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PRELIMINARY STUDY ON THE *PODOSPHAERA SP.* CAUSATIVE AGENT OF POWDERY MILDEW ON PRUNUS CERASIFERA IN BULGARIA

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Abstract

The rootstock Myrobalan 29C (*Prunus cerasifera* Ehrh.) is largely used as rootstocks of plum and apricot planting material. In May 2020, symptoms of powdery mildew were observed on leaves and young stems of Myrobalan 29C rootstocks. On the adaxial and abaxial leaf surface white colonies with white superficial mycelium and conidia were present. Chasmothecia appeared in some injured leaf tissues. Microscopic examination showed globose chasmothecia with appendages arising from the upper half of the fruiting bodies. Usually, the appendages were one to four times longer than the chasmothecial diameter and had two to four dichotomous branches. A single ascus observed in chasmothecium was broadly ellipsoid-ovoid and contained six to eight ascospores. The shape of the ascospores was ellipsoid-ovoid, too. Conidia formed in true chains on erect conidiophores, which were ellipsoid, hyaline and measured 24.6-33.6 × 10.8-20.8 µm (average 29.6 × 15.8 µm). Pathogenicity was confirmed by inoculation of healthy Myrobalan 29C, apricot (*P. armeniaca* L.) and cherry (*P. avium* L.) plants. Disease signs on all of the inoculated experimental species appeared as white powdery coating very similar to the powdery coating on natural infected Myrobalan 29C. Based on the morphological characteristics of the conidia, chasmothecia and results of the pathogenicity tests, the causative agent of the symptoms observed was presumed to belong to *Podosphaera* sp. (Ascomycetes). To the best of our knowledge, previously no detailed characterization of *Podosphaera* sp. in *P. cerasifera* was made in Bulgaria.

Keywords: *Prunus cerasifera*, powdery mildew, *Podosphaera* sp.

INTRODUCTION

Stone fruits (*Prunus* spp.) are economically important fruit trees worldwide. The genus comprises plum (*P. domestica* L.), myrobalan (*P. cerasifera* Ehrh.), peach (*P. persica* Batch.), apricot (*P. armeniaca* L.), almond (*Amigdalus communis* L.), sweet and sour cherries (*P. avium* L. and *P. cerasus* L.), etc. Traditionally, in the production of stone fruits, the planting material - seedling rootstocks, have been used but currently more commercial clonal *Prunus* rootstocks have been introduced in application (Beckman & Lang, 2003). Myrobalan 29C (*P. cerasifera*) is a type of a clonal plum and apricot rootstock, recently grown and used in Bulgaria. The rootstock is reported as tolerant to roots asphyxia, phytopathogens of the genera *Armillaria*, *Phytophthora*, *Agrobacterium* and *Verticilium* and resistant to nematodes of *Meloidogyne* spp. (Carmona-Martin & Petri, 2020).

The causative agents of powdery mildews are obligate biotrophic fungal plant pathogens belonging to the phylum Ascomycota and the family Erysiphaceae; they comprise 900 species that infect more than 10,000 dicot and monocot plant species globally (Braun & Cook, 2012). Many species of the family Erysiphaceae cause economically important diseases of cultivated crops, including fruit trees (Glawe, 2008). In fruit trees in Bulgaria, the powdery mildews inflict significant injuries on apple (*Malus domestica*) and peach (*Prunus persica).*

In September 2019, on the leaves of several Myrobalan 29C rootstocks, grown in a greenhouse, sporadic white dusty patterns were recorded. In the next year, in May 2020, typical signs of powdery mildew in high degree were observed. Our library search on the occurrence of powdery mildew in myrobalan in Bulgaria resulted in finding a short note by Christoff (1939); he suggested *Podoshaera oxyacanthae* as a causative agent of powdery mildew in myrobalan without providing detailed data on the characteristics and pathogenicity of the suspected pathogen. Besides the cited short note from the first half of 20th century, no other reports on powdery mildew in myrobalan in Bulgaria are available. Although powdery mildew is considered as a lesser disease on plum, apricot, sweet and sour cherries, almond, myrobalan, etc., in our country, according to Kiss (2005) and Desprez-Loustau et al. (2010), some powdery mildews can become invasive in different parts of the world affecting plant health, biosecurity and leading to biodiversity risks (Jones & Baker, 2007; Brasier, 2008; Desprez-Loustau et al., 2010).

The aim of the present study is to characterize and identify the pathogen causing the powdery mildew on Myrobalan 29C.

MATERIALS AND METHODS

The studied Myrobalan 29C rootstocks were *in vitro* propagated, acclimatized to *ex vitro* conditions and grown in a greenhouse located in Plovdiv region. In September 2019, sporadic white dusty patterns on the leaves of several rootstocks were recorded. After dormancy, in May 2020, typical signs of powdery mildew, i.e. white powdery colonies with white superficial mycelium and conidia on the adaxial and abaxial leaf surfaces and young stems of inspected Myrobalan 29C rootstocks were observed. Samples were taken from symptomatic plants for investigation. Three Myrobalan 29C plants with symptoms of powdery mildew were kept for 2 weeks under lower humidity with the purpose of formation of the sexual form of fungal pathogens.

Morphological examination

The morphological examination for characterization of the powdery mildew causative agent was based on the teleomorph (chasmothecium) and the morphology of anamorph (conidiophores and conidia). Several leaves covered with white coating as well leaves with formed chasmothecia were observed under stereomicroscope and fungal structures (chasmothecia, conidiophores and conidia) were mounted in a drop of sterile distilled water on a glass slide. For fungal microscopic structure, 30 measurements were made using Leica DM 500 compound microscope (Germany) with a 40× objective lens. Calculations were implemented at the 95% confidence level. Fungal structures were analysed using software of Leica ICC50 HD camera.

The anamorphic criteria used in the morphological identification were: the presence of characteristic conidial chains, mycelium layer on leaf surfaces and additional characteristics as described by Lange (1996). A major morphological distinction of asexual form examined included the pattern of the conidia formation on conidiophores, i.e. singly or in chains.

The main morphological characteristics of chasmothecia examined included size of fruiting body, number of asci in the chasmothecium, number of ascospores per ascus, number and arrangement of appendages.

Pathogenicity test

Pathogenicity tests were carried out by inoculation of young leaves of healthy Myrobalan 29C, apricot and cherry plants. Before inoculation the leaves of the experimental plants were surface disinfected with 50% alcohol by spray and washed three times with sterile distilled water.

Two approaches for artificial inoculation were applied. First approach was carried out by scattering of a small part the mycelium from the symptomatic leaves on young healthy apricot leaves. Conidia pieces taken from vigorously growing conidia chains were aseptically transferred onto apricot (*P. armeniaca*). The second approach was conducted by putting of leaf discs, each of diameter of 5 mm, cut out of vigorously growing conidia. In that manner, the last three fully developed young leaves of Myrobalan 29C and cherry rootstock (*P. avium*) were inoculated, placing one disk on each leaf. The plants inoculated by these two approaches were maintained in a moist chamber at 23°C.

Additionally, buds from plum cultivar *Stanley* were grafted onto Myrobalan 29C and after that maintained in a greenhouse. The grafted plants were inspected for appearance of disease symptoms on the plum scions sprouted from grafted buds. The pots were placed in a room at room's temperature. The experiment used a completely randomized design with three replications (three inoculated leaves and control - three not inoculated leaves from each plant).

The inoculation was rated as positive if the colonies were visually observed or as negative if no visual symptoms were there. The incubation period was determined for all the studied plants.

RESULTS AND DISCUSSION

As noted above, the studied Myrobalan 29C rootstocks were *in vitro* propagated, acclimatized to *ex vitro* conditions and grown in a greenhouse. Initially, the symptoms of powdery mildew were detected on single leaves. However, in the next stages, the percentage of infected Myrobalan 29C plants gradually increased and in July the spread of diseases was calculated at the level of 97.5%. The symptoms of local infection were recognized mainly on young leaves covered on the upper side with white powdery surface growth (Fig. 1). The systemic form of diseases was not reported.

Fig. 1. Symptoms on Myrobalan 29C grown in greenhouse.

Morphological examination

The microscope observations visualized conidia formed in true chains on erect conidiophores. Conidia were ellipsoid, hyaline and measured at 24.6-33.6 × 10.8-20.8 µm (av. 29.6 x 15.8 µm). Mycelium on powdery mildew symptomatic leaves was superficial, white and greyish-white, delicate, amphigenous. Mycelial hyphae were septate, hyaline, branched. Catenescent conidia, ellipsoidal to short sub-cylindrical, 1-celled, with fibrosin bodies, 24.6-measured at 33.6 × 10.8-20.8 µm (av. 29.6 × 15.8 µm), were produced on erect conidiophores, with cylindrical foot cells followed by 1-4 short cells, arising from epiphytic hyphae on both surfaces of leaves (Fig. 2 E-F). Mature chasmothecia were spherical, gregarious or scattered on infected leaves, brown to black-brown (Fig. 2A), measured at 83-101 µm (av. 95 µm). Chasmothecial appendages were 2-4, smooth or slightly curved, brown to paler toward the apex, 201-342 µm (av. 271 µm) long, one to four times longer than the chasmothecial diameter;

with two to four dichotomous branches (Fig. 2C). A single ascus of each chasmothetium was broadly ellipsoid-ovoid, 62-92 × 58-73 µm, containing six to eight hyaline, ellipsoid-subglobose ascospores, measured at 18-24 × 14-16.5 µm (Fig. 2D).

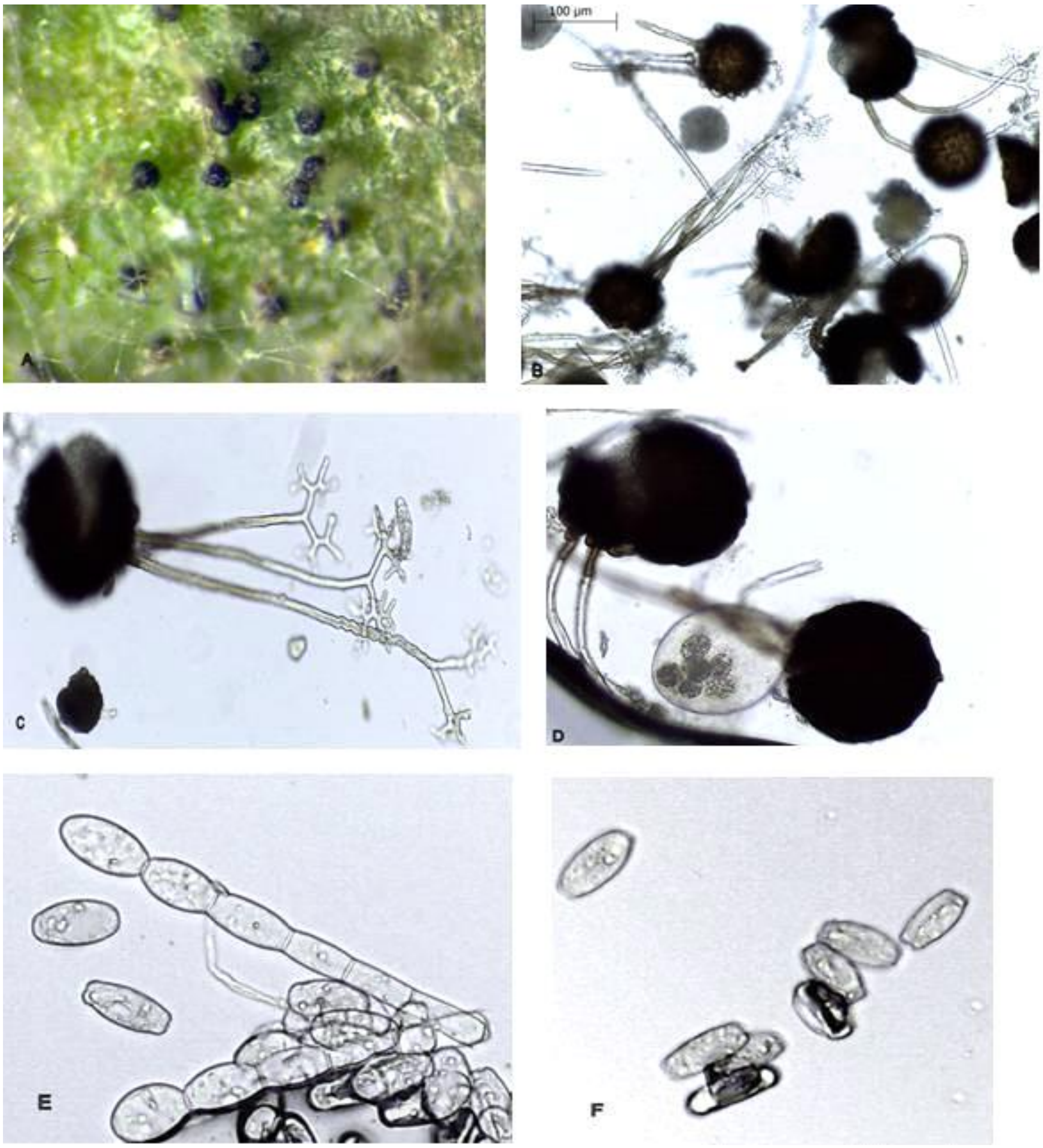

Fig. 2. A. Chasmothecia on infected leaf. **B.** *Podosphaera* chasmothecia. **C.** Chasmothecial smooth or slightly curved appendages with dichotomous branches at their ends. **D.** Single ascus with ascospores of *Podosphaera* chasmothecia; **E.** Erect conidiophores with conidia; **F.** Conidia with fibrosin bodies.

Pathogenicity test

Symptoms of powdery mildew on artificially inoculated Myrobalan 29Cand cherry leaves were observed after eight days post-inoculation period. Disease signs on the inoculated apricot leaves appeared 30 days after the inoculation.

White powdery surface growth observed on the inoculated leaves of all experimental species was very similar to the powdery surface growth on natural infected Myrobalan 29C (Fig. 3).

Powdery mildew symptoms on plum cultivar *Stanley*, grafted on infected Myrobalan 29C, were recorded three months after the developing of the grafted scions.

Fig. 3. Symptoms of powdery mildew after inoculation with *Podosphaera sp.*
(On the apricot leaves - left, on the cherry leaves – right)

The pathogenic fungus present on the inoculated Myrobalan 29C, apricot and cherry plants was identical morphologically to that originally observed on diseased Myrobalan 29C plants.

In comparison to all other crop pathogenic fungi, the variety of powdery mildews appear to be spreading most rapidly in a global scale and are predicted to reach all available hosts by 2050 in many countries of the world (Bebber et al., 2014, 2019; Bebber & Gurr, 2015). Climate changes and continuous evolution of pathogens require new research on phytopathogen virulence, plant genotypes susceptibility and plant protection practices.

According to Braun & Cook (2012) the morphological characteristics of the conidia, chasmothecia as well as the results of the pathogenicity tests, the causative agent of the symptoms observed on Myrobalan 29C was presumed to be *Podosphaera* sp. (Erysiphaceae).

Molecular phylogenetic analyses, started in the early 2000s, have shown that the traditional generic concept of the Erysiphales does not mirror their phylogeny based on the morphological characteristics of the sexual morphs (chasmothecia) (Braun et al., 2002). Surprisingly, grouping species according to the characteristics of their asexual morphs reflect their molecular phylogeny (Takamatsu, 2013b). That discovery led to major taxonomic revisions (Braun et al., 2002) and the currently accepted generic concept is now based on a combination of the morphological traits of the asexual and sexual morphs (Braun, 2011; Takamatsu, 2013a). The most recent monographs recognized 17 genera of Erysiphaceae as powdery mildews phytopathogens (Braun & Cook, 2012; Kiss, 2020). According to the above cited authors, within the well known morphologic species of powdery mildews there are usually many subspecies, forms, varieties, biotypes or races which cannot be distinguished morphologically or which can be distinguished by only slight morphological differences. More recently the study on the phylogeny of the *P. tridactyla*, carried out by Meeboon et al. (2020) suggested that *P. tridactyla* is a complex consisting of at least 12 different species. Based on detailed morphological examinations and molecular sequence analyses, they have proposed dividing *P. tridactyla* into 10 species, encompassing 7 new species.

For the first time in Bulgaria, *P. tridactyla* was reported as a causative agent of powdery mildew in plum by Savoff (1923). Later, Christoff (1939) noticed *P. tridactyla* as the causative agent of powdery mildew on blackthorn (*P. spinosa*) and suggested *P. oxyacanthae* as a causative agent of the disease on plum, apricot, myrobalan and almond. Nevertheless, the cited authors did not describe in details the morphology of the causative agents and their potential host range, i.e. their pathogenicity and virulence. To the best of our knowledge, this study is the first detailed report of morphological characteristic and pathogenecity of *Podosphaera* sp. on *P. Cerasifera* in Bulgaria.

CONCLUSION

The preliminary identification of the causal pathogen of powdery mildew in rootstocks Mirobalan 29C was performed by examination of morphological features. The data of the morphological and pathogenicity analysis are almost identical to the typical characteristic of the species *Podosphaera tridactyla* but the final conclusion about the species identification could not be based solely on morphological characteristics. Samples from symptomatic plants were taken for molecular assay and the analysis is currently in progress.

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