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Light microscopy of apple powdery mildew (*Podosphaera leucotricha*) and influence of climatic conditions on primary infections in nursery and orchard

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Abstract Apple powdery mildew (APM) is a very destructive pathogen, it produces damages in fruit orchards but also severe attacks are recorded in nursery also when climatic conditions ar favorable for the development of the disease. Apple powdery mildew infects young tissues in the plant, starting from buds,blossoms leaves until twigs and even branches causing specific symptoms. The environmental conditions have a major role in the disease epidemics but also the biological reserve, susceptibility of cultivars. In the present paper we investigated the microscopy of the early spring conidial infection and the meteorological factors affecting some susceptible cultivars. Results showed that the environmental conditions, the rapid increasing of temperatures in early spring favored the development of the fungi. The climate changes observed in recent years during winter, the warmer spring temperatures which occurred in the present year 2016 favored the appearance of the disease. The asexual conidia measures 2,5-2,6 µm in length, 1,4 µm in width , surface area 3,16 μm^2. The shape of the conidia in most cases is oval (Figs.8-9), sometimes rounded, thus revealed the miscoscopy images. A medium number of conidia were present in microscopy slides, in early disease development we observed 450-500 conidia.

Key words

apple powdery mildew, infection, environment, conidia

Apple powdery mildew (APM) is a very destructive pathogen, it produces damages in fruit orchards but also severe attacks are recorded in nursery also. Powdery mildew infects young tissues in the plant, starting from buds,blossoms leaves until twigs and even branches causing specific symptoms. The environmental conditions have a major role in the disease epidemics but also the biological reserve, susceptibility of cultivars. Several researchers studied the fungus *Podosphaera leucotricha*, [1,4,5,7] in their researches showed that infections occure in overwintering buds during the vegetation period, primary infections causing secondary infections which than form a white mycelium which covers the annual shoots, blossoms, leaves. Conidia arises on specialized organs called conidiophores, conidia are asexual spores which produce the primary infections which are the most dangerous. The leaves develop lesions after the white mycelium spreads on the entire leaf on the upper side, they are curling and folding and finaly turn into dark brown and die. The vigor of tree in the orchard or in the nursery can be affected in a high degree. The aim of the study was to elucidate the APM conidia micromorphology, shape and principal geometrical descriptors, density of the APM conidia resulting from leaves from orchard and from nursery.

Materials and Methods

Experiences were effectuated in open field orchard and nursery at Fruit Research and Development Station Bistrita in spring 2016 in both variants using Jonathan cultivar sensible to apple powdery mildew infection. Infected leaves with mycelia and conidia were transported in sterile plastic bags to Babes-Bolyai University from Cluj / Electron Microscopy Center to investigate conidial characteristics and micromorphology of the pathogen agent. The investigation was effectuated with an Olympus BX 51 light microscope. The mean daily temperature, relative air humidity, cumulative rainfall was recorded at the meteorological station of FRDS Bistrita during december-march 2016 with focus on the primary climatic conditions of march 2016. There were used conidia traps from microscopy glass slides greased with white vaseline, put on metal holders in order to count the infection progress.Conidia were counted and the slides were investigated concerning the micromorphology.

Results and Discussions

Results showed that the environmental conditions, the rapid increasing of temperatures in early spring favored the development of the fungi. The climate changes observed in recent years, the warmer spring temperatures which occurred in the present year 2016 favored the appearance of the disease first in the orchard (Fig.1, Fig.2).

Fig.1 Sporulating white mycelium of apple powdery mildew on the attacked leaf

Fig.2 Infected blossoms diseased by apple powdery mildew

Grafted experimental trees (Fig.3) had apple powdery mildew symptoms early in this spring 2016, showing that the fluctuating weather conditions favored the apearance of the disease however for short period, because temperatures droped again at the end of march and in the symptomatology of APM there have been observed a certain withdrawal.

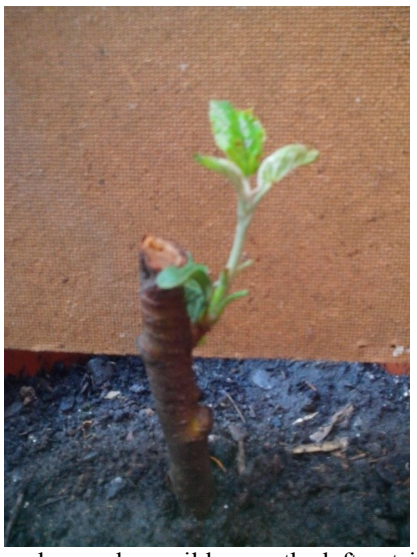

Fig.3 White mycelium of apple powdery mildew on the left outside curling leaves in grafted tree

Nuseries which are close in distance to infected orchards through wind dispersal could be affected by apple powdery mildew. Phenological observations in this spring showed the rapid expansion and unfolding of the phenological stages, the warm spring temperature in february before mouse ear stage favored the rapid apearance of the conidia and the white mycelium. New green leaves are the most susceptible to the disease, the disease attacked very early the unfolded developing fresh new leaves. The relatively moderate mild winter conditions in december 2015-february 2016 does not reduced in the orchard the biological reserve of the fungi, thus the mycelium, so the reducing of the infection source does not occurred.

The infection attack on diseased leafs were high, in the optical light microscopy we suceeded to find several conidiophores, specialized stalks which produce new emerging conidia (Figs 4-9).

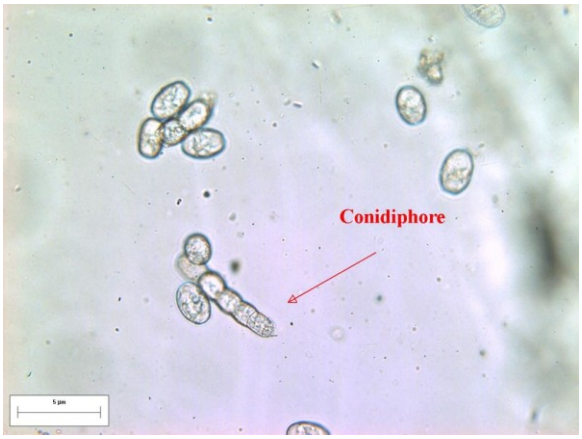

Fig. 4 Conidiophore and emerging asexual conidia
(scale bar represents 5µm, magnification 40x)

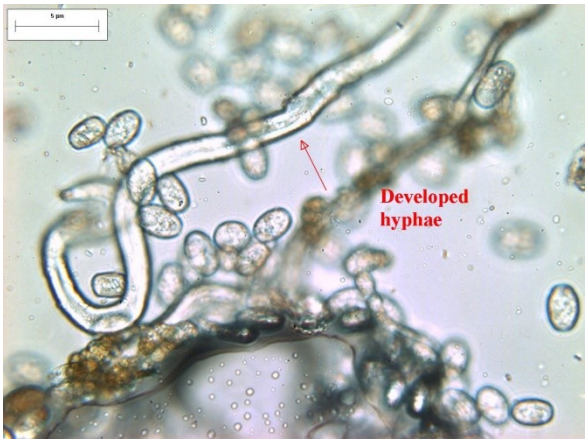

Fig. 5 Developed hyphae prepared to attack leaf tissue
(scale bar represents 5µm, magnification 40x)

Fig 6 Apple powdery mildew conidia, hyaline
and containing fibrosin inclusions
(scale bar represents 5µm, magnification 40x)

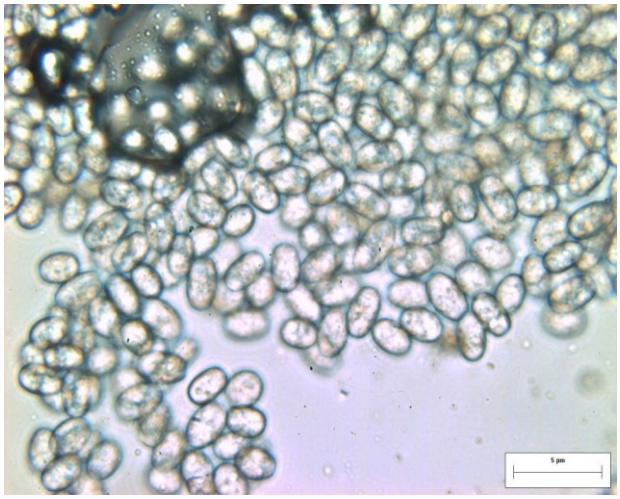

Fig 7 Apple powdery mildew conidia colony
(scale bar represents 5µm)
(scale bar represents 5µm, magnification 40x)

Fig. 8 Detail image of conidiophore
(scale bar represents 2µm,
magnification 100x-immersion)

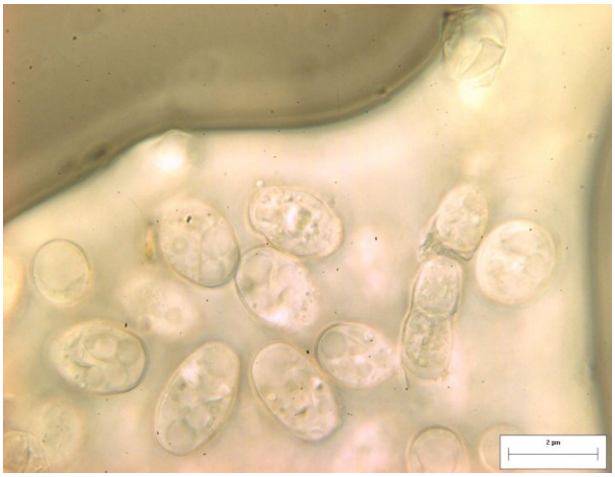

Fig 9. Detail of conidia with evident fibrosin
(scale bar represents 2µm,
magnification 100x-immersion)

The asexual conidia measures 2,5-2,6 µm in length, 1,4 µm in width , surface area 3,16 µm^2. The shape of the conidia in most cases is oval (Figs.8-9), sometimes rounded, revealed the miscoscopy images. A medium number of conidia were present in microscopy slides, in early disease development we observed 450-500 conidia.

The infection source can come from infected terminal shoots from near orchards through dispersal of conidia with wind, infected buds and branches which were not eliminated from tree crown.

Nuseries which are close in distance to infected orchards through wind dispersal could be affected by apple powdery mildew.

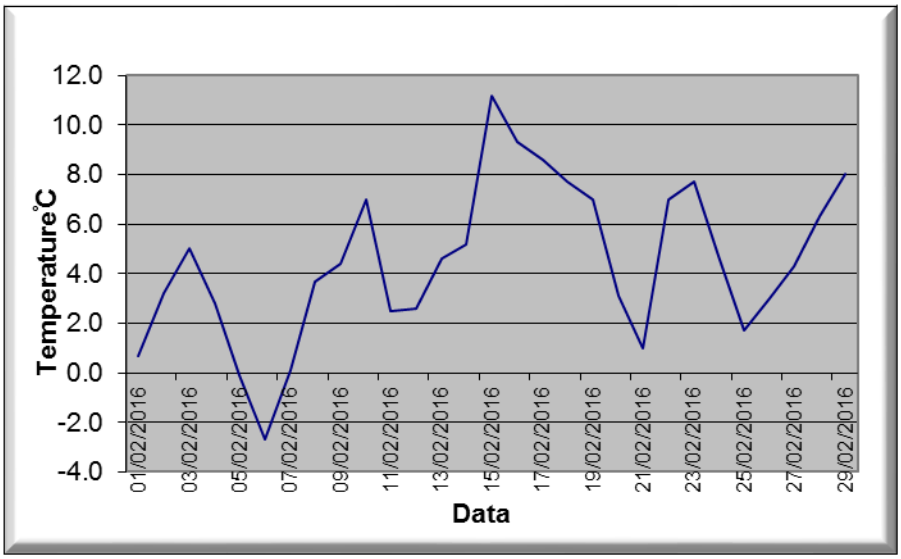

Fig. 10 Mean temperatures at SCDP Bistrita in February 2016

Mean temperature of month February 2016 was 4,5℃ with 1,3 ˚ C more than the multiannual average recorded during 1961-1990 in Bistrita and it is visible in the chart from Fig. 10 that between days 13.02-19.02 the average temperatures achieved 11,2℃ in 15.02 and in the same day it was recorded the absolute maximum of 17.5℃ unusual for this month.

The tendency of warming in this period contributed to optimal development of APM fungi.

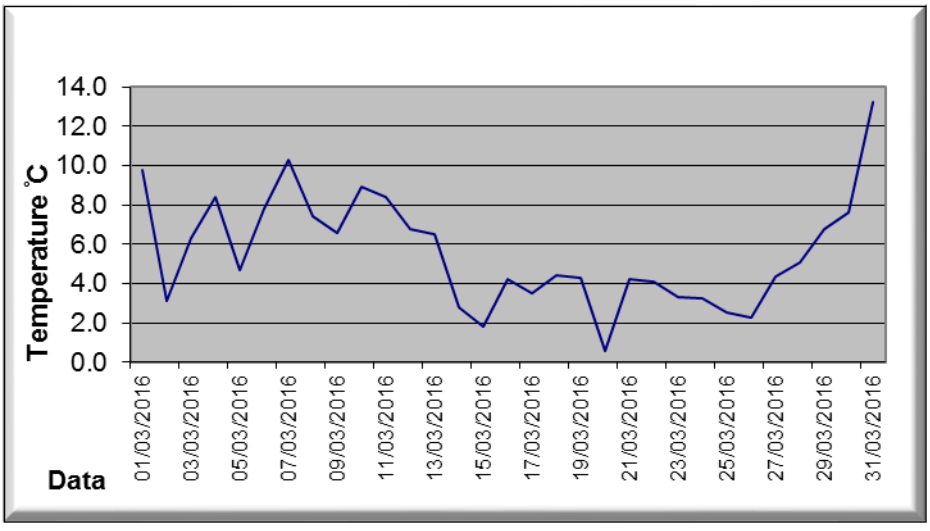

Fig. 11 Mean temperatures at SCDP Bistrita in March 2016

Analyzing Fig.11 one can observe that in the beginning of the month higher temperatures were recorded reaching 10.5℃ in 07.03 followed by a drop down of temperatures registering 0.6℃ average temperature in 20.03. This decreasing of temperatures influenced negatively the further development of the fungi, a certaing withdrawal being observed.

Primary infections in silver tip stage occure when infected dormant terminal buds are present in the tree. In the microscopy images with several germ tubes were also observed, primary and secondary hyphae. Several researchers pointed out that both temperature and atmospheric moisture influences the primary colony development. Warm and moist periods or cool damp sunless weather favored the disease also [2]. Experiments showed that Podosphaera leucotricha could germinate over a wide range of temperatures, observation stated by researchers Meenu Gupta and S.K. Sharma also[2]. They observed that in laboratory conditions in a climate chamber the maximum germination of conidia occurred at 25℃ (49,4 %) followed by 30 ℃ (47 %) and 20℃ (46.19%). Minimum germination was recorded at 5℃ and 35℃. Conidial germination was the highest at 95.6 % RH and 89,9 % RH. The observation of Woodward [8] is that the apple powdery mildew favored the warm and dry periods as favorable development in direct relationship with meteorological conditions. This hypothesis is confirmed by Sutton [6] who states that the concentration of the conidia in air was positively corelated with wind, temperature , solar radiation and negatively with RH and leaf wetness. The infection epidemics is strongly bound with mean air temperature, relative humidity and cumulative rainfall. Several researches observed that the factors act not only in single manner but collectively, several other reserchers concluded that a relatively mild dry climate influences the occurrence of the disease, conidiophores beeing dispersed by the rainfall, which can damage the conidiophores.

Acknowledgements

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