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RESEARCH REGARDING THE IDENTIFICATION OF THE FUNGUS *PHOMOPSIS MALI* ROBERTS (*PHOMOPSIS* FRUIT TREE CANKER) IN A TWO YEARS OLD ECOLOGICAL APPLE ORCHARD - CASE STUDY

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Abstract

Phomopsis mali Roberts (Phomopsis fruit tree canker) is a fungus that infects the fruit tree trunks, branches and sprigs. There were described more than 60 species of Phomopsis. Every of the species is identified in general after the size of the conidia and after the host from which was isolated, the precise identification being sometimes difficult. Phomopsis is a fungus that can produce serious damages in production because it affects the fruitful sprigs. On the other hand, in case of massive attacks it can lead to the fruit trees decline. In the young orchards cultivated in ecologic super-intensive system, the young trees can die in the case of severe infections. The purpose of this research was to identify the Phomopsis fungus in an ecologic super-intensive apple orchard from Arad County (western Romania), in the first two years after the plantation. The biological material used was consisting in six samples sets (sprigs, stems, branches and roots) collected from four apple varieties (Primiera/M_9, Crimson Crisp/M_9, Golden Orange/M_9 and GoldRush/M_9). The identification of the disease and of the pathogen was done using visual and laboratory methods. In laboratory was identified the fungus with the humid chamber method and by placement of diseased tissue samples on culture medium followed by incubation at 23 - 24°C for seven days. The branches, sprigs and stems were analysed at stereomicroscope too. Under the cracked bark were identified numerous pycnidia of black colour and pear shaped, specific to the Phomopsis mali fungus. There were noticed young and old fructifications, the old ones were from the previous year or even from the precedent years. The obtained results after the visual analysis of the trees in the orchard and after the laboratory analyses highlighted the presence of the fungus Phomopsis mali. There were highlighted at the microscope the alpha and beta conidia, typical for this fungus. In the orchard all the trees were presenting symptoms specific to the Phomopsis fruit tree canker. The trees covered with numerous canker lesions were died. At the assessment time the dead trees rate on varieties was the following: Primiera – 16.5%, Crimson Crisp – 1.7%, Golden Orange – 16.1%, and Gold Rush – 17.2%. From all the analysed apple varieties only Crimson Crisp had reacted well to the attack of the Phomopsis mali fungus.

Key words*: *Phomopsis mali, apple, varieties, pycnidia, Phomopsis fruit tree canker, ecologic orchard

INTRODUCTION

Phomopsis fruit tree canker is produced by the fungus *Diaporthe perniciosa* Marchal sin. *Diaporthe eres* Nitschke (sexual life cycle), with the conidian form or asexual life cycle *Phomopsis mali* Roberge, sin. *Phomopsisperniciosa* Grove. The fungus *Phomopsis mali* is famed from taxonomic point of view in the Kingdom *Fungi*, Phylum *Ascomycota*, Class *Sordariomycetes*, Order *Diaporthales*, Family *Diaporthaceae*, Genus *Phomopsis* (Sacc.) Bubák 1905, Species *Phomopsis mali* (Schultzer & Sacc.) Died.

(syn. *Phomopsis prunorum* (Cooke) Grove [10][18]. Even the scientific community recommends the use of the name *Diaporthe* for *Phomopsis*, in practice is used most often the name *Phomopsis* because the anamorph stage of the fungus is found more often in nature instead the teleomorph one [23]. According with Shenoy *et al*. [27], the use of two names for the same pathogen is useless and can lead often to confusions. Thus, the name of the anamorph stage is used in practice very often for other pathogens too [31].

Phomopsis fruit tree canker produced by the fungus *Phomopsis mali* on apple tree is a disease found more and more often in the young orchards from western Romania cultivated in ecological system. The symptoms produced by the pathogen are recognized relatively easily, but many times they are confused with those produced by *Cytospora* sp., *Botryosphaeria* sp. or even by *Erwinia amylovora* [19][7].

In the case of apple trees, *Phomopsis mali* attacks the sprigs, branches stems, and fruits [15]. At the surface of the attacked organs can be noticed a change of the bark colour becoming reddish – orange or even blackish in advanced phases of the pathogen evolution. The first necroses appear in the area of the vegetative buds, floral buds but also of the petioles area. Together with these symptoms can be observed the appearance of depressed areas in the bark with an irregular shape that often cracks (cankers). In massive attacks the fungus passes from bark the wood entering in the vascular tissues and the attacked organs are fading first and later will have a burn appearance [21][24][14]. The necrosed sprigs and branches are visible from distance. Exceptionally, in the very severe cases the fungus can enter even in thick trunks and branches. In the young trees *Phomopsis mali* can enter very easily in the wood of the thin stems, killing them [21][15][28][25]. When are forming the fruiting bodies (pycnidia and perithecia), at the surface of the bark appear small swellings that are giving a rugous aspect to the attacked organs [21][15].

The pycnidia and the perithecia are developing in the infected tissues. The perithecia (teleomorph fruiting bodies) are forming rarely in nature in comparison with the pycnidia (anamorph fruiting body) that are observed more frequently. Both fruiting bodies types are important in the identification of the species *Phomopsis* that has produced the disease together with the host [31][1]. The pycnidia have ostioles, are black, pear shaped and are producing two types of conidia: alpha (aseptate, spindle like, hyaline, guttulate or not-guttulate) and beta (aseptate, filiform, hyaline, not-guttulate) [8][29][20][6]. In conditions of humidity the pycnidia are releasing a mucilaginous mass white in colour at the beginning, full of spores, that in contact with the air is solidifying and can take different shapes. In the presence of water, the spores will produce new infections.

The perithecia can appear solitaire or in groups and are sunken into the substrate. The asci from the inside are unitunicate, clavate and contain ascospores that can have different shapes from spindle like, elliptic, linear, cylindric or curved. The ascospores are hyaline and septate [33][13][17][15].

Phomopsis fruit tree canker can evolve during the entire year in favourable climatic conditions, but especially during the summer and autumn when can be registered maxim attack intensities [21][15][32][26].

In general, all the species of *Phomopsis* are producing infections in conditions of cool weather (temperatures between 15 - 24 ^{o}C) and rainy weather (rain helps the production of the spores, dispersion and initiation of the infection) [15][26]. Over the winter season the pathogen survives in the infected organs, mostly in branches and sprigs. There was noticed that in the perennial herbaceous plants the fungus can overwinter on senescent leaves and fruit remains. According with Schilder [26], often the pathogen can reach in orchard with the planting material. The climate conditions in continuous change, the neglecting of the prevention methods (mostly phytosanitary hygiene), are favouring the infections with *Phomopsis* in the young apple orchards cultivated in ecological system [7][2][9].

There is interesting the fact that numerous *Phomopsis* species have potential in the

control of the invasive weeds from agricultural crops. The myco-herbicide effect is due to their hemi-biotrophic till to necrotrophic living, their capacity to persist in the environment and due to its extended sporulation during the entire duration of a year [16][3][22][4][30].

The control of the *Phomopsis* tree canker of the apple tree is difficult when the pathogen is already installed, mostly for the very young trees in the second year of life. In such situations the pathogen can enter in the stems with small diameter. There is recommended the harmonious jointing of the cultural hygiene measures (destroying of the diseased biological material) with the chemical methods (use of fungicides). There are vital the treatments applied in spring (at the budding phenophase) and those applied in autumn (October, after the fruit harvesting and after the leaf fall [26]. For a good control of the disease is necessary the early diagnosis of the fungus. The right identification of the *Phomopsis* species is based mainly on morphological and cultural features and the association with the host, but sometimes is difficult to realise it. Why is necessary the precise identification of the pathogen? For the elimination of the confusions with other pathogens and for the setting of the proper control measures[5][24][11][12][31].

The branch canker produced by the fungus *Phomopsis* sp. is more and more present in the last years in the nurseries and orchards from Romania. Very young orchards of one or two years are diseased, some being even in the very severe situation when nothing can be done to save the plants, mostly where they are cultivated sensitive varieties. The most severe problems are in the ecologic orchards created with diseased planting material.

The approached topic from this work is very actual and is very interesting for the fruit growers that are confronting more often in the last years with this disease.

The main purpose of this study was the correct identification of the pathogen from a young apple orchard cultivated in super-intensive ecologic system, from western Romania. There were applied laboratory analyses that were done on sections of diseased tissue and their introduction in humid chambers where the evolution of the fungus was analysed.

MATERIALS AND METHODS

The biological material was sampled directly from an apple orchard from Arad County in April 2021 (Photo 1). The apple orchard is cultivated in super-intensive ecologic system (2000 trees/hectare) and it was planted during the years 2019-2020. There were collected samples from plants consisting in branches, trunks, sprigs and roots (entire plants) from every variety cultivated in the orchard, respectively Primiera, Crimson Crisp, Gold Orange and GoldRush. The samples were brought in the laboratory where first were analysed at stereomicroscope. The photographs were taken with a Nikon camera with micro-objective. The frequency of the dry trees due to the disease was set by their counting ant reporting to the total number of the analysed trees. In the same time there were analysed and photographed the external symptoms at every 20 trees from every variety.

Photo 1. Aspect from the apple orchard during the sample collection (in Arad County, Romania).
Source: Original photo by Cotuna O. (2021).

Parts of sprigs, branches and stems were studied at the stereomicroscope for the

observation of the specific fruiting bodies (pycnidia).

The humid chamber method was used for the stimulation of the pycnidia to release the mucilaginous masses in which are forming the conidia of the fungus *Phomopsis mali*. In this way, there were cut segments of sprigs, stems and branches with the length of about 10 cm.

They were washed with tap water and after that were rinsed with distilled water. The sterilization on surface of the plant segments was done using sodium hypochlorite 3% (1 minute) and ethylic alcohol 96% (1 minute). The rinsing was done with distilled water for 2 minutes. The segments prepared in this way were placed in humid chambers and incubated at 24 - 25 ^{o}C for several days. the humid chambers were done in plastic dishes (20/10 cm) with sterile filter paper on which was dropped sterile water. After two days the pycnidia from the tissues from the humid chambers were erupted and were released mucilaginous masses specific to the analysed pathogen. With the sterile needle were extracted drops of mucilage that were introduced in lactophenol blue and were analysed at the microscope (Axion Zeiss).

In parallel from the infected tissues were detached pycnidia that were placed directly in lactophenol blue and left there for an hour. Through this simple method there was possible to analyse at the microscope in a shorter time the alpha and beta conidia of the pathogen.

RESULTS AND DISCUSSIONS

The partial identification of the pathogen present in the apple orchard was done with the occasion of a phytosanitary control. With this occasion was noticed that the apple trees have obvious signs of disease manifested on stems, branches and sprigs. The symptoms aspect was as rugous dry cankers on the bark surface. Due to the obvious symptoms noticed on sight the suspected pathogen was the fungus of *Phomopsis* tree canker *Diaporthe eres* Nitschke (sin. *Diaporthe perniciosa* Marchal, teleomorf form) with the anamorph form *Phomopsis perniciosa* Grove (sin. *Phomopsis mali* Roberge).

The trees from the orchard were presenting symptoms specific to *Phomopsis sp.* in different evolution stages. Also, at the spring break many trees with symptoms of Phomopsistree canker were died. The dead trees were covered in totality by rugous cankers, cracks, under those were hundreds of black pycnidia that will spread the pathogen on the new grown parts of the young trees. It is well known that this pathogen produces infections during the entire year, with a higher intensity during the summer and autumn months.After the evaluation proceeded in the apple orchard, the rate of dead trees found was the following: Primiera - 16.5%; Crimson Crisp - 1.7%; Golden Orange - 16.1% and GoldRush - 17.2% (Figure 1).

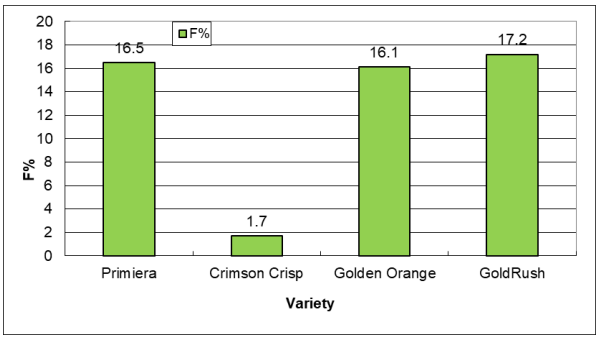


Fig. 1. Frequency of the dead apple trees due to the pathogen *Phomopsis mali* in April 2021 (%)
Source: Original graph generated on the base of the calculated experimental values.

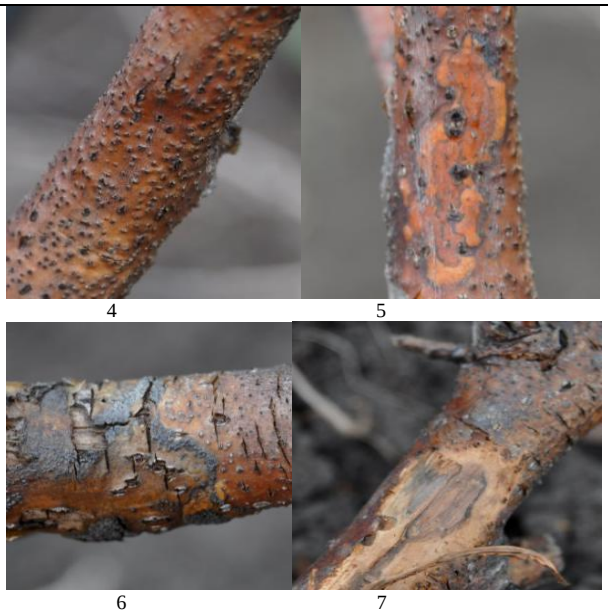
There was noticed that in the variety Crimson Crisp the frequency of the dead apple trees is very low in comparison with the other apple varieties cultivated in the orchard. In the other varieties the frequency of the dead trees has varied between 16.1 % and 17.2 %.

The visual analysis of the symptoms in the samples of 20 trees from every variety was highlights several types of symptoms: sunken spots with dark colour in the bark tissue (located in the dud area and the petiole scars); open wounds with calluses on the edges located at the joint of the branches, on trunk and on the sprigs from the previous year with canker aspect; dead branches (necrosed) presenting ulcerations (the bark of the branches has rugous aspect); bark with colour changes (orange – brick red, black, grey); deformation and swelling of the bark (initial symptoms produced by the pathogen); in the

areas with swollen bark, under the bark were found dozens of fruiting bodies of the fungus; old ulcerations, rugous, verrucous, black (at their surface were placed the black coloured fruiting bodies of the fungus); blacken xylem tissue in section; cracks in the bark tissue from the branches (in the cracks were small black bodies, respectively the fungal pycnidia); great parts of ligneous tissue browned in trunks and branches joints; the sprigs with healthy appearance were having browned areas under the bark that was surrounding the buds; the cortical tissue of the roots was rotten and was brown in colour in transversal section; radicular system with obvious diseased signs (browning); browning of the central cylinder of the lateral root system (Photo 2-8).

Photo 2. Swollen bark, reddish and with young fructifications specific to the fungus *Phomopsis mali*.
Source: Original photo by Cotuna O. (2021).

Photo 3. Bark with rugous appearance
Source: Original photo by Cotuna O. (2021)



4 5 6 7

Photo 4 - 7. Reddish – orange bark, rugous, cracked, xylem necrosis.
Source: Original photo by Cotuna O. (2021).

Photo 8. Necrosed wood of the apple tree stem.
Source: Original photo by Cotuna O. (2021).

At steremycroscope were analysed the branches, sprigs and stems. There were observed the open lesions, the cracks from the bark and the swellings.
Under the cracked bark were present numerous pyriform black pycnidia characteristic to the fungus *Phomopsis mali*. There were noticed young and old fructifications, the old ones being produced in the previous years (Photos 9 – 15).

Photo 9. Agglomeration of pycnidia under bark
Source: Original photo by Cotuna O. (2021).

10 11

12 13

Photo 10 - 13. In the presence of humidity from the pycnidia were expulsed white-cream mucilaginous masses. In contact with the air takes different shapes
Source: Original photo by Cotuna O. (2021).

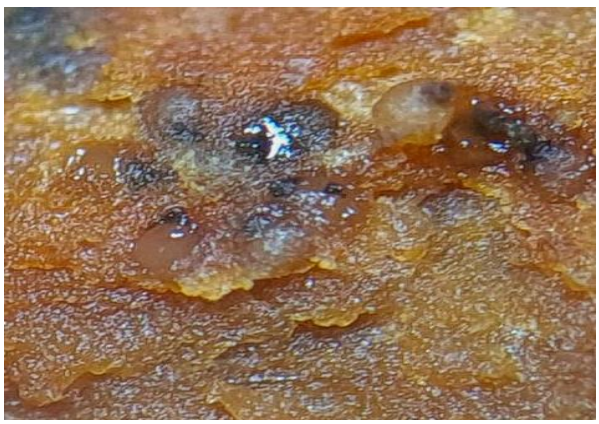


Photo 14. Young pycnidia.
Source: Original photo by Cotuna O. (2021).

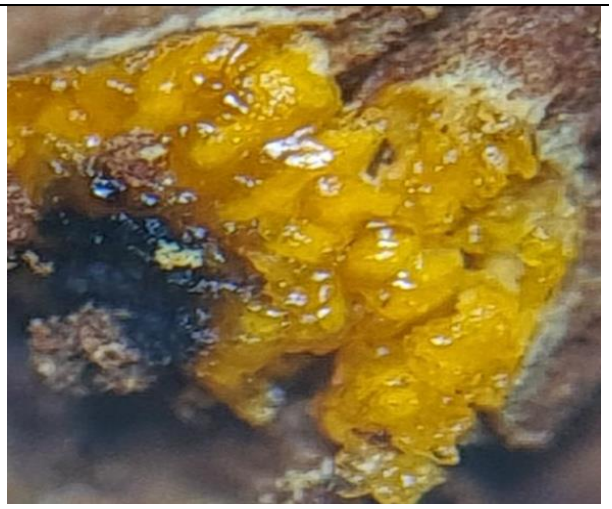


Photo 15. Pycnidia in formation.
Source: Original photo by Cotuna O. (2021).

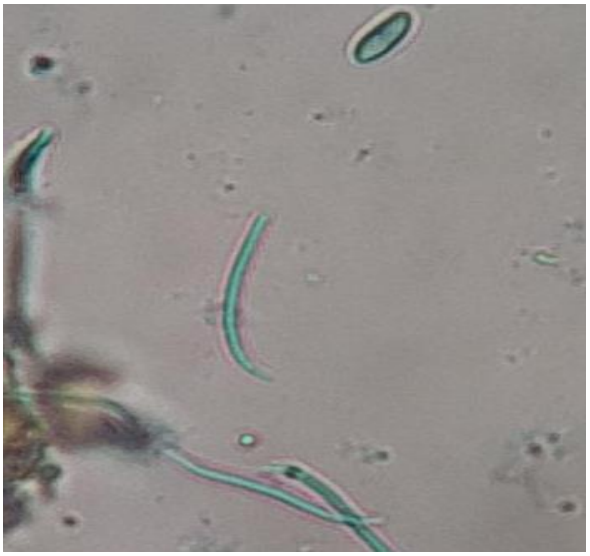


Photo 16. Alpha and beta conidia at the microscope (x40)
Source: Original photo by Cotuna O. (2021).

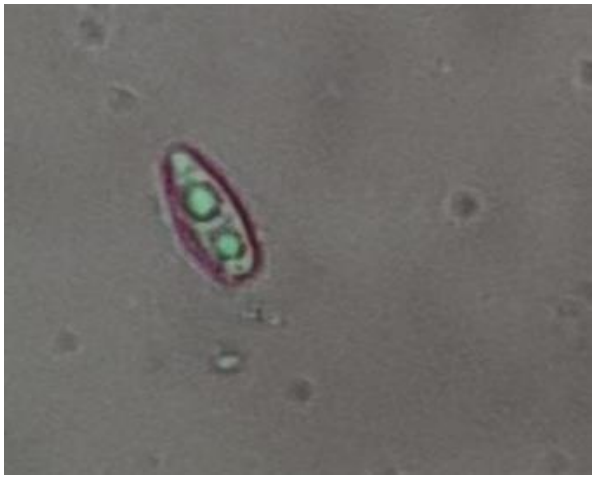


Photo 17. Alpha conidia at the microscope (x40).
Source: Original photo by Cotuna O. (2021).

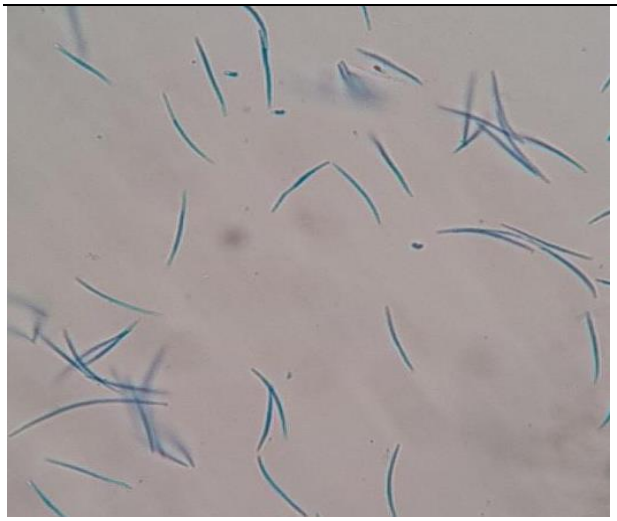


Photo 18. Beta and alpha conidia at the microscope (x40)
Source: Original photo by Cotuna O. (2021).

laboratory analyses is confirmed the diagnosis of *Phomopsis* tree canker produced by the fungus *Phomopsis mali***.**The correct diagnosis is necessary to exclude other fungi that are producing similar symptoms (*Botryosphaeria* sp., *Cytospora* sp.).

We cannot know for sure if the *Phomopsis* species identified in this research is certainly *Phomopsis mali*. Thus, the external symptoms, the host, the present fructifications, the shape of the conidia, the colour of the gelatinous masses that were drained from the pycnidia are conducting us to this species, although quite present in the orchards from Romania.

The pathogen *Phomopsissp*. is a destructive fungus, with potential in production of important loses in the fruit yield (the spur sprigs are killed by the disease). In the very severe situations, with high attack intensities the trees will be in decline. The most severely affected can be the young ecological plantations where the plantings will die. For the *Phomopsis sp.* fungus the tree age doesn't matter. The pathogen can attack at any age of the three. Sometimes it sets even in the young trees from the nurseries.

CONCLUSIONS

After the proceeding of analyses from the field and from the laboratory we can assume that the pathogen identified in the apple tree orchard from Arad County cultivated in ecological system is a species of *Phomopsis*, possibly *Phomopsis mali.*

The most attacked apple variety was GoldRush, followed by Primiera and Golden Orange. In these varieties the trees were died in a rate between 16-17%. The young ecological orchards are susceptible to the attack of the destructive pathogens as is *Phomopsis* sp. The control possibilities in these orchards are practically inexistant, because the application of the chemical substances is forbidden and the biological treatments doesn't have curative effect.

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