

BIOLOGICAL PARAMETERS OF SOME PLANT PATHOGENIC FUNGI USED AS MODEL IN THE PRECISION MANAGEMENT OF SUGAR BEET DISEASES IN ROMANIAMaria Iamandei^{1*}, Maria Oprea¹, Mihaela Cioloca²¹Research-Development Institute for Plant Protection- Bucharest, Romania²National Institute of Research and Development for Potato and Sugar Beet-Brasov

*Correspondence address:

Romania, Bucharest 013813

Blv. Ion Ionescu de la Brad no. 8

Tel. 004-021-2693231, 2693234, fax: 004-021-2693239

E-mail: maria_iamandei@yahoo.com

Abstract: The biological parameters of *Cercospora beticola* and *Ramularia beticola* fungi were studied at R.D.I.P.P. Bucharest Mycology laboratory, using a modified Tuite method. It was established how the fungi colonies' development were influenced by: abiotic factors (temperature, relative humidity), the pH values of the substrate on which they grow, the duration of the incubation period, the energetic and plastic sources. Knowing these elements a prognosis model can be established related to the attack of the two fungi in field conditions, and used in the precision management of the sugar beet crop's diseases.

Key words: *Cercospora beticola*, *Ramularia beticola*, sugar beet, precision management

INTRODUCTION

Annually, the Romanian sugar beet crops are affected by plant pathogenic fungi that negatively influence the quality and lead to the decrease of the production. Among these, the cercosporiosis produced by *Cercospora beticola* fungus is found in all the areas with sugar beet crops, it is favored by warm and rainy summers, and it can be found mainly in the sprayed crops, where it produces the repeated defoliation of plants, followed by a massive decrease of roots production and of sugar content (Dumitrascu & Sesan, 1988; Doncila et al., 1992). Cercospora Leaf Spot caused by *Cercospora beticola* is one of the most destructive foliar disease of sugar beets in all sugar beet-growing areas with world-wide economic importance (Malandrakis et al., 2006; Yonggang L. & Fengming M., 2011). Another pathogen fungus, *Ramularia beticola*, affects the crops in the summers that register lower temperatures and not many rains, leading to the sterilization of 10 – 20% of the flowers and to a decrease of 5 – 10% of the production.

In natural conditions, the evolution of pathogens depends on the environment factors, that's why the first step in researches is the study of the two fungi's biological parameters, in order to establish the needs in regard to certain abiotic factors (temperature, relative atmospheric humidity), pH values of the substrate they grow on, the duration of the incubation period, the energetic and plastic sources. The study is necessary for anticipating the moment when the first infections appear and their evolution in the field, depending on the year's climatic conditions. Both species will be used on the models conceived for the precision management of sugar beet crop diseases.

MATERIAL AND METHOD

The two fungi were isolated from sugar beet leaves that presented symptoms of the disease (figure 1 and 2), collected from various sugar beet crop fields located in Brasov area. Under laboratory conditions, at R.D.I.P.P. Bucharest Mycology Laboratory, the abiotic factors that influence the development of fungi were established by applying the modified Tuite method (M. Oprea & M. Iamandei, unpublished).

RESULTS

Cercospora beticola Saac. fungus cultivated on a PDA (Potato-Dextrose-Agar) medium formed in the first phases hyaline hyphae, with rare septa and rich content, and at maturity the hyphae colored in

brown, remaining septal and branched. The in vitro development activity of the fungus was influenced by the conditions created in the laboratory, as following:

- ❖ *Influence of the temperature:* The minimum temperature for the formation of the colonies was 2°C, these developed in the shape of a lax mycelium, light brown colored, a yellowish reverse; the fructifications were absent. The aspect was similar at temperatures of 6°C. The 8°C temperature determined a better development of the colonies, thus the mycelium is compact, with a soft aspect, brown-yellowish colored, with a light brown reverse; the presence of the conidia was noticed, which were rare at the surface of the mycelium. A change was registered at 16°C, when the colonies formed a vegetative mass and a good sporulation. The optimum temperature was comprised between 18 – 24°C, the colonies formed on the medium had the specific characteristics of a well developed culture. Over 26°C the development of the colonies was weaker, as well as the number of the formed conidia, and the maximum temperature for forming the colonies was of 32°C, when these appeared like a mycelium, without forming conidia. The lethal temperature was considered 36°C, when the fungus was totally inhibited in its growth (see diagram from figure 3).
- ❖ *The relative humidity* represents an important factor in the fungus' evolution (graphic 2). At values of 15%, the conidia didn't develop. Under an relative humidity of over 36,8%, the formed mycelium was lax, and the conidia didn't form. Under values of 66 – 72%, the formed colonies had a felty aspect, light brown colored with white borders, and the fructifications didn't form. Starting with values of over 75,6% the conidia started to form, developed at the surface of the colonies. As the values of the relative humidity increase, the development of the colonies is very good, the vegetative mass is dense, felty, brown colored, and the fructifications are sometimes abundant (figure 4)
- ❖ *The influence of the pH values on the Cercospora beticola fungus colonies development.* pH values of the substrate where the fungus developed comprised a large spectrum, the colonies formed a good vegetative mass and the conidia appeared starting with the pH value of 3; the optimal values were comprised between 4 and 7. Along with the alkalization of the medium, the fungus developed less vegetative, but continued to form numerous conidia. The fungus has a large development area on substrates with pH values from acid to strong alkaline (figure 5).
- ❖ *The establishment of the incubation duration in the artificial infections depending on the temperature.* The temperature and the relative humidity were important factors in the appearance of the first symptoms. After the artificial infections, for the entire duration of the experiment, a high atmospheric humidity was maintained by keeping the infected organs in moist chambers. *On infected and unwounded leaves* the incubation period was of 14 days under temperatures of 2°C and 4°C. Along with the increase of temperature, the incubation period decreased to 7 days between 8°C and 14°C, then to 2 days between 18°C and 28°C. Over the value of 32°C the infections were stopped (figure 6).
- ❖ *The plastic and energetic sources* are determined in the development of the colonies of the *Cerospora beticola* because the basic elements necessary for nutrition are carbon and azoth. In table 1 it can be noticed that the fungus metabolizes very well the carbon from the monosaccharide: glucose, dextrose, fructose, maltose, mannose, trehalose, arabinose, manit, levulose and ribose. On a substrate with polysaccharide content, like cellulose, the development of the fungus colonies is low. The carbon is metabolized from starch relatively well. In table 2 it can be noticed that the fungus easily metabolizes the azoth from the inorganic compounds like ammonium nitrate and less from potassium nitrate and ammonium phosphate. This proves that the presence of the ammonium nitrate administrated in the soil favors the production of the infections due to *Cerospora beticola*. The same is with the urea from which the fungus easily metabolizes the azoth.

Under laboratory conditions, the fungus *Ramularia beticola* Fautr.et.Lamb. is influenced in its development as follows:

- ❖ *The temperature* – the influence of this parameter in the formation and development of the fungus colonies was presented in graphic 5. The minimum temperature for the formation of the colonies was 2°C, these being represented by a lax mycelium, arachnoid, without producing conidia. The optimal temperature was of 16 – 20°C, when a very good vegetative mass was formed and the formed conidia were abundant. The maximum temperature was of 24°C, the fungus colonies were developed in a small manner, without fructification, and the lethal temperature was of 26°C, over this threshold the colonies stopped forming (figure 7).
- ❖ *The relative humidity* represents an important factor in the fungus evolution. In our experiment, at values of 15%, the colonies didn't develop. Under an relative humidity of over 36,8%, the formed mycelium was lax, and the conidia didn't develop. Under values of 70%, the colonies had a felty appearance, light brown colored with white borders, and the number of the fructifications was pretty high. Starting with values of over 75,6%, the development of conidia was noticed, formed at the surface of the colonies. Along with the increase of the relative humidity the development of the colonies is very good, the vegetative mass is dense, felty, gray, and the fructifications are numerous (figure 8).
- ❖ *The influence of the pH values:*The pH values of the substrate on which the fungus develops influences the growth and the fructification. We have observed that there is a large spectrum, the colonies are forming a good vegetative mass with the appearance of conidia at the pH value of 3, the optimal values are between 4 and 7. Along with the alkalization of the medium, the fungus developed less from a vegetative point of view, but it continued very well to produce fructifications. The fungus represents a large aria of development on substrates with pH values from acid to strong alkaline (figure 9).

CONCLUSIONS

By studying the influence of the temperature on the development of the colonies of *C. beticola*, the following values were observed: the minimum temperature (2°C), the optimal interval (24 – 28°C), the maximum temperature of 32°C and the lethal one for the conidia germination of 36°C, and for *R. beticola* the values obtained were: the minimum temperature of 2°C, the optimal interval of 16 – 20°C, the maximum temperature of 24°C and the lethal one for the conidia germination of 26°C.

Regarding the influence of the air relative humidity on the two fungi's colonies development it was noticed that in both cases the values higher than 75% favor the formation of conidia, the vegetative mass becomes dense, felty, and the fructifications are numerous.

Related to the artificial infections with *C. beticola* it was established that the duration of the incubation depending on temperature, at higher vales of the atmospheric humidity, was of 2 days, in the 18 – 28°C temperature interval.

Knowing these elements a prognosis model can be established related to the attack of the two fungi in field conditions, anticipating a prevalence of the *R. beticola* for the first part of the crop's vegetation, and of the *C. beticola* in the second half of the sugar beet crop's vegetation.

For the other studied parameters (the influence of pH values, of light and plastic and energetic sources from the crop medium) the optimal values were established and they will be used in cultivating the fungi colonies used for the tests under laboratory conditions of the phytosanitary use products destined to control them.

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Fig. 1 *Cercospora* Leaf Spot disease on sugar beet leaves



Fig. 2 *Ramularia beticola* symptoms of attack on sugar beet leaves

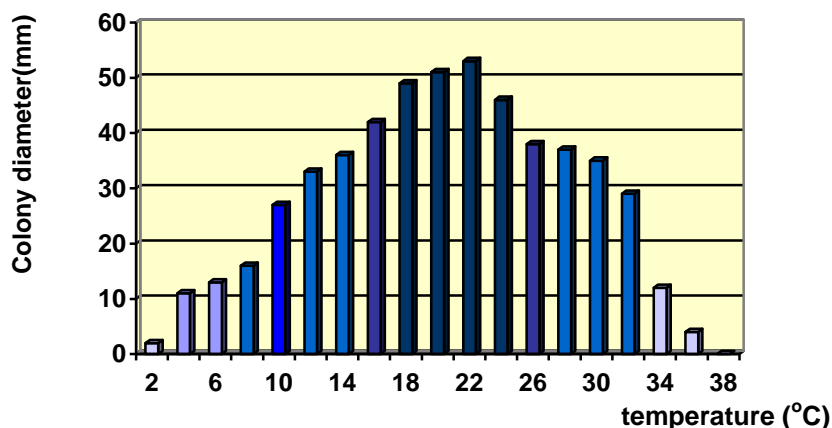


Fig. 3 The influence of the temperature on the *Cercospora beticola* colonies development

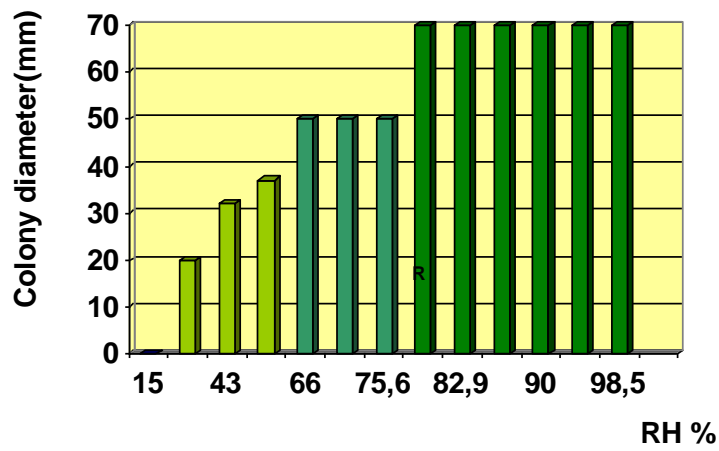


Fig. 4 The influence of the relative humidity on the *Cercospora beticola* colonies development

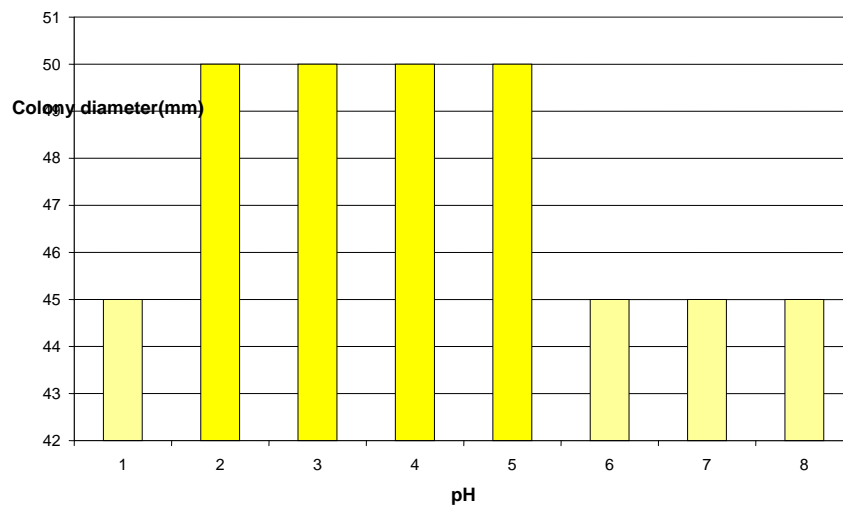


Fig. 5 The influence of the pH on the *Cercospora beticola* colonies development

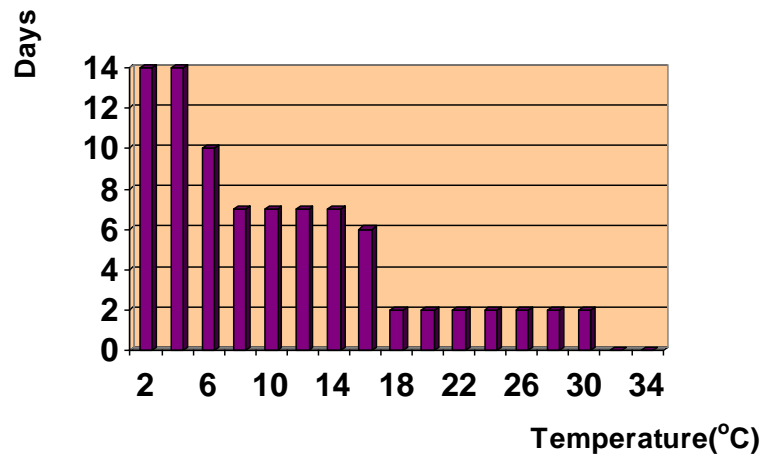


Fig. 6 The establishment of the incubation duration in the artificial infections depending on the temperature

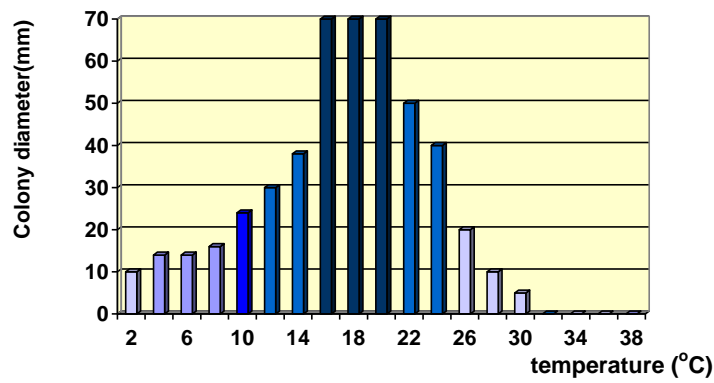


Fig. 7 The influence of the temperature on the *Ramularia beticola* colonies development

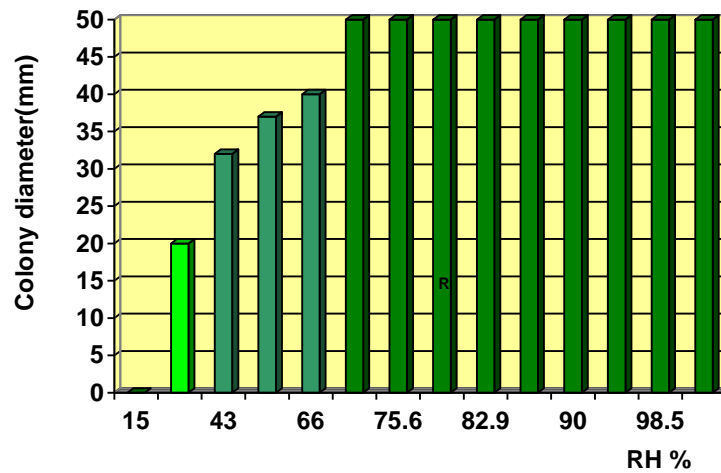


Fig. 8 The influence of the relative humidity on the *Ramularia beticola* colonies development

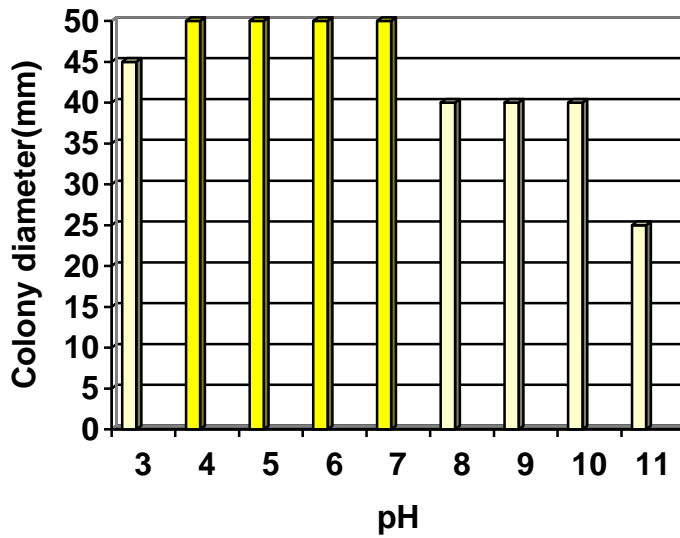


Fig. 9 The influence of the pH on the *Ramularia beticola* colonies development

The growth of *Cercospora beticola* fungus colonies on various carbon sources

Table 1

Carbon sources	<i>Cercospora beticola</i> colonies development
Monosaccharidae	
Glucose, dextrose, fructose, maltose, mannose, trehalose, arabinose, manit, levulose and ribose	Vegetative mass of the formed colony was rich, the mycelium was felty, good sporulation
Polysaccharidae	
Cellulose	Colonies with lower vegetative mass, the mycelium developed in the substrate, with a soft aspect, brown-yellowish colored, lower sporulation
Starch	The mycelium was less developed, colony margins are lobated, very weak pigmentation, conidium arranged concentrically

The growth of *Cercospora beticola* fungus colonies on various azoth sources

Table 2

Azoth sources	<i>Cercospora beticola</i> colonies development
Inorganic compound	
Potassium nitrate	Vegetative mass with felty mycelium of light grey, good sporulation
Ammonium nitrate	Very good vegetative mass, felty and light grey, weaker sporulation
Ammonium phosphate	Fungus developed less vegetative, colonies had a felty aspect, light brown colored, and the conidia did not form
Organic compound	
Urea	Very good vegetative mass, felty and light grey, good sporulation
Asparagine	Rich vegetative mass, felty light grey, good sporulation