cultivar Yalova 1 showed the lowest susceptibility to bacterial blight (4.77%), making it the most resistant of the cultivars tested. Other walnut cultivars Silistrenski, Valmit, Izvor 10, Sebin and Valeris were distributed as moderately susceptible and showed similar susceptibility levels, ranging from 6.85% to 8.67%. There were statistically non-significant differences in susceptibility to the bacterial blight. Similar results were published by other authors in which studies have shown that Bulgarian local walnut cultivars are generally susceptible to bacterial blight and exhibit slight susceptibility to walnut blight (Gandev et al., 2014; Blagoeva, 2022). In organic-grown orchards incidence of walnut leaf spots was reported Sebin was more susceptible from Izvor 10 (Zhelev et al., 2016) in this study both cultivars reacted almost similarly.

CONCLUSIONS

Global climate change, marked by rising temperatures, shifts in precipitation patterns, and an increasing frequency of extreme weather events, exerts a complex and multifaceted influence on the physiological processes, growth, and development of all fruit trees, including walnuts. These adverse factors can disrupt photosynthesis, and impair nutrient uptake ultimately impacting the productivity and longevity of these trees. In the comparative analysis, it is clear that Yalova 1 is the most resistant cultivar to anthracnose and bacteriosis compared to other studied walnut cultivars. The cultivars Valeris and Silistrenski were reported with the lowest percentage of infection and classified as having a low susceptibility to anthracnose. This makes them a good choice for areas where these diseases are prevalent. Other Rumanian and Turkish cultivars can be grown in Bulgaria conditionals with additional plant protection programs.

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