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Grapevine powdery mildew (Uncinula necator (Schw.) Burr.) – a permanent issue concerning the health status of grapes cenosis in Bulgaria

Mariana B. Nakova[1], Boris K. Nakov[1], and Miroslav Tityanov[2]

[1] Department of phytopathology, Agriculture University Plovdiv, Plovdiv, Bulgaria
[2] SAB Sumi-Agro, Bulgaria

Abstract. Grapes powdery mildew appeared permanently in the grapes growing regions of Bulgaria. The disease was reported in our country by Kostov in 1900. More than a century the powdery mildew disease has been causing less or greater yield losses depending on the climate. In the past when the vineyards were small and scattered, the disease was not an economic problem. Later, during the second half of the 20th century, the grapes growing areas consolidated and enlarged, and the concentration and intensification of production took place. The pathogen spread permanently to epidemic in the vineyards and the disease became destructive and economically important. During that period commercial varieties were grown susceptible to the causal agent of the powdery mildew, and organic fungicides were applied in the disease control system. Mistakes in the grapes growing technology are observed and the disease management strategies applied are not always scientifically proved. The statements that in Bulgaria there exist conditions suitable for the appearance and development of grapes powdery mildew only in the Black sea region are disproved. A new research is necessary to be done to answer the questions about: the sources of primary inoculum, the influence of the ecological conditions on the appearance and spread of the powdery mildew, and the timing for the disease control. In the survey the overwintering of the pathogen, the appearance of the first symptoms and the dynamics of the disease spread have been discussed. As for the ecological conditions in Bulgaria, it is considered that the fungus mainly survives as mycelia in the buds and on the shoots of the vines serving as a source of a permanent infection background. The studies carried through during 1994–2002 proved that the pathogen influenced by the ecological conditions could also form cleistothecia and they could be the source of the initial infection. The effect of the leaves removal around the clusters on the spread of the grapes powdery mildew and the field resistance of some varieties were studied. Critical stages of the disease appearance and spread are defined based on the research data. Management strategies are proposed including alternative approach practices.

The grapes powdery mildew permanently appears in the grapes cenosis in Bulgaria. More than a century it has been causing less or greater yield losses. The disease was reported by Kostov in 1900. In the past, when the vineyards were small and scattered, the disease was not an economic problem. But after the consolidation of the areas, the concentration and intensification of the production during the second half of the 20th century, the development of the disease became permanent and, not rarely, epiphytotic and economically important. The problem is due to the growing of vine varieties sensitive to the pathogen causing the disease, to the introduction of organic fungicides in the production, to the mistakes done in the technology applied and to methods for control not always scientifically based.

In Bulgaria the studies of the biological characteristics, the epidemiology, the prediction and the control are limited and not unidirectional. Debatable are the questions concerning the overwintering and the source of the primary inoculum; the influence of the ecological conditions, as well as the development dynamics and the prediction of the powdery mildew; and questions concerning the moments and methods of control.

The history data show that the powdery mildew is the oldest grapevine disease known to science and practice. It comes from North America where the disease has always existed [1], but the grown grapevine varieties were comparatively resistant, with low yield losses, so the disease did not attract the attention of the specialists.

As far as Europe is concerned the fungus was first brought to England in 1845. In 1847 Tucker and Berkeley report independently a new cryptogamic parasite on grapes. Berkeley identifies it as *Oidium tuckeri.*

Leclerc (1853) [2] reports the oidium to be noticed in France in 1847 and only for two years it had managed to spread throughout the country. In 1850 the vineyards around Paris were destructed, i.e. the fungus caused the first epiphytothic after being transferred to Europe.

In Italy the disease was found in 1849 [3]. During 1851–1852 the powdery mildew spread to devastating proportions in several European and Mediterranean countries. Within 1853–1856 the disease caused serious economic problems: eradicated vineyards; selling-off lands; resettlement of the poorest people; raising of the wine prices; 2 to 3 times crop reduction in comparison with the good years [4].

Thus, by the end of the 19th century the grapevine powdery mildew and its causal agent had become a worldwide issue. The outbreak of the epidemic drew the scientists' attention resulting in an intensive research work on the symptoms, the biological features and the measures for control.

Viala (1853, 1877) [5] is the first to state that the earliest symptoms can be found on the vines during bud break and later the symptoms spread on the whole surface of the new shoots. This he calls "flag shoots". According to him the fungus remains as a micelium in the dormant buds under their scale. The infection happens in the autumn. Appel (1903) [6] confirms Viala's thesis. Amici (1852) [7] and Mohl (1853) [8] report damages caused on the inflorescences. Arnaud (1931) [1], Rendu (1853) [9] and Viala (1887) [10] add some information about infections and serious symptoms on the grape berries up to the berry touch period. Pearson and Gartel (1985) [11] find out that the buds become infected mainly from the 3rd to the 5th internodes.

Jossifovitch (1923) [12], Frezal (1953) [13] and Molnar (1914) [14] also state that in mild winters the micelium stays alive and restores during the following spring. Arnaud (1931) [1] rejects that possibility. Viala (1893) [10] finds the micelium on "the newly opened buds". Wortmann (1900) [15] and Ravaz (1921) [16] confirm the statement of "the presence of the micelium" in the buds and the fact that out of the infected buds come shoots "fully covered with the micelium and the spores of the fungus".

Boublas (1961) [17] finds out that the buds having micelium are located from the 3rd to the 6th node. Lafon (1967) [18], Bulit and Lafon (1978) [19], Sall and Wrysinski (1982) [20] also observe that "the early symptoms of the oidium appear from the infected buds". Pearson and Gartel (1985) [11] report that "the disease happens in large scale from the infected buds during April and May". Rumbolz et al. (2002) [21], Rugner et al. (2002) [22], Ypema and Gubler (2000) [23] also state the same data of the fungus overwintering as a micelium. The tempetatures and the humidity in June favour the mass spread of the oidium.

The slight curl on the periphery of the leaf blades is the first direct symptom of the powdery mildew appearance followed by a rapid invasion of the micelium and the abundant spore formation. The late infections establish conditions for inoculations and the survival of the parasite till the next vegetation season.

There are discussions on the role of the teleomorph in the life cycle of the powdery mildew causal agent. In Europe the teleomorph is reported by Couderc (1893) [24]. Cleistothecia are observed in large numbers in September-October 1893, after a dry and hot summer followed by the drop in temperatures to 4°–8°C [25]. Pacottet (1903) [26] also assumes the preservation of the pathogen via teleomorph. According to Foex (1911) [27] the run out of the nutrient substances is a very important factor for the cleistothecia formation. Laibach (1930) [28] states the role of the "worsened physiological conditions of the host plants, and especially the lack of water to stimulate the teleomorph formation". Jossifovitch (1923) [12] proves that the cleistothecia put on dry leaves or wet sand, during October-February, can survive till March. Nedeltscheff (1924) [29] and Geoffrion (1960) [30] also support the thesis that dry weather favours the formation of the cleistothecia. Schnathorst (1965) [31] reveals the correlation between the environmental conditions and the development of the teleomorph. According to Gadoury and Pearson (1988, 1988) [32,33] the ascospores are discharged during the stages of the bud break and flowering, at 4°–32°C and rain – 2.5 mm. The first spots can be found during the last week of May, when shoots are 7–15 cm high. In certain regions of California [23] the fungus is stated to survive as micelia and cleistothecia. In South Africa the sources of the initial infection are the micelium in the infected buds and the cleistothecia [34]. Gee et al. (2000) [35] conclude that the cleistothecia are the source of the additional inoculum in Australia. Miazzi et al. (2003, 2008) [36,37] announce the existence of A and B genetic groups in South Italy developed in separate agro-cenosis during the vegetation period. They correlate this to the cleistothecia formation. Gadoury et al. (2012) [38] survey the spread of these two groups in the USA and think they appear to be the parental forms of those groups registered in Italy and introduced separately in Europe. A certain additional research should be carried out in order to define the mechanism of the appearance of both groups.

The status of the grapevine powdery mildew issue in Bulgaria

The scientific researches on the biological features of the oidium causal agent in our literature are not enough. The first report is made by Nedeltscheff (1923/24) [39]. The author establishes that cleistothecia are formed comparatively rarely and they do not play an essential role in the life cycle of the the fungus. Atanasov (1934) [40] explains that the question of the overwintering is debatable: cleistothecia or dormant mycelia on the shoots. Hristov (1956) [41] states that the causal agent overwinters with cleistothecia ripening after the first or the second overwintering. According to him the large scale appearance of the disease in Europe can be explained with the formation of the dormant mycelium on the annual shoots in autumn but not with the cleistothecia. That micelium overwinters and causes the disease during the next spring. It is also accepted that there are favourable conditions for the development of the powdery mildew only along the Black sea cost line and in the regions along the Danube River [42]. Perhaps the authors are not aware of the surveys carried out in the end of the 19th and the beginning of the 20th centuries on the overwintering of the fungus in the buds and the early development of the disease [10,11,15,16].

Contradictory are the data in some Bulgarian articles on the proper period of the first spraying. Martinov (1938, 1940) [43,44] suggests three sprayings with sulphur, at 20°C, after the end of the flowering. According to Nedeltscheff and Kondarev (1949) [45] the first treatment with sulphur should be done after the fruit set. The next applications has to be done every other 15 days, if it is necessary. The authors support their thesis explaining the fact that "in Bulgaria the oidium does not appear before the fruit set. Thus the early treatments are useless". Raykov and Nenov (1959) [42] are the first who give in 1953 some data about the damages of the disease in the Black Sea region reaching up to 50% of the yield, or even "the full devastation of the grapes crops". The authors advise how to

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limit the losses: the first sulphur application should be done in the end of the flowering stage, just at the very beginning of the disease development. “The end of the flowering” means the moment when the last caps fall.

All these instructions how to control the oidium are based on the real observations on the first symptoms of the disease, without considering the studies on the biological features of the pathogen described in the foreign literature, i.e. the early development of the pathogen causing infections with the very beginning of the buds break. The first latent period lasts about 3–4 weeks coinciding with the stages of forming the inflorescence and flowering when the first symptoms are discovered.

After 1960 comes a period of introducing the organic fungicides replacing the traditional copper and sulphur active ingredients. The scientific circles and the practices do not consider the fact that the zineb fungicides show an effect to the downy mildew (*Plasmopara viticola*), and at the same time they strongly stimulate the development of the powdery mildew (*Uncinula necator*). The very first experiments in Bulgaria during 1958–1960, show that with the zineb treated variants, symptoms of powdery mildew are registered on 80% of the leaves and the clusters. While with the copper and sulphur treated variants the registered spots are few [46]. The oidium starts its development in large scales permanently. During 1965–1970 the disease becomes epidemic in some villages in the region of Plovdiv (the villages of Markovo, Belashtitsa, Brestnik), as well in the towns of Nessebar, Pomorie and the entire region of Bourgas (Nakov, not published data). Iliev (1969) [47] writes about great damages of the powdery mildew in the region of Bourgas.

The carried out investigations of the vineyards, greatly affected by the powdery mildew, have established the fact that the first centers of infection appear from the “flag shoots” developed from the infected buds. The microscopic analysis of the infected buds shows that there are micelia and spores right before the buds break, just under the scales. After being cut and coloured with cotton-blue dye the bud tissues show the micelium hyphae and the chains of conidia at 6–8°C. The surveys done in the region of the town of Nessebar show that the infected shoots develop from inoculated buds [48]. The author assumes that spores might be transferred from the southern regions, i.e. from Turkey. According to him the cleistothecia are formed but they can not ripen. The observations and surveys confirm the information in the literature that the infected bud is the source of the initial inoculums. Thus, it is necessary for early treatments to be done, just after the bud break, when the shoots are 0.5 – 2 – 3 cm long.

After 1970–1980 the systemic (therapeutical) fungicides become popular in the plant protection. Their ability to penetrate into the plants’ tissues; to go through the organism and kill the pathogen is considered to be a revolutionary period in science [49]. When the treatment is done in the stage of the bud break and the the first leaves unfolded, the systematic fungicides penetrate inside and kill the micelium. The late teatments omit the earliest infections which lead to sporulation and spread of the pathogen.

The authors of this article present the results of their surveys on the influence of certain epidemiological factors on the appearance and development of the teleomorph of the pathogen. The data show the fact that during the last decade of the 20th century there appear some natural phenomena, such as: extremely high temperatures, dry and hot weather during the vegetation season, provoking stress situations and disturbance not only in the host plant physiology but in the fungus life cycle. The damages of the powdery mildew increase. The existent complicated situation requires constant observations on the disease symptoms, the sources of the initial infection and the critical moments furthering its intensive development. The meteorological data are received from the automised system EnviroCaster (Neogen corporation, Michigan, USA), having an installed module forecasting the powdery mildew. The disease development dynamics is registered with the variety Brestovitsa vines, not being treated with fungicides. Every three days they are monitored for the appearance and development of the disease symptoms on the leaves, the shoots and the grapes. The growth of the micelium on the newly formed leaf spots is measured, in mm, every day. Periodically the spots on the shoots, from the 1st to the 8th nodes, are reported in numbers, while the spots on the berries are reported in percentage. Observations of the cleistothecia formation on the infected parts are done regularly. The forming of the ascospores and their ripening is monitored in the experimental field of the Agriculture University Plovdiv and in the laboratory [50].

The development of the powdery mildew is examined with the following varieties too: Bolgar, Super early Bolgar, Rousalka, Hamburgski Misket, Cardinal, Aligote, Dimyat, Cabernet Sauvignon, Mavrud during 1994–1997 on the experimental field of the Agriculture University Plovdiv. These varieties were treated with fungicides and leaf removal around the clusters (under, above, and in contact with cluster), in the stages “end of the flowering to fruit set”.

During 1994–1995 vines in the village of Brestnik (the University experimental field) were observed for the cleistothecia formation. During the end of July and the beginning of August the damages of the powdery mildew on the leaves with the Brestovitsa variety reached 63.92%, while the damages on the fruits were 47.32%. The mass formation of the cleistothecia on the leaves, the leaf stalks and the shoots is registered during the second half of September and the beginning of October. This period (1994–1995) had dry and hot summer, with extremely high temperatures reaching 38.6°C, while is certain periods up to 39–41°C. In the afternoons, for 3-4 hours, the relative humidity dropped to 29–33%. There is observed a depression in the plant development; the leaves with the oidium spots grow older fast, and perhaps they cannot serve as an adequate pathogen nurishing source. That makes the pathogen start the developement of the teleomorph. This process is stimulated by the sudden dramatic temperature drop to 8°–12°C. Similar are the characteristics of years 1996, 1997, 1999, 2001, 2002 [50]. During 1994–1995 the development of the cleistothecia is studied in the laboratory – at 4°–8°C, with and without moistering leaves, with cleistothecia, on steriled sand. During 2002 and 2003 the experiments are carried outdoor, on the experimental field of the Agriculture University Plovdiv – the leaves with cleistothecia were placed on the soil surface covered with 1–2 cm sand. With both experiments the development of the teleomorph, the formation of the asci and ascospores, happens during the last decade of October and in November. Ascospores were formed in the end

Figure 1. Powdery mildew symptoms – "flag shoots".

of November and the beginning of December. Part of the ascospores put in a wet chamber germinated. The ripened ascospores preserve their fertility, at the variable temperatures (2–8°C) and humidity, till the beginning of the vine vegetation, i.e. till the end of March and during April (1995, 2003). During the inoculation of the young vines with ripened cleistothecia (in pots and on leaves) in a wet chamber, at 26°C and high relative humidity, above 76%, symptoms appear after 6–7 days (May 1995; May 2003). The received data coincide with the data present by Viala (1893) [10], Pacottet (1903) [26], Yossifovitch (1923) [12], Pearson and Gadoury (1987) [51], Geoffrion (1960) [30], Foex (1911) [27], Gee et al., (2000) [35], i.e. cleistothecia can be formed in different regions under unfavourable meteorological conditions and they might be a source of an additional infection on the grapevines cenosis and serve as an initial infection background.

Surveys on the prediction of the powdery mildew development were carried out during 1993–1999 with the help of the EnviroCaster system (Neogen corporation, Michgan, USA). The results show that in the grapevine cenosis there exists a permanent initial infection background. The most strongly infected are the buds from the 3rd to the 6th node, but the infection can also be seen on the 8th node. The flag shoots growing out of the infected buds are covered with mycelia and conidia. At the end of May there appear shoots with smaller young leaves looking distorted and slightly curled up (Fig. 1). On the upper leaf surface light green asymmetrical small spots can be found. These spots resemble "oil spots" and are covered spares mycelia and spores (Fig. 2).

The data coincide with the publications of Gadoury et al. (2012) [38], Halleen and Holz (2000) [34] developing the hypothesis that these are spots caused by the ascospores of the pathogen. The studies on the oidium in Bulgaria show that these symptoms can be seen in the years, when during the last vegetation period, the fungus cleistothecia were massively formed.

During the research work the registered relative humidity is in the following intervals:

April – 45–70–82%;
May – 48–50–85%;
June – 42–50–70–85%;
July – 55–60–70–80%;

Figure 2. Powdery mildew symptoms – ascospore infections ("oil spots").

Table 1. 'Critical periods' in the powdery mildew development.

Temperatures, °C from ….. to……	Relative humidity, %	Leaf wetness period, in hours
9.2–12.4	62–96	21–27
14.0–15.2	60 –95	11–16
16.0–19.9	61–94	8–10
22.5–26.5	60–86	5–6
26.9–29.1	76–92	9–11

Table 2. Development of the incubation periods, in days.

Temperatures, °C from .... to	Duration of the incubation period, in days
9.8–10.7	24–26
1.2–12.9	18–19
13.5–15.1	14–16
19.8–20.6	8 - 9
26.9–28.1	5–6

August – 40–45–70–85%.

During the hot daily hours in the second half of July and in August the humidity drops down to 29%–30%, for one to three hours, at 39°–41°C. The relative humidity data compared to the data published by other authors show that in the grapevine cenosis such humidity favours the powdery mildew development during the vegetation period.

Based on the information about the influence of the temperature, the relative humidity and the leaf wetness period /in hours/ on the pathogen development, several periods of risk (critical periods) are defined. The critical stages could be used to predict the powdery mildew appearance and spread (Tables 1, 2).

During the years 1994–1997 a lot of reseach work was done concerning the effect of the leaf removal around the cluster (under, above and in contact) on the powdery mildew development (Table 3). With the Brestovitsa variety showing the highest sensitivity towards the powdery mildew causal agent, the difference between the treatments with the leaf removal and without the leaf removal is from 10.5 to 20%. With the other varieties, having 'green operations' and sprayings with fungicides applied, the differences in the the powdery mildew development on the clusters are: from 5.5 to 5.67% (variety Bolgar); from 13.4 to 21% (variety Dimyat), growth stage

Table 3. The influence of the partial leaf removal around the cluster on the development of *Uncinula necator* with the different varieties in the region of Plovdiv (average data) (1996–1997).

Variety	Disease severity on berries - % of infected berries, growth stages			
	"berries pea-sized", bunches hang		"beginning of berry touch"	
	Without leaf removal	With leaf removal	Without leaf removal	With leaf removal
Brestovitsa	2.32	0.32	77.8–95.3	67.3–75.3
Bolgar	2.36	0.21	17.2–19.1	11.53–13.60
Dimyat	1.80	0.0	22.38–29.99	8.98–8.98
Cabernet Sauvignon	2.43	0.0	1.80–2.29	0.32–1.05
Mavrud	1.10	0.0	2.32-2.32	0.0–1.02
Кардинал	3.42	1.32	2.76–2.76	0.37–0.64
Super early Bolgar	2.78	0.014	4.33–6.49	2.64–3.54
Rousalka	1.40	0.0	1.09–2.07	0.31–0.62
Hamburgski Misket	1.39	0.0	3.26–3.26	0.72–0.72
Aligote	0.5	0.0	0.12–0.55	0.0

beginning of the berry ripening. This tendency is the same with the rest of the varieties.

Conclusion

The conclusions based on the research work on certain biological features of the powdery mildew causal agent (*Uncinula necator*) are as follows:

- The pathogen overwinters mainly as a micelium in the buds, from the 3rd to the 6th node, causing the first early infections on the bud break and the later development of the systemic infection 'flag shoots'.
- Cleistothecia are formed under the influence of certain conditions such as: dry weather and extremely high temperatures in July and August followed by a dramatic drop of temperatures in September–October. Asci and ascospores are formed at the end of November and in December. Part of the ascospores stay alive till the beginning of the next vines vegetation period. The ascospores can cause later infections and serve as an infection background of the agrocenosis.
- Critical periods (periods with high infection risk) during the powdery mildew development are defined, which could be used for powdery mildew prediction and signaling.
- The effect of the leaf removal practices, around the grapes clusters, on the powdery mildew development is studied.

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