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# THE INFLUENCE OF PESTICIDES ON THE GROWTH OF THE FUNGUS *DIPLOCARPON ROSAE WOLF*.

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***Abstract***
*The fungus Diplocarpon rosae (anamorph =* Marssonina rosae *Lind.) is the causal organism of rose black spot, which is the most damaging disease of rose worldwide.On rose, black spots with fringed margins appear on either leaf surface but predominantly the upper surface of leaves. Infected leaves turn yellow, except for the black spots, and drop from the plant. Similar symptoms occur on petioles and fruit. Flower petals develop red spots and some distortion. Canes may become infected during the first year of development. The influence of different pesticides used in rosa on the growth of the fungus Diplocarpon rosae, a natural pathogen of Rosa, was evaluated under the laboratory conditions. The pathogen was grown on PDA (potato - glucose - agar) and Czapek Dox culture media in order to track its dynamics and preferences over nutrients. The fungicides studies were difenoconazol (Score 250 EC), miclobutanil (Systhane 12 E), cooper (Champ 77 WG) and boscalid + piraclostrobin (Signum 50 WP). Good results were obtained with difenoconazol and boscalid-piraclostrobin which inhibited growth of fungus.*

*Keywords: Diplocarpon, fungicides, Rosa*

## 1. INTRODUCTION

The pathogen *Diplocarpon rosae* is part of the Fungi Kingdom, the *Ascomycota* lineage, the *Leotiomycetes* class, the *Helotiales* order, the *Dermataceae* family, the *Diplocarpon* genus.
The fungus *Diplocarpon rosae* (anamorph = *Marssonina rosae* Lind.) is the causal organism of rose black spot, which is the most damaging disease of rose worldwide (Dobbs, 1984). Symptoms include lesions on leaves and stems as well as frequent leaf yellowing and defoliation that significantly compromise plant growth and appearance. Consequently, numerous topical and systemic fungicides are used by homeowners and landscapers to combat this disease. *Diplocarponrosae* is classified as an ascomycete in the family Dermateaceae and is a hemibiotrophic fungus that is restricted to the genus *Rosa* L. (Blechert and Debener, 2005). It is spread primarily through waterborne, two-celled asexual spores (conidia) that require free water to germinate. Visual symptoms may develop in susceptible cultivars in as little as 4 d after spore germination and penetration. The sexual stage of this fungus has been reported only twice in North America and twice in England (Horst, 1983), although frequency of sexual recombination in *D. rosae* populations has not been determined.The fungus attacks the leaves, the young branches, the strips, the sepals and the petals, which cause the appearance of blackish spots, sometimes of a purplish hue, of circular shape with the precisely delimited edges, at first small. Usually, the spots are visible on the upper side of the leaf, but in some cases these spots can be seen on the lower side.



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They can reach a diameter of 12-18 mm and can come together and occupy a large part of the attacked organism. Over time, the stains take on the gray-blackish color, and on their surface small, prominent spots appear, visible to the naked eye. The strongly attacked leaves are easily detached from the branches and fall. Quite often the appearance of black spots on the young branches. On the stamens, petals and sepals, the attack of the fungus is less frequent (Agrios, 1997).

## 2. MATERIALS AND METHODS

The isolates used in this study were obtained from leaves of *Rosa* spp. The isolates were cultivated on culture media, namely: potato dextrose agar (PDA) and Czapek Dox. The experiments included five variants for each medium and minimum four repetitions per variant (Petre, 2015).
The pesticides studied were selected from wide spectrum of the pesticides commonly used in strawberry cultivation. Pesticides were added to sterilised medium after cooling to approximately $40^0$ C. Plates were incubated at $24^0$ C. Colony diameter was measured every three days for 21 days.

## 3. RESULTS AND DISCUSSIONS

Höhnel showed that the type of the genus *Gloeosporium*, namely *Gloeosporium chestnut* Desm. & Mont, has the conidia 1-septate, the species being described by Magnus in 1906 within the genus *Marssonina*. Von Arx considers the two genera identical, but opts for the conservation of the genus *Marssonina*.
A complete revision of the *Marssonina* genus has not yet been performed and it is likely that this genus will be fragmented into smaller generic units based on the variability of conidial morphology, conidiogenesis and teleomorphic connection.
The mycelium is submerged in the substrate, hyaline, branched, septate, forming blackish stroma on which the hoppers differentiate (fig.1.). The hoppers are subcuticular, dark-brown, isolated, sometimes associated, with a pseudoparenchymatic subiculum, 56.5-156 x 14.5-45.5 μm. Conidiophores are hyaline, irregularly branched, septa, smooth, compressed in the canopy. Conidiogenesis is anelid holoblastic. The conidia are hyaline, 1-septate, strangled by the septum, with the cells unequal, the base truncated, the apical cell rounded at the end is slightly thicker than the lower cell which is narrowed slightly towards the base, gutted, slightly curved, of (-10-) 15.5 - 17.5 (-19) x (-4-) 7 (-8) μm (after Sutton 13.5 - 16.5 x 4.5-5.5 μm, 1980).

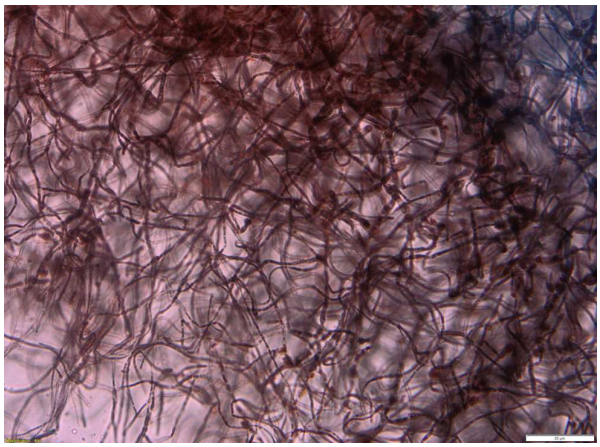

***Figure 1. Mycelium hyphae of Diplocarpon rosae***



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The pathogen was grown on PDA (potato - glucose - agar) and Czapek Dox culture media in order to track its dynamics and preferences over nutrients.
On the PDA environment, the fungus presents a whitish mycelium with a circular development from the inoculation point. In the center of the growth point, the mycelium hyphae have a yellowish color on a diameter of 3 cm (fig. 2). On this culture medium (rich in starch and glucose, two carbohydrates essential in the metabolism of fungi), the growth of the fungus is very fast, in 6 days it reaches a diameter of 6/6 cm, after 10 days the surface of the Petri dish is completely covered.
The mycelial hyphae are hyaline, septate, richly branched.
As an acervular fungus forms acervular conidiomes. Conidiophores are hyaline, irregularly branched, septa, smooth, compressed into the acervuli. The conidia are hyaline, 1-septate, strangled by the septum, with unequal cells.
On the Czapek Dox environment, the fungus presents a reddish mycelium with a circular development from the point of inoculation. Being an environment rich in mineral and sucrose elements, the dynamics of the pathogen was clearly superior to that of the PDA environment. The difference in color of the hyphae is explained by the additional presence in the Czapek Dox environment of iron, sodium and potassium (fig. 3, 4)

***Figure 2. Diplocarpon rosae on PDA medium***

***Figure 3. Diplocarpon rosae on  Czpek Dox medium***


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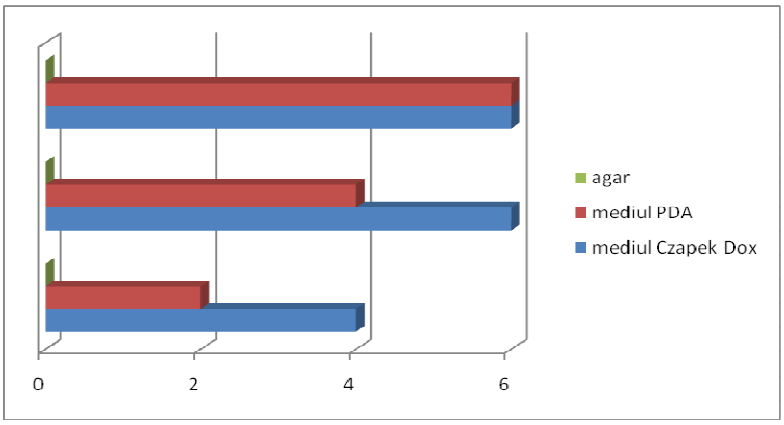


***Figure 4. The influence of the culture environment on the growth and development of the Diplocarpon rosae***

The Score 250 EC (difenoconazol 250 g/l) fungicid (cloro – fenoxi – clorofenil – metil – dioxalan – metil – triazol, *IUPAC* - *cis-trans*-3-chloro-4-[4-methyl-2-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether, *CAS* - 1-[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole) it is of Swiss origin, the molecule of which was obtained in the laboratories of Ciba-Geigy. Its active substance, in its pure state, is a white crystalline powder, very little soluble in water, soluble in organic solvents. It is a local systemic fungicide, with a very broad spectrum of action, especially active on pathogens of the genera: *Alternaria, Septoria, Cercospora, Ramularia, Venturia, Phoma, Erysiphe, Pseudopeziza, Uncinula, Puccinia, Uromyces, Podosphaera* etc., preventing their entry into the host, formation of breakers and growth of mycelium. It is widely used in the control of foliar diseases in cereals, but also in fruit and vegetable growing. It is not toxic to mammals and bees, but it is dangerous to aquatic organisms, especially to fish.
Applied in the concentration of 0.05% in our experiments, from the analysis of figures 5, the strong fungistatic character on the pathogen is observed.

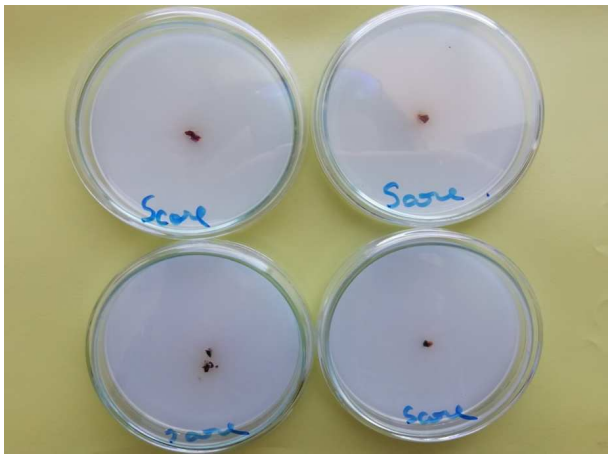


***Figure 5. Score 250 EC – culture of 10 days***

The Systhane 12 CE (miclobutanil 125 g / l) - (RS) -2- (4-chlorophenyl) -2- (1H-1,2,4-triazol-1-ylmethyl) hexanenitrile, CAS - α-butyl-α - (4-chlorophenyl) -1H-1,2,4-triazole-1-propanenitrile,. is



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a local systemic fungicide of American origin (Rohm and Haas Company / Phil.), from the group of demethylation inhibitors (DMI) in the ergosterol biosynthesis process. The active substance is a light yellow crystalline powder, soluble in water (142 mg / l at 250C) and in most organic solvents.It has a wide spectrum of action, acting preventively and curatively on many diseases, especially powders, rusts, monilose, fennel.
Slightly toxic to humans, but irritating to eyes and harmful if swallowed. It has minor ecotoxicological effects, but being toxic to aquatic organisms, it can have direct and indirect adverse effects in these ecosystems.
Applied in the concentration of 0.03% in our experiments, from the analysis of figures 6, the slightly fungistatic character on the pathogen is observed.

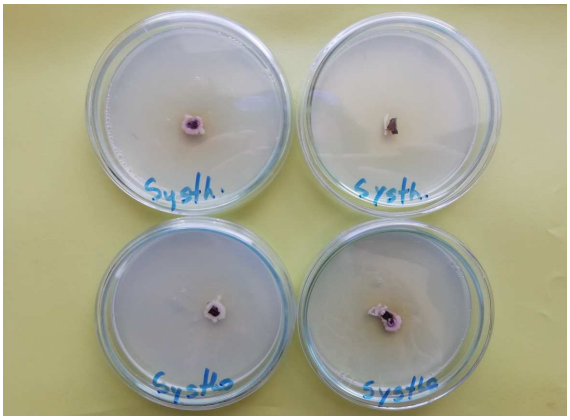


***Figure 6. Systhane Forte – culture of 10 days***

The Champ 70 WP (50% copper hydroxide) copper (II) hydroxide, IUPAC - copper (II) hydroxide or copper (2+) hydroxide or copper hydroxide, CAS - copper hydroxide (Cu (OH) 2) is the only well-defined copper hydroxide. It can be obtained by adding alkaline hydroxide to the solution of a copper (II) salt. By drying it is transformed into an amorphous powder, with a variable water content. Crystallized copper (II) hydroxide can only be obtained at low temperatures and under special conditions. It is more stable than amorphous.
Copper (II) hydroxide is considered the most active copper compound, being the basis of numerous formulations with fungicidal and bactericidal action, both alone and in various mixtures with different inorganic (sulfur) and organic fungicides (mancozeb, maneb, metiram). zinc, captan, fenamidon, folpet, etc.).
Applied in the concentration of 0.3% in our experiments, from the analysis of figures 7, the slightly fungistatic character on the pathogen is observed.
Signum fungicide 50 WP (26.7% boscalid + 6.7% piraclostrobin) this fungicide combines two new substances, with different modes of action: piraclostrobin, from the strobilurin group and boscalide, from the carboxamide group. Signum has a double movement in the plant: translaminar and acropetal, systemically local. It acts by inhibiting the germination of the spores and the growth of the germinal tube, but also on the sporulation. The special, different, mode of action of the active substances, together with their combinatorial effect, determines the achievement of an unusually broad spectrum of control against pathogens. Also, due to its selectivity, it can be used in several cultures.


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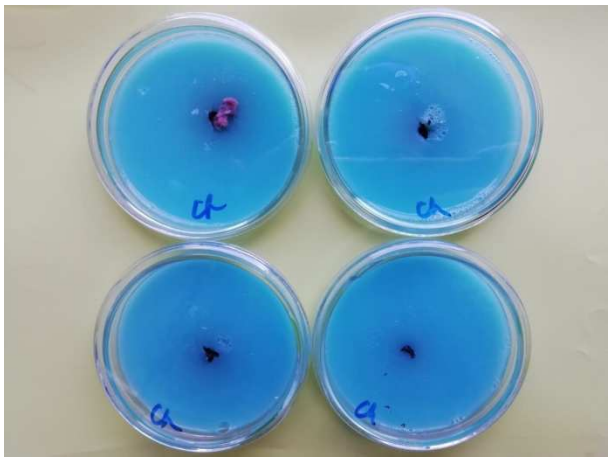

***Figure 7. Champion 50 WP – culture of 10 days***

Early application in the concentration of 0.15%, especially preventive, results in excellent efficacy against pathogens (fig. 8).

***Figure 8. Signum® – culture of 10 days***

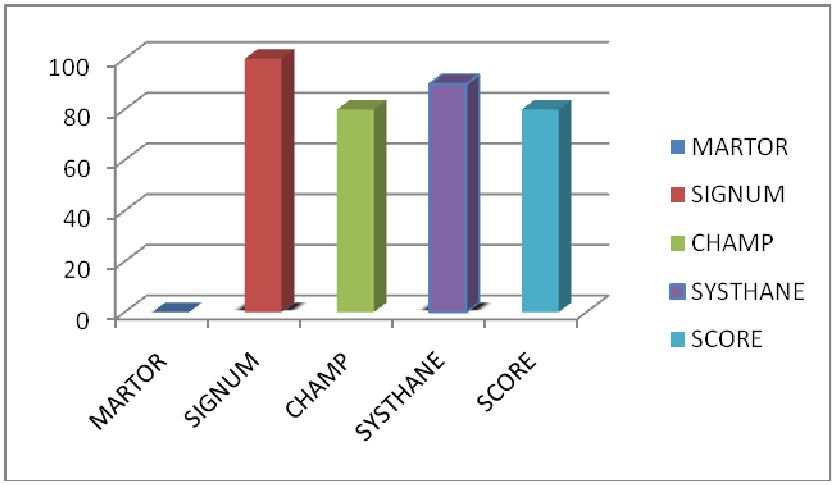


***Figure 9. Effectiveness of treatments against the pathogen Diplocarpon rosae***



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## 4. CONCLUSIONS

In assessing the overall effectiveness of a plant protection product, other aspects, positive or negative, are taken into consideration: the duration of its biological activity (persistence of action), compatibility with different protection strategies or cultural practices, application facilities, etc.
The net result of the positive and negative effects of a new plant protection product should be a sufficient agricultural benefit to justify its introduction and use in production (fig. 9).
The direct biological efficacy of plant protection products depends on many factors, namely: the chemical nature and mode of action of the active substance, the concentration or dose of the active substance or commercially applied product, the form of pesticide conditioning, the duration of action of the active substance, the conditions environment (temperature, humidity, light, etc.), the stage of development of the pathogen, the application equipment for the plant protection product etc.
The direct effectiveness is evaluated under conditions as close as possible to those of use in the agricultural practice of the product. This means, depending on the case, the evaluation in field experiments or in greenhouses.

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