

COMPORTAREA UNOR SOIURI DE NUC ÎN FAZĂ JUVENILĂ LA ATACUL BACTERIOZEI ȘI ANTRACNOZEI

BEHAVIOR OF SOME WALNUT VARIETIES UNDER JUVENILE PHASE TO BACTERIOSE AND ANTRACNOSE ATTACK

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Abstract

Walnut (*Juglans regia* L.) is an important species for the European and Romanian fruit growing, but, despite its resilience, it can be attacked by specific diseases such as bacteriose (*Xanthomonas arboricola* pv. *juglandis* (Pierce) Dye) and anthracnose (*Gnomonia leptostyla* D.C. Trav) which due to the climatic changes became more active than in the past decades. This paper presents results on the behavior of 48 walnut varieties preserved into the new collection established at RIFG Pitesti Romania to the field infections with these pathogens, their resilience in juvenile phase and their overall classification. The data collected reveal that the microclimate conditions during years of the study (2018-2020) were very favorable for the walnut diseases attack. The correlations monthly average temperatures (*C) -- average leaf wetness hours sum (h/month) and monthly average precipitation sum (mm) -- average leaf wetness hours sum (h/month), contributed to the infections speed and diseases development on the studied biologic material. For these the coefficients $R^2=0.6086^{***}$ ($r=0.7801^{***}$, $n=35$) and $R^2=0.5584^{***}$ ($r=0.7472^{***}$, $n=35$) were calculated. Under such circumstances, the best behavior on the walnut blight had the varieties: 'Fălești', 'Geoagiu 453', 'Jupânești', 'Miroslava', 'Novaci', 'Timval', 'Valcor', 'Valrex', 'Velnița', 'Victoria' and 'Vlădești' with damages degree DD% on leaves no higher than 0,03. Also, The most resilient on anthracnose attack on leaves were the varieties: 'Argeșean', 'Gemenea 20', 'Pestișani', 'Pecianski', 'Schinoasa', (DD%=0.13), 'Valcris' (DD%=0.11), 'Codrene', 'Secular RM', 'Sibișel 265' (DD%=0.10) and 'Valrex' (DD%=0.05).

Cuvinte cheie: nuc, fază juvenilă, bacterioză, antracnoză.

Key words: nuc, juvenile phase, bacteriose, antracnose.

1. Introduction

Solemn presence in our national culture and history, the walnut (*Juglans regia* L.) is a constant presence in the Romanian landscape and national fruit growing, being appreciated for its fruits, whom kernel is consumed both fresh and processed, also for his high quality wood, and for the ecological and aesthetical attributes of the tree. It is also very important the use of walnut as source of pharmaceutical active substances. (Cociu et al., 2003; Coman, 2019; Parnia et al. 1997, Sumedrea et al., 2014). For these reasons and due the increasing demand for walnut kernel and wood, in Romania of las decades there is a real interest for the modernization of the walnut assortment, plantings, growing technologies and new phytoprotection programs. Due to the running of the program for fruit growing development the surface cultivated intensively (1,591 ha; 35,725 t nuts in shell / year, Faostat, 2018) with high valuable walnuts varieties is going to increase with more than 2010 ha, from which 1568 ha (78%) will be conducted under organic farming system (Coman, 2019).

Although the walnut is vulnerable on more than 30 species of pathogens and pests, in all countries with a strong tradition in walnut culture and in our country as well, the most damaging diseases are the walnuts blight and anthracnose.

Walnut blight, caused by the bacteria *Xanthomonas arboricola* pv. *juglandis* overwinters in buds, catkins, and diseased fruits enters in the young organs of the walnuts where causes necroses. The infected buds and catkins and young nuts fall down. The growing nuts are affected by the diseases which affect the kernel or the entire fruits which decreases their commercial value. On the leaves the attack manifest as spots surrounded by yellow tissue which evaluates in necrosis. On the infected shoots the necroses progress daily, the shoots die progressively or are improper to be used for grafting.

Anthracnosis is caused by a fungi *Gnomonia leptostyla* which overwinters trees buds and falling leaves and attack leaves shoots and fruits during the next growing seasons. On the attacked leaves polygonal spots occurs, first yellow, then turn in light brown with dark brown edges. The heavy attacked

leaves, becomes yellow or brown and fall down premature. The damaged shoots present long and sunken spots and are improper to be used for grafting. The fruits remains small deformed and fall down earlier.

During last decades, researches regarding walnut blight and anthracnose and their containment were conducted in Spain, France, Italy, Turkey, USA, China etc., and were focused towards, pathogens rapid isolation and identification, their ecology, germplasm screening for tolerant genotypes, assessment of their photosynthetic and production potential, diseases forecast and better positioning of the diseases treatments by using pathogens antagonists and new active ingredients (other than common copper and mancozeb formulations on which resistant strains were reported), long term studies on assortment improvement and on varieties resilience. (Aleta et al. 1997, 1999; Belisario et al., 1997; Buchaner et al. 2010; Dumitrescu and Botu, 2000; Gironde and Manceau, 2011; Ginibre et Prunet; 1999; Ozaktan et al., 2011; Maria, 1997; Martins, 1996; Moragrega et al., 2011, Rovira, et al., 2018).

Also, in Romania, due the climatic changes occurred in last decades, long term researches on walnut varieties and specific pest and diseases and the efforts for their containment are necessary, prior the increase of the cultivated surfaces and drive them to profitability.

2. Material and methods

The studies were carried out during 2018-2020 at RIFG Pitesti-Maracineni Romania, and has had as objectives the evaluation of some Romanian and foreign walnut cultivars on anthracnose (*Gnomonia leptostila* D.C. Trav) and walnut blight (*Xanthomonas campestris* p.v. *juglandis* (Pierce) Dye), in the juvenile phase, when the trees are the most sensitives.

The experimental device was located on an alluvial soil unit from the right shore of the Budeasa creek, an affluent of the Doamnei River. The annual average temperature (1969-2020): 10.0 °C; the absolute maximum temperature in July: 38.8 °C; the absolute minimum temperature in January: -24.0°C; the average precipitation amount (1969-2020): 678 mm / year. First autumn frost occurs at the end of October, and the latest spring frost in the second decade of the April and accidentally in the last decade of the month).

The biological material consisted in 48 varieties, 3-4 years old walnut trees among which 36 were Romanian ones (including 3 generative rootstocks) and 12 foreign varieties from which 9 were from Republic of Moldavia and 3 from USA, each planted in two replicates. The planting distances were 10 m between trees rows and 7 m among the trees, the maintenance works being the usual ones for the young walnut orchards, the water and fertilizers were provided either by microaspiration or drip fertirrigation, at a rate of 0.4 l/tree/day.

The data related to local microclimate were collected using the semi-automate weather station WatchDog (Spectrum Technologies Inc.), and were stored, processed and analyzed using the facilities of MS Office Excel 2010 and Specware Pro 9.0 module for early warning.

To assess the biological material behavior to the diseases on leaves, shoots and green fruits, an evaluation scale with six steps and intermediary steps (0 - 5 notes) was used, the area of the lesions being converted in percentage (see Table 3).

Attack frequency - F [%], attack intensity - I notes [0-7], damages degree - DD [%] were calculated every year according the following formulas:

$$F [\%] = (n / N) \times 100,$$

where F [%] = attack frequency; n=number of diseased organs; N=total number of examined organs.

$$DD\% [\%] = ((\text{sum } (f\% \times I \text{ notes}) / n) / 100)$$

where DD [%] = damages degree, f%=class attack frequency; I=attack intensity scored with 0--7 notes; n=number of attack classes.

The experimental data were stored, processed and analyzed, using MS Office Excel 2010 facilities.

3. Results and discussions

3.1. Results on the microclimatic potential on walnuts diseases development during the study period

The data collected reveal that the microclimate conditions during years of the study were very favorable for the walnut diseases attack.

Assessment of the figure 1 reveal that the monthly average temperatures ranged between 10.9-15.3°C in April up to 22.0-22.8°C in August to drop at 17.1-18.9°C in September. However, the in October 2018 the monthly average temperatures was 20.1°C which insured good conditions to the pathogens inoculum survival and to develop in the spring of 2019.

The examination of the figure 2 shows that leaf wetness played an important role for walnut blight and anthracnose development. One can see the precipitation deficit in April, but leaf wetness was ranging between 79-130 h/month in May, 168.8-188.3 h/month in June and 98.3-139.3 h/month in July which were very favorable for walnut blight exponential development and infection. In 2018 and 2020, in August and September, the leaf wetness ranged between 79.2-94.0 and 79.2-81.0 h/month, good conditions to accentuate the anthracnose symptoms. Based on processed data, many correlation coefficients were calculated, which proves that the microclimate was favorable for walnut diseases development and also for varieties evaluation (see Tables 1 and 2). Among the correlations, four were the most significant (see figures 3--6). The correlations monthly average temperatures (MEDT °C)--average leaf wetness hours sum (LWET h/month) and monthly average precipitation sum (PRECIP mm)--average leaf wetness hours sum (LWET h/month), contributed to the infections speed and diseases development on the studied biologic material. For these were calculated the coefficients $R^2=0.6086^{***}$ ($r=0.7801^{***}$, $n=35$) and $R^2=0.5584^{***}$ ($r=0.7472^{***}$, $n=35$).

This fact was proven also by the forecasting system, in the case of the walnut blight *Xanthomonas campestris* p.v. *juglandis* (Pierce) Dye, when calculates the epiphytic infection potential (EIP 0-500) and the mean infection risk (MIR 0-3).

In the figure 7 we can see, that in 2018, the infection risk 2.5 in April and May and 3.0 during the summer to drop again at 2.5 in September, which mean that also the inoculum was present for the infection on the biological material in 2019.

The figure 8 reveal that under the microclimate conditions of 2019, the walnut blight infection risk was nearly constant 3.0, from mid of May to mid of September.

In 2020, the microclimate conditions lead to a constant infection risk rated with note 3.0 in the 3rd decade of May and since June to September (See figure 9).

3.2. Results on walnuts genotypes behavior on walnut blight

Assessment of the data regarding the walnut blight damages degree occurred on young walnuts foliage (see figure 10) reveal that the most affected varieties were: 'Recea' (DD%=1,17-1.18), 'Ciumești 77' (DD%=0.83), 'Orăștie' (DD%=0,75) and 'Germisara' (DD%=0.48).

A good behavior had the intermediate and lateral bearing varieties, originated in USA, 'Hartley' (DD%=0.38) 'Chandler' (DD%=0.20) and 'Thehama' (DD%=0.05). They overpass the varieties 'Claudia' and 'Geoagiu 65', on which damages degree were DD%=0.40.

The best behavior on the walnut blight had the varieties: 'Fălești', 'Geoagiu 453', 'Jupânești', 'Miroslava', 'Novaci', 'Timval', 'Valcor', 'Valrex', 'Velnița', 'Victoria' and 'Vlădești' with damages degree DD% on leaves no higher than 0,03.

3.3 Results on walnuts genotypes behavior on anthracnose

Assessment of the data regarding the anthracnose damages degree occurred on young walnuts foliage (see figure 11), reveal that the most affected varieties were: 'Jupânești' (DD%=1.37-1.38), 'Orăștie' (DD%=1.17-1.18), 'Anica' (DD%=0.75), 'Chișinău' (DD%=0.75), 'Ciprian' (DD%=0.75), 'Valcor' (DD%=0.75) and 'Verisval' (DD%=0.48).

A good behavior had the intermediate and lateral bearing varieties, originated in USA, 'Hartley' (DD%=0.13) 'Chandler' (DD%=0.20) and 'Tehama' (DD%=0.05). They overpass the varieties 'Sarmis', 'Sibișel 50', 'Sușita', 'Velnița' and 'Victoria' (DD%=0.48), 'Sibișel Precoce' (DD%=0.40), 'Vlădești' (DD%=0.40) and 'M-44-39 Târgu Jiu' (DD%=0.32).

The best behavior on anthracnose attack on leaves had the varieties: 'Argesean', 'Gemenea 20', 'Pestișani', 'Pecianski', 'Schinoasa', (DD%=0.13), 'Valcris' (DD%=0.11), 'Codrene', 'Secular RM', 'Sibișel 265' (DD%=0.10) and 'Valrex' (DD%=0.05).

The results obtained during this study revealed the vulnerability of some walnut varieties and their different behavior to the blight and anthracnose in the juvenile phase.

For comparison, the results obtained in 1996-2009 with some Romanian and foreign walnut cultivars during their early fruit yielding period, highlighted that attack intensity I offered some information related to the plant resistance from the infections to the visible symptoms of the diseases, but at the same time, the attack frequency F (%), offered some information regarding the plant susceptibility to be infected and also about crown and orchard distribution of the diseases, the facts being noticed previously by Martins J.M.S., et all in 1995. In cold and wet springs, the blight attack on walnuts is more severe. The climatic accidents such as late frosts, storms and hails can aggravate the frequency and the intensity of the anthracnose and walnut blight attack.

In order to protect the preserved biological material for further studies and to prevent and contain the diseases infections, we aim to carry late pruning to complete the young trees canopy, to apply moderate fert-irrigation according the trees needs in juvenile phase, as well as integrated treatments including new formulations of copper and mancozeb fungicides, pathogens antagonists and innovative

products which trigger the trees defense mechanisms, all measures being correlated with the microclimate, varieties phenology, pathogens life cycle and products action mode.

4. Conclusions

During 2018-2020, the microclimate conditions during years of the study were very favorable for the walnuts diseases attack.

Based on processed data, many correlation coefficients were calculated which proves that the microclimate was favorable for walnut diseases development and also for varieties evaluation.

Attack severity of main walnut diseases is influenced by initial inoculum, infection frequency, speed and severity is driven by temperature and leaf wetness, especially in the spring and first part of the summer; this fact was proven also by the forecasting system in the case of the walnut blight.

The primary inoculum of walnut blight is available for the entire vegetation period, the amount of inoculum may increase from year to year.

Attack intensity I offered some information related to the plant resistance from the infections to the visible symptoms of the diseases, but at the same time, the attack frequency F (%), offered some information regarding the plant susceptibility to be infected and also about crown and orchard distribution of the diseases.

The best behavior on the walnut blight had the varieties: 'Făleşti', 'Geoagiu 453', 'Jupânești', 'Miroslava', 'Novaci', 'Timval', 'Valcor', 'Valrex', 'Velnița', 'Victoria' and 'Vladești' with damages degree DD% on leaves no higher than 0,03.

The best behavior on anthracnose attack on leaves had the varieties: 'Argesean', 'Gemenea 20', 'Pestișani', 'Pecianski', 'Schinoasa', (DD%=0.13), 'Valcris' (DD%=0.11), 'Codrene', 'Secular RM', 'Sibișel 265' (DD%=0.10) and 'Valrex' (DD%=0.05).

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Tables and Figures

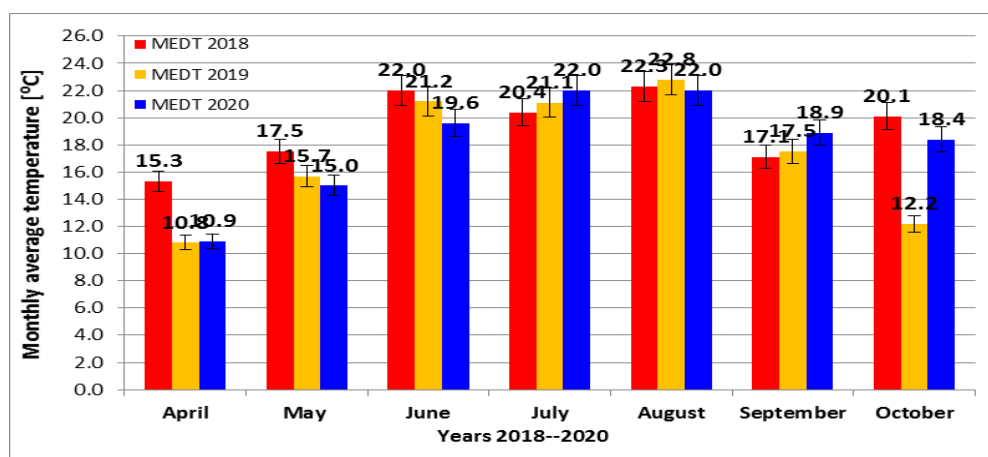


Fig. 1. Monthly average temperatures which favoured walnuts diseases attack RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m

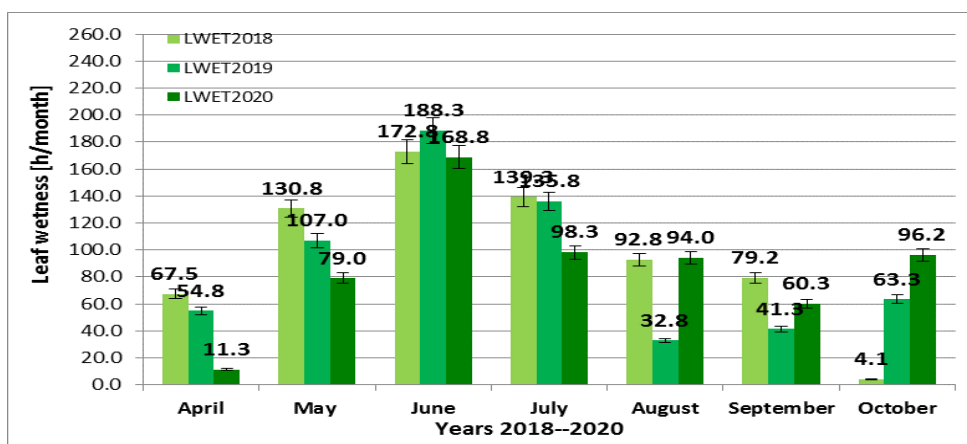


Fig. 2. Monthly leaf wetness hours which favored walnuts diseases attack RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m

Table 1. Correlation coefficients R^2 obtained between several microclimate parameters RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m 2019-2020

R2	MAXT [°C]	MEDT [°C]	PRECIP [mm]	LWET [h]	WIND [km/]	GUST[km/h]
MAXT [°C]	x	0.5829***	0.0450	0.3515***	0.0072	0.0017
MEDT [°C]	0.5829***	x	0.1890**	0.6086***	0.0017	0.0087
PRECIP [mm]	0.0450	0.1890**	x	0.5584***	0.0000	0.0005
LWET [h]	0.3515**	0.6086***	0.5584***	x	0.0025	0.0042
WIND [km/]	0.0073	0.0000	0.0000	0.0025	x	0.4916***
GUST[km/h]	0.0017	0.0087	0.0005	0.0420	0.4916***	x

Table 2. Correlation coefficients r obtained between several microclimate parameters RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m 2019-2020

r	MAXT [°C]	MEDT [°C]	PRECIP [mm]	LWET [h]	WIND2 [km/]	GUST[km/h]
MAXT [°C]	x	0.7634***	0.2121	0.5928***	0.0848	0.0412
MEDT [°C]	0.7634***	x	0.4347**	0.7801***	0.1304	0.0932
PRECIP [mm]	0.2121	0.4347**	x	0.7472***	0.0000	0.0223
LWET [h]	0.5929***	0.7801***	0.7472***	x	0.0500	0.0648
WIND2 [km/]	0.2701	0.0000	0.0000	0.0500	x	0.7011***
GUST[km/h]	0.1303	0.1303	0.0223	0.0648	0.7011***	x

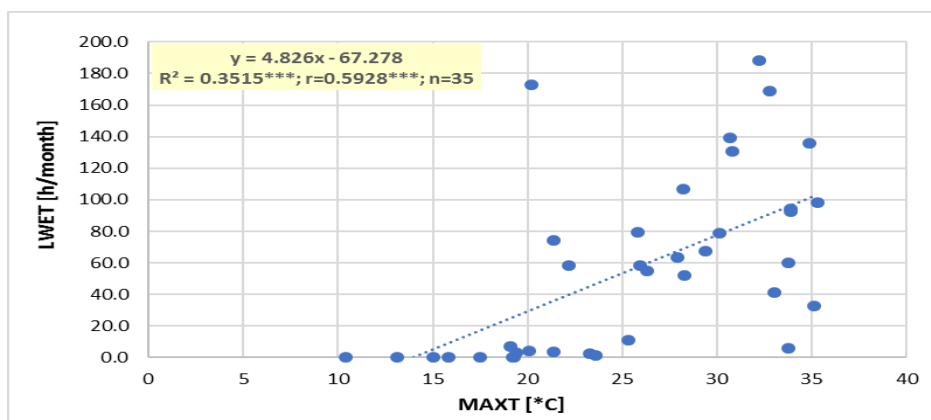


Fig. 3. Correlation between monthly average of maximum temperature and leaf hour's sum per month, RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m, 2018-2020

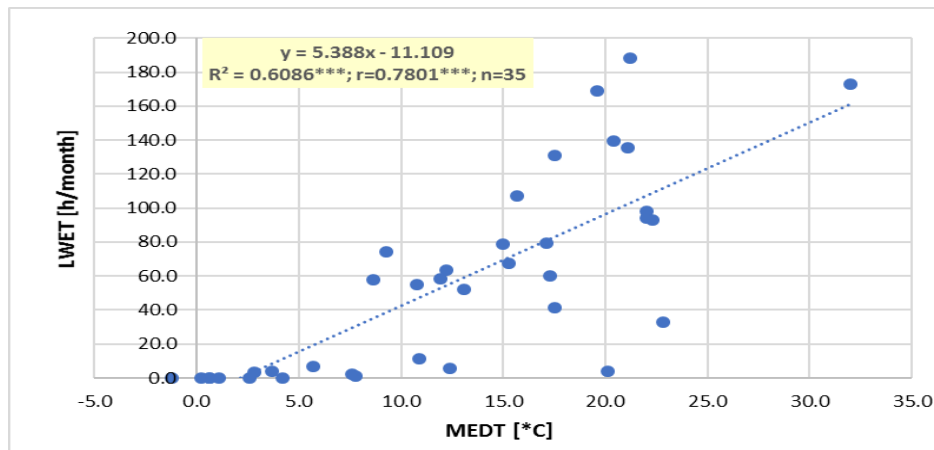


Fig. 4. Correlation between monthly average temperature and leaf hours sum per month, RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m, 2018-2020

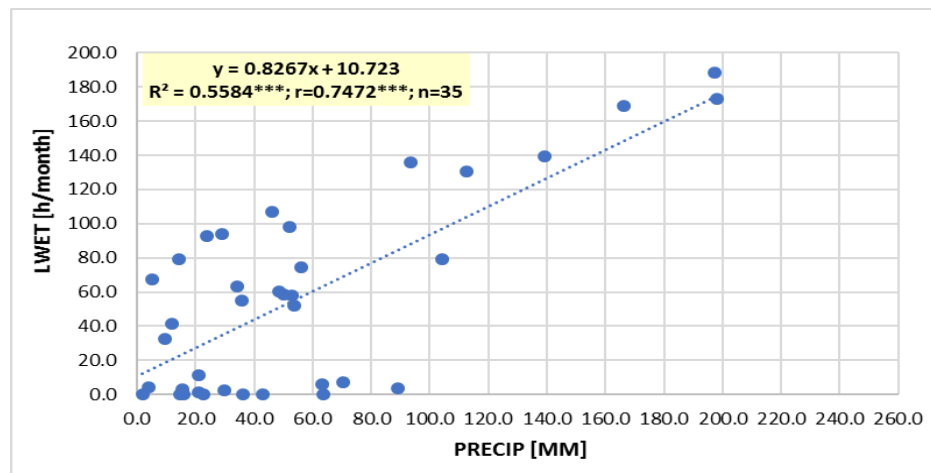


Fig. 5. Correlation between monthly average precipitations and leaf hours sum per month, RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m, 2018-2020

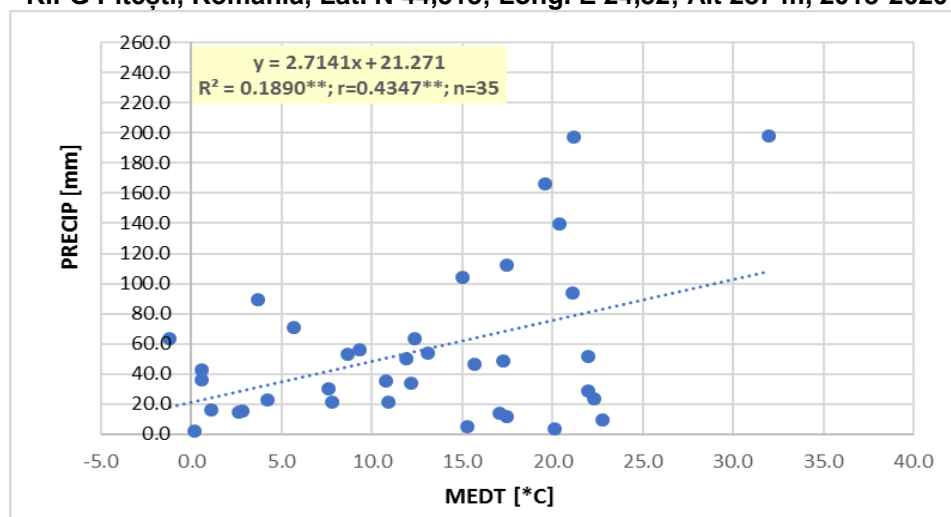


Fig. 6. Correlation between monthly average temperature and average precipitations per month, RIFG Pitești, Romania, Lat. N 44,513; Long. E 24,52; Alt 287 m, 2018-2020

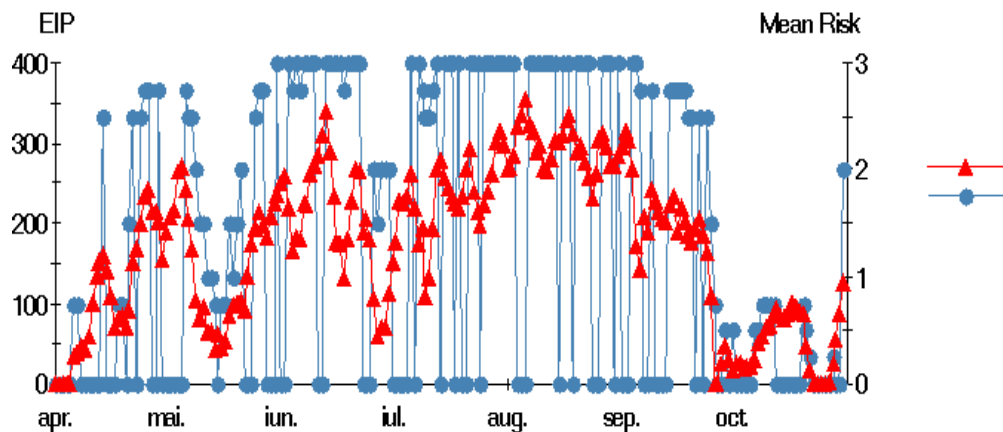


Fig. 7. Epifitic infection potential (red) and mean attack risk (blue) of walnut blight - *Xanthomonas arboricola* pv. *juglandis* RIFG Pitești, Romania, 2018 Lat. N 44,513; Long. E 24,52; Alt 287 m

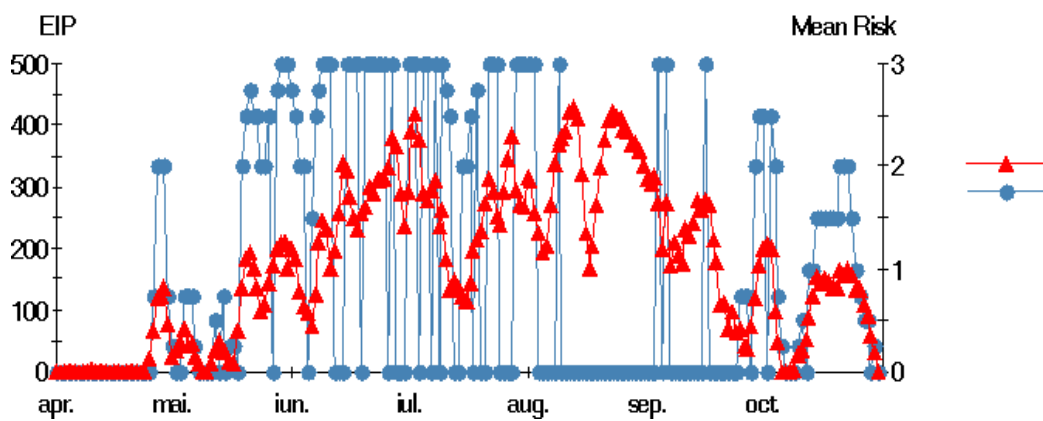


Fig. 8. Epifitic infection potential (red) and mean attack risk (blue) of walnut blight - *Xanthomonas arboricola* pv. *juglandis* RIFG Pitești, Romania, 2019 Lat. N 44,513; Long. E 24,52; Alt 287 m

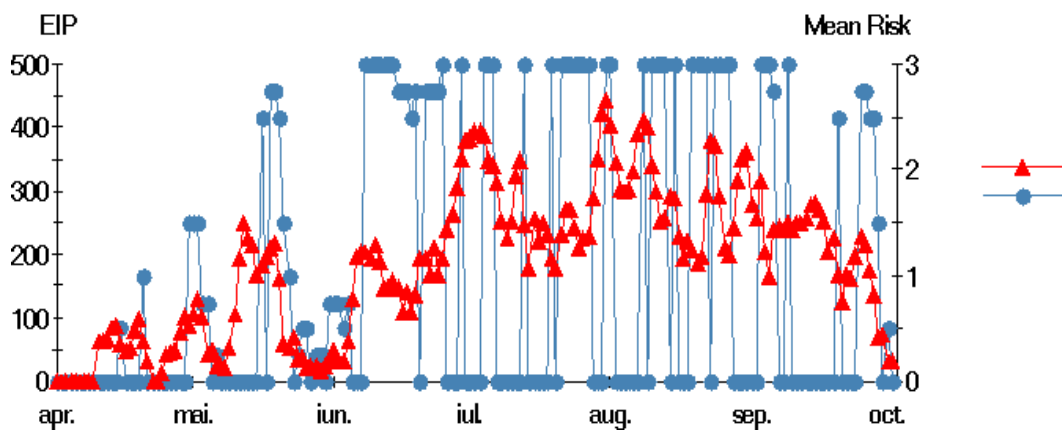


Fig. 9. Epifitic infection potential (red) and mean attack risk of walnut blight (blue) of walnut blight - *Xanthomonas arboricola* pv. *juglandis* RIFG Pitești, Romania, 2020 Lat. N 44,513; Long. E 24,52; Alt 287m

Table 3. The assessment scale for diseases attack on walnuts cultivars

Attack intensity in the orchard [notes]	Necrosis area attack frequency F [%]	Observations
0.0	0	1. The value of the attack frequency F [%] on leaves was obtained by direct observations on 100-200 leaflets followed by indicator calculation according the formula: $F(\%) = (n \times 100)/N$ where, n = number of attacked leaflets; N = total number of observed leaflets. 2. On walnuts is very difficult to compare the spots numbers on leaflets of different size. In the case of spots confluence it was took into consideration the necrosis area. The same work was done for the green fruits
0.5	2	
1.0	3	
1.5	6	
2.0	10	
2.5	18	
3.0	26	
3.5	38	
4.0	50	
4.5	64	
5.0	75	x

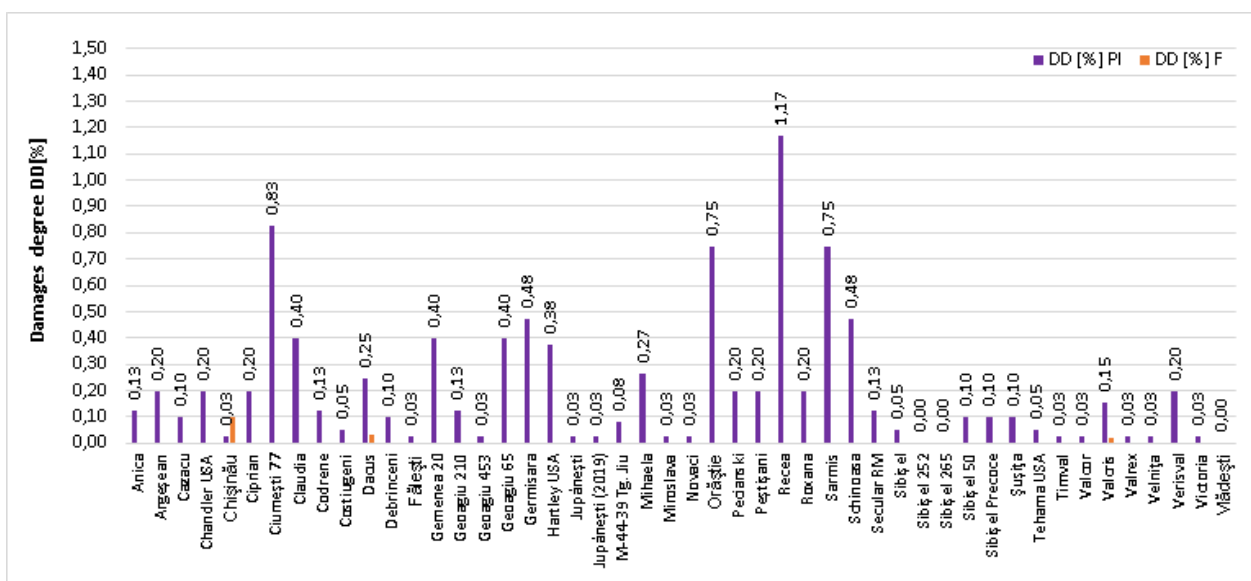


Fig. 10. Behavior of studied varieties on walnut blight *Xanthomonas arboricola* pv. *juglandis* RIFG Pitești, Romania, 2019-2020 Lat. N 44,513; Long. E 24,52; Alt 287m

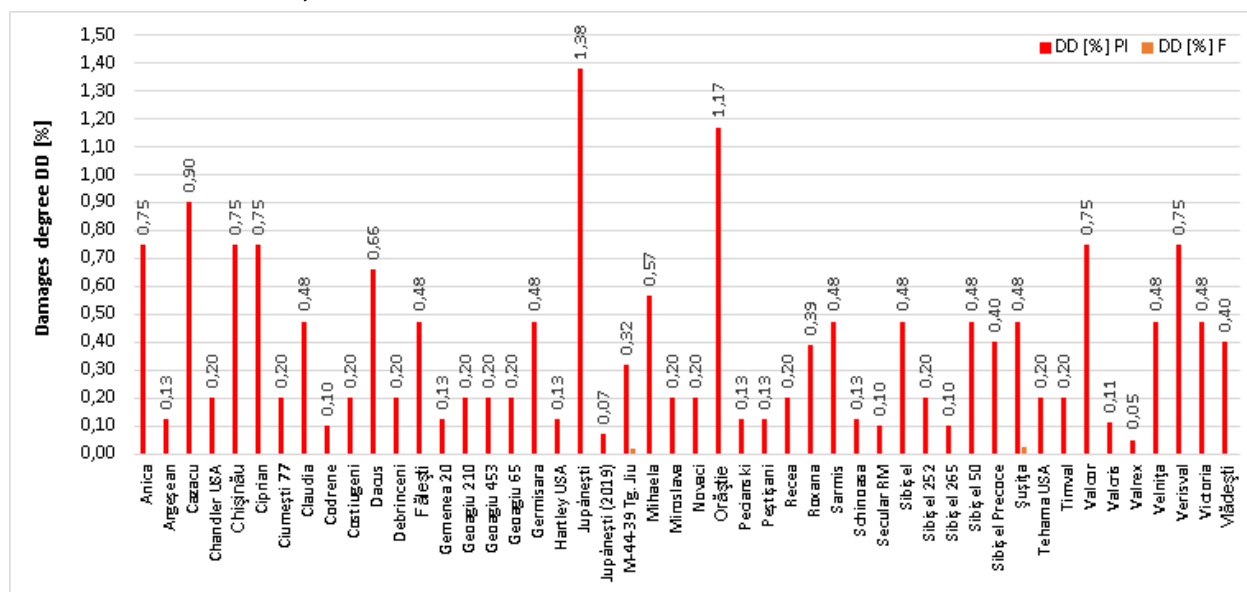


Fig. 11. Behavior of studied varieties on walnut blight *Gnomonia leptostyla* D.C. Trav RIFG Pitești, Romania, 2019-2020 Lat. N 44,513; Long. E 24,52; Alt 287m



Fig. 12. Young walnut from 'Recea' variety affected on top by *Xanthomonas arboricola* pv. *juglandis*, RIFG Pitești, Romania, 2019-2020, Lat. N 44,513; Long. E 24,52; Alt 287m.



Fig. 13. Growing shoots severe affected by *Xanthomonas arboricola* pv. *juglandis*, RIFG Pitești, Romania, 2020, Lat. N 44,513; Long. E 24,52; Alt 287m.



Fig. 14. Growing fruits affected by *Xanthomonas arboricola* pv. *juglandis*, RIFG Pitești, Romania, 2020, Lat. N 44,513; Long. E 24,52; Alt 287m.



Fig. 15. Leaves affected by *Gnomonia leptostyla* D.C. Trav, RIFG Pitești, Romania, 2020, Lat. N 44,513; Long. E 24,52; Alt 287m.