IDENTIFICATION OF THE PHYTO - PATHOGENIC FUNGUS *CYTOSPORA LEUCOSTOMA* (PERS.) SACC. IN CHERRY TREES FROM WESTERN ROMANIA (CASE STUDY)

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Abstract. *Chronic wood infections caused by Cytospora species can be devastating to fruit trees. The Cytospora canker can be produced by many species of the genus Cytospora. Cytospora fungi commonly found in orchards are Cytospora cincta and Cytospora leucostoma. From a morphological point of view, the two species are difficult to differentiate. Cytospora species mainly affect branches, but can cause destructive infections in trunks and large branches, severely limiting the longevity and productivity of orchards. This cancer is considered serious in declining or poorly maintained orchards, where the trees are no longer vigorous. Due to infections, the trees can dry out and the economic losses are major. It can be found frequently in the improper managed orchards or old orchards were is producing important loses by the drying of the branches and even the death of the trees following the infection through lesions produced by hailstone, frost, insects and tools. Usually the most sensitive trees are the weakened ones. In the last years the fungi from the genus Cytospora become more frequent in the cherry orchards from Romania. It is problematic the fact that frequently the infected trees are young. This research is a case study based on the infection with a fungus from Cytospora genus from a cherry orchard from western Romania. The main purpose of the paper was to correctly identify the above mentioned orchard pathogen by laboratory analyses (macroscopic and microscopic). Tissue samples from branches and sprouts were placed on the culture medium for the observation of the mycelia. The prepared samples were incubated at a temperature of 24 °C, for 5 days. The results obtained show that the fungus responsible for the cherry tree death belongs to the genus Cytospora. The form and colour of the pycnidia from beneath the bark is specific to the species Cytospora leucostoma, this species being reported by other researchers too as present in the orchards from Romania. The perfect form of the fungus (Valsa) wasn't observed.*

Keywords*: Cytospora leucostoma, Cytospora canker, pycnidia, cherry, pycniospores, perithecium, lesions.*

INTRODUCTION

Canker of the branches or *Cytospora* canker or *Leucostoma* canker can be caused by many species of the fungal genus *Cytospora*. This disease is considered serious in the old orchards or in the improperly managed orchards where the trees have lost their vigour. Due to *Cytospora* canker the trees can die, this fact determining major economic loses. In the vigorous orchards where the threes are healthy, usually this pathogen doesn't appear [MINOIU *et* LEFTER, 1987]. The infections produced by *Cytospora* species can be devastating for the fruit trees (*Prunus persica, P. armeniaca, P. avium, Malus spp., Juglans spp., etc.*) and for many other ligneous species [BIGGS *et* GROVE, 2005; WANG *et al.* 2011; FAN *et al.*, 2015a]. The researches in field have shown that more than 85 ligneous plant species can be infected by this fungus [SINCLAIR *et al.*, 1987, ADAMS *et al.*, 2005, 2006; SPIELMAN, 1983; BILLS, 1996; CHRISTENSEN, 1940]. According with *Index Fungorum*, there were described about 612 species until nowadays. KIRK *et al.* (2008) have enumerated about 110 accepted species of *Cytospora*,

while all the other species names were considered synonyms of taxa described previously or treated as non-*Cytospora* species before the appearance in 2011 of the rule *one fungus – one name* [Hawksworth, 2011]. The most frequent fungi from orchards that are determining *Cytospora* canker in fruit trees are *Leucostoma cinctum* and *L. persoonii* (ascomycetes from the order *Diaporthales*, family *Valsaceae*). The anamorphic forms of the mentioned fungi are *Cytospora cincta* (= *Leucocytospora cinctum*) and respectively *C. leucostoma* (= *L. persoonii*). The morphologic differences between these two species are often difficult to be differenced. The asexual structures of the fungi (pycnidia) are forming in the canker lesions from branches and stalk, under the death bark. At the beginning pycnidia are evident as small excrescences and their top are breaking easy to expose the stromae disk-like. The disk of *L. persoonii* is white, while in the species *L. cinctum* is grey. In humid conditions the mature pycnidia produces orange or red gummy exudates that contain thousands of conidia. The conidia are released as gelatinous strings after few minutes after wetting. The teleomorphic reproducing structures (perithecia with ascospores) are forming much later, often after 2 or 3 years from the initial formation of the pycnidia [BIGGS, 1989: BIGGS *et* GROVE, 2005].

In the orchards infected with *Cytospora*, the first disease signs that can be observed easily are the chlorosis of the leaves, the drying of the shoots tips and the drying of some branches. The dry branches are becoming obvious at the summer end. At a careful examination of the branches there can be noticed bark cankers of dark colour with the centre destroyed. Usually this pathogen attacks the trees weakened by the attack of the pathogens and pests or affected by the unfavourable climate conditions and the wrong application of some technologies [MINOIU *et* LEFTER, 1987]. Usually the fungi from the genus *Cytospora* are producing severe cankers in threes and shrubs that are determining finally the drying of the branches and even the threes' death. The stalk and branch cankers appear as areas lightly sunken in bark, elongated and discoloured. The affected bark has uneven, exaggerated great lenticels, slimy leaks *etc.* most of the times the discolouring isn't very evident because the fungus kills rapidly the bark. Sometimes the fungus is developing very fast on stressed trees and the lesions aren't evident or aren't forming. The bark from above the infected cambium appears as sunken. The infected area can have different colours from yellow, brown, brown-reddish, grey till to black, depending by the *Cytospora* species. The infected interior bark and the cambium become brown-reddish and even black. Due to the infection the tissues become waterish, spongious and smelly, having macerated aspect. The wood beneath the cambium is colouring in brown [SPIELMAN, 1983].

In cherry, the colour of the wood is: light brown, brown and sometimes grey. Near to these symptoms appear oily slimy leaks on the bark surface that can have different colours. Thus, there can be observed the asexual fruiting bodies of the fungus (pycnidia) under the bark. These fruiting bodies have a hard consistence, conic form and blackish colour. During their formation is visible a whitish point in the top and it is surrounded by an irregular white area (stromae disc). This colouring disappears later they becoming black. *Cytospora* fungi can have different colours. The pycnidia are easy visible with the naked eye when the bark is detached. The infected bark is detaching very easy from the wood because is already destroyed by the pathogen. In humid conditions (but not excessive) the spores from these structures are passing into the slimy exudates with amber-like colour (in some species) or orange – reddish (in cherry). The oily-slimy drops can take different forms, but most often are looking like fine strings that are overlapping one after each other or not. Sometimes it forms curls or waves. Usually they are positioned at the edge of the cankers. The death bark can remain attached on the tree even several years and then falls dawn, detaching from the stalk in big pieces. The wood of the infected branches and stalk is browned in section. The browned area can be greater

or smaller, depending by the evolution of the infection. The edges of the browned area are usually steep [SINCLAIR *et al*., 1987; BERTRAND, 1976].

Cytospora canker has started to create problems in cherry in many orchards from Romania. Young orchards of 5 and 8 years are diseased, some of them being in severe condition when there can't be done anything to save the plants, and mostly when are cultivated sensitive varieties. The suspicion is that in these orchards aren't applied correctly the chemical treatments and the prevention measures aren't respected. Due to the lack of the specialists in field, the disease isn't identified in time. On the present climatic background (with hot summers) highly favourable for the fungal infections the *Cytospora* canker kills young trees. As is known, this is a summer disease, the maximal growth of the fungus takes place even at temperatures of 32ºC. The canker is developing best at high temperatures (July – September), when the growth of the trees is low. If in the past this disease was specific to the old trees, nowadays the disease is reported more often on young trees. From the *Cytospora* species reported in Romania, the most frequent is reported *Cytospora leucostoma* (Pers.) Sacc. Its presence was reported in the past mainly in old and improper managed orchards [MINOIU *et* LEFTER, 1987; LEFTER *et* MINOIU, 1990]. *Cytospora leucostoma* was and is still considered a relatively weak pathogen from the point of view of the infection capacity, because isn't able to infect health tissues, only the damaged ones. The spores of this fungus are spread by the raindrops and wind, being able to infect any type of lesion from the bark of the trees. The bark lesions can be due to solar burns, old bacterial cankers, holes produced by the bark and stalk insects *etc.* There must be noticed the fact that this pathogen isn't affecting the healthy tissue and bark without holes produced by the bark pests that are considered vectors [BERTRAND *et* ENGLISH, 1976; SCHULZ *et* SCHMIDLE, 1983].

The main purpose of the work was to identify correctly the pathogen from a cherry orchard from western Romania by laboratory analyses (macroscopic and microscopic) that have supposed the realisation of sections from the diseased tissue and the cultivation on culture medium.

MATERIAL AND METHODS

Biological material was sampled from a cherry orchard from western Romania. There were collected plant samples from branches, stalk and bark from a cherry from the last stage of disease. The suspicion was that the disease was set in orchard with 5 - 6 years before, considering the evidences at the moment of sample collection. The samples were analysed in laboratory macroscopic and using stereomicroscope (4x). In the first stage the samples were analysed visually for the confirmation of the diagnostic of *Cytospora* canker. At the surface of the bark were present excrescences and cankers specific to this disease. Under the bark were evidenced the asexual fructifications of the fungus named pycnidia. There were cut branch sections with different diameters having in view to observe the fungus mycelia. The sections were detached from the branches with visible browning of the wood

The stages of the sample preparations were the following: washing of the sections detached with tap water; rinsing twice with distilled water; cutting of sections with 5 mm diameter; sterilization of the tissues with alcohol 96%; rinsing twice the sections with distilled water for 5 minutes; drying of the sterilised sections on absorbent paper; preparation of the Petri dishes by sterilisation in autoclave; and preparation of the culture medium (agar). After the preparation and cooling of the culture medium, it was poured in Petri dishes and immediately the tissue samples were distributed on the plates using a pincers and the dishes were covered rapidly. The prepared Petri dishes were introduced in incubator at 24 ºC for 5 days. After 5 days of incubation there were made observations there being noticed that the

fungus started to grow on tissue samples. The examination of the tissues with mycelia was done using a stereomicroscope (4x), it being completed with microscopic examination of native samples (at 10x and 20x).

The pycnidia from beneath the bark were analysed with the stereomicroscope. For the observation of the conidia there were detached the pycnidia with a scalpel from the surface of the wood. After detaching they were washed twice with distilled water and then were introduced in sterile water for 30 minutes. In humid conditions there are released the conidia as slimy strings that can be observed at the microscope.

RESULTS AND DISCUSSIONS

Identification of the pathogen was realised by the visual analysis of the samples in laboratory. There were examined young sprouts and thick branches that have detached bark. The visual analysis was followed by the microscopic analysis (with stereomicroscope and microscope).

At the examination of the branches surface there were noticed bark cankers with dark colour and with the centre destroyed. Usually, this pathogen attacks the trees weakened by the attack of the pathogens and pests in unfavourable climate conditions and the wrong application of some technologies. The bark from above the infected cambium appears as sunken. Under the diseased bark were present pycnidia. These fruiting have hard consistence, conic form and black colour.

The visual analysis of the samples of the sprouts, branches, stalk and bark had evidenced the following symptoms: numerous excrescences at the surface of the bark that determinate a rough bark surface, exaggerate great lenticels on bark, canker lesions with different colours depending by the lesion age (yellow, brown and black) from the surface of the bark. With these symptoms appear oily, gummy leaks at the edge of the canker lesions, these being characteristic for *Cytospora* canker. These exudates are transformed in flexuous sleeves. These can be observed easily on the diseased bark. Thus, they can take different forms; respectively they can lease together or can take the aspect of waves and curls. The interior bark was affected and was presenting brown-reddish colour, patchy even black; and the wood from beneath the cambium was browned. At the level of stalk the browned tissues have macerated, spongy aspect and emitted a specific smell. In section the wood appear necrosed in different rates and forms. Under the diseased bark are evident the pycnidia of the fungus *Cytospora leucostoma*in a great number. They have black colour and are surrounded by a irregular edge of white colour (stromae disk-like) (from figure 1 to figure 10).

Fig. 1. Excrescences on the surface of cherry bark *(photo: Cotuna Otilia, 2019)*

Fig. 2. Lenticels with exaggerates dimension on cherry bark *(photo: Cotuna Otilia, 2019)*

Fig. 3. Canker lesions with different colours depending by the age of the lesion (*photo: Cotuna Otilia, 2019)*

Fig. 4. Oily and gummy leakes with different forms found at the edge of the canker lesions (*photo: Cotuna Otilia, 2019)*

Fig. 5. Drowning and decaying of the cherry bark *(photo: Cotuna Otilia, 2019)*

Fig. 6. Browning and decaying of the cherry wood *(photo: Cotuna Otilia, 2019)*

Fig. 7. Browning and necrosis in cherry wood *(photo: Cotuna Otilia, 2019)*

Fig. 8. Mycelia in wood at the branch edges *(photo: Cotuna Otilia, 2019)*

Fig. 9. Diseased bark is detaching easily *(photo: Cotuna Otilia, 2019)*

Fig. 10. Pycnidia of the fungus *Cytospora sp.* black colours, surrounded by a irregular white edge, the white disk-like is specific for the species ***leucostoma*** *(photo: Cotuna Otilia, 2019)*

The white colour of the disk-like is specific for the species *leucostoma*. According with MINOIU *et* LEFTER (1987) the pycnidia of the fungus *Cytospora leucostoma* are grouped under the bark, and have dimensions between 1000 – 1500 x 600-800 µ. Other symptoms typical for the development of *Cytospora* disease there were noticed mycelia in wood at the edge of the branches. Randomly the bark was decayed and detached. On the stalk surface the apparently health bark was detaching easily.

Analysis with stereomicroscope and microscope

This analysis was done after the preparation of the tissue samples and their introduction on culture medium (agar solution) and placed for 5 days in incubator at a temperature of 24ºC. On the prepared sample tissues was growth the white mycelium specific for the fungus *Cytospora sp.*. For the microscopic analysis of the conidia there were detached the pycnidia from beneath the bark. The pycnidia were introduced in sterile water for 30 minutes. In the presence of water the conidia (pycniospores) were released from pycnidia and it was possible to analyse them to the microscope (from figure 11 to figure 13).

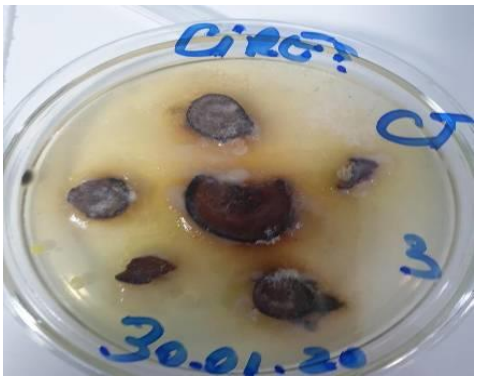


Fig. 11. White-gray mycelia growth on the surface of the sections after 5 days of incubation *(photo: Cotuna Otilia, 2019)*

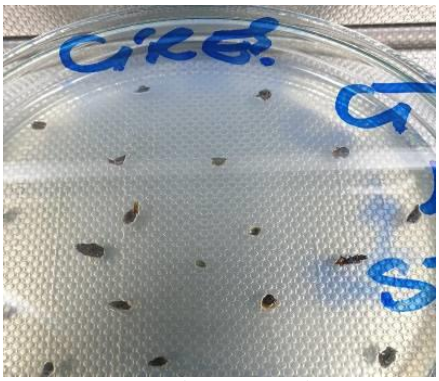
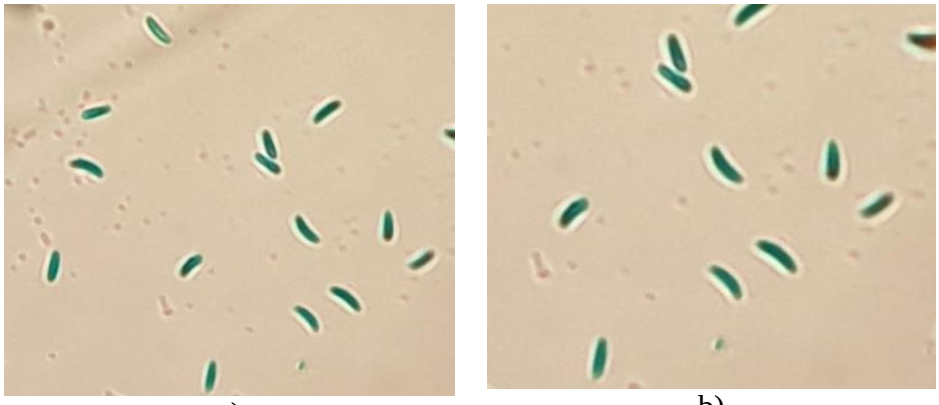


Fig. 12. Detaching of the pycnidia from the cherry wood and placing in sterile water for 30 minutes for the microscopic analysis of the conidia *(photo: Cotuna Otilia, 2019)*



a) b)

Fig. 13. *Cytospora leucostoma* conidia at microscope (a – 10x, b – 20x) *(photo: Cotuna Otilia, 2019)*

Pycniospores are unicellular, hyaline and their size can be comprised among 4 – 7.8 x 1.2 – 1. 5 µ. In rainy weather conditions the pycniospores are released as limy strings of several millimetres length [MINOIU *et* LEFTER, 1987]. After the laboratory analysis the diagnosis of ***Cytospora* canker** produced by the fungus ***Cytospora leucostoma*** in the analysed cherry trees is confirmed.

The mycelium of the fungus growths in phloem and xylem and is obstructing them. The bark of the trees can be attacked during the spring, autumn and winter. The susceptible trees to infection are those affected by drought, solar burns, herbicides or mechanical injuries. The most susceptible are the trees with the lesions at the level of root system and the transplanted ones.

Cytospora canker is a disease that unfortunately cannot be controlled chemically even the efficiency of the chemical treatments is intensely studied nowadays. The results of these studies show that there are differences between treated and non-treated. But with all of these the pathogen cannot be chemically controlled. Even the efficiency of some fungicides wasn't confirmed, it isn't refused too. There are many reasons that are determining the inefficiency of the chemical control. The main reason from those reasons is the great production of spores released by the fungus that covers a long time interval. The climatic conditions are the ones that can favour and disfavour the spores production, there being well known that during the periods with high temperatures and rainfall amounts the spores production grows and decrease during the periods with low temperatures and rainfall amounts.

The canker of the branches is considered critical in the old orchards or in the improperly managed ones, respectively where the trees have lost their vigour. In the healthy orchards, where the treatments against diseases and pests and maintenance works are executed properly, this pathogen isn't installing.

CONCLUSIONS

The results obtained after the laboratory analysis are supporting the *Cytospora* canker diagnostic, the identified fungus as responsible for the cherry tree drying are belonging to the genus *Cytospora*. The form and colour of the pycnidia from beneath the bark is characteristic for the species *Cytospora leucostoma*, this species being reported by other researchers too as being present in the orchards from Romania. From the pycnidia introduced in sterile water

there was released the unicellular pycniospores, with hyaline aspect as is characteristic for this fungus. There weren't noticed the perithecia, respectively the perfect form of the fungus.

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Aknowledgements

This research was possible with the logistic support of the laboratory "***Invasive Species Monitoring Unit***" from the Agriculture Faculty of Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara.