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PRELIMINARY RESEARCH ON THE LEAVES MICROBIOTA OF EDIBLE CLIMBING ROSE UNDER ORGANIC MANAGEMENT

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Abstract

Little is known about the fungal communities of rose leaves, the interactions between and with the main pathogens or their response to abiotic and biotic stress. In this context, a preliminary study on microbiota of healthy and infected leaves by Diplocarpon rosae and Podosphaera pannosa was carried out in the organic edible climbing rose plantation of Faculty of Horticulture - USAMV Bucharest. The composition and incidence of fungal isolates was variable in time as well as in presence or absence of pathogens. The fungal community of rose leaves was represented by Alternaria, Aureobasidium, Cladosporium, Chaetomium, Epicoccum, Fusarium, Penicillium and Sordaria isolates. Our study highlights the presence of some well knowns natural antagonists as Chaetomium globosum, Epicoccum nigrum, Sordaria fimicola and Aureobasidium pullulans isolates. Further, presently ongoing studies shall reveal the antagonistic properties of our isolates and their colonizing ability (in vitro and in vivo assays). Our further studies will contribute to a better understanding of the composition and diversity of rose leaves fungal community and how the microbiota can play a role in plant protection

Key words*: edible rose, leaves fungal microbiota, organic management.*

INTRODUCTION

Rose is one of the most important ornamental horticultural crops all over the world, being historically associated with beautiful gardens. However, there is a worldwide trend of using roses as an ingredient in various spices and functional foods (Park et al., 2005), as raw material for anti-inflammatory drugs or as a potential source of antioxidant compounds promoting human health (Ge et al., 2013; Lee et al., 2015; Choi et al., 2015; Haejo & Shin, 2017; Butcaru et al., 2019).

Rose is susceptible to a large number of diseases that impact negatively on overall plant performance, reduce flower yield, quality, marketing and production costs (Chalova et al., 2017).

Podosphaera pannosa (powdery mildew), and *Diplocarpon rosae* (black spot) are the most common and damaging fungal pathogens in roses (Debener & Byrne, 2014; Byrne et al., 2019). Both diseases affect susceptible cultivars worldwide (Gochomo et al., 2006; Munnenkhoff et al., 2017), leading to development of immature and stunted plants, yellowing and premature defoliation or reduced rose flower production.

Organic edible roses are grown using environmentally friendly practices. Cultivation of healthy plants in organic farming is a challenge due to the lack of resistance of *Rosa damascena* to the main diseases and pests (Chalova et al., 2017). Cultural practices as planting in well - drained soil amended with organic matter, providing air circulation, avoiding overhead watering or maintaining good sanitation are basic guidelines for disease management. Also, organic products (biological control agents, plant extracts) are available and effective in rose disease control, having a preventive rather than eradicating function (Gupta & Dikshit, 2010). However, some botanical pesticides may express certain levels of toxicity and therefore extensive research is needed prior to their practical application (Chalova, 2017).

To protect edible roses against fungal infections it is important to explore novel strategies. Leaves has been recognized as an important habitat for microorganisms, including bacteria, yeasts and filamentous fungi. Knowledge of leaves microbiota is important for the management of foliar diseases. Indigenous fungal and bacterial populations have been found efficient in limiting pathogen population, and thereby minimizing the disease severity in different crops (Suman, 2008).

There are several studies thar have been reported a wide range of beneficial effects of microbiota members on plant health including disease suppression, induction of systemic resistance, increased nutrient acquisition, increased tolerance to abiotic stresses or adaptation to environmental variations (Hassani et al., 2018).

Despite the increasing interest in leaves microbiota, little is known about the diversity and community structure of fungi associated with *Rosa* spp. as well as its ecological roles. Also, little is known about the fungal communities of edible rose leaves, the interactions between and with the main pathogens or their response to abiotic and biotic stress. In this context, a preliminary study on microbiota of healthy and infected edible climbing rose leaves by *Diplocarpon rosae* and *Podosphaera pannosa* was carried out.

MATERIALS AND METHODS

Biological material.

A survey of black spot and powdery mildew was conducted in an organic edible climbing rose plantation established in 2015 at University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture.

Typical symptoms of black spot that include circular dark brown spots with irregular margins measuring up to 15 mm were observed.

Powdery mildew was identified as a white to grayish spots or patches and powdery growth on the upper surface of the leaves.

Isolation of microbiota from healthy and infected leaves.

Healthy and infected rose leaves with black spot and powdery mildew symptoms (Falstaff cultivar) were collected in June and November 2020.

The leaves were cut into small pieces (0.5 cm), surface sterilized using 70% ethanol and rinsed for three times. Tissues were blotted dried and plated on potato dextrose agar (PDA). Plates were incubated at 22°C until growing of colonies. Fifty leaves/variant have been collected and one hundred fragments have been plated. The experiments were repeated twice.

Results are expressed as isolates incidence (%).

Fungal isolates were identified based on their macroscopic (colony colour, pigmentation) and microscopic characteristics.

RESULTS AND DISCUSSIONS

Fungal community associated with healthy or infected edible rose leaves was assessed in July and November 2020.

In July, the fungal community of edible climbing rose leaves has been represented by *Alternaria*, *Aureobasidium*, *Cladosporium*, *Chaetomium*, *Epicoccum*, *Fusarium*, *Penicillium* and *Sordaria* isolates. Bacterial strains were also present.

The composition and incidence of fungal isolates was variable in time as well as in presence or absence of pathogens (Figure 1).

In healthy leaves, the highest incidence was recorded for *Chaetomium globosum* isolates (48%) in July, followed by *Aureobasidium pullulans* (17%) and *Sordaria fimicola* isolates (13%). All isolates belong to species that are recognized as natural antagonists. In November, the fungal community associated with healthy leaves was represented by *Alternaria alternata* (25%), *Epicoccum nigrum* and *Cladosporium cladosporioides* (3%) isolates. Bacterial colonies were also present (3%).

In July, in leaves with black spot symptoms, the highest incidence was recorded for *S. fimicola* isolates (22%). Other isolates detected were *A. alternata* (18%), *Fusarium* sp. (8%), *C. globosum* (6%) and *Aureobasidium pullulans* (6%). In November, *A. alternata* isolates was recorded with the highest incidence (84%). Like for healthy leaves, isolates of *E. nigrum* have been recorded, but with low incidence (3%). *Penicillium* sp. isolates (3%) and bacterial colonies (7%) were also been present in leaves with black spot symptoms (Figure 1).

The leaves community associated with powdery mildew attack have been represented by *S. fimicola* isolates (49%), *A. pullulans* (28%), *C. globosum* (21%), *A. alternata* (18%), *Fusarium* sp. (14%), and *Monilia (Neurospora) sitophila* (10%). Bacterial strains were also present (8%). In November, from leaves with powdery mildew symptoms only *A. alternata* (67%) and *E. nigrum* isolates (27%) have been detected and isolated (Figure 1).

Different microorganisms have been isolated from rose phylloplane (leaf surface) with powdery mildew symptoms, but by a different method than ours (wash leaf method). Kumar and Chandel (2018) reported the presence of *Fusarium*, *Botrytis*, *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Trichothecium*, *Trichoderma* and two bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.). Some of these isolates have been recorded in our study, too.

Gosh & Shamsi (2014) reported five fungal species that have been associated with black spot symptom of *Rosa centifolia*. The associated fungi were *C. cladosporioides, C. oxysporum, Marssonina rosea*, *Penicillium* sp. and *P. guepinii*.

Our study highlights the presence of *C. globosum*, *E. nigrum, S. fimicola* and *A. pullulans* isolates with potential antagonistic properties.

Chaetomium globosum isolates have been detected only in July, both in healthy and infected leaves (Figure 2), with the highest prevalence in healthy tissues. *Chaetomium globosum* is a saprophytic fungus which is widely distributed on plant, soil, straw and dung as well as an endophytic one. *Chaetomium* species have been documented to be potential antagonists of several soil- and seed-borne plant pathogens (Yue et al., 2018).

Epicoccum nigrum isolates were detected only in November (Figure 2), with low incidence in healthy and black spoted leaves and with a higher prevalence in leaves with powdery mildew symptoms. *Epiccocum nigrum* is a ubiquitous saprophytic hyphomycete found on various substrates (soils and plants). Also, it was isolated as an endophytic fungus. *Epicoccum* species are famous for their application in the biocontrol of many fungal pathogens but also for their capability to produce biologically active compounds with medical applications (antioxidant, antimicrobial, and anticancer agents) and pigments with industrial application (Ogórek & Pląskowska, 2011; Elkhateeb & Daba, 2019).

Sordaria fimicola isolates have been detected only in July, in healthy and infected leaves (Figure 2) with higher incidence in leaves with powdery mildew and black spot symptoms compared to healthy leaves.

The genus *Sordaria* has important saprophytic species that grow well and fast on organic materials (Kavak, 2012). The main habitat of *S. fimicola* is decaying organic matter and dung of plant-eating animals. This fungus, a model for studying genetics and meiosis in ascomycetes has been also isolated as endophyte or from the surface of necrotic leaves colonized by fungal pathogens (Kavak, 2012; Newcombe et al., 2016). Recent studies focusing on novel biocontrol agents, reported the antagonistic activity of *S. fimicola* in wheat (Er, 2010) and maize (Abdallah et al., 2018) against *Fusarium graminearum*.

Isolates of *A. pullulans* have been detected only in July (Figure 2), with a low prevalence in healthy leaves. A higher incidence, compared to healthy leaves was observed in leaves with powdery mildew symptoms followed by those with black spot attack. *Aureobasidium pullulans* is a saprophytic yeast-like fungus, well documented, found in plants, soil, rocks and water which is well known for his antagonistic activity, especially against postharvest pathogens (Bozoudi & Tsaltas, 2018).

These observed dynamics/increase of indigenous beneficial populations in diseased leaves could be the result of the pathogens activity or a direct relationship between them and pathogen. The host plant also provides metabolic capabilities which leads to the adaptation of niche specialized inhabitants (Thrall et al., 2007). Effective colonization, large population size and the viability of beneficials are important for successful biocontrol of plant diseases. Increasing the size of a constituent of the indigenous microbiota of leaves when the constituent is used as a biocontrol agent is one of our further research objectives. When the size of indigenous beneficial population is low, application of bio-products based on biological control agents could lead to a better control of diseases.

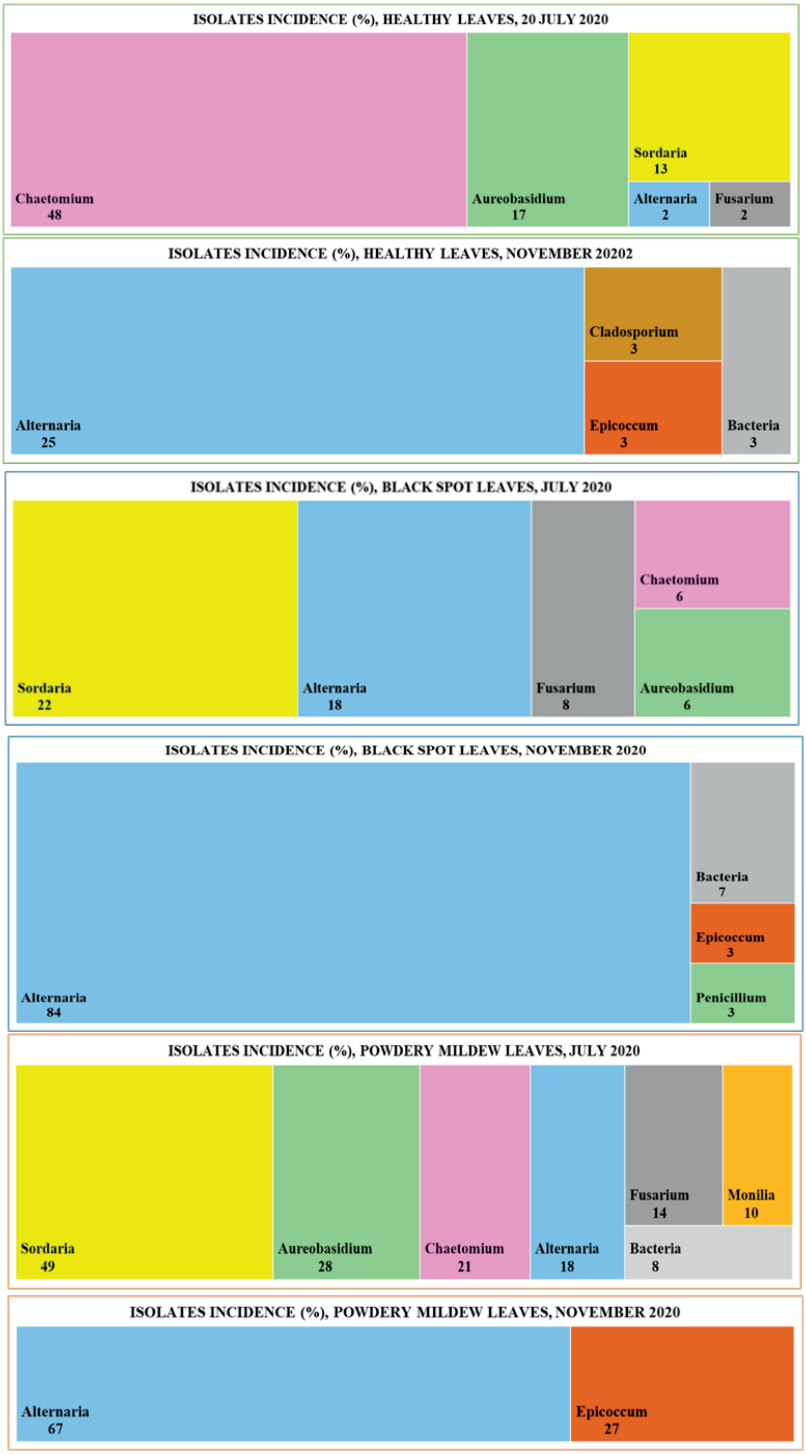

Figure 1. Incidence of detected isolates on organic climbing rose leaves, healthy or affected by black spot and powdery mildew

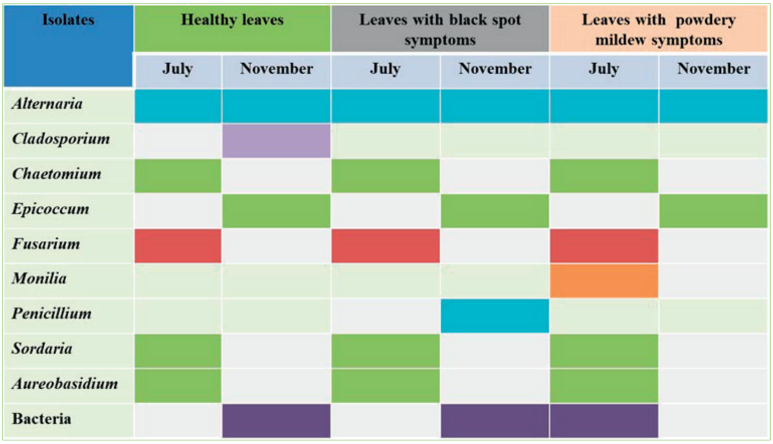

Figure 2. Occurrence of detected isolates in July and November on organic climbing rose leaves, healthy or affected by black spot and powdery mildew

Among the other isolates that have been detected, we mention those belonging to *A. alternata*, which have been present both in July and November, in all variants (Figure 2). In healthy leaves, the prevalence of *A. alternata* isolates was low in July and higher in November. In leaves with black spot or powdery mildew symptoms, the incidence of *A. alternata* isolates was higher than in healthy leaves, in July. In November, these tissues have been colonized mostly by *A. alternata* isolates.

CONCLUSIONS

Our preliminary results provide a first glimpse into the microbial community associated with healthy or diseased leaves of organic edible climbing rose. The microbiota composition and incidence of isolates was variable in time as well as in presence or absence of *D. rosae* or *P. pannosa* pathogens.

Since leaves of organic edible climbing rose are naturally colonized by resident microorganisms known for their antagonistic activity such as *A. pullulans*, *C. globosum, E. nigrum*, and *S. fimicola* the present challenge is to understand their biocontrol potential and how such microorganisms can be successfully integrated in the control main diseases.

Further studies are needed to better understand how the composition and diversity of edible rose microbial community can interacts or influence the pathogens. Research is currently underway to test their antagonistic properties and colonizing ability on the phylloplane (*in vitro* and *in vivo* assays). Also, studies are underway for the identification of recurring patterns in the dynamics of the microbial populations, and the acquisition of knowledge on the mechanisms that generate these patterns.

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