RESEARCH REGARDING THE PATHOGEN AGENT *Dothistroma septosporum* (Dorog.) M. Morelet ON *Pinus cembra* L.

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Abstract

The asexual form of the fungus *Mycosphaerella* attacks most species of pine trees and produces major damage in the woody nursery. This pathogen has had only seven years after the first infection in Tanzania to reach nurseries in central or eastern Africa. Global losses resulting from epidemics in the world were major. There were losses of 67% to *Pinus radiata* trees 7-8 years of age in California, total losses of *P. ponderosa* trees in most eastern states of the United States of America and 40% loss of trees *P. flexilis* in Montana (Taylor, Schwandt, 1998). Within this paper observations were performed in the laboratory and in the field, determinations were made based on symptoms and morphological characteristics of the fungus. To achieve these observations and measurements samples were taken every ten days for three months from The Botanical Garden of Iaşi, from the species *Pinus nigra* and *Pinus cembra* damaged by Dothistroma septosporum.

Key words: *Dothistroma septosporum*, *Mycosphaerella pini*, *Pinus* sp., red band needle blight.

Asexual form of the fungus *Mycosphaerella pini* was first described in Russia under the name of *Cytosporina septospora* (Doroguin G., 1911). Thirty years later in 1941 Hulbar reports this fungus in Illinois, under the name of *Dothistroma pini* (Hulbary R.L., 1941). Moerelet considered the two forms as identical and gived a new name *Dothistroma septospora*, this nomenclature has also been accepted by Sutton in 1980 (Sutton B.C., 1980).

Depending on the length of the conidias three conidial forms were determined by Thyr and Shaw in 1964, *Dothistroma pini* var. *pini* with the conidia length between 15.4-28.0 µm and *Dothistroma pini* var. *linearis* with the conidia length between 23.0-42.0 µm (Thyr D.D., 1964). *Dothistroma pini* var. *pini* is found in central and eastern North America, England, New Zealand, Australia and Chile, while *Dothistroma pini* var. *linearis* is found in western Canada and the United States of America (Ivory M.H., 1967, Peterson G.W.,1974, Edwards D.W., 1978). The third conidial form, *Dothistroma pini* var. *keniensis* is one of intermediate size 13.0 - 47.5 µm, and the name was given by Ivory in 1967, after the country where this form can be observed predominantly, namely Kenya (Ivory M.H., 1967). In 1980, Sutton changes the name of this fungus, and *Dothistroma pini* var. *linearis* becomes *D. septospora* var. *lineare*, *Dothistroma pini* var. *pini* becomes *D. septospora* var. *septospora* and *Dothistroma pini* var. *keniensis* becomes *D. septospora* var. *keniense* (Sutton B. C., 1980).

In 1967, Gadgil, reveals large variations of such conidial length and starts to doubt this classification system based on the length of conidia, therefore proposed that one conidial form will be accepted regardless of the length (Gadgil P.D., 1967). In the same year, Ivory, reveals that the length of conidia of isolated cultures is greater than the length of the conidia determined on infected needles (Ivory M.H., 1967). In recent years similar studies have been conducted but none of them has made a clear distinction between these varieties based on length of conidia, or based on DNA analysis (Edwards D.W., 1978, Roux C., 1984, Bradshaw et al., 2000).

Mycosphaerella genus is considered by many as polifiletic because more than 40 forms of asexual fungi are associated with this genre. Barr in 1996 separates the asexual species *Lecanosticta* and *Dothistroma*, and fits them into a new genre called *Eruptio*, based on the idea that these two species differ from other species of the genra *Mycosphaerella* (Barr M.E., 1996). After the ITS analysis it was concluded that the *Mycosphaerella* genre is actually monophyletic and sexual forms of species *Dothistroma* and *Lecanosticta* should remain within this genra (Goodwin S. B., et al 2001).

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Since 1950 this pathogen has spread rapidly, from it's first discovery in 1957 in Tanzania it only took seven years for the disease to be observed in all the *Pinus radiata* nurseries in Central and East Africa. The causes of the spread of this pathogen are not very well known but key factors are wind, raindrops and sapling from nursery already infected (Gibson I.A.S., 1974).

Regarding the case of New Zealand, following repeated tests proved that almost all samples infested with the pathogen are clones, suggesting that one infestation was necessary to spread the disease at an entire country (Hirst P. et. al., 1999). In Germany it was determined both the sexual and asexual form on *Pinus mugo* in 1983 and the cause was probably the introduction of infected *Pinus nigra* seedlings in nurseries (Butin H., 1983).

Most host plants are *Pinus* species, but to this list were added other species such as *Pseudotsuga menziesii* by Gibson in 1979 (Gibson I.A.S., 1979), in 1987 *Larix decidua* and *Picea abies* were added by Lang (Lang, K.J., 1987). The most susceptible species of pine in the EPPO region to this pathogen agent are: *P. canariensis*, *P. contorta*, *P. halepensis*, *P. muricata*, *P. nigra*, *P. pinea*, *P. ponderosa*, *P. radiata*, *P. thunbergii*. Many Central American pine species are resistant or immune to this pathogen, but *Pinus nigra* proved to be particularly susceptible to climatic conditions and culture in Europe, while *Pinus sylvestris* was attacked rarely even then when it was located in proximity to infected *P. nigra*. (Lang K.J., 1987). *Pinus radiata* is one of the most important pine species in economic terms, but it's also one of the most susceptible, some individuals develop resistance with age while some species such as *P. ponderosa* remain susceptible during their entire life (Gibson I.A.S., 1972).

Dothistroma septosporum, the asexual form of *Mycosphaerella pini*, is widespread in the countries in wich susceptibile species to this pathogen agent are in different conditions from those of their natural habitat. The sexual form of the fungus was determined in a narrower area predominantly in Northern Hemisphere countries including: Canada (Funk A., 1966), United States of America (Cobb F.W., 1968, Peterson G.W., 1974, Peterson G.W., 1976), Germany (Butin and Richter, 1983), Serbia (Karadzic D., 1989), Poland (Kowalski T., 1998) and Portugal (Fonseca N., 1997).

The largest economic losses were seen in countries such as New Zealand, Chile and South Africa, where some pine species susceptible (*Pinus radiata*) to *M. pini* were cultivated intensively. In New Zealand were recorded for example losses of 10% from the total of 45,000 hectares (New D., Griffith J.A., 1989).

In 1963 in Kenya over 1,500 hectares of P. radiata were destroyed by the disease, and in their place were planted other pine species less susceptible to this fungus (Gibson I.A.S., et. al, 1964).

MATERIAL AND METHOD

Samples of *Pinus cembra* and *Pinus nigra* were taken every 10 days for three months starting from February. Macroscopic observations and sampling of *Pinus* spp. were performed in the Botanical Garden of Iaşi. Pine needles that show symptoms were placed in humid chamber at a temperature of 28ºC for 24 hours, and the attacked areas were sectioned in 0.5 mm portions, which were examined under a microscope to determine the morphology of the pathogen agent. Based on morphological characters the systematic classification was performed and afterwards the determining of the fungus.

RESULTS AND DISCUSSIONS

From March a series of symptoms were observed on the studied *Pinus* spp. specimens from the Botanical Garden of Iaşi.

On the infected needles the pathogens fructifications are observed that are initially white and subepidermal. Over time these fructifications changes color to dark brown, and as the pathogen develops the epidermis breaks.

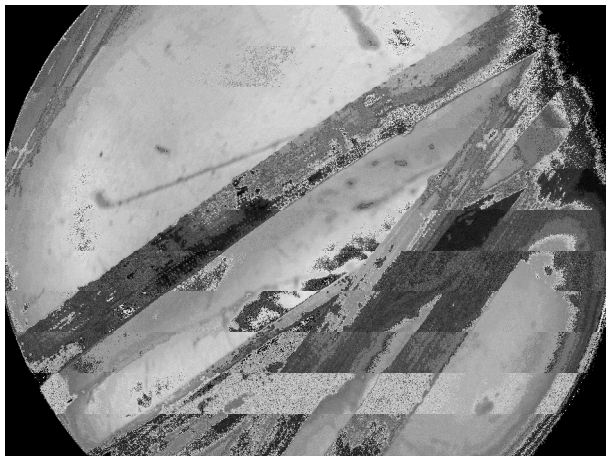

Figura 1 ***Dothistroma septosporum* fructifications on *Pinus cembra*** (original)

In the first stage on the needles yellow bands with a small area of necrosis at the top appear. Next to these necrotic areas a lignification occurs due to excess resin in response to the attack of the host plant. In 20-30 days, the yellow bands, due to the presence dothistrominei, turned deep brick-red,

and the needle turns fully brown. After the browning within 10 days the needles shuddered.

On *Pinus nigra* the symptoms were observed on the basal branches first and then they forwarded to the tip shaft, while on *Pinus cembra* initial symptoms were observed inside the crown, and with time they have extended to the outer branches.

Symptoms are described in the literature on basal branches first, then they advance towards the interior of the shaft, specimens were analyzed almost completely shaken although these trees age was just 3 years.

On the surface these bands can be seen fructifications reddish spherical black, round or oval with dimensions between 300-1500 µm long and 300 µm wide (fig.1). The symptoms observed in the field coincide with those described by Edwards and Walker in 1978.

The pathogen agent *Dothistroma septosporum* is part of the kingdom *Fungi*, phylum *Ascomycota*, subphylum *Pezizomycotina*, class *Dothideomycetes*, order *Capnodiales*, family *Mycosphaerellaceae*.

In the infected tissues hyaline, filiform, smooth, thin-walled, slightly curved, with 1, 3 or 5 tanks conidia were determined, ranging in size from 18-31 x 2-2,5 µm (fig. 2). The size and morphology is included in those cited in the literature.

Figura 2 ***Dothistroma septosporum*** **conidia** (original)

Regarding integrated control, planting seedlings free of pathogens or resistant species were recomended as phytosanitary measures and if there was attack in the nursery, a minimum of six months before the establishment of a new culture would be recommended (Bradshaw R. E., 2004).

Chemical control is performed with products based on copper oxychloride such as ALCUPRAL 50 PU (WP) in concentration 0,3% and CURENOX 50 (WP) in concentration 0,4%. In the previous studies copper oxychloride was observed to destroy *Dothistroma septosporum* spores (Koltay A., 2001).

Dothistroma septosporum is a quarantine pathogen included in the A2 list of EPPO association since 1992 and the main phytosanitary risk is the importation or exportation of seedlings.

CONCLUSIONS

In the climatic conditions of 2015 in Moldavia, pathogen *Dothistroma septosporum* produced significant damage to crops of *Pinus* sp.

Specimens damaged by this pathogen presents a series of brick-red strips, about 1-2 mm wide and spherical black, round or oval fructifications with a size between 300-1500 µm long and 300 µm wide can be observed on the reddish surface of these bands.

Although symptoms are described in the literature on the basal branches first, and then they advance towards the interior of the shaft, the analyzed specimens were almost completely shaken although they were only 3 years old.

Observations under a microscope showed hyaline conidia, filiform, smooth, thin-walled, slightly curved, with 1, 3 or 5 tanks, with dimensions between 18-31 x 2-2.5 µm falling within those cited in the literature.

Regarding integrated control, planting seedlings free of pathogens or resistant species were recomended as phytosanitary measures and if there was attack in the nursery, a minimum of six months before the establishment of a new culture would be recommended.

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